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# Genetic Testing

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This Clinical Policy Bulletin addresses genetic testing.

### I. Medical Necessity

A. Aetna considers genetic testing medically necessary to establish a molecular diagnosis of an inheritable disease when all of the following are met:

1. The member displays clinical features, or is at direct risk of inheriting the mutation in question (pre-symptomatic); and
2. The result of the test will directly impact the treatment being delivered to the member; and
3. After history, physical examination, pedigree analysis, genetic counseling, and completion of conventional diagnostic studies, a definitive diagnosis remains uncertain, and one of the following diagnoses is suspected (this list is not all-inclusive); and
4. Disease-specific criteria met.

See Appendix for [List 1](#): Suspected Diagnoses.

### B. Familial Colorectal, Endometrial, or Gastric Cancer Testing

Aetna considers germline genetic testing for familial high risk colorectal cancer, endometrial cancer, or gastric cancer medically necessary when National Comprehensive Cancer Network (NCCN) testing criteria for individual syndromes are met. NCCN testing criteria for individual syndromes are included in NCCN Genetic/Familial High-Risk Assessment: Colorectal, Endometrial, and Gastric (access to NCCN Guidelines is free with registration). The criteria are found in the following sections:

1. Lynch Syndrome- HRS-3

2. Adenomatous Polyposis-POLYP-1
3. Juvenile Polyposis Syndrome- JPS-1
4. Peutz-Jeugers Syndrome- PJS1
5. Hereditary Diffuse Cancer syndrome- HGAST1
6. Serrated Polyposis Syndrome (SPS1)
7. Li-Fraumeni Syndrome and Cowden Syndrome testing criteria are found in the NCCN Genetic/Familial High-Risk Assessment: Breast, Ovarian, and Pancreatic Guidelines.

Note: Genetic testing is considered medically necessary for the syndrome specific gene or genes noted in the NCCN guidelines. Multi-gene panel tests that include additional genes are considered medically necessary only for individuals with a personal or family history of a related cancer, more than one potential inherited cancer syndrome could be responsible for the clinical presentation, and the panel is more efficient than multiple single gene or targeted panel tests. All other uses of multi-gene panel tests, panel tests that include RNA analysis, or polygenic risk scores are considered experimental, investigational, or unproven.

See also [CPB 0516 - Colorectal Cancer Screening \(..../500\\_599/0516.html\)](#).

#### C. Prenatal and Preconception Carrier Screening

Aetna considers prenatal and preconception carrier screening medically necessary when the following criteria are met:

1. Carrier screening: Panels must include cystic fibrosis, spinal muscular atrophy, and/or hemoglobinopathies for individuals/couples who are pregnant or planning a pregnancy; and
2. Results of the test will be used in management of the pregnancy, potentially affected fetus, or for family planning.

Note: Aetna considers genetic carrier testing experimental, investigational, or unproven for all other indications because its effectiveness for indications other than the ones listed above has not been established. Aetna considers repeat carrier screening experimental, investigational, or unproven.

#### D. Specific Genetic Tests:

##### 1. Androgen insensitivity syndrome (AIS):

Aetna considers AR (androgen receptor) gene sequence analysis for AIS as medically necessary for the following indications below. (Testing strategy: Begin with sequence analysis of AR. If pathogenic variant in AR is not identified, gene-targeted deletion/duplication analysis may be considered),

- a. Individual to be tested exhibits signs or symptoms of AIS (e.g., undermasculinization of the external genitalia, impaired spermatogenesis with otherwise normal testes, absent or rudimentary müllerian structures (i.e., fallopian tubes, uterus, and cervix) and results will directly impact clinical management; or
- b. Carrier screening of female reproductive partner planning a pregnancy and has family history of AIS consistent with X-linked inheritance (i.e., affected 46,XY individuals, manifesting heterozygous females (46,XX)); or
- c. Prenatal testing in the offspring of the biological parent with confirmed AR mutation.

Aetna considers genetic testing for AIS experimental, investigational, or unproven for the identification of AR carriers in the general population and for all other indications because there is inadequate evidence in the published peer-reviewed clinical literature regarding its effectiveness.

2. Angelman syndrome:

Aetna considers genetic testing medically necessary to confirm a diagnosis of AS when the following criteria are met:

- a. Presence of gait ataxia and/or tremulous movement of the limbs; and
- b. Presence of severe developmental delay or intellectual disability; and
- c. Presence of severe speech impairment; and
- d. Presence of unique behavior with inappropriate happy demeanor that includes frequent laughing, smiling and excitability.

AS Testing Strategy:

- a. Testing begins with DNA methylation analysis chromosome 15 (15q11-q13)
- b. If DNA methylation analysis is normal, then proceed to UBE3A sequence analysis
- c. If DNA methylation analysis is abnormal, then proceed to deletion/duplication analysis/ FISH/CMA
- d. If deletion/duplication analysis/FISH/CMA is normal, then proceed to uniparental disomy (UPD) study
- e. If UPD is normal, then proceed to imprinting defect (ID) study.

3. Arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C):

Comprehensive genetic testing (DSC2, DSG2, DSP, JUP, PKP2, and TMEM43) is considered medically necessary for members with confirmed or suspected diagnosis of arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C) (i.e., persons who have at least two minor criteria or one major Task Force criteria) (see [Appendix](#)). Mutation-specific genetic testing for ARVC is considered medically necessary for first or second-degree relatives of the index case following the identification of the ARVC-causative mutation in the index case.

Genetic testing for ARVD/C is considered experimental, investigational, or unproven for all other indications.

4. Ashkenazi Jewish testing panel:

Aetna considers medically necessary preconception or prenatal carrier screening for persons of Ashkenazi Jewish ancestry with a panel of genetic tests recommended by the American College of Medical Genetics (ACMG):

- a. Tay Sachs disease
- b. Canavan disease
- c. Cystic fibrosis
- d. Familial dysautonomia
- e. Familial hyperinsulinism
- f. Joubert syndrome
- g. Maple syrup urine disease
- h. Bloom syndrome
- i. Fanconi anemia
- j. Niemann-Pick disease
- k. Gaucher disease
- l. Glycogen storage disease type 1
- m. Mucolipidosis IV
- n. Nemaline myopathy.

If only one partner is of Ashkenazi Jewish ancestry, then testing of that partner is considered medically necessary. Testing of the other partner is considered medically necessary only if the result of testing of the Ashkenazi Jewish partner is positive.

5. Autosomal dominant polycystic kidney disease (ADPKD):

Genetic testing for ADPKD (PKD1, PKD2) is considered medically necessary for adults with multiple cysts on appropriate imaging studies (ultrasound, CT or MRI) in persons who are at high risk for rapid progression to end-stage renal disease (ESRD) as determined by their treating nephrologist who are being considered for tolvaptan (Jynarque).

6. CADASIL:

Aetna considers DNA testing for CADASIL medically necessary for either of the following indications:

- a. Pre-symptomatic individuals where there is a family history consistent with an autosomal dominant pattern of inheritance and there is a known mutation in an affected member of the family; or
- b. Symptomatic individuals who have a family history consistent with an autosomal dominant pattern of inheritance of this condition (clinical signs and symptoms of CADASIL include stroke, cognitive defects and/or dementia, migraine, and psychiatric disturbances).

Aetna considers CADASIL genetic testing experimental, investigational, or unproven for all other indications because its effectiveness for indications other than the ones listed above has not been established.

7. Catecholaminergic polymorphic ventricular tachycardia (CPVT):

Aetna considers genetic testing for CPVT medically necessary for the following indications:

- a. Member has either of the following:
  - i. A first-degree relative (i.e., parent, full-sibling, child) with a confirmed CPVT mutation (Note: Test for known familial mutation); or
  - ii. A structurally normal heart and presents with syncope or cardiac arrest due to VT or ventricular fibrillation (VF) precipitated by exercise-, catecholamine, or emotional stress; and
- b. Testing will be targeted to genes associated with CPVT (e.g., RYR2, CASQ2, TRDN, TECRL, CALM1, CALM2, CALM3). Note: A cardiac ion channelopathy genomic sequencing panel followed if negative by reflex duplication/deletion gene analysis panel is considered an equally acceptable alternative to serial single-gene testing for this indication.

8. Corneal dystrophy:

Aetna considers genetic testing of the TGFB1 (transforming growth factor, beta-induced) gene medically necessary for the following indications:

- a. Members have been evaluated by an ophthalmologist and corneal dystrophy is suspected; and
- b. Member has a parent affected with corneal dystrophy or is a known carrier of a TGFB1 mutation; and
- c. Results of genetic testing will either:

- i. confirm diagnosis that may not be clear (e.g., granular corneal dystrophy type II (GCD2)) based on clinical findings (e.g., slit lamp exam) and results will directly impact clinical management; or
- ii. affect member's reproductive decisions when individual is the reproductive partner of an individual affected with corneal dystrophy or is a known carrier of a TGFBI mutation.

Aetna considers genetic testing for corneal dystrophy experimental, investigational, or unproven for the identification of TGFBI carriers in the general population and for all other indications because there is inadequate evidence in the published peer-reviewed clinical literature regarding its effectiveness.

9. Cystic fibrosis:

- a. Aetna considers genetic carrier testing for cystic fibrosis medically necessary for members in any of the following groups:
  - i. Persons and their reproductive partners seeking prenatal care; or
  - ii. Persons and their reproductive partners who are planning a pregnancy; or
  - iii. Persons with a family history of cystic fibrosis; or
  - iv. Persons with a first degree relative identified as a cystic fibrosis carrier; or
  - v. Reproductive partners of persons with cystic fibrosis; or
  - vi. Positive newborn screen for CF, or signs and symptoms of CF are present, and sweat chloride test is positive, intermediate, inconclusive or cannot be performed (eg, infant is too young to produce adequate volumes of sweat).

- b. Aetna considers a core panel of 25 mutations that are recommended by the American College of Medical Genetics (ACMG) medically necessary for cystic fibrosis genetic testing. The standard CF transmembrane regulator (CFTR) mutation panel is as follows: (Available at: [American College of Medical Genetics and Genomics \(https://www.acmg.net/\)](https://www.acmg.net/)):

Standard CF Transmembrane Regulator (CFTR) Mutation

ΔF508	ΔI507	G542X	G551D	W1282X	N1303K
R553X	621+1G→T	R117H	1717-1G→A	A455E	R560T
R1162X	G85E	R334W	R347P	711+1G→T	1898+1G→A
2184delA	1078delT	3849+10kbC→T	2789+5G→A	3659delC	I148T
3120+1G→A					

- c. Aetna considers full gene sequencing for cystic fibrosis (CF) medically necessary only in members presenting with a positive newborn screen, bronchiectasis, symptoms of CF, or a positive family history for CF and sweat chloride values in the intermediate range (between 30 and 59 mmol/L) on 2 separate occasions.

Aetna considers genetic carrier testing for cystic fibrosis experimental, investigational, or unproven for all other indications because its effectiveness for indications other than the ones listed above has not been established.

Aetna considers screening for cystic fibrosis mutations that extend beyond the standard mutation panel recommended by the ACMG experimental,

investigational, or unproven.

10. Dilated cardiomyopathy (DCM):

Aetna considers single- or multi-gene genetic testing medically necessary to confirm diagnosis of hereditary DCM diagnosis when either criteria is met:

- a. Member has confirmed DCM with no identifiable cause (e.g., autoimmune disease, drug-related, endocrine disorders, infections, toxins); and
  - i. Testing will be targeted to genes related to DCM (e.g., BAG3, DSP, FLNC, LMNA, MYH7, RBM20, TNNT2, TTN); and
  - ii. Member meets any of the following:
    - Member has cardiac conduction disease (first-, second-, or third-degree block); or
    - Sudden unexplained cardiac death in a first- or second-degree blood relative 40 years of age or younger; or
    - Testing will inform prognosis, treatment selection, or reproductive management; or
- b. Cascade genetic testing in member considered at-risk for DCM when all of the following criteria are met:
  - i. First-degree relative has confirmed hereditary DCM established by genetic testing or had sudden unexplained cardiac death prior to age 40; and
  - ii. Testing will be targeted to genes related to DCM; and
  - iii. Testing will inform prognosis, treatment selection, or reproductive management.

11. Duchenne muscular dystrophy and Becker muscular dystrophy:

Aetna considers DMD gene testing medically necessary when the following criteria are met:

- a. Carrier screening when the individual to be tested is an asymptomatic female and has an affected blood relative in whom a disease-causing DMD or BMD mutation has been identified. (Testing Strategy: Test for known mutation); or
- b. Individual to be tested\* exhibits characteristic features of DMD or BMD (eg, progressive symmetric muscular weakness (proximal greater than distal) often with enlargement of calf muscles, wheelchair dependency before age 13 years for DMD and after age 16 years for BMD); and individual has elevated serum creatine kinase (CK) concentration. (Note: Normal value ranges may vary slightly among different labs. Some labs use different measurements or test different samples. In males with DMD, serum CK levels are >10 times normal and in BMD five times normal. Some female carriers of DMD or BMD have levels two to 10 times normal).

\* Note on Testing Strategy: Testing begins with deletion/duplication analysis of DMD gene. Sequence analysis of DMD gene is considered medically necessary for individuals with a negative deletion/duplication result.

12. Ehlers-Danlos syndrome:

Aetna considers sequence analysis\*\* of COL3A1 gene for EDS vascular type when the following criteria are met:

- a. Asymptomatic individuals with a first-degree blood relative (ie, parent, full-sibling, child) who has been diagnosed with EDS vascular type in whom the

- disease-causing mutation has been identified; or
- b. Symptomatic individuals to confirm a diagnosis of EDS vascular type when the following criteria are met:

i. Presence or history of at least one of the following:

- Arterial rupture; or
- First-degree blood relative (ie, parent, full-sibling, child) diagnosed with EDS vascular type; or
- Intestinal rupture; or
- Uterine rupture during pregnancy; or

ii. Presence or history of at least two of the following:

- Acrogeria (aged appearance to extremities, particularly hands); or
- Arteriovenous carotid cavernous sinus fistula; or
- Characteristic facial appearance (thin lips and philtrum, small chin, thin nose, large eyes); or
- Chronic joint subluxations/dislocations; or
- Clubfoot; or
- Congenital dislocation of the hips; or
- Early-onset varicose veins; or
- Easy bruising (spontaneous or with minimal trauma); or
- Gingival recession; or
- Hypermobility of small joints; or
- Pneumothorax/pneumohemothorax; or
- Tendon/muscle rupture; or
- Thin, translucent skin (especially noticeable on chest/abdomen).

\*\*Note: Testing begins with sequence analysis of COL3A1 gene. Biochemical (protein-based) testing may be considered for individuals with a negative sequencing result or when a sequence variant of unknown significance (VUS) is found.

Genetic testing for EDS is considered experimental, investigational, or unproven for all other indications, including the following:

- a. An at-risk (unaffected) individual when an affected family member has been tested for mutations and received a result of VUS (also known as unclassified variant or variant of uncertain significance); or
- b. Deletion/duplication analysis of COL3A1 gene; or
- c. EDS, arthrochalasia (COL1A1, COL1A2 genes); or
- d. EDS, dermatosparaxis (ADAMTS2 gene); or
- e. EDS, hypermobility type (TNXB gene); or
- f. EDS, kyphoscoliotic type (PLOD1); or
- g. EDS, classic type (COL5A1 and COL5A2 genes); or
- h. General population screening.

13. Factor V Leiden:

Aetna considers Factor V Leiden genetic testing medically necessary for members with an abnormal activated protein C (APC) resistance assay result~~sss~~ and any of the following indications:

- a. Asymptomatic female who is planning pregnancy or is currently pregnant and not taking anticoagulation therapy and either of the following:
  - i. First-degree blood relative (ie, parent, full-sibling, child) with a history of high-risk thrombophilia (eg, antithrombin deficiency, double

- heterozygosity or homozygosity for FVL or prothrombin G20210A); or
- ii. First-degree blood relative (ie, parent, full-sibling, child) with venous thromboembolism (VTE) before age 50 years; or
  - b. First unprovoked (eg, from an unknown cause) VTE at any age (especially age less than 50 years); or
  - c. Individual with a first VTE AND a first-degree blood family member (ie, parent, full-sibling, child) with a VTE occurring before age 50 years; or
  - d. Individual with history of recurrent VTE; or
  - e. Venous thrombosis at unusual sites (eg, cerebral, mesenteric, portal and hepatic veins); or
  - f. VTE associated with the use of oral contraceptives or hormone replacement therapy (HRT); or
  - g. VTE during pregnancy or the puerperium; or
  - h. For Factor V Leiden genetic testing for recurrent pregnancy loss, see [CPB 0348 - Recurrent Pregnancy Loss \(./300\\_399/0348.html\)](#).

§§§ The requirement to have an abnormal activated protein C (APC) resistance assay result is waived if the member is on direct oral anticoagulants (e.g., apixaban [Eliquis], dabigatran [Pradaxa], rivaroxaban [Xarelto], or edoxaban [Savaysa]).

Aetna considers Factor V Leiden genetic testing experimental, investigational, or unproven for all other indications because its effectiveness for indications other than the ones listed above has not been established.

14. Familial hypercholesterolemia:

Based upon a consensus statement on familial hypercholesterolemia (FH) from the European Atherosclerosis Society (Nordestgaard, et al., 2013), Aetna considers genetic testing for familial hypercholesterolemia medically necessary in persons who meet any of the following criteria:

- a. Persons with a definite or probable diagnosis of FH (DLCN > 5) \*\*\*; or
- b. First degree relative with a causative FH mutation\*\*\*; or
- c. Plasma total cholesterol  $\geq 310$  mg/dL ( $> 8$  mmol/L) in an adult or adult family member(s)† or  $> 230$  mg/dL ( $> 6$  mmol/L) in a child or child family member(s)† ); or
- d. Premature clinical CHD†† in the subject or family member(s)†; or
- e. Tendon xanthomas in the subject or family member(s)†; or
- f. Sudden premature cardiac death in a family member†.

Note: Genetic testing for FH includes LDL-R mutations (approximately 75% of FH mutations), Apo-B mutations (approximately 20%) and PCSK9 mutations (approximately 5%).

\*\*\* The European Atherosclerosis Society recommends using the DLCN criteria (available at [European Heart Journal \(http://eurheartj.oxfordjournals.org/content/34/45/3478.long\)](#)) to establish the clinical diagnosis of FH. Among individuals with a definite or probable diagnosis of FH (DLCN > 5), and particularly those with an obvious clinical diagnosis with xanthoma and/or high cholesterol plus a family history of premature CHD, molecular genetic testing is strongly recommended. When a causative mutation is found in the index case, a genetic test should be offered to all first-degree relatives

† In cases of probable or definite FH, cascade screening using LDL cholesterol measurement in the family should be conducted and the subject referred for genetic testing if available, with subsequent cascade testing in the family if a causative mutation is found. Initial family members to be tested are biological

first-degree relatives, namely parents, siblings, and children. Biological second-degree relatives including grandparents, grandchildren, uncles, aunts, nephews, nieces, and half-siblings should also be considered.

†† Premature CHD signifies CHD before age 55 in males and before age 60 in female first-degree relatives, while in second-degree relatives, the corresponding ages are 50 and 55. Clinical CHD is defined by a history of an AMI, silent MI, unstable angina, coronary revascularization procedure (PCI or CABG) or clinically significant atherosclerotic cardiovascular disease diagnosed by invasive or noninvasive testing (such as coronary angiography, stress test using treadmill, stress echocardiography, or nuclear imaging).

15. Familial hypocalciuric hypercalcemia:

Aetna considers familial hypocalciuric hypercalcemia (FHH) medically necessary in any of the following:

- a. Atypical cases where no family members are available for testing; or
- b. Families with familial isolated hyperparathyroidism; or
- c. Infants or children under 10 years of age in whom neonatal hyperparathyroidism, neonatal severe hyperparathyroidism, and FHH are the commonest causes of parathyroid hormone-dependent hypercalcemia; or
- d. Individuals with overlap in the calcium/creatinine (Ca/Cr) clearance ratio, namely between 0.01 and 0.02; or
- e. Individuals with the phenotype of FHH whose parents are both normocalcemic (i.e., FHH possibly caused by a de novo CaSR mutation).

16. Familial nephrotic syndrome (NPHS1, NPHS2):

- a. Aetna considers genetic testing for an NPHS1 mutation medically necessary for children with congenital nephrotic syndrome (nephrotic syndrome appearing within the first month of life) who are of Finnish descent or who have a family history of congenital nephrotic syndrome. Genetic testing for NPHS1 mutations are considered experimental, investigational, or unproven for screening other persons with nephrotic syndrome and for all other indications because its effectiveness for other indications other has not been established.
- b. Aetna considers genetic testing for an NPHS2 mutation medically necessary for children with steroid resistant nephrotic syndrome (SRNS) and for children who have a family history of SRNS. Genetic testing for NPHS2 is considered experimental, investigational, or unproven for persons with steroid-responsive nephrotic syndrome and for all other indications because its effectiveness for indications other than the ones listed above has not been established.

Aetna considers genetic testing for familial nephrotic syndrome experimental, investigational, or unproven for all other indications.

17. Fragile X:

- a. Aetna considers genetic testing of the FMR1 gene medically necessary for members in any of the following risk categories where the results of the test will affect a member's clinical management or reproductive decisions:
  - i. Individuals with developmental delay/intellectual disability, autism or primary ovarian insufficiency (POI)†† (also known as premature ovarian failure); or
  - ii. Individual with progressive cerebellar ataxia and intention tremor in individuals older than 50 years of age;

iii. Individuals planning a pregnancy who have either of the following:

- A family history of fragile X syndrome, or
- A family history of unexplained developmental delay/intellectual disability, autism or primary ovarian insufficiency (POI)<sup>†††</sup>; or

iv. Fetuses of known carrier mothers. Prenatal testing of a fetus by amniocentesis or chorionic villus sampling is indicated following a positive Fragile X carrier test in the mother.

<sup>†††</sup> POI is defined as female younger than 40 years of age with FSH levels in the postmenopausal range and at least three months of amenorrhea, oligomenorrhea or dysfunctional uterine bleeding.

b. The following is considered medically necessary:

Fragile X DNA testing for members with:

- i. a negative cytogenetic test for fragile X if they have any physical or behavioral characteristics of fragile X syndrome and have a family history of fragile X syndrome or undiagnosed developmental delay/intellectual disability;
- ii. a phenotype that is not typical for fragile X syndrome who have a cytogenetic test that is positive for fragile X.

Aetna considers population-based fragile X syndrome screening of individuals who are not in any of the above-listed risk categories experimental, investigational, or unproven because its effectiveness for indications other than the ones listed above has not been established.

18. Hereditary ataxia panel:

Aetna considers a hereditary ataxia panel medically necessary when the following criteria are met:

- a. Member has signs and symptoms of a hereditary ataxia; and
- b. Other causes of ataxia have been considered and ruled out, if possible (e.g., head trauma, alcoholism, vitamin deficiencies (such as low vitamin B12), multiple sclerosis, stroke, brain tumors, ear infections); and
- c. Clinical presentation, including completed additional lab and clinical testing, does not fit a well-described syndrome for which single-gene testing or a symptom-based small targeted panel is available; and
- d. If there is a known hereditary ataxia pathogenic variant in the family, single-gene testing for that pathogenic variant has been performed and is negative.

19. Hereditary hearing loss panel:

Aetna considers genetic testing medically necessary to confirm diagnosis of hereditary hearing loss when all of the following criteria are met:

- a. Environmental causes has been ruled out and etiology remains unclear; and
- b. Testing will be targeted to genes related to hereditary hearing loss (e.g., OtoGenome, OtoSeq); and
- c. Testing will inform prognosis, treatment selection, or reproductive management.

20. Hereditary hemochromatosis:

Aetna considers genetic testing for HFE gene mutations medically necessary for persons who meet any of the following criteria:

- a. Member who has symptoms consistent with iron overload and member who has 2 consecutive transferrin saturations of 45% or more or 2 consecutive or elevated ferritins (ie, >200 ng/mL in men; >150 ng/mL in women); or
- b. Member has a first degree blood relative (i.e., parent, full-sibling, child) diagnosed with hereditary hemochromatosis; or
- c. Member has a first degree blood relative with known HFE sequence variants consistent with HH.

Genetic testing for hereditary hemochromatosis is considered experimental, investigational, or unproven for general population screening and for all other indications because its effectiveness for indications other than the ones listed above has not been established.

21. Hereditary hemoglobinopathies:

Aetna considers genetic testing for hemoglobinopathies and thalassemias (includes, but not limited to: Sickle Cell Anemia [HBB Gene], Alpha Thalassemia [HBA1/HBA2 Genes] and Beta Thalassemia [HBB Gene]) medically necessary for persons planning pregnancy or at the initial prenatal visit if no prior testing results are available for interpretation. Hemoglobinopathy testing may be performed using hemoglobin electrophoresis or molecular genetic testing (e.g., carrier screening that includes sickle cell disease [SCD] and other hemoglobinopathies). The use of non-invasive prenatal diagnosis for SCD with cell-free fetal DNA is considered experimental, investigational, or unproven; and currently not recommended by the American College of Obstetricians and Gynecologists (ACOG).

22. Hereditary hemorrhagic telangiectasia (HHT) (Osler-Weber-Rendu syndrome)

Aetna considers genetic testing to confirm diagnosis of HHT medically necessary when the following criteria is met:

- a. Member is a child or young adult who meet all of the following criteria:
  - i. Member is asymptomatic; and
  - ii. Biological parent has confirmed diagnosis of HHT; and
  - iii. Testing will be targeted to disease-causing mutations in the ACVRL1, ENG, GDF2, or SMAD4 gene; or
- b. Member has not previously had genetic testing for HHT and meets all of the following criteria:
  - i. Member has two or more of the following:
    - Cutaneous or mucosal telangiectasias
    - Spontaneous and recurrent epistaxis
    - Multiple mucocutaneous telangiectasia at characteristic sites
    - Visceral involvement (e.g., gastrointestinal telangiectasia; pulmonary, cerebral, or hepatic arteriovenous malformations [AVMs])
    - A first-degree blood relative (i.e., parent, sibling, child) with HHT; and
  - ii. Testing will be targeted to disease-causing mutations in the ACVRL1, ENG, GDF2, or SMAD4 gene; and
  - iii. Testing will inform prognosis, treatment selection, or reproductive management.

23. Hereditary pancreatitis (PRSS1):

Aetna considers genetic testing for hereditary pancreatitis (PRSS1 mutation) medically necessary in symptomatic persons with any of the following indications:

- a. A family history of pancreatitis in a first-degree (parent, sibling, child) or second-degree (aunt, uncle, grandparent) relative; or
- b. An unexplained episode of documented pancreatitis occurring in a child that has required hospitalization, and where there is significant concern that hereditary pancreatitis should be excluded; or
- c. Recurrent (2 or more separate, documented episodes with hyperamylasemia) attacks of acute pancreatitis for which there is no explanation (anatomical anomalies, ampullary or main pancreatic strictures, trauma, viral infection, gallstones, alcohol, drugs, hyperlipidemia, etc.); or
- d. Unexplained (idiopathic) chronic pancreatitis.

This policy is based upon guidelines from the Consensus Committees of the European Registry of Hereditary Pancreatic Diseases, the Midwest Multi-Center Pancreatic Study Group and the International Association of Pancreatology (Ellis et al, 2001).

Aetna considers genetic testing for hereditary pancreatitis experimental, investigational, or unproven for all other indications because its effectiveness for indications other than the ones listed above has not been established.

24. Huntington disease

Aetna considers genetic testing for Huntington disease (HD) medically necessary for either of the following indications:

- a. Predictive testing for CAG repeat length in asymptomatic individuals from families in which there is a history of HD to define risk of transmission; or
- b. Prenatal testing for CAG repeat length in fetuses from families in which there is a history of HD.

Genetic testing for Huntington disease is considered experimental, investigational, or unproven for indications other than those listed above.

25. Hypertrophic cardiomyopathy (HCM):

Aetna considers single- or multi-gene genetic testing medically necessary to confirm diagnosis of hereditary HCM diagnosis when either criteria is met:

- a. Member has confirmed HCM with no identifiable cause (e.g., hypertension, infiltrative disease, metabolic and endocrine disorders, neuromuscular disorders, valvular disease); and
  - i. Testing will be targeted to genes related to HCM (e.g., MYBPC3, MYH7, MYL2, MYL3, TNNI3, TNNT2); and
  - ii. Member meets either of the following:
    - Sudden unexplained cardiac death in a first-degree blood relative 40 years of age or younger; or
    - Testing will inform prognosis, treatment selection, or reproductive management; or
- b. Cascade genetic testing in member considered at-risk for HCM when all of the following criteria are met:

- i. First-degree relative has confirmed hereditary HCM established by genetic testing, had sudden unexplained cardiac death (SCD) prior to age 40, or SCD occurred at any age with an established diagnosis of HCM; and
- ii. Testing will be targeted to genes related to HCM; and
- iii. Testing will inform prognosis, treatment selection, or reproductive management.

Aetna considers genetic testing for HCM experimental, investigational, or unproven for all other indications because its effectiveness for indications other than the one listed above has not been established.

26. Hypophosphatasia:

Aetna considers genetic testing for a pathogenic variant in ALPL medically necessary for confirmation of the diagnosis of hypophosphatasia.

27. Inherited bone marrow failure syndromes (IBMFSs) panel:

Aetna considers an inherited bone marrow failure syndromes (IBMFSs) panel medically necessary for the evaluation of unexplained bone marrow failure with cytopenia when all of the following criteria are met:

- a. The member exhibits single or multi-lineage cytopenias, aplastic anemia, or myelodysplastic syndrome; and
- b. The member and family history have been evaluated by a Board-Certified or Board-Eligible Medical Geneticist; and
- c. Alternate etiologies have been considered and ruled out when possible (e.g., environmental exposure, injury, infection); and
- d. Clinical presentation does not fit a well-described syndrome for which single-gene testing is available; and
- e. If there is a known inherited bone marrow failure pathogenic variant in the family, single-gene testing for that pathogenic variant has been performed and is negative.

28. Interstitial lung disease (ILD):

Aetna considers genetic testing of SP-C and ABCA3 mutations medically necessary for interstitial lung disease (ILD) in infants presenting with acute respiratory failure in the absence of other explanations, or in older children with chronic presentation or family history of ILD, especially if the diagnostic imaging (radiographic patterns or bronchoscopic findings) is consistent with ILD.

29. Kennedy disease (spinal and bulbar muscular atrophy [SBMA]):

Aetna considers AR (androgen receptor) CAG trinucleotide repeat gene testing for SBMA medically necessary for the following indications:

- a. Individual to be tested exhibits signs or symptoms of SBMA (e.g., gynecomastia, fasciculation of the tongue, lips or perioral region, dysarthria, dysphagia, muscle weakness of the limbs) and results will directly impact clinical management; or
- b. Carrier screening of female reproductive partner planning a pregnancy and has family history of SBMA; or
- c. Prenatal testing in the offspring of the biological parent with confirmed AR CAG trinucleotide repeat mutation.

Aetna considers genetic testing for SBMA experimental, investigational, or unproven for the identification of AR carriers in the general population and for all other indications because there is inadequate evidence in the published peer-

reviewed clinical literature regarding its effectiveness.

30. Left ventricular noncompaction (LVNC):

Aetna considers genetic testing to confirm LVNC medically necessary when all the following criteria are met:

- a. Member is symptomatic with evidence of LVNC (e.g., echocardiogram); and
- b. Testing will be targeted to genes common in LVNC (i.e., MYH7, MYBPC3, and TTN); and
- c. Testing will inform prognosis, treatment selection, or reproductive management.

Aetna considers mutation-specific genetic testing medically necessary for first degree relatives of persons with a known disease-causing gene variant.

31. Legius syndrome:

Aetna considers genetic testing for Legius syndrome (SPRED1) medically necessary when all of the following criteria are met:

- a. Member has multiple cafe-au-lait spots; and
- b. Member does not meet diagnostic criteria for neurofibromatosis type 1 (NF1); and
- c. Testing will inform prognosis, treatment selection, or reproductive management.

32. Loeys-Dietz syndrome (LDS):

Aetna considers TGFBR1 and TGFBR2 gene testing for LDS medically necessary when the following criteria are met:

- a. Asymptomatic individual who has an affected first-degree blood relative (i.e., parent, full-sibling, child) with a known deleterious or suspected deleterious mutation (Testing strategy: Test for known familial mutation); or
- b. To confirm or establish a diagnosis of LDS in an individual with characteristics of LDS (eg, aortic/arterial aneurysms/tortuosity, arachnodactly, bicuspid aortic valve and patent ductus arteriosus, blue sclerae, camptodactly, cerebral, thoracic or abdominal arterial aneurysms and/or dissections, cleft palate/bifid uvula, club feet, craniosynostosis, easy bruising, joint hypermobility, ocular hypertelorism, pectus carinatum or pectus excavatum, scoliosis, talipes equinovarus, thin skin with atrophic scars, velvety and translucent skin, widely spaced eyes) (Testing strategy: Begin with sequence analysis of TGFBR2. If a mutation is not identified, proceed with sequence analysis of TGFBR1).

33. Long QT syndrome:

Aetna considers genetic testing for familial long QT syndrome (LQTS) medically necessary when acquired causes have been ruled out (e.g., drug-induced, electrolyte abnormalities, eating disorders, coronary artery disease, and bradyarrhythmias) and one of the following criteria is met:

- a. Symptomatic member with a prolonged corrected QT interval (QTc) greater than or equal to 460 ms on electrocardiogram (ECG); or
- b. QTc greater than or equal to 480 ms on repeated ECG with or without symptoms; or
- c. Asymptomatic member without a family history of congenital LQTS but who have serial ECGs with QTc greater than or equal to 460 ms before puberty or

- greater than or equal to 480 ms post-puberty; or
- d. Member has a high probability Schwartz score greater than or equal to 3.5 (see [Appendix](#)); or
  - e. Member has an intermediate probability of congenital LQTS based on family history, ECG findings, and has an intermediate Schwartz score of 1.5 to 3 (see [Appendix](#)); or
  - f. Analysis of specific genes for members with prolonged QTc and a specific diagnosis as follows:
    - i. KCNQ1 and KCNE1 in when Jervell and Lange-Nielsen syndrome is suspected; or
    - ii. CACNA1C when Timothy syndrome is suspected; or
    - iii. KCNJ2 when Andersen-Tawil syndrome is suspected; or
    - iv. TRDN when Triadin knockout syndrome is suspected; or
    - v. CALM1, CALM2, KCNQ1, KCNH2, SCN5A and TRDN genes when Romano Ward syndrome is suspected.

Aetna considers a cardiac ion channelopathy genomic sequencing panel and duplication/deletion gene analysis panel medically necessary alternative to single gene testing.

Aetna considers genetic testing for long QT syndrome experimental, investigational, or unproven for all other indications because its effectiveness for indications other than the ones listed above has not been established.

34. Malignant hyperthermia susceptibility:

Aetna considers genetic testing for malignant hyperthermia susceptibility (MHS) medically necessary for either of the following indications:

- a. Screening clinically confirmed MHS persons for variants in the RYR1 gene that are considered causative for MH by the European Malignant Hyperthermia Group (EMHG) to facilitate predictive testing in at-risk relatives; or
- b. Screening at-risk relatives of persons with clinically confirmed MHS for a known familial variant in the RYR1 gene that is considered causative for MH by the EMHG.

Aetna considers genetic testing for malignant hyperthermia susceptibility (MHS) experimental, investigational, or unproven for all other indications.

35. Marfan syndrome:

Aetna considers FBN1 gene testing for Marfan syndrome (MFS) medically necessary for the following indications:

- a. Marfan syndrome is suspected, but the clinical diagnostic criteria (see Appendix for [List 2 \(appendixlist2\)](#): Clinical Diagnostic Criteria for Marfan Syndrome) have not led to a confirmed diagnosis of Marfan syndrome, and both of the following criteria are met:
  - i. No known FBN1 mutation in the family; and
  - ii. Presence of ectopia lentis with any aortic dilation or significant aortic dilation (Z score greater than or equal to two)/dissection only;

Testing strategy: Begin with sequencing of FBN1 gene. Deletion/duplication analysis of FBN1 gene is considered medically necessary if a mutation is not identified by sequence analysis.

- b. Testing of an asymptomatic individual who has an affected first-degree blood

- relative (i.e., parent, full-sibling, child) with a known deleterious or suspected deleterious mutation. (Testing strategy: Test for known familial mutation); or
- c. The prenatal diagnosis or PGD of Marfan syndrome in the offspring of parents with known disease-causing variants.

Genetic testing for Marfan syndrome (MFS) is considered experimental, investigational, or unproven for any other indications, including but not limited to:

- a. The use of FBN1 gene testing in the diagnostic evaluation of Marfan syndrome in individuals exhibiting only minor features of the condition, according to the Ghent diagnostic criteria;
- b. The use of TGFBR2 gene testing to facilitate the diagnosis of Marfan syndrome in individuals testing negative for FBN1 gene variants;
- c. The use of TGFBR1 gene testing to facilitate the diagnosis of Marfan syndrome in individuals testing negative for FBN1 and TGFBR2 gene variants;
- d. The use of Marfan syndrome gene testing in individuals fulfilling the Ghent diagnostic criteria who will not be using the information for reproductive decision making or facilitating the diagnosis of Marfan syndrome in at-risk relatives.

See Appendix for [List 2: Clinical Diagnostic Criteria for Marfan Syndrome](#) and [Table 1 \(appendixtable1\): Calculation of the Systemic Score for Marfan Syndrome](#).

36. Maturity onset diabetes of the young (MODY):

Aetna considers genetic testing of glucokinase gene (GCK), hepatic nuclear factor 1- $\alpha$  (HNF1- $\alpha$ ), and hepatic nuclear factor 4- $\alpha$  (HNF4- $\alpha$ ) for maturity-onset diabetes of the young (MODY) medically necessary for the diagnosis of MODY2 or MODY3 in persons with hyperglycemia or non-insulin-dependent diabetes who have a family history of abnormal glucose metabolism in at least 2 consecutive generations, with the individual or  $\geq 1$  family members diagnosed before age 25.

Aetna considers genetic testing for maturity-onset diabetes of the young (MODY) experimental, investigational, or unproven for all other indications.

37. Menkes disease:

Aetna considers genetic testing medically necessary for the diagnosis of Menkes disease in children with low serum copper (0 to 55  $\mu\text{g}/\text{dL}$ ) and low serum ceruloplasmin (10 to 160 mg/L) concentrations.

38. Myotonic dystrophy type 1 and type 2:

Aetna considers genetic testing for myotonic dystrophy type 1 (DM1) (DMPK gene) and myotonic dystrophy type 2 (DM2) (CNBP gene) when the following criteria are met:

- a. Individual to be tested exhibits characteristic features of DM1 or DM2 (eg, muscle weakness, muscle pain, myotonia). (Testing Strategy: Targeted mutation analysis of DMPK and/or CNBP gene. CNBP sequence analysis for DM2 is considered experimental, investigational, or unproven); or
  - b. Individual to be tested is asymptomatic; and
    - i. At least 18 years old; and
    - ii. Has an affected first-degree blood relative (ie, parent, full-sibling, child) in whom a disease-causing DM1 or DM2 mutation has been identified.
- (Testing Strategy: Test for known familial mutation); or

- c. Individual is the reproductive partner of an individual affected with or carrier of DM1 or DM2.

39. Neurofibromatosis:

Genetic testing for neurofibromatosis is considered medically necessary for persons who meet the following criteria:

- a. Displays a sign of or has clinical features of the NF; or
- b. Has a 50% risk of inheriting NF (pre-symptomatic); and
- c. A definitive diagnosis remains uncertain despite a complete family/personal history, physical examination and conventional diagnostic studies; and
- d. Confirmation of the diagnosis will impact treatment.

Genetic testing for neurofibromatosis is considered experimental, investigational, or unproven for all other indications.

40. Noonan syndrome:

Aetna considers genetic testing of PTPN11, SOS1, or KRAS genes medically necessary for the diagnosis of Noonan syndrome in persons with characteristic features to assist in reproductive planning.

41. Oculopharyngeal muscular dystrophy (OPMD):

Aetna considers PABPN1 gene testing for OPMD medically necessary for the following indications:

- a. Individual to be tested exhibits signs or symptoms of OPMD (e.g., ptosis, dysphagia, tongue weakness, proximal weakness in the extremities) and results will directly impact clinical management; or
- b. Carrier screening of persons planning a pregnancy and the following criteria are met:
  - i. Individual to be tested has a family history of OPMD or is a known carrier of a PABPN1 mutation; or
  - ii. Individual to be tested is the reproductive partner of an individual affected with OPMD or is a known carrier of a PABPN1 mutation; and
  - iii. Results of genetic testing will affect member's reproductive decisions.

Aetna considers genetic testing for OPMD experimental, investigational, or unproven for the identification of PABPN1 carriers in the general population and for all other indications because there is inadequate evidence in the published peer-reviewed clinical literature regarding its effectiveness.

42. Osteogenesis imperfecta (OI):

Genetic testing for COL1A1 and COL1A2 gene sequencing in the management of osteogenesis imperfecta types I to IV, is considered medically necessary for the following indications:

- a. Genetic testing for sequence variants in COL1A1/2 to confirm the presence of mosaicism in the asymptomatic parent of a child with OI caused by sequence variants in COL1A1/2 for reproductive decision making purposes; or
- b. Preimplantation genetic diagnosis or prenatal diagnosis for sequence variants in COL1A1/2 in persons in which 1 or both reproductive partners have OI caused by sequence variants in COL1A1/2; or
- c. Genetic testing for sequence variants in COL1A1/2 for the diagnosis of OI when clinical and radiological examination and family history provide

- inadequate information for diagnosis of OI; or
- d. Genetic testing for sequence variants in COL1A1/2 in children diagnosed with OI to aid in reproductive planning for unaffected parents seeking to have additional children.

Genetic testing for COL1A1 and COL1A2 gene sequencing is considered experimental, investigational, or unproven in any other circumstances, including, but not limited to:

- a. Testing for sequence variants in COL1A1/2 to confirm diagnosis of OI when clinical and radiological examination and family history provide adequate information for diagnosis of OI.-
- b. Genetic testing for COL1A1/2 is considered experimental, investigational, or unproven for all other indications.

43. Peutz-Jeghers syndrome:

STK11 (LKB1) gene testing may be considered for individuals with a suspected or known clinical diagnosis of Peutz-Jeghers syndrome, or a known family history of a STK11 (LKB1) mutation. Testing may be considered for individuals whose medical and/or family history is consistent with any of the following:

- a. A relative with a known deleterious STK11 (LKB1) gene mutation; or
- b. A clinical diagnosis of PJS based on at least 2 of the following features:
  - i. At least 2 PJS-type hamartomatous polyps of the small intestine;
  - ii. Mucocutaneous hyperpigmentation of the mouth, lips, nose, eyes, genitalia, or fingers;
  - iii. A family history of PJS.

44. Prader-Willi syndrome (PWS):

Aetna considers genetic testing medically necessary to confirm a diagnosis of PWS when the following criteria are met:

- a. Individual is age birth to two years with hypotonia with poor suck; or
- b. Individual is age two to six years with the following characteristics:
  - i. Hypotonia with history of poor suck; and
  - ii. Global developmental delay (GDD); or
- c. Individual is age six to 12 years with the following characteristics:
  - i. History of hypotonia with poor suck (hypotonia often persists); and
  - ii. GDD; and
  - iii. Excessive eating with central obesity if uncontrolled; or
- d. Individual is age 13 years to adulthood with the following characteristics:
  - i. Cognitive impairment, usually mild intellectual disability; and
  - ii. Excessive eating with central obesity if uncontrolled; and
  - iii. Hypothalamic hypogonadism.

PWS Testing Strategy:

- a. Testing begins with DNA methylation analysis
- b. If DNA methylation analysis is abnormal, then proceed to FISH/CMA
- c. If FISH/CMA is normal, then proceed to uniparental disomy (UPD) study
- d. If UPD is normal, then proceed to imprinting defect (ID) study.

45. Primary dystonia (DYT1):

Aetna considers genetic testing for DYT1 medically necessary for the following indications:

- a. Parents of children with an established DYT1 mutation, for purposes of family planning; or
- b. Persons with onset of primary dystonia other than focal cranial-cervical dystonia after age 30 years who have an affected relative with early onset (before 30 years); or
- c. Persons with primary dystonia with onset before age 30 years.

Aetna considers DYT-1 testing experimental, investigational, or unproven for all other indications, including the following because its effectiveness for indications other than the ones listed above has not been established:

- a. Asymptomatic individuals (other than parents of affected children), including those with affected family members (genetic testing for dystonia (DYT-1) is not sufficient to make a diagnosis of dystonia unless clinical features show dystonia); or
- b. Persons with onset of symptoms after age 30 years who either have focal cranial-cervical dystonia; or
- c. Persons with onset of symptoms after age 30 years who have no affected relative with early onset dystonia.

This policy is adapted from guidelines from the European Federation of Neurological Societies.

46. Prothrombin G20210A thrombophilia (F2 Gene):

Aetna considers F2 gene testing for prothrombin G20210A thrombophilia when the following criteria are met:

- a. Asymptomatic female who is planning pregnancy or is currently pregnant and not taking anticoagulation therapy, and either of the following:
  - i. First-degree blood relative (ie, parent, full-sibling, child) with a history of high-risk thrombophilia (eg, antithrombin deficiency, double heterozygosity or homozygosity for FVL or prothrombin G20210A); or
  - ii. First-degree blood relative (ie, parent, full-sibling, child) with VTE before age 50 years; or
- b. First unprovoked (eg, from an unknown cause) VTE at any age (especially age less than 50 years); or
- c. Individual with a first VTE and a first-degree blood family member (ie, parent, full-sibling, child) with a VTE occurring before age 50 years; or
- d. Individual with history of recurrent VTE; or
- e. Venous thrombosis at unusual sites (eg, cerebral, mesenteric, portal and hepatic veins); or
- f. VTE associated with the use of oral contraceptives or hormone replacement therapy (HRT); or
- g. VTE during pregnancy or the puerperium.

Aetna considers F2 gene testing experimental, investigational, or unproven for all other indications because its effectiveness for indications other than the ones listed above has not been established.

47. RFC1 repeat expansion variant analysis (RFC1 repeat expansion test):

Aetna considers RFC1 (replication factor C subunit 1) repeat expansion variant analysis (RFC1 Repeat Expansion Test) medically necessary for persons with adult-onset gait imbalance and impairment of the oculocephalic reflex (doll's eyes or visually enhanced vestibulo-ocular reflex) for whom CANVAS (cerebellar ataxia, neuropathy, and vestibular areflexia syndrome) is suspected.

48. SB3F2:

Aetna considers genetic testing for SF3B2 not medically necessary for evaluation of hemi-facial microsomia (also known as crano-facial microsomia, and Goldenhar syndrome) unless identification of a mutation in the affected child would provide the opportunity to test the parent to determine if there was an increased risk of recurrence in future children.

49. SHOX-related short stature:

Aetna considers genetic testing for SHOX-related short stature medically necessary for children and adolescents with any of the following features:

- a. Above-average body mass index (BMI); or
- b. Cubitus valgus (increased carrying angle); or
- c. Dislocation of the ulna at the elbow; or
- d. Increased sitting height/height ratio; or
- e. Madelung deformity of the forearm; or
- f. Muscular hypertrophy; or
- g. Reduced arm span/height ratio; or
- h. Short or bowed forearm.

Aetna considers genetic testing for SHOX-related short stature experimental, investigational, or unproven for all other indications because its effectiveness for indications other than the ones listed above has not been established.

50. Spinal muscular atrophy:

Aetna considers genetic testing for SMN1 and SMN2 medically necessary for the following indications:

- a. Carrier screening of persons and their reproductive partners who are seeking prenatal care or who are planning a pregnancy; or
- b. Individual to be tested exhibits symptoms of SMA (eg, symmetrical proximal muscle weakness, absent or markedly decreased deep tendon reflexes); or
- c. Carrier screening when the individual to be tested is asymptomatic and any of the following criteria are met:
  - i. Individual has a family history of SMA or SMA-like disease<sup>##</sup>; or
  - ii. Individual has an affected or carrier blood relative in whom a disease-causing SMA mutation has been identified. (Testing Strategy: Test for familial mutation); or
  - iii. Individual is the reproductive partner of an individual affected with or carrier of SMA or SMA-like disease; or
- d. The prenatal diagnosis or preimplantation genetic diagnosis of SMA in the pregnancy of two known carriers.

<sup>##</sup> Note: SMA includes arthrogryposis multiplex congenita-SMA (AMC-SMA), congenital axonal neuropathy (CAN), SMA0, SMA I (Werdnig-Hoffmann disease), SMA II, SMA III (Kugelberg-Welander disease) and SMA IV.

Aetna considers genetic testing for spinal muscular atrophy (SMA) experimental, investigational, or unproven for the identification of SMN1 deletion carriers in the general population and for all other indications because there is inadequate evidence in the published peer-reviewed clinical literature regarding its effectiveness.

51. Spinocerebellar ataxia (SCA):

Aetna considers genetic testing of SCA1 (ATXN1 gene), SCA2 (ATXN2 gene), SCA3 (ATXN3 gene), SCA6 (CACNA1A gene), SCA7 (ATXN7) and DRPLA (ATN1 gene) medically necessary to aid in the diagnosis of SCA when the following criteria are met:

- a. Individual to be tested exhibits signs and symptoms of SCA such as progressive gait and limb incoordination, imbalance, dysarthria and disturbances of eye movements; and
- b. Non-genetic causes of ataxia have been excluded (eg, alcoholism, multiple sclerosis, primary or metastatic tumors or paraneoplastic diseases associated with occult carcinoma of the ovary, breast or lung, vascular disease, vitamin deficiencies) Note: Initially, testing for SCA1, SCA2, SCA3, SCA6, SCA7 and DRPLA are considered medically necessary.

If results are normal, and a high index of suspicion remains for SCA based on clinical findings, testing for the following additional genes is considered medically necessary: SCA5 (SPTBN2); SCA8 (ATXN8/ATXN8OS); SCA10 (ATXN10); SCA12 (PPP2R2B); SCA13 (KCNC3); SCA14 (PRKCG); SCA17 (TBP); and SCA27 (FGF14).

52. Tay-Sachs disease:

Aetna considers genetic testing of the HEXA gene<sup>###</sup> medically necessary for carrier screening for Tay-Sachs disease for persons planning pregnancy or seeking prenatal care when any of the following criteria are met:

- a. Individual to be tested has an abnormal or inconclusive beta-hexosaminidase A enzyme activity; or
- b. Individual to be tested has an affected or carrier family member in whom a mutation has been identified; or
- c. Individual to be tested is of Ashkenazi Jewish descent or the reproductive partner of an individual of Ashkenazi Jewish descent; or
- d. Individual to be tested is the reproductive partner of an individual affected with or carrier of Tay-Sachs disease.

<sup>###</sup> Note: Testing begins with a targeted mutation panel. If negative, sequence analysis may be considered.

53. Thoracic aortic aneurysms and dissections (TAAD):

Aetna considers multigene panel testing medically necessary for members who have confirmed aortic root/ascending aortic aneurysm or aortic dissection and any of the following risk factors for heritable thoracic aortic disease (HTAD):

- a. Thoracic aortic disease (TAD) and syndromic features of Marfan syndrome, Loeys-Dietz syndrome, or vascular Ehlers-Danlos syndrome; or
- b. TAD presenting at age less than 60 years; or
- c. A family history of either TAD or peripheral/intracranial aneurysm in a first- or second-degree relative; or
- d. A history of unexplained sudden death at a relatively young age in a first- or second-degree relative.

Aetna considers genetic testing for TAAD experimental, investigational, or unproven for all other indications because its effectiveness for indications other than the one listed above has not been established.

54. Tuberous sclerosis complex:

Aetna considers genetic testing of TSC1/ TSC2 medically necessary for confirmation of tuberous sclerosis complex.

55. Unverricht-Lundborg disease (EPM1):

Aetna considers CSTB (cystatin B) gene testing for EPM1 medically necessary for the following indications:

- a. Individual to be tested exhibits signs or symptoms of EPM1 (e.g., involuntary, stimulus-sensitive myoclonus, tonic-clonic epileptic seizures, abnormal EEG, marked photosensitivity, ataxia, dysarthria) and results will directly impact clinical management; or
- b. Carrier screening of persons planning a pregnancy and the following criteria are met:
  - i. Individual has a family history of EPM1 or is a known carrier of a CSTB mutation; or
  - ii. Individual is the reproductive partner of an individual affected with EPM1 or is a known carrier of a CSTB mutation; and
  - iii. Results of genetic testing will affect member's reproductive decisions; or
- c. Prenatal testing in the offspring when both biological parents have confirmed CSTB mutations.

Aetna considers genetic testing for EPM1 experimental, investigational, or unproven for the identification of CSTB carriers in the general population and for all other indications because there is inadequate evidence in the published peer-reviewed clinical literature regarding its effectiveness.

56. Versiti aHUS Genetic Evaluation Panel:

Aetna considers Versiti aHUS Genetic Evaluation Panel medically necessary for diagnosis of aHUS.

57. Versiti Autosomal Dominant Thrombocytopenia Panel:

Aetna considers Versiti Autosomal Dominant Thrombocytopenia Panel medically necessary when the following criteria are met:

- a. Member has a family history consistent with a dominantly inherited form of thrombocytopenia and single-gene or targeted testing for that pathogenic variant has been performed and is negative; or
- b. Clinical presentation does not fit a well-described syndrome for which single gene or targeted testing is available; and
- c. Acquired, nongenetic causes have been considered and ruled out, if possible (e.g., environmental exposure, infection).

58. Versiti Congenital Neutropenia Panel:

Aetna considers Versiti Congenital Neutropenia Panel medically necessary to confirm diagnosis of severe congenital neutropenia (SCN) when both criteria are met:

- a. Clinical presentation is consistent with SCN (e.g., recurrent infections/fevers, blood absolute neutrophil count (ANC) less than 500/microL); and
- b. Testing will inform prognosis and treatment selection.

59. von Hippel-Lindau disease (VHL):

Aetna considers VHL gene testing medically necessary for VHL syndrome when the following criteria are met:

- a. Individual to be tested has a first- (ie, parent, full-sibling, child) or second-degree (ie, aunt, uncle, grandparent, grandchild, niece, nephew, half-sibling) blood relative with a known deleterious or suspected deleterious VHL gene mutation (Testing strategy: test for specific familial mutation); or
- b. Individual to be tested has a personal history of one or more of the following (Testing strategy: Testing begins with sequence analysis of the VHL gene. Deletion/ duplication analysis of the VHL gene may be considered for individuals with a negative sequencing analysis result):
  - i. Clear cell renal cell carcinoma
  - ii. Endolymphatic sac tumor
  - iii. Epididymal or adnexal papillary cystadenoma
  - iv. Hemangioblastoma
  - v. Multiple renal and/or pancreatic cysts
  - vi. Pancreatic neuroendocrine tumors
  - vii. Pancreatic serous cystadenomas
  - viii. Pheochromocytoma or paraganglioma
  - ix. Retinal angioma.

60. von Willebrand disease (VWD):

Aetna considers targeted genetic testing for VWD medically necessary for the following indications:

- a. To diagnose type 2B VWD for individuals suspected of type 2A or 2B in need of additional testing; or
- b. For individuals with suspected type 2N VWD in need of additional testing.

61. Whole exome sequencing (WES) or whole genome sequencing (WGS):

- a. Whole exome sequencing (WES) or whole genome sequencing (WGS) is considered medically necessary for the evaluation of unexplained congenital or neurodevelopmental disorder when all of the following criteria are met:
  - i. A genetic etiology is considered the most likely explanation for the phenotype, based on any of the following:
    - a. Multiple congenital abnormalities affecting unrelated organ systems; or
    - b. Bilateral sensorineural hearing loss without syndromic findings and targeted hearing loss panel test is negative or inconclusive; or
    - c. Autism spectrum disorder with syndromic features (e.g., congenital anomalies, seizures, severe/profound intellectual disability~~or~~) and comparative genomic hybridization (CGH) or targeted panel testing is inconclusive (For additional information on CGH, see [CPB 0787 Comparative Genomic Hybridization \(CGH\)](#) ([./700\\_799/0787.html](#)); or
  - d. Two of the following criteria are met:
    - i. Structural or functional abnormality affecting at minimum a single organ system;

- ii. Global developmental delay<sup>△</sup>, intellectual disability<sup>○○</sup>, symptoms of a complex neurodevelopmental disorder (e.g., self-injurious behavior, reverse sleep-wake cycles, dystonia, hemiplegia, spasticity, muscular dystrophy), and/or severe neuropsychiatric condition (e.g., schizophrenia, bipolar disorder, Tourette syndrome)
  - iii. Epilepsy (e.g., intractable, early onset, epileptic encephalopathy)
  - iv. Family history strongly suggestive of a genetic etiology, including consanguinity
  - v. Period of unexplained developmental regression (unrelated to epilepsy or autism)
  - vi. Biochemical findings suggestive of an inborn error of metabolism, and
- 
- ii. The member and family history have been evaluated by a Board-Certified or Board-Eligible Medical Geneticist, and
  - iii. Member receives pre- and post-test counseling by an appropriate independent provider (not an employee of the genetics testing laboratory), such as an American Board of Medical Genetics or American Board of Genetic Counseling-certified Genetic Counselor, or an Advanced Practice Nurse in Genetics (APGN) credentialed by either the Genetic Nursing Credentialing Commission (GNCC) or the American Nurses Credentialing Center (ANCC), and
  - iv. Alternate etiologies have been considered and ruled out when possible (e.g., environmental exposure, injury, infection), and
  - v. Clinical presentation does not fit a well-described syndrome for which single-gene or targeted panel testing is available, and
  - vi. WES or WGS is more efficient than the separate single-gene tests or panels that would be recommended based on the differential diagnosis (e.g., genetic conditions that demonstrate a high degree of genetic heterogeneity), and
  - vii. A diagnosis cannot be made by standard clinical work-up, excluding invasive procedures such as muscle biopsy, and
  - viii. WES or WGS is predicted to have an impact on health outcomes, including:
    - a. Guiding prognosis and improving clinical decision-making, which can improve clinical outcome by:
      - i. application of specific treatments as well as withholding of contraindicated treatments for certain rare genetic conditions
      - ii. surveillance for later-onset comorbidities
      - iii. initiation of palliative care
      - iv. withdrawal of care; or
    - b. Reducing diagnostic uncertainty (e.g., eliminating lower-yield testing and additional screening testing that may later be proven unnecessary once a diagnosis is achieved); or
    - c. For persons planning a pregnancy, informing genetic counseling related to recurrence risk and prenatal diagnosis options.
  - b. Family trio testing (WES or WGS of the biologic parents or sibling of the affected child) is considered medically necessary when criteria for WES or WGS of the child are met.
  - c. Data re-analysis of WES or WGS is considered medically necessary where above listed medical necessity criteria for WES or WGS are met at the time the reanalysis is considered.
  - d. Rapid genome sequencing or exome sequencing (rGS) is considered medically necessary for the evaluation of acutely-ill members 21 years of age or younger when all of the above medical necessity criteria for WES or WGS are met.
  - e. Rapid genome sequencing or exome sequencing (rGS) is considered not

medically necessary for any of the following:

- i. Isolated transient neonatal tachypnea
  - ii. Isolated unconjugated hyperbilirubinemia
  - iii. Isolated hypoxic ischemic encephalopathy with clear precipitating event
  - iv. Isolated meconium aspiration
  - v. Isolated prematurity
  - vi. Infection/sepsis with normal response to therapy.
- f. Fetal testing for prenatal diagnosis via amniocentesis, chorionic villus sampling (CVS), or percutaneous umbilical blood sampling (PUBS), using exome or genome sequencing is considered medically necessary when all of the following criteria are met:
- i. The fetus is affected with non-immune hydrops fetalis; and
  - ii. The member's current pregnancy has had a karyotype and/or microarray performed and the results were uninformative; and
  - iii. Alternate etiologies have been considered and ruled out when possible (e.g., environmental exposure, injury, infection, maternal condition).

All other indications for fetal exome or genome testing are considered experimental, investigational, or unproven for evaluation of a fetus during pregnancy or a terminated fetus.

◊ For the purpose of this policy, global developmental delay is defined as members under age 5 years who fail to meet developmental milestones and clinical severity cannot be reliably assessed.

◊◊ For the purpose of this policy, intellectual disability (ID) is characterized by limited attainment in one or more of general mental abilities, such as reasoning, problem solving, planning, abstract thinking, judgment, academic learning, and learning from experience. Individuals require support for in one or more aspects of daily living activities. Individuals with severe/profound ID require support in all aspects of daily living.

Note: The diagnostic yield of WGS after a non-diagnostic WES is negligible and due mostly to a re-analysis effect. In the absence of specific information regarding advances in the knowledge of mutation characteristics and the value of WGS after WES for a specific clinical presentation, WGS after a non-diagnostic WES is considered not medically necessary.

62. Repeat Germline Genetic Testing:

Aetna considers repeating germline genetic test medically necessary if the existing result is inconsistent with the individual's clinical presentation or if the test methodology has changed and may yield a different result from the original report that could impact patient management.

II. Experimental, Investigational, or Unproven

A. Aetna considers genetic testing experimental, investigational, or unproven for any of the following:

- Age-related macular degeneration
- Brugada syndrome
- Choroidal neovascularization (e.g., Retnagene)
- Congenital stationary night blindness
- Coronary artery disease (except testing for familial hypercholesterolemia)
- Costello syndrome (HRAS gene)
- Diamond-Blackfan anemia

- Epidermolytic hyperkeratosis
- Essential tremor
- Facioscapulohumeral muscular dystrophy (FSHD)
- Familial Alzheimer disease
- Familial amyotrophic lateral sclerosis (C9orf72, FUS, TARDBP mutations). For SOD1 mutation, see [CPB 0715 - Pharmacogenetic and Pharmacodynamic Testing \(..700\\_799/0715.html\)](#)
- Familial cold urticaria/familial cold autoinflammatory syndrome
- Familial partial lipodystrophy [FPLD2]
- Frizzled class receptor 6 (FZD6) gene sequencing for diagnosis of nail dystrophy
- Genetic testing CLCN1 for congenital myotonia
- Genetic testing for GNE and VCP for Ehlers-Danlos syndrome and osteogenesis imperfecta
- Genetic testing for interstitial lung disease in adults
- Genetic testing of the titin gene for familial dilated cardiomyopathy
- Genetic testing panels for colon cancer syndromes
- Genetic testing panels for the assessment and treatment of autism and other pervasive developmental disorders other than CGH
- Genetic testing panels for X-linked intellectual disability
- Glioblastoma multiforme
- Glomerulopathy Gene Set
- HADHB for breast cancer
- Hemiplegic migraine (HM)
- Hemophilia C (F11 (Factor XI))
- Heterotaxy
- Klippel-Feil syndrome
- Lactose intolerance
- Malignant melanoma (CDKN2A/p16) (e.g., Melaris)
- May-Hegglin anomaly
- McCune-Albright syndrome
- Mowat-Wilson syndrome (ZEB2 gene)
- MTHFR testing for essential hypertension
- Multiple mitochondrial respiratory chain complex deficiencies
- Myoclonus-dystonia (epsilon-sarcoglycan gene (SCGE) deletion analysis)
- Migrainous vertigo
- Narcolepsy
- Next-generation sequencing for the diagnosis of learning disabilities in children
- Osteoporosis
- Parkinson disease
- Polycystic liver disease
- Seizure disorders other than identified as medically necessary above (e.g., creatine transporter 1 sequencing for testing parents of individuals with seizures; GABRG2 mutations for infantile febrile seizures; Generalized epilepsy with febrile seizures plus (GEFS+))
- Short Multiply Aggregated Sequence Homologies (SMASH) (Marvel Genomics)
- Sleep-walking
- Townes-Brocks syndrome (SALL1 gene)
- Type 2 diabetes (other than MODY)
- Very long chain acylCoA dehydrogenase deficiency (VLCADD)
- von Willebrand factor gene testing (except to diagnose type 2B VWD for individuals suspected of type 2A or 2B in need of additional testing or for individuals suspected of type 2N VWD in need of additional testing).

B. Tests:

- deCODE AF
- deCODE BreastCancer
- deCODE Glaucoma
- deCODE MI

- deCODE PrCa
- deCODE T2
- Epigenetic methylation assay (e.g., EpiSign)
- EpiSEEK test for epilepsy/seizures
- Genetic Addiction Risk Score (GARSPREDX™)
- GeneticsNow Comprehensive
- Home genetic tests
- Lifetime Genomics Risk Assessment for venous thromboembolism (VTE)
- MTHFR genetic testing for risk assessment of hereditary thrombophilia
- Multigene panels to predict the risk of several inherited disorders (other than identified as medically necessary above), including but not limited to hereditary cancer panels (not an all-inclusive list):

- BreastNext
- CancerNext
- CancerNext Expanded
- ColoNext
- Coloseq
- Invitae Common Hereditary Cancers Panel
- Invitae Gastric Cancer Panel
- Invitae Hereditary Cancer Syndromes Panel
- Invitae Hereditary Paraganglioma-Pheochromocytoma Panel
- Invitae Melanoma Panel
- Invitae Melanoma-Pancreatic Cancer Panel
- Invitae Multi-Cancer Panel
- Invitae Pancreatic Cancer Panel
- Invitae Thyroid Cancer Panel
- MelanomaNext (next-generation sequencing panel) for prediction of increased risk for melanoma and other cancers
- MyPhenome Hungry Gut
- myRisk Hereditary Cancer Panel
- OncoGeneDx Comprehensive Cancer Panel
- OncoGeneDx Custom Panel
- OncoGeneDx High/Moderate Risk Panel
- OncoGeneDx Pancreatic Cancer Panel
- OvaNext
- PancNext
- Panexia
- ProstateNow
- VistaSeq Hereditary Cancer Panel

- Nuclear encoded mitochondrial genomic sequencing panel
- Optical genome mapping for cancer and genetic diseases
- OtoSCOPE Genetic Hearing Loss Panel
- Plasminogen activator inhibitor-1 (PAI-1) for inherited thrombophilia
- POLG1 for mitochondrial recessive ataxia syndrome
- PROSTOX ultra
- Septo-optic Dysplasia Spectrum Sequencing Panel (HESX1 (3p14.3), OTX2 (14q22.3), PAX6 (11p13), PROP1 (5q35.3), SOX2 (3q26.33))
- Single nucleotide polymorphisms for breast cancer (Oncovue, Brevagen)
- SLCO1B1 testing for statin induced myopathy
- SLT1 testing for Asperger syndrome
- Versiti Panels (Note: these are different proprietary panels which may evaluate some of the same genes. For information on these panels, see Background section.)

- Versiti Coagulation Disorders Panel
- Versiti Comprehensive Bleeding Disorder Panel
- Versiti Comprehensive Platelet Disorder Panel

- Versiti Fibrinolytic Disorder Panel
- Versiti Inherited Thrombocytopenia Panel
- Versiti Platelet Function Disorder Panel
- Versiti Red Cell Genotyping Panel
- Versiti Thrombosis Panel

- Whole mitochondrial genome sequencing.
- Whole transcriptome sequencing for unexplained constitutional or other heritable disorders or syndromes;

- C. APC messenger RNA (mRNA) sequence analysis (CustomNext + RNA, Ambry Genetics) because its clinical value has not been established;
- D. Factor V HR2 allele DNA mutation analysis because its effectiveness has not been established;
- E. Genetic testing for central core disease (CCD) because there is inadequate evidence in the peer-reviewed published literature regarding its effectiveness.

### III. Policy Limitations and Exclusions

Genetic testing of Aetna members is excluded from coverage under Aetna's benefit plans if the testing is performed primarily for the medical management of other family members who are not covered under an Aetna benefit plan. In these circumstances, the insurance carrier for the family members who are not covered by Aetna should be contacted regarding coverage of genetic testing. Occasionally, genetic testing of tissue samples from other family members who are not covered by Aetna may be required to provide the medical information necessary for the proper medical care of an Aetna member. Aetna covers genetic testing for heritable disorders in non-Aetna members when all of the following conditions are met:

- The information is needed to adequately assess risk in the Aetna member; and
- The information will be used in the immediate care plan of the Aetna member; and
- The non-Aetna member's benefit plan, if any, will not cover the test (a copy of the denial letter<sup>+</sup> from the non-Aetna member's benefit plan must be provided).

+ Aetna may also request a copy of the certificate of coverage from the non-member's health insurance plan if: (i) The denial letter from the non-member's insurance carrier fails to specify the basis for non-coverage; (ii) The denial is based on a specific plan exclusion; or (iii) The genetic test is denied by the non-member's insurance carrier as not medically necessary and the medical information provided to Aetna does not make clear why testing would not be of significant medical benefit to the non-member.

### IV. Related Policies

- [CPB 0189 - Genetic Counseling \(../100\\_199/0189.html\)](#)
- [CPB 0227 - BRCA Testing, Prophylactic Mastectomy, and Prophylactic Oophorectomy \(../200\\_299/0227.html\)](#)
- [CPB 0282 - Noninvasive Down Syndrome Screening \(../200\\_299/0282.html\)](#)
- [CPB 0348 - Recurrent Pregnancy Loss \(../300\\_399/0348.html\)](#)
- [CPB 0349 - Alzheimer's Disease Tests \(../300\\_399/0349.html\)](#)
- [CPB 0464 - Serum and Urine Marker Screening for Fetal Aneuploidy \(../400\\_499/0464.html\)](#)
- [CPB 0516 - Colorectal Cancer Screening \(../500\\_599/0516.html\)](#)
- [CPB 0648 - Autism Spectrum Disorders \(../600\\_699/0648.html\)](#)
- [CPB 0715 - Pharmacogenetic and Pharmacodynamic Testing \(../700\\_799/0715.html\)](#)
- [CPB 0787 - Comparative Genomic Hybridization \(CGH\) \(../700\\_799/0787.html\)](#)

## Applicable CPT / HCPCS / ICD-10 Codes

CPT codes covered if selection criteria are met:

<b>Code</b>	<b>Code Description</b>
Genetic testing for dominant polycystic kidney disease (PKD1 and PKD2) - no specific code:	
0012U	Germline disorders, gene rearrangement detection by whole genome next-generation sequencing, DNA, whole blood, report of specific gene rearrangement(s)
0102U	Hereditary breast cancer-related disorders (eg, hereditary breast cancer, hereditary ovarian cancer, hereditary endometrial cancer), genomic sequence analysis panel utilizing a combination of NGS, Sanger, MLPA, and array CGH, with mRNA analytics to resolve variants of unknown significance when indicated (17 genes [sequencing and deletion/duplication])
0103U	Hereditary ovarian cancer (eg, hereditary ovarian cancer, hereditary endometrial cancer), genomic sequence analysis panel utilizing a combination of NGS, Sanger, MLPA, and array CGH, with mRNA analytics to resolve variants of unknown significance when indicated (24 genes [sequencing and deletion/duplication], EPCAM [deletion/duplication only])
0214U	Rare diseases (constitutional/heritable disorders), whole exome and mitochondrial DNA sequence analysis, including small sequence changes, deletions, duplications, short tandem repeat gene expansions, and variants in non-uniquely mappable regions, blood or saliva, identification and categorization of genetic variants, proband
0215U	Rare diseases (constitutional/heritable disorders), whole exome and mitochondrial DNA sequence analysis, including small sequence changes, deletions, duplications, short tandem repeat gene expansions, and variants in non-uniquely mappable regions, blood or saliva, identification and categorization of genetic variants, proband
0218U	Neurology (muscular dystrophy), DMD gene sequence analysis, including small sequence changes, deletions, duplications, and variants in non-uniquely mappable regions, blood or saliva, identification and characterization of genetic variants
0231U	CACNA1A (calcium voltage-gated channel subunit alpha 1A) (eg, spinocerebellar ataxia), full gene analysis, including small sequence changes in exonic and intronic regions, deletions, duplications, short tandem repeat (STR) gene expansions, mobile element insertions, and variants in non-uniquely mappable regions
0233U	FXN (frataxin) (eg, Friedreich ataxia), gene analysis, including small sequence changes in exonic and intronic regions, deletions, duplications, short tandem repeat (STR) expansions, mobile element insertions, and variants in non-uniquely mappable regions
0234U	MECP2 (methyl CpG binding protein 2) (eg, Rett syndrome), full gene analysis, including small sequence changes in exonic and intronic regions, deletions, duplications, mobile element insertions, and variants in non-uniquely mappable regions
0236U	SMN1 (survival of motor neuron 1, telomeric) and SMN2 (survival of motor neuron 2, centromeric) (eg, spinal muscular atrophy) full gene analysis, including small sequence changes in exonic and intronic regions, duplications and deletions, and mobile element insertions
0237U	Cardiac ion channelopathies (eg, Brugada syndrome, long QT syndrome, short QT syndrome, catecholaminergic polymorphic ventricular tachycardia), genomic sequence analysis panel including

	ANK2, CASQ2, CAV3, KCNE1, KCNE2, KCNH2, KCNJ2, KCNQ1, RYR2, and SCN5A, including small sequence changes in exonic and intronic regions, deletions, duplications, mobile element insertions, and variants in non-uniquely mappable regions
0449U	Carrier screening for severe inherited conditions (eg, cystic fibrosis, spinal muscular atrophy, beta hemoglobinopathies [including sickle cell disease], alpha thalassemia), regardless of race or self-identified ancestry, genomic sequence analysis panel, must include analysis of 5 genes (CFTR, SMN1, HBB, HBA1, HBA2)
81161	DMD (dystrophin) (eg, duchenne/becker muscular dystrophy) deletion analysis, and duplication analysis, if performed
81162	BRCA1 (BRCA1, DNA repair associated), BRCA2 (BRCA2, DNA repair associated) (eg, hereditary breast and ovarian cancer) gene analysis; full sequence analysis and full duplication/deletion analysis (ie, detection of large gene rearrangements)
81177	ATN1 (atrophin 1) (eg, dentatorubral-pallidoluysian atrophy) gene analysis, evaluation to detect abnormal (eg, expanded) alleles
81178	ATXN1 (ataxin 1) (eg, spinocerebellar ataxia) gene analysis, evaluation to detect abnormal (eg, expanded) alleles
81179	ATXN2 (ataxin 2) (eg, spinocerebellar ataxia) gene analysis, evaluation to detect abnormal (eg, expanded) alleles
81180	ATXN3 (ataxin 3) (eg, spinocerebellar ataxia, Machado- Joseph disease) gene analysis, evaluation to detect abnormal (eg, expanded) alleles
81181	ATXN7 (ataxin 7) (eg, spinocerebellar ataxia) gene analysis, evaluation to detect abnormal (eg, expanded) alleles
81182	ATXN8OS (ATXN8 opposite strand [non-protein coding]) (eg, spinocerebellar ataxia) gene analysis, evaluation to detect abnormal (eg, expanded) alleles
81183	ATXN10 (ataxin 10) (eg, spinocerebellar ataxia) gene analysis, evaluation to detect abnormal (eg, expanded) alleles
81184	CACNA1A (calcium voltage-gated channel subunit alpha1 A) (eg, spinocerebellar ataxia) gene analysis evaluation to detect abnormal (eg, expanded) alleles
81185	CACNA1A (calcium voltage-gated channel subunit alpha1 A) (eg, spinocerebellar ataxia) gene analysis; full gene sequence
81186	CACNA1A (calcium voltage-gated channel subunit alpha1 A) (eg, spinocerebellar ataxia) gene analysis; known familial variant
81187	CNBP (CCHC-type zinc finger nucleic acid binding protein) (eg, myotonic dystrophy type 2) gene analysis, evaluation to detect abnormal (eg, expanded) alleles
81200	ASPA (aspartoacylase)(eg, Canavan disease) gene analysis, common variants (eg, E285A, Y231X)
81205	BCKDHB (branched-chain keto acid dehydrogenase E1, beta polypeptide) (eg, Maple syrup urine disease) gene analysis, common variants (eg, R183P, G278S, E422X)
81209	BLM (Bloom syndrome, RecQ helicase-like) (eg, Bloom syndrome) gene analysis, 2281del6ins7 variant
81220 - 81221	CFTR (cystic fibrosis transmembrane conductance regulator) (eg, cystic fibrosis) gene analysis; common variants (eg, ACMG/ACOG guidelines) and with known familial variants
81223	CFTR (cystic fibrosis transmembrane conductance regulator) (eg, cystic fibrosis) gene analysis; full gene sequence
81238	F9 (coagulation factor IX) (eg, hemophilia B), full gene sequence

81240	F2 (prothrombin, coagulation factor II) (eg, hereditary hypercoagulability) gene analysis, 20210G>A variant
81242	FANCC (Fanconi anemia, complementation group C) (eg, Fanconi anemia, type C) gene analysis, common variant (eg, IVS4+4A>T)
81243	FMR1 (Fragile X mental retardation 1) (eg, fragile X mental retardation) gene analysis; evaluation to detect abnormal (eg, expanded) alleles
81244	FMR1 (fragile X mental retardation 1) (eg, fragile X mental retardation) gene analysis; characterization of alleles (eg, expanded size and methylation status)
81250	G6PC (glucose-6-phosphatase, catalytic subunit) (eg, Glycogen storage disease, Type 1a, von Gierke disease) gene analysis, common variants (eg, R83C, Q347X)
81251	GBA (glucosidase, beta, acid) (eg, Gaucher disease) gene analysis, common variants (eg, N370S, 84GG, L444P, IVS2+1G>A)
81252 - 81253	GJB2 (gap junction protein, beta 2, 26kDa; connexin 26) (eg, nonsyndromic hearing loss) gene analysis; full gene sequence
81255	HEXA (hexosaminidase A [alpha polypeptide]) (eg, Tay-Sachs disease) gene analysis, common variants (eg, 1278insTATC, 1421+1G>C, G269S)
81256	HFE (hemochromatosis) (eg, hereditary hemochromatosis) gene analysis, common variants (eg, C282Y, H63D)
81257	HBA1/HBA2 (alpha globin 1 and alpha globin 2 (eg, alpha thalassemia, Hb Bart hydrops fetalis syndrome, HbH disease), gene analysis, for common deletions or variant (eg, Southeast Asian, Thai, Filipino, Mediterranean, alpha3.7, alpha4.2, alpha20.5, and Constant Spring)
81258 - 81259	HBA1/HBA2 (alpha globin 1 and alpha globin 2) (eg, alpha thalassemia, Hb Bart hydrops fetalis syndrome, HbH disease), gene analysis
81260	IKBKAP (inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase complex-associated protein) (eg, familial dysautonomia) gene analysis, common variants (eg, 2507+6T>C, R696P)
81269	HBA1/HBA2 (alpha globin 1 and alpha globin 2) (eg, alpha thalassemia, Hb Bart hydrops fetalis syndrome, HbH disease), gene analysis; duplication/deletion variants
81271	HTT (huntingtin) (eg, Huntington disease) gene analysis; evaluation to detect abnormal (eg, expanded) alleles
81274	HTT (huntingtin) (eg, Huntington disease) gene analysis; characterization of alleles (eg, expanded size)
81284	FXN (frataxin) (eg, Friedreich ataxia) gene analysis; evaluation to detect abnormal (expanded) alleles
81285	FXN (frataxin) (eg, Friedreich ataxia) gene analysis evaluation; characterization of alleles (eg, expanded size)
81286	FXN (frataxin) (eg, Friedreich ataxia) gene analysis; full gene sequence
81288	MLH1 (mutL homolog 1, colon cancer, nonpolyposis type 2) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; promoter methylation analysis
81289	FXN (frataxin) (eg, Friedreich ataxia) gene analysis evaluation; known familial variant(s)
81290	MCOLN1 (mucolipin 1)(eg, Mucolipidosis, type IV) gene analysis, common variants (eg, IVS3-2A>G, del6, 4kb)
81292 - 81294	MLH1 (mutL homolog 1, colon cancer, nonpolyposis type 2) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; full sequence analysis or known familial variants or duplication/deletion variants

81295 - 81297	MSH2 (mutS homolog 2, colon cancer, nonpolyposis type 1) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; full sequence analysis or known familial variants or duplication/deletion variants
81298 - 81300	MSH6 (mutS homolog 6 [E. Coli]) (eg, jeredotaru mpm-polyposis colorectal cancer, Lynch syndrome) gene analysis; full sequence analysis or known familial variants or duplication/deletion variants
81301	Microsatellite instability analysis (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) of markers for mismatch repair deficiency (eg, BAT25, BAT26), includes comparison of neoplastic and normal tissue, if performed
81302	MECP2 (methyl CpG binding protein 2) (eg, Rett syndrome) gene analysis; full sequence analysis
81310	NPM1 (nucleophosmin) (eg, acute myeloid leukemia) gene analysis, exon 12 variants
81317	PMS2 (postmeiotic segregation increased 2 [S. cerevisiae]) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; full sequence analysis or known familial variants or duplication/deletion variants
81318	PMS2 (postmeiotic segregation increased 2 [S. cerevisiae]) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; known familial variants
81319	PMS2 (postmeiotic segregation increased 2 [S. cerevisiae]) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; duplication/deletion variants
81324 - 81326	PMP22 (peripheral myelin protein 22) (eg, Charcot-Marie-Tooth, hereditary neuropathy with liability to pressure palsies) gene analysis; duplication/deletion analysis, full sequence analysis, known familial variant
81329	SMN1 (survival of motor neuron 1, telomeric) (eg, spinal muscular atrophy) gene analysis; dosage/deletion analysis (eg, carrier testing), includes SMN2 (survival of motor neuron 2, centromeric) analysis, if performed
81330	SMPD1 (sphingomyelin phosphodiesterase 1, acid lysosomal) (eg, Niemann-Pick disease, Type A) gene analysis, common variants (eg, R496L, L302P, fsP330)
81331	SNRPN/UBE3A (small nuclear ribonucleoprotein polypeptide N and ubiquitin protein ligase E3A) (eg, Prader-Willi syndrome and/or Angelman syndrome), methylation analysis
81332	SERPINA1 (serpin peptidase inhibitor, clade A, alpha-1 antiproteinase, antitrypsin, member 1) (eg, alpha-1-antitrypsin deficiency), gene analysis, common variants (eg, *S and *Z)
81336	SMN1 (survival of motor neuron 1, telomeric) (eg, spinal muscular atrophy) gene analysis; full gene sequence
81337	SMN1 (survival of motor neuron 1, telomeric) (eg, spinal muscular atrophy) gene analysis; known familial sequence variant(s)
81343	PPP2R2B (protein phosphatase 2 regulatory subunit Bbeta) (eg, spinocerebellar ataxia) gene analysis, evaluation to detect abnormal (eg, expanded) alleles
81344	TBP (TATA box binding protein) (eg, spinocerebellar ataxia) gene analysis, evaluation to detect abnormal (eg, expanded) alleles
81361 - 81364	HBB (hemoglobin, subunit beta) (eg, sickle cell anemia, beta thalassemia, hemoglobinopathy)
81401	Molecular pathology procedure, Level 2 (eg, 2-10 SNPs, 1 methylated

variant, or 1 somatic variant [typically using nonsequencing target variant analysis], or detection of a dynamic mutation disorder/triplet repeat)

81404	Molecular pathology procedure, Level 5 (eg, analysis of 2-5 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 6-10 exons, or characterization of a dynamic mutation disorder/triplet repeat by Southern blot analysis) [covered for glucokinase gene (GCK), hepatic nuclear factor 1- $\alpha$ (HNF1- $\alpha$ ), and hepatic nuclear factor 4- $\alpha$ (HNF4- $\alpha$ ) for maturity-onset diabetes of the young (MODY)]
81405	Molecular pathology procedure, Level 6 (eg, analysis of 6-10 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 11-25 exons, regionally targeted cytogenomic array analysis) [covered for glucokinase gene (GCK), hepatic nuclear factor 1- $\alpha$ (HNF1- $\alpha$ ), and hepatic nuclear factor 4- $\alpha$ (HNF4- $\alpha$ ) for maturity-onset diabetes of the young (MODY)]
81406	Molecular pathology procedure, Level 7 (eg, analysis of 11-25 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 26-50 exons, cytogenomic array analysis for neoplasia [covered for glucokinase gene (GCK), hepatic nuclear factor 1- $\alpha$ (HNF1- $\alpha$ ), and hepatic nuclear factor 4- $\alpha$ (HNF4- $\alpha$ ) for maturity-onset diabetes of the young (MODY)]
81408	Molecular pathology procedure, Level 9 (eg, analysis of >50 exons in a single gene by DNA sequence analysis) ABCA4 (ATP-binding cassette, sub-family A [ABC1], member 4) (eg, Stargardt disease, age-related macular degeneration), full gene sequence ATM (ataxia telangiectasia mutated) (eg, ataxia telangiectasia), full gene sequence CDH23 (cadherin-related 23 [FBN1 sequencing]
81412	Ashkenazi Jewish associated disorders (eg, Bloom syndrome, Canavan disease, cystic fibrosis, familial dysautonomia, Fanconi anemia group C, Gaucher disease, Tay-Sachs disease), genomic sequence analysis panel, must include sequencing of at least 9 genes, including ASPA, BLM, CFTR, FANCC, GBA, HEXA, IKBKAP, MCOLN1, and SMPD1
81413	Cardiac ion channelopathies (eg, Brugada syndrome, long QT syndrome, short QT syndrome, catecholaminergic polymorphic ventricular tachycardia); genomic sequence analysis panel, must include sequencing of at least 10 genes, including ANK2, CASQ2, CAV3, KCNE1, KCNE2, KCNH2, KCNJ2, KCNQ1, RYR2, and SCN5A
81414	Cardiac ion channelopathies (eg, Brugada syndrome, long QT syndrome, short QT syndrome, catecholaminergic polymorphic ventricular tachycardia); duplication/deletion gene analysis panel, must include analysis of at least 2 genes, including KCNH2 and KCNQ1
81434	Hereditary retinal disorders (eg, retinitis pigmentosa, Leber congenital amaurosis, cone-rod dystrophy), genomic sequence analysis panel, must include sequencing of at least 15 genes, including ABCA4, CNGA1, CRB1, EYS, PDE6A, PDE6B, PRPF31, PRPH2, RDH12, RHO, RP1, RP2, RPE65, RPGR, and USH2A
81435	Hereditary colon cancer disorders (eg, Lynch syndrome, PTEN hamartoma syndrome, Cowden syndrome, familial adenomatous polyposis); genomic sequence analysis panel, must include sequencing of at least 10 genes, including APC, BMPR1A, CDH1, MLH1, MSH2, MSH6, MUTYH, PTEN, SMAD4, and STK11
81436	duplication/deletion analysis panel, must include analysis of at least 5 genes, including MLH1, MSH2, EPCAM, SMAD4, and STK11
81439	Hereditary cardiomyopathy (eg, hypertrophic cardiomyopathy, dilated cardiomyopathy, arrhythmogenic right ventricular cardiomyopathy), genomic sequence analysis panel, must include sequencing of at least

	Genomic sequence analysis panel, must include sequencing of at least 5 cardiomyopathy-related genes (eg, DSG2, MYBPC3, MYH7, PKP2, TTN).
81442	Noonan spectrum disorders (eg, Noonan syndrome, cardio-facio-cutaneous syndrome, Costello syndrome, LEOPARD syndrome, Noonan-like syndrome), genomic sequence analysis panel, must include sequencing of at least 12 genes, including BRAF, CBL, HRAS, KRAS, MAP2K1, MAP2K2, NRAS, PTPN11, RAF1, RIT1, SHOC2, and SOS1
81448	Hereditary peripheral neuropathies (eg, Charcot-Marie-Tooth, spastic paraplegia), genomic sequence analysis panel, must include sequencing of at least 5 peripheral neuropathy-related genes (eg, BSCL2, GJB1, MFN2, MPZ, REEP1, SPAST, SPG11, SPTLC1)
83080	b-Hexosaminidase, each assay
88245 - 88269	Chromosome analysis
88271 - 88275	Molecular cytogenetics
88323	Consultation and report on referred material requiring preparation of slides
88341	Immunohistochemistry or immunocytochemistry, per specimen; each additional single antibody stain procedure (List separately in addition to code for primary procedure)
88342	initial single antibody stain procedure
88344	each multiplex antibody stain procedure
88360 - 88361	Morphometric analysis, tumor immunohistochemistry (eg, Her-2/neu, estrogen receptor/progesterone receptor), quantitative or semiquantitative, each antibody; manual or using computer-assisted technology
CPT codes not covered for indications listed in the CPB:	
Frizzled class receptor 6 (FZD6) gene sequencing, Glomerulopathy gene set - no specific code:	
0094U	Genome (eg, unexplained constitutional or heritable disorder or syndrome), rapid sequence analysis
0101U	Hereditary colon cancer disorders (eg, Lynch syndrome, PTEN hamartoma syndrome, Cowden syndrome, familial adenomatous polyposis), genomic sequence analysis panel utilizing a combination of NGS, Sanger, MLPA, and array CGH, with mRNA analytics to resolve variants of unknown significance when indicated (15 genes [sequencing and deletion/duplication], EPCAM and GREM1 [deletion/duplication only])
+0130U	Hereditary colon cancer disorders (eg, Lynch syndrome, PTEN hamartoma syndrome, Cowden syndrome, familial adenomatous polyposis), targeted mRNA sequence analysis panel (APC, CDH1, CHEK2, MLH1, MSH2, MSH6, MUTYH, PMS2, PTEN, and TP53) (List separately in addition to code for primary procedure)
+0136U	ATM (ataxia telangiectasia mutated) (eg, ataxia telangiectasia) mRNA sequence analysis (List separately in addition to code for primary procedure)
+0137U	PALB2 (partner and localizer of BRCA2) (eg, breast and pancreatic cancer) mRNA sequence analysis (List separately in addition to code for primary procedure)
0156U	Copy number (eg, intellectual disability, dysmorphology), sequence analysis [Short Multiply Aggregated Sequence Homologies (SMASH) (Marvel Genomics)]
+0157U	APC (APC regulator of WNT signaling pathway) (eg, familial adenomatous polyposis [FAP]) mRNA sequence analysis[Short Multiply Aggregated Sequence Homologies (SMASH) (Marvel Genomics)]

+0158U	MLH1 (mutL homolog 1) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) mRNA sequence analysis (List separately in addition to code for primary procedure)
+0159U	MSH2 (mutS homolog 2) (eg, hereditary colon cancer, Lynch syndrome) mRNA sequence analysis (List separately in addition to code for primary procedure)
+0160U	MSH6 (mutS homolog 6) (eg, hereditary colon cancer, Lynch syndrome) mRNA sequence analysis (List separately in addition to code for primary procedure)
+0161U	PMS2 (PMS1 homolog 2, mismatch repair system component) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) mRNA sequence analysis (List separately in addition to code for primary procedure)
+0162U	Hereditary colon cancer (Lynch syndrome), targeted mRNA sequence analysis panel (MLH1, MSH2, MSH6, PMS2) (List separately in addition to code for primary procedure) [Short Multiply Aggregated Sequence Homologies (SMASH) (Marvel Genomics)]
0209U	Cytogenomic constitutional (genome-wide) analysis, interrogation of genomic regions for copy number, structural changes and areas of homozygosity for chromosomal abnormalities
0212U	Rare diseases (constitutional/heritable disorders), whole genome and mitochondrial DNA sequence analysis, including small sequence changes, deletions, duplications, short tandem repeat gene expansions, and variants in non-uniquely mappable regions, blood or saliva, identification and categorization of genetic variants, proband
0213U	Rare diseases (constitutional/heritable disorders), whole genome and mitochondrial DNA sequence analysis, including small sequence changes, deletions, duplications, short tandem repeat gene expansions, and variants in non-uniquely mappable regions, blood or saliva, identification and categorization of genetic variants, each comparator genome (eg, parent, sibling)
0260U	Rare diseases (constitutional/heritable disorders), identification of copy number variations, inversions, insertions, translocations, and other structural variants by optical genome mapping
0264U	Rare diseases (constitutional/heritable disorders), identification of copy number variations, inversions, insertions, translocations, and other structural variants by optical genome mapping
0265U	Rare constitutional and other heritable disorders, whole genome and mitochondrial DNA sequence analysis, blood, frozen and formalin-fixed paraffin-embedded (FFPE) tissue, saliva, buccal swabs or cell lines, identification of single nucleotide and copy number variants
0266U	Unexplained constitutional or other heritable disorders or syndromes, tissue-specific gene expression by whole-transcriptome and next-generation sequencing, blood, formalin-fixed paraffin-embedded (FFPE) tissue or fresh frozen tissue, reported as presence or absence of splicing or expression changes
0267U	Rare constitutional and other heritable disorders, identification of copy number variations, inversions, insertions, translocations, and other structural variants by optical genome mapping and whole genome sequencing
0270U	Hematology (congenital coagulation disorders), genomic sequence analysis of 20 genes, blood, buccal swab, or amniotic fluid
0272U	Hematology (genetic bleeding disorders), genomic sequence analysis of 51 genes, blood, buccal swab, or amniotic fluid, comprehensive

0276U	Hematology (inherited thrombocytopenia), genomic sequence analysis of 23 genes, blood, buccal swab, or amniotic fluid
0299U	Oncology (pan tumor), whole genome optical genome mapping of paired malignant and normal DNA specimens, fresh frozen tissue, blood, or bone marrow, comparative structural variant identification
0300U	Oncology (pan tumor), whole genome sequencing and optical genome mapping of paired malignant and normal DNA specimens, fresh tissue, blood, or bone marrow, comparative sequence analyses and variant identification
0413U	Oncology (hematolymphoid neoplasm), optical genome mapping for copy number alterations, aneuploidy, and balanced/complex structural rearrangements, DNA from blood or bone marrow, report of clinically significant alterations
0425U	Genome (eg, unexplained constitutional or heritable disorder or syndrome), rapid sequence analysis, each comparator genome (eg, parents, siblings)
0426U	Genome (eg, unexplained constitutional or heritable disorder or syndrome), ultra-rapid sequence analysis
0454U	Rare diseases (constitutional/heritable disorders), identification of copy number variations, inversions, insertions, translocations, and other structural variants by optical genome mapping
0469U	Rare diseases (constitutional/heritable disorders), whole genome sequence analysis for chromosomal abnormalities, copy number variants, duplications/deletions, inversions, unbalanced translocations, regions of homozygosity (ROH), inheritance pattern that indicate uniparental disomy (UPD), and aneuploidy, fetal sample (amniotic fluid, chorionic villus sample, or products of conception), identification and categorization of genetic variants, diagnostic report of fetal results based on phenotype with maternal sample and paternal sample, if performed, as comparators and/or maternal cell contamination
0529U	Hematology (venous thromboembolism [VTE]), genome-wide single-nucleotide polymorphism variants, including F2 and F5 gene analysis, and Leiden variant, by microarray analysis, saliva, report as risk score for VTE
0534U	Oncology (prostate), microRNA, single-nucleotide polymorphisms (SNPs) analysis by RT-PCR of 32 variants, using buccal swab, algorithm reported as a risk score
81222	CFTR (cystic fibrosis transmembrane conductance regulator) (eg, cystic fibrosis) gene analysis; duplication/deletion variants
81291	MTHFR (5,10-methylenetetrahydrofolate reductase) (eg, hereditary hypercoagulability) gene analysis, common variants (eg, 677T, 1298C)
81312	PABPN1 (poly[A] binding protein nuclear 1) (eg, oculopharyngeal muscular dystrophy) gene analysis, evaluation to detect abnormal (eg, expanded) alleles
81377	HLA Class II typing, low resolution (eg, antigen equivalents); one antigen equivalent, each
81383	HLA Class II typing, high resolution (ie, alleles or allele groups); one allele or allele group (eg, HLA-DQB1*06:02P), each
81410	Aortic dysfunction or dilation (eg, Marfan syndrome, Loeys Dietz syndrome, Ehler Danlos syndrome type IV, arterial tortuosity syndrome); genomic sequence analysis panel, must include sequencing of at least 9 genes, including FBN1, TGFBR1, TGFBR2, COL3A1, MYH11, ACTA2, SLC2A10, SMAD3, and MYLK
81411	duplication/deletion analysis panel, must include analyses for

## IGFBR1, IGFBR2, MYH11, and CUL3A1

81419	Epilepsy genomic sequence analysis panel, must include analyses for ALDH7A1, CACNA1A, CDKL5, CHD2, GABRG2, GRIN2A, KCNQ2, MECP2, PCDH19, POLG, PRRT2, SCN1A, SCN1B, SCN2A, SCN8A, SLC2A1, SLC9A6, STXBP1, SYNGAP1, TCF4, TPP1, TSC1, TSC2, and ZEB2
81440	Nuclear encoded mitochondrial genes (eg, neurologic or myopathic phenotypes), genomic sequence panel, must include analysis of at least 100 genes, including BCS1L, C10orf2, COQ2, COX10, DGUOK, MPV17, OPA1, PDSS2, POLG, POLG2, RRM2B, SCO1, SCO2, SLC25A4, SUCLA2, SUCLG1, TAZ, TK2, and TYMP
81443	Genetic testing for severe inherited conditions (eg, cystic fibrosis, Ashkenazi Jewish-associated disorders [eg, Bloom syndrome, Canavan disease, Fanconi anemia type C, mucolipidosis type VI, Gaucher disease, Tay-Sachs disease], beta hemoglobinopathies, phenylketonuria, galactosemia), genomic sequence analysis panel, must include sequencing of at least 15 genes (eg, ACADM, ARSA, ASPA, ATP7B, BCKDHA, BCKDHB, BLM, CFTR, DHCR7, FANCC, G6PC, GAA, GALT, GBA, GBE1, HBB, HEXA, IKBKAP, MCOLN1, PAH)
81449	Solid organ neoplasm, genomic sequence analysis panel, 5-50 genes, interrogation for sequence variants and copy number variants or rearrangements, if performed; RNA analysis
81451	Hematolymphoid neoplasm or disorder, genomic sequence analysis panel, 5-50 genes, interrogation for sequence variants, and copy number variants or rearrangements, or isoform expression or mRNA expression levels, if performed; RNA analysis
81455	Solid organ or hematolymphoid neoplasm or disorder, 51 or greater genes, genomic sequence analysis panel, interrogation for sequence variants and copy number variants or rearrangements, or isoform expression or mRNA expression levels, if performed; DNA analysis or combined DNA and RNA analysis
81456	RNA analysis
81460	Whole mitochondrial genome (eg, Leigh syndrome, mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes [MELAS], myoclonic epilepsy with ragged-red fibers [MERFF], neuropathy, ataxia, and retinitis pigmentosa [NARP], Leber hereditary optic neuropathy [LHON]), genomic sequence, must include sequence analysis of entire mitochondrial genome with heteroplasmy detection
81465	Whole mitochondrial genome large deletion analysis panel (eg, Kearns-Sayre syndrome, chronic progressive external ophthalmoplegia), including heteroplasmy detection, if performed
81470	X-linked intellectual disability (XLID) (eg, syndromic and non-syndromic XLID); genomic sequence analysis panel, must include sequencing of at least 60 genes, including ARX, ATRX, CDKL5, FGD1, FMR1, HUWE1, IL1RAPL, KDM5C, L1CAM, MECP2, MED12, MID1, OCRL, RPS6KA3, and SLC16A2
81471	duplication/deletion gene analysis, must include analysis of at least 60 genes, including ARX, ATRX, CDKL5, FGD1, FMR1, HUWE1, IL1RAPL, KDM5C, L1CAM, MECP2, MED12, MID1, OCRL, RPS6KA3, and SLC16A2

Other CPT codes related to the CPB:

81400 - 81408	Molecular pathology procedures
83020	Hemoglobin fractionation and quantitation; electrophoresis
83021	chromatography
96040	Medical genetics and genetic counseling services, each 30 minutes face-to-face with patient/family

HCPCS codes covered if selection criteria are met:

83010	DNA analysis for germline mutations of the RET proto oncogene for
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S3840	DNA analysis for germline mutations of the RET proto-oncogene for susceptibility to multiple endocrine neoplasia type 2
S3841	Genetic testing for retinoblastoma
S3842	Genetic testing for von Hippel-Lindau disease
S3844	DNA analysis of the connexin 26 gene (GJB2) for susceptibility to congenital, profound deafness
S3845	Genetic testing for alpha-thalassemia
S3846	Genetic testing for hemoglobin E beta-thalassemia
S3850	Genetic testing for sickle cell anemia
S3853	Genetic testing for myotonic muscular dystrophy
S3866	Genetic analysis for a specific gene mutation for hypertrophic cardiomyopathy (HCM) in an individual with a known HCM mutation in the family
HCPCS codes not covered for indications listed in the CPB:	
S3861	Genetic testing, sodium channel, voltage-gated, type V, alpha subunit (SCN5A) and variants for suspected Brugada syndrome
S3865	Comprehensive gene sequence analysis for hypertrophic cardiomyopathy
Other HCPCS codes related to the CPB:	
G0461	Immunohistochemistry or immunocytochemistry, per specimen; first single or multiplex antibody stain
G0462	each additional single or multiplex antibody stain (list separately in addition to code for primary procedure)
S0265	Genetic counseling, under physician supervision, each 15 minutes
ICD-10 codes covered if selection criteria are met:	
C16.0 - C16.9	Malignant neoplasm of stomach [with 2 HNPCC-related cancers]
C17.0 - C17.9	Malignant neoplasm of small intestine [with 2 HNPCC-related cancers]
C18.0 - C21.8	Malignant neoplasm of colon [persons with serrated polyposis syndrome with at least some adenomas]
C22.0 - C22.9	Malignant neoplasm of liver and intrahepatic bile ducts [with 2 HNPCC cancers]
C34.00 - C34.92	Malignant neoplasm of bronchus and lung
C40.00 - C41.9	Malignant neoplasm of bone and articular cartilage [osteosarcoma]
C49.0 - C49.9	Malignant neoplasm of other connective and soft tissue [soft tissue sarcoma]
C50.011 - C50.929	Malignant neoplasm of breast
C54.0 - C54.9	Malignant neoplasm of corpus uteri
C56.1 - C56.9	Malignant neoplasm of ovary [with 2 HNPCC cancers]
C64.1 - C64.9	Malignant neoplasm of kidney, except pelvis [renal cell cancer syndrome]
C65.1 - C65.9	Malignant neoplasm of renal pelvis [transitional cell for microsatellite instability (MSI) testing and MLH1 and MLH2 sequence analysis for HNPCC]
C66.1 - C66.9	Malignant neoplasm of ureter [transitional cell for microsatellite instability (MSI) testing and MLH1 and MLH2 sequence analysis for HNPCC]
C67.0 - C67.9	Malignant neoplasm of bladder [transitional cell for microsatellite instability (MSI) testing and MLH1 and MLH2 sequence analysis for HNPCC]
C69.20 - C69.22	Malignant neoplasm of retina [retinoblastoma]
C71.0 - C71.9	Malignant neoplasm of brain [except glioblastoma multiforme]
C73	Malignant neoplasm of thyroid gland [medullary thyroid carcinoma]

	[cribriform-morular variant or papillary thyroid cancer]
C74.00 - C74.92	Malignant neoplasm of adrenal gland [adrenocortical carcinoma]
C91.00 - C95.92	Leukemias
D02.3	Carcinoma in situ of other parts of respiratory system [hereditary leiomyomatosis]
D12.7 - D12.9	Benign neoplasm of rectosigmoid junctn, rectum, anus and anal canal
D35.00 - D35.02	Benign neoplasm of adrenal gland [hereditary paraganglioma (SDHS, SDHB)]
D55.21 - D55.29	Anemia due to disorders of glycolytic enzymes [pyruvate kinase (PK) deficiency anemia]
D57.00 - D57.819	Sickle cell disorders
D58.2	Other hemoglobinopathies [hb-C disease] [Hereditary hemoglobinopathies]
D66	Hereditary factor VIII deficiency [hemophilia A/VWF]
D67	Hereditary factor IX deficiency [hemophilia B]
D68.2	Hereditary deficiency of other clotting factors [deficiency of factor II (prothrombin), 20210A mutation]
D69.42	Congenital and hereditary thrombocytopenia purpura [amegakaryocytic]
D70.0	Congenital agranulocytosis [congenital neutropenia] [cyclic]
D70.4	Cyclic neutropenia [congenital]
E11.00 - E11.9	Type 2 diabetes mellitus [covered for maturity-onset diabetes of the young (MODY)]
E23.0	Hypopituitarism [Kallman's syndrome] (FGFR1)
E25.0	Congenital adrenal hyperplasia
E31.21	Multiple endocrine neoplasia [MEN] type I
E70.0	Classical phenylketonuria
E70.30 - E70.39	Albinism
E71.0	Maple syrup urine disease
E71.311	Medium chain acyl CoA dehydrogenase deficiency [MCAD]
E74.04	McArdle's disease
E74.20 - E74.29	Disorders of galactose metabolism
E75.02	Tay-Sachs disease
E75.21 - E75.22, E75.240 - E75.249	Other sphingolipidosis [Fabry (-Anderson, Gaucher, Niemann-Pick disease (sphingomyelin phosphodiesterase))]
E75.29	Other sphingolipidosis [Canavan's disease (aspartoacylase A)]
E76.01 - E76.03	Mucopolysaccharidosis, type I [(MPS-1)]
E78.01	Familial hypercholesterolemia
E83.110	Hereditary hemochromatosis
E83.40 - E83.49	Disorders of magnesium metabolism [Gitelman's syndrome]
E83.52	Hypercalcemia (familial hypocalciuric)
E84.0 - E84.9	Cystic fibrosis [CTFR]
E85.2	Heredofamilial amyloidosis, unspecified [hereditary amyloidosis (TTR variants)]
E88.01	Alpha-1-antitrypsin deficiency
E88.41	MELAS syndrome [mitochondrial encephalopathy (MTTL1, tRNAleu)]
F70 - F79	Intellectual disabilities
F84.2	Rett's syndrome [MECP2]
G10	Huntington's disease

G11.10 - G11.19	Early-onset cerebellar ataxia [Friedreich's ataxia] [frataxin]
G11.3	Cerebellar ataxia with defective DNA repair
G11.4	Hereditary spastic paraparesis [hereditary spastic paraparesis 3 (SPG3A) and 4 (SPG4, SPAST)]
G11.8	Other hereditary ataxias [spinocerebellar atrophy (ataxin, CACNA1A)]
G11.9	Hereditary ataxia, unspecified [hereditary cerebellar atrophy NOS] [spinocerebellar atrophy (ataxin, CACNA1A)] [SCA types 8, 10, 17 and DRPLA]]
G12.1, G12.8 - G12.9	Spinal muscular atrophy [Kennedy disease (SBMA) (SMN)]
G24.1	Genetic torsion dystonia [primary TOR1A (DYT1)]
G31.82	Leigh's disease
G40.301 - G40.319	Generalized idiopathic epilepsy and epileptic syndromes [nonspecific myoclonic epileptic seizures (MERRF) (MTTK) (tRNAlys)]
G40.401 - G40.419	Other generalized epilepsy and epileptic syndromes, not intractable
G47.35	Congenital central alveolar hypoventilation syndrome
G60.0	Hereditary motor and sensory neuropathy [Charcot-Marie-Tooth disease]
G71.00 - G71.09, G71.20 - G71.29	Muscular dystrophy and congenital myopathies [benign (Becker) muscular dystrophy] [limb-girdle muscular dystrophy (LGMD1, LGMD2)] [not covered for oculopharyngeal muscular dystrophy (OPMD)] [severe (Duchenne) muscular dystrophy]
G71.11 - G71.19	Myotonic disorders [myotonic dystrophy (CMPPK, ZNF-9)]
G72.89	Other specified myopathies [dysferlin]
G90.1	Familial dysautonomia [Riley-Day]
H35.50	Unspecified hereditary retinal dystrophy. [bi-allelic RPE65 mutation-associated retinal dystrophy]
H35.52	Pigmentary retinal dystrophy [retinitis pigmentosa]
H47.22	Hereditary optic atrophy [Leber's optic atrophy (LHON)]
H90.0 - H91.93	Hearing loss [hereditary (Connexin-26, GJB2)]
I11.0 - I11.9	Hypertensive heart disease [premature CHD]
I20.0	Unstable angina [premature CHD]
I20.1 - I20.9	Angina pectoris [premature CHD]
I21.01 - I21.9	ST elevation (STEMI) and non-ST elevation (NSTEMI) myocardial infarction [premature CHD]
I21.A1	Myocardial infarction type 2
I21.A9	Other myocardial infarction type
I24.0 - I24.9	Other acute ischemic heart diseases [premature CHD]
I25.110 - I25.119	Atherosclerotic heart disease of native coronary artery with angina pectoris [premature CHD]
I25.2	Old myocardial infarction [premature CHD]
I25.3	Aneurysm of heart [premature CHD]
I25.41 - I25.42	Coronary artery aneurysm and dissection [premature CHD]
I25.5	Ischemic cardiomyopathy [premature CHD]
I25.6	Silent myocardial ischemia [premature CHD]
I25.700 - I25.799	Atherosclerosis of coronary artery bypass graft(s) and coronary artery of transplanted heart with angina pectoris [premature CHD]
I25.810 - I25.9	Other forms of chronic ischemic heart disease [premature CHD]
I42.0	Dilated cardiomyopathy [hereditary]

I42.1 - I42.2	Obstructive hypertrophic and other hypertrophic cardiomyopathy
I42.8	Other cardiomyopathies [arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C)] [left ventricle non compaction genetic testing]
I47.20 - I47.29	Ventricular tachycardia [persons that display exercise- or emotion-induced polymorphic ventricular tachycardia or ventricular fibrillation, occurring in a structurally normal heart] [catecholaminergic polymorphic]
I49.01	Ventricular fibrillation
I71.010 - I71.03	Dissection of thoracic aorta
I71.10 - I71.13	Thoracic aortic aneurysm, ruptured
I71.20 - I71.23	Thoracic aortic aneurysm, without rupture
I78.0	Hereditary hemorrhagic telangiectasia
J47.0 - J47.9	Bronchiectasis
K62.0 - K62.1	Anal and rectal polyp
K85.00 - K85.92	Acute pancreatitis [unexplained episode in a child requiring hospitalization with significant concern that hereditary pancreatitis (PRSS1) should be excluded]
K86.1	Other chronic pancreatitis [unexplained (idiopathic) for hereditary pancreatitis (PRSS1)]
L60.3	Nail dystrophy
N04.0 - N04.9	Nephrotic syndrome [congenital (NPHS1, NPHS2)]
Q04.3	Other reduction deformities of brain [lissencephaly (classical)] [Joubert syndrome]
Q61.11 - Q61.3	Polycystic kidney
Q61.5	Medullary cystic kidney [nephronophthisis]
Q61.9	Cystic kidney disease, unspecified [Meckel-Gruber syndrome]
Q75.001 - Q75.1	Other congenital malformations of skull and face bones [craniosynostosis] [Crouzon's disease (CTFR), Saethre-Chotzen syndrome (TWIST, FGFR2)]
Q77.1	Thanatophoric short stature
Q77.4	Achondroplasia
Q78.0	Osteogenesis imperfecta
Q79.60 - Q79.69	Ehlers-Danlos syndrome
Q79.8	Other congenital malformations of musculoskeletal system [Jackson-Weiss syndrome] [Muencke syndrome (FGFR2)]
Q82.8	Other specified congenital malformations of skin [Bloom syndrome]
Q85.01	Neurofibromatosis, type 1 [von Recklinghausen's disease] [neurofibromin] [not covered for Legius syndrome]
Q85.02	Neurofibromatosis, type 2 [acoustic neurofibromatosis] [Merlin]
Q85.81, Q85.83, Q85.89	Other phakomatoses, not elsewhere classified [von Hippel Lindau syndrome (VHL)]
Q87.0	Congenital malformation syndrome predominantly affecting facial appearance [acrocephalosyndactyly] [Pfeiffer syndrome (FGFR1)]
Q87.11 - Q87.19	Congenital malformation syndromes predominantly associated with short stature [Prader-Willi syndrome] [GABRA, SNRPN] [Noonan syndrome]
Q87.40 - Q87.43	Marfan's syndrome
Q93.51	Angelman syndrome
Q93.81	Velo-cardio-facial syndrome [22q11 deletion syndrome (CATCH-22)]

Q99.2	Fragile X chromosome [Fragile X syndrome]
R62.52	Short stature (child) [SHOX-related]
R74.8	Abnormal levels of other serum enzymes [abnormal level of amylase] [hyper-amylasemia]
T88.3xx+	Malignant hyperthermia due to anesthesia
Z13.228	Encounter for screening for other metabolic disorders [phenylketonuria, galactosemia] and other inborn errors of metabolism
Z13.29	Encounter for screening for other suspected endocrine disorder [thyroid]
Z13.5	Encounter for screening for eye and ear disorders
Z14.01	Asymptomatic hemophilia A carrier
Z14.02	Symptomatic hemophilia A carrier
Z14.1	Cystic fibrosis carrier
Z14.8	Genetic carrier of other disease
Z15.01	Genetic susceptibility to malignant neoplasm of breast [Li-Fraumeni syndrome] [covered for Li-Fraumeni syndrome testing other than OncoVue]
Z31.430	Encounter of female for testing for genetic disease carrier status for procreative management
Z31.440	Encounter of male for testing for genetic disease carrier status for procreative management
Z80.0	Family history of malignant neoplasm of digestive organs
Z81.0	Family history of intellectual disabilities
Z82.0	Family history of epilepsy and other diseases of the nervous system
Z82.41	Family history of sudden cardiac death (SCD)
Z82.49	Family history of ischemic heart disease and other diseases of the circulatory system [premature CHD]
Z82.79	Family history of other congenital malformations, deformations and chromosomal abnormalities
Z83.49	Family history of other endocrine, nutritional and metabolic diseases [first degree relative with a causative FH mutation]
Z83.79	Family history of other diseases of the digestive system [pancreatitis]
Z84.81	Family history of genetic disease carrier
Z85.030 - Z85.038	Personal history of malignant neoplasm of large intestine [with HNPCC related cancers]
Z85.040 - Z85.048	Personal history of malignant neoplasm of rectum, rectosigmoid junction, and anus
Z85.42	Personal history of malignant neoplasm of other parts of uterus
Z95.1	Presence of aortocoronary bypass graft [premature CHD]
Z95.5	Presence of coronary angioplasty implant and graft [premature CHD]
Z98.61	Coronary angioplasty status [premature CHD]
ICD-10 codes not covered for indications listed in the CPB:	
C43.0 - C43.9	Malignant melanoma of skin
C61	Malignant neoplasm of prostate
D37.1 - D37.5	Neoplasm of uncertain behavior of stomach, intestines, colon and rectum [desmoid tumor]
D61.01	Constitutional (pure) red blood cell aplasia
D68.01	Von Willebrand disease, type 1
D68.022	Von Willebrand disease, type 2M
D68.03	Von Willebrand disease, type 3

D68.04	Acquired von Willebrand disease
D68.1	Congenital factor XI deficiency [hemophilia C]
D72.0	Genetic anomalies of leukocytes
E71.310	Long chain/very long chain acyl CoA dehydrogenase deficiency
E73.0 - E73.9	Lactose intolerance
E88.1	Lipodystrophy [familial partial lipodystrophy]
E88.89	Other specified metabolic disorders [multiple mitochondrial respiratory chain complex deficiencies]
F51.3	Sleepwalking [somnambulation]
G20.A1 - G20.C	Parkinson's disease
G25.0	Essential tremor [benign]
G25.3	Myoclonus [dystonia]
G43.401 - G43.419	Hemiplegic migraine
G47.411 - G47.429	Narcolepsy and cataplexy
H35.051 - H35.059	Retinal neovascularization
H35.30 - H35.33	Degeneration of macula
H53.63	Congenital night blindness
I20.0 - I20.89	Angina pectoris
I21.01 - I21.4	ST elevation (STEMI) and non-ST elevation (NSTEMI) myocardial infarction
I24.0 - I25.9	Other acute and chronic forms of ischemic heart disease
I25.10 - I25.9	Chronic ischemic heart disease
M81.0 - M81.8	Osteoporosis without current pathological fracture
Q24.8	Other specified congenital malformations of heart [Brugada syndrome]
Q44.6	Cystic disease of liver
Q61.11 - Q61.3	Polycystic kidney
Q76.1	Klippel-Feil syndrome
Q78.1	Polyostotic fibrous dysplasia [McCune-Albright syndrome]
Q80.0 - Q80.9	Congenital ichthyosis [epidemolysis hyperkatoisis]
Q89.9	Congenital malformation, unspecified [heterotaxy]
R42	Dizziness and giddiness [migrainous vertigo]
R56.00 - R56.01	Febrile convulsions
Z13.6	Encounter for screening for cardiovascular disorders
Z80.3	Family history of malignant neoplasm of breast
Hereditary ataxia panel:	
CPT codes covered for indications listed in the CPB:	
0216U	Neurology (inherited ataxias), genomic DNA sequence analysis of 12 common genes including small sequence changes, deletions, duplications, short tandem repeat gene expansions, and variants in non-uniquely mappable regions, blood or saliva, identification and categorization of genetic variants
0217U	Neurology (inherited ataxias), genomic DNA sequence analysis of 51 genes including small sequence changes, deletions, duplications, short tandem repeat gene expansions, and variants in non-uniquely mappable regions, blood or saliva, identification and categorization of genetic variants
81188	CSTB (cystatin B) (eg, Unverricht-Lundborg disease) gene analysis; evaluation to detect abnormal (eg, expanded) alleles
81189	full gene sequence
81190	known familial variant(s)

81284	FXN (frataxin) (eg, Friedreich ataxia) gene analysis; evaluation to detect abnormal (expanded) alleles
81285	characterization of alleles (eg, expanded size)
81286	full gene sequence
81460	Whole mitochondrial genome (eg, Leigh syndrome, mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes [MELAS], myoclonic epilepsy with ragged-red fibers [MERFF], neuropathy, ataxia, and retinitis pigmentosa [NARP], Leber hereditary optic neuropathy [LHON]), genomic sequence, must include sequence analysis of entire mitochondrial genome with heteroplasmy detection
81465	Whole mitochondrial genome large deletion analysis panel (eg, Kearns-Sayre syndrome, chronic progressive external ophthalmoplegia), including heteroplasmy detection, if performed

ICD-10 codes covered if selection criteria are met:

G11.0 - G11.9 Hereditary ataxia

ICD-10 codes not covered for indications listed in the CPB:

C71.0 - C71.9 Malignant neoplasm of brain

E50.0 - E50.9 Vitamin A deficiency

F10.120 - F10.988 Alcohol related disorders

G35 Multiple sclerosis

H65.00 - H66.9 Non-suppurative otitis media

H66.001 - H66.93 Suppurative and unspecified otitis media

H67.1 - H67.9 Otitis media in diseases classified elsewhere

I63.00 - I63.9 Cerebral infarction

S00.00XA - Injuries to the head

S09.93XS

Hereditary hearing loss panel:

CPT codes covered for indications listed in the CPB:

81430 Hearing loss (eg, nonsyndromic hearing loss, Usher syndrome, Pendred syndrome); genomic sequence analysis panel, must include sequencing of at least 60 genes, including CDH23, CLRN1, GJB2, GPR98, MTRNR1, MYO7A, MYO15A, PCDH15, OTOF, SLC26A4, TMC1, TMPRSS3, USH1C, USH1G, USH2A, and WFS1

81431 Hearing loss (eg, nonsyndromic hearing loss, Usher syndrome, Pendred syndrome); duplication/deletion analysis panel, must include copy number analyses for STRC and DFNB1 deletions in GJB2 and GJB6 genes

ICD-10 codes covered if selection criteria are met:

H90.0 - H91.93 Hearing loss [Hereditary]

Legius syndrome (SPRED1):

CPT codes covered for indications listed in the CPB:

Legius syndrome (SPRED1) -no specific code

ICD-10 codes covered if selection criteria are met:

L81.3 Cafe au lait spots

Q85.00 - Q85.09 Neurofibromatosis (nonmalignant) [Legius syndrome (SPRED1)]

COL1A1/COL1A2:

CPT codes covered for indications listed in the CPB:

81408 Molecular pathology procedure, Level 9 (eg, analysis of >50 exons in a single gene by DNA sequence analysis)

ICD-10 codes covered if selection criteria are met:

Q78.0 Osteogenesis imperfecta

Whole exome sequencing (WES) or Whole genome sequencing (WGS):

CPT codes covered if selection criteria are met:

81415 Exome (eg, unexplained constitutional or heritable disorder or

	syndrome); sequence analysis
81416	sequence analysis, each comparator exome (eg, parents, siblings) (List separately in addition to code for primary procedure)
81417	re-evaluation of previously obtained exome sequence (eg, updated knowledge or unrelated condition/syndrome)
81425	Genome (eg, unexplained constitutional or heritable disorder or syndrome); sequence analysis
81426	sequence analysis, each comparator genome (eg, parents, siblings) (List separately in addition to code for primary procedure)
81427	re-evaluation of previously obtained genome sequence (eg, updated knowledge or unrelated condition/syndrome)
HCPCS codes related to the CPB:	
S3870	Comparative genomic hybridization (cgf) microarray testing for developmental delay, autism spectrum disorder and/or intellectual disability
ICD-10 codes covered for indications listed in the CPB (not all-inclusive):	
F72	Severe intellectual disabilities [Autism]
F73	Profound intellectual disabilities [Autism]
F82	Specific developmental disorder of motor function
F84.0 - F84.9	Pervasive developmental disorders [only covered with syndromic features]
F89	Unspecified disorder of psychological development
G40.001 - G40.89	Epilepsy and recurrent seizures [Autism]
H90.3	Sensorineural hearing loss, bilateral
Q00.0 - Q99.9	Congenital malformations, deformations and chromosomal abnormalities
ICD-10 codes not covered for indications listed in the CPB (not all-inclusive):	
000.00- O9A.519	Pregnancy, childbirth and the puerperium
Factor V Leiden:	
CPT codes covered if selection criteria are met:	
81241	F5 (coagulation Factor V) (eg, hereditary hypercoagulability) gene analysis, 20210G>A variant
CPT codes related to the CPB:	
85307	Activated Protein C (APC) resistance assay
HCPCS codes related to the CPB:	
Apixaban, Dabigatran, Rivaroxaban, Edoxaban -no specific code	
ICD-10 codes covered if selection criteria are met:	
I21.01 - I22.9	Myocardial infarction
I26.01 - I26.99	Pulmonary embolism
O22.20 - O22.33	Superficial thrombophlebitis and deep phlebothrombosis in pregnancy
Z82.49	Family history of ischemic heart disease and other diseases of the circulatory
Z86.711 -Z86.72	Personal history of venous thrombosis and embolism
SP-C/ABCA3 mutation - no specific code:	
ICD-10 codes covered if selection criteria are met [for infants and children]:	
J84.841 - J84.848	Other interstitial lung diseases of childhood
Z83.6	Family history of other diseases of the respiratory system
ICD-10 codes not covered for indications listed in the CPB (not all-inclusive) [for adults]:	
J84.10 - J84.83,	Other interstitial pulmonary diseases
J84.89 - J84.9	
TSC1/TSC2 - no specific code:	
ICD-10 codes covered if selection criteria are met:	
Q85.1	Tuberous sclerosis

CLCN1 genetic testing - no specific code:

ICD-10 codes not covered for indications listed in the CPB (not all-inclusive):

G71.12 Myotonia congenital

Genetic testing for GNE and VCP:

CPT codes not covered for indications listed in the CPB (not all-inclusive):

GNE and VCP testing – no specific code

ICD-10 codes not covered for indications listed in the CPB (not all-inclusive):

Q78.0 Osteogenesis imperfecta

Q79.60 - Q79.69 Ehlers-Danlos syndromes

EpiSEEK:

No specific code

ICD-10 codes not covered for indications listed in the CPB:

G40.009 - G40.919 Epilepsy and recurrent seizures

POLG1:

No specific code

ICD-10 codes not covered for indications listed in the CPB:

G11.8 Other hereditary ataxias [mitochondrial recessive ataxia syndrome]

SF3B1 and TET2:

No specific code

ICD-10 codes not covered for indications listed in the CPB:

D46.0 - D46.9 Myelodysplastic syndromes

C9orf72 genetic testing - no specific code:

ICD-10 codes not covered for indications listed in the CPB (not all-inclusive):

G12.21 Amyotrophic lateral sclerosis

TARDB:

CPT codes not covered for indications listed in the CPB:

81405 Molecular pathology procedure, Level 6 (eg, analysis of 6-10 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 11-25 exons, regionally targeted cytogenomic array analysis)

ICD-10 codes not covered for indications listed in the CPB (not all-inclusive):

G12.21 Amyotrophic lateral sclerosis

FUS:

CPT codes not covered for indications listed in the CPB:

81406 Molecular pathology procedure, Level 7 (eg, analysis of 11-25 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 26-50 exons, cytogenomic array analysis for neoplasia)

ICD-10 codes not covered for indications listed in the CPB (not all-inclusive):

G12.21 Amyotrophic lateral sclerosis

HADHB:

CPT codes not covered for indications listed in the CPB:

81406 Molecular pathology procedure, Level 7 (eg, analysis of 11-25 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 26-50 exons, cytogenomic array analysis for neoplasia)

ICD-10 codes not covered for indications listed in the CPB (not all-inclusive):

C50.011 - C50.929 Malignant neoplasm of breast

MTHFR:

CPT codes not covered for indications listed in the CPB:

81291 MTHFR (5,10-methylenetetrahydrofolate reductase) (eg, hereditary hypercoagulability) gene analysis, common variants (eg, 677T, 1298C).

ICD-10 codes not covered for indications listed in the CPB (not all-inclusive):

I10 Essential (primary) hypertension.

Coloseq:

CPT codes not covered for indications listed in the CPB:

81435 Hereditary colon cancer disorders (eg, Lynch syndrome, PTEN hamartoma syndrome, Cowden syndrome, familial adenomatous polyposis): genomic sequence analysis panel must include sequencing

Panel, genomic sequence analysis panel, must include sequencing of at least 10 genes, including APC, BMPR1A, CDH1, MLH1, MSH2, MSH6, MUTYH, PTEN, SMAD4, and STK11 [Not covered for Coloseq]

81436 Hereditary colon cancer disorders (eg, Lynch syndrome, PTEN hamartoma syndrome, Cowden syndrome, familial adenomatous polyposis); duplication/deletion analysis panel, must include analysis of at least 5 genes, including MLH1, MSH2, EPCAM, SMAD4, and STK11 [Not covered for Coloseq]

88381 Microdissection (ie, sample preparation of microscopically identified target); manual [Not covered for Coloseq]

Panexia:

81216 BRCA2 (breast cancer 2) (eg, hereditary breast and ovarian cancer) gene analysis; full sequence analysis [Not covered for Panexia]

81217 BRCA2 (breast cancer 2) (eg, hereditary breast and ovarian cancer) gene analysis; known familial variant [Not covered for Panexia]

81406 Molecular pathology procedure, Level 7 (eg, analysis of 11-25 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 26-50 exons, cytogenomic array analysis for neoplasia) [Not covered for Panexia]

There are no specific codes for the genetic testing listed :

Exome Sequencing, BrevaGen, Septo-optic Dysplasia Spectrum Sequencing Panel (HESX1 (3p14.3), OTX2 (14q22.3), PAX6 (11p13), PROP1 (5q35.3), SOX2 (3q26.33)

Long QT Testing:

CPT codes covered if selection criteria are met:

0237U Cardiac ion channelopathies (eg, Brugada syndrome, long QT syndrome, short QT syndrome, catecholaminergic polymorphic ventricular tachycardia), genomic sequence analysis panel including ANK2, CASQ2, CAV3, KCNE1, KCNE2, KCNH2, KCNJ2, KCNQ1, RYR2, and SCN5A, including small sequence changes in exonic and intronic regions, deletions, duplications, mobile element insertions, and variants in non-uniquely mappable regions

81413 Cardiac ion channelopathies (eg, Brugada syndrome, long QT syndrome, short QT syndrome, catecholaminergic polymorphic ventricular tachycardia); genomic sequence analysis panel, must include sequencing of at least 10 genes, including ANK2, CASQ2, CAV3, KCNE1, KCNE2, KCNH2, KCNJ2, KCNQ1, RYR2, and SCN5A

81414 Cardiac ion channelopathies (eg, Brugada syndrome, long QT syndrome, short QT syndrome, catecholaminergic polymorphic ventricular tachycardia); duplication/deletion gene analysis panel, must include analysis of at least 2 genes, including KCNH2 and KCNQ1

ICD-10 codes covered if selection criteria are met:

I45.81 Long QT syndrome

I46.9 Cardiac arrest, cause unspecified

I47.2 Ventricular tachycardia

I49.01 Ventricular fibrillation

R94.31 Abnormal electrocardiogram [ECG] [EKG]

Z82.41 Family history of sudden cardiac death

Z82.49 Family history of ischemic heart disease and other diseases of the circulatory system

SLCO1B1:

CPT codes not covered for indications listed in the CPB:

81328 SLCO1B1 (solute carrier organic anion transporter family, member 1B1) (eg, adverse drug reaction), gene analysis, common variant(s) (eg, \*5)

ICD-10 codes not covered for indications listed in the CPB:

G72.0 Drug-induced myopathy [statin]

Versiti Comprehensive Platelet Disorder Panel:

CPT codes not covered for indications listed in the CPB:	
0274U	Hematology (genetic platelet disorders), genomic sequence analysis of 62 genes and duplication/deletion of PLAU, blood, buccal swab, or amniotic fluid
ICD-10 codes not covered for indications listed in the CPB:	
D69.41 - D69.49	Other primary thrombocytopenia
D69.51 - D69.59	Secondary thrombocytopenia
D69.6	Thrombocytopenia, unspecified
Versiti Fibrinolytic Disorder Panel:	
CPT codes not covered for indications listed in the CPB:	
0273U	Hematology (genetic hyperfibrinolysis, delayed bleeding), analysis of 9 genes (F13A1, F13B, FGA, FGB, FGG, SERPINA1, SERPINE1, SERPINF2, by next generation sequencing, and PLAU by array comparative genomic hybridization), blood, buccal swab, or amniotic fluid
ICD-10 codes not covered for indications listed in the CPB:	
D65	Disseminated intravascular coagulation [defibrination syndrome]
Versiti Platelet Function Disorder Panel:	
CPT codes not covered for indications listed in the CPB:	
0277U	Hematology (genetic platelet function disorder), genomic sequence analysis of 40 genes and duplication/detection of PLAU, blood, buccal swab, or amniotic fluid
ICD-10 codes not covered for indications listed in the CPB:	
D69.1	Qualitative platelet defects
Versiti Red Cell Genotyping Panel:	
CPT codes not covered for indications listed in the CPB:	
0282U	Red blood cell antigen typing, DNA, genotyping of 12 blood group system genes to predict 44 red blood cell antigen phenotypes
ICD-10 codes not covered for indications listed in the CPB:	
D55.0 - D59.39	Hemolytic anemias
R71.0 - R71.8	Abnormality of red blood cells
Versiti Thrombosis Panel:	
CPT codes not covered for indications listed in the CPB:	
0278U	Hematology (genetic thrombosis), genomic sequence analysis of 14 genes, blood, buccal swab, or amniotic fluid
ICD-10 codes not covered for indications listed in the CPB:	
D68.51 - D68.59	Primary thrombophilia
D68.61 - D68.69	Other thrombophilia
ALPL:	
CPT codes covered for indications listed in the CPB:	
Pathogenic variant in ALPL - no specific code:	
ICD-10 codes covered if selection criteria are met:	
E83.30 - E83.39	Disorders of phosphorus metabolism and phosphatases [hypophosphatasia]
Androgen receptor (AR) mutation:	
CPT codes covered for indications listed in the CPB:	
0230U	AR (androgen receptor) (eg, spinal and bulbar muscular atrophy, Kennedy disease, X chromosome inactivation), full sequence analysis, including small sequence changes in exonic and intronic regions, deletions, duplications, short tandem repeat (STR) expansions, mobile element insertions, and variants in non-uniquely mappable regions
81405	Molecular pathology procedure, Level 6 (eg, analysis of 6-10 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 11-25 exons, regionally targeted cytogenomic array analysis)
ICD-10 codes covered if selection criteria are met:	
E34.50 - E34.52	Androgen insensitivity syndrome

## G12.0 - G12.9 Spinal muscular atrophy and related syndromes [Kennedy disease]

CSTB mutation:

CPT codes covered for indications listed in the CPB:

0232U	CSTB (cystatin B) (eg, progressive myoclonic epilepsy type 1A, Unverricht-Lundborg disease), full gene analysis, including small sequence changes in exonic and intronic regions, deletions, duplications, short tandem repeat (STR) expansions, mobile element insertions, and variants in non-uniquely mappable regions
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81188	CSTB (cystatin B) (eg, Unverricht-Lundborg disease) gene analysis; evaluation to detect abnormal (eg, expanded) alleles
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81189	CSTB (cystatin B) (eg, Unverricht-Lundborg disease) gene analysis; full gene sequence
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81190	CSTB (cystatin B) (eg, Unverricht-Lundborg disease) gene analysis; known familial variant(s)
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ICD-10 codes covered if selection criteria are met:

G40.301 - G40.319	Generalized idiopathic epilepsy and epileptic syndromes [Unverricht-Lundborg disease]
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TGFBI mutation:

CPT codes covered for indications listed in the CPB:

83520	Immunoassay for analyte other than infectious agent antibody or infectious agent antigen; quantitative, not otherwise specified [for TGFBI mutation]
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ICD-10 codes covered if selection criteria are met:

H18.501 - H18.599	Hereditary corneal dystrophies
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PABPN1 mutation for oculopharyngeal muscular dystrophy (OPMD):

CPT codes covered for indications listed in the CPB:

81312	PABPN1 (poly[A] binding protein nuclear 1) (eg, oculopharyngeal muscular dystrophy) gene analysis, evaluation to detect abnormal (eg, expanded) alleles
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ICD-10 codes covered if selection criteria are met:

G71.09	Other specified muscular dystrophies [oculopharyngeal muscular dystrophy (OPMD)]
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Versiti Autosomal Dominant Thrombocytopenia Panel:

CPT codes covered for indications listed in the CPB:

0269U	Hematology (autosomal dominant congenital thrombocytopenia), genomic sequence analysis of 14 genes, blood, buccal swab, or amniotic fluid
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ICD-10 codes covered if selection criteria are met:

D59.31 - D59.39	Hemolytic-uremic syndrome [Atypical]
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D69.41 - D69.49	Other primary thrombocytopenia
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D69.51 - D69.59	Secondary thrombocytopenia
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D69.6	Thrombocytopenia, unspecified
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Versiti Congenital Neutropenia Panel:

CPT codes covered for indications listed in the CPB:

0271U	Hematology (congenital neutropenia), genomic sequence analysis of 24 genes, blood, buccal swab, or amniotic fluid
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Other CPT codes related to the CPB:

85004	Blood count; automated differential WBC count
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85048	Blood count; leukocyte (WBC), automated
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ICD-10 codes covered if selection criteria are met:

D70.0 - D70.9	Neutropenia
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D72.810 -	Decreased white blood cell count
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D72.819	
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Versiti aHUS Genetic Evaluation Panel:

CPT codes covered for indications listed in the CPB:

0268U	Hematology (atypical hemolytic uremic syndrome [aHUS]), genomic
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sequence analysis of 15 genes, blood, buccal swab, or amniotic fluid

ICD-10 codes covered if selection criteria are met:

D59.31 - D59.39 Hemolytic-uremic syndrome [Atypical]

Von Willebrand disease (VWD) factor testing for type 2B and 2N:

CPT codes covered for indications listed in the CPB:

0283U Von Willebrand factor (VWF), type 2B, platelet-binding evaluation, radioimmunoassay, plasma

0284U Von Willebrand factor (VWF), type 2N, factor VIII and VWF binding evaluation, enzyme-linked immunosorbent assays (ELISA), plasma

ICD-10 codes covered if selection criteria are met:

D68.00 Von Willebrand disease, unspecified

D68.020 Von Willebrand disease, type 2A

D68.021 Von Willebrand disease, type 2B

D68.023 Von Willebrand disease, type 2N

D68.029 Von Willebrand disease, type 2, unspecified

D68.09 Other von Willebrand disease

Inherited bone marrow failure syndromes panel:

CPT codes covered for indications listed in the CPB:

81441 Inherited bone marrow failure syndromes (IBMFS) (eg, Fanconi anemia, dyskeratosis congenita, Diamond-Blackfan anemia, Shwachman-Diamond syndrome, GATA2 deficiency syndrome, congenital amegakaryocytic thrombocytopenia) sequence analysis panel, must include sequencing of at least 30 genes, including BRCA2, BRIP1, DKC1, FANCA, FANCB, FANCC, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCL, GATA1, GATA2, MPL, NHP2, NOP10, PALB2, RAD51C, RPL11, RPL35A, RPL5, RPS10, RPS19, RPS24, RPS26, RPS7, SBDS, TERT, and TINF2

ICD-10 codes covered if selection criteria are met:

D60.0 – D60.9 Acquired pure red cell aplasia [erythroblastopenia]

D61.01 – D61.9 Other aplastic anemias and other bone marrow failure syndromes

D46.0 – D46.Z Myelodysplastic syndromes

Z83.2 Family history of diseases of the blood and blood-forming organs and certain disorders involving the immune mechanism

Z86.2 Personal history of diseases of the blood and blood-forming organs and certain disorders involving the immune mechanism

RFC1 (replication factor C subunit 1) repeat expansion variant analysis (RFC1 Repeat Expansion Test):

CPT codes covered if selection criteria are met:

0378U RFC1 (replication factor C subunit 1), repeat expansion variant analysis by traditional and repeatprimed PCR, blood, saliva, or buccal swab

ICD-10 codes covered if selection criteria are met:

G32.81 Cerebellar ataxia in diseases classified elsewhere [CANVAS syndrome]

H55.89 Other irregular eye movements [doll's eyes]

R26.0 – R26.9 Other abnormalities of gait and mobility

SF3B2 Testing:

CPT codes not covered for indications listed in the CPB (not all-inclusive):

SF3B2 Testing –no specific code

ICD-10 codes not covered for indications listed in the CPB (not all-inclusive):

Q67.0 Congenital facial asymmetry [Hemi-facial microsomia]

Q67.1 Congenital compression facies [Hemi-facial microsomia]

Q67.4 Other congenital deformities of skull, face and jaw [Hemi-facial microsomia]

Q87.0 Congenital malformation syndromes predominantly affecting facial appearance [Goldenhar syndrome]

CPT codes not covered for indications listed in the CPB (not all-inclusive):

Ambry Melanoma Next -no specific code

ICD-10 codes not covered for indications listed in the CPB (not all-inclusive):

C43.0 – C43.9	Malignant melanoma of skin
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Experimental and Investigational:

Epigenetic methylation assay (e.g., EpiSign), GeneticsNow Comprehensive, MyPhenome Hungry

Gut, ProstateNow

CPT codes not covered for indications listed in the CPB:

0318U	Pediatrics (congenital epigenetic disorders), whole genome methylation analysis by microarray for 50 or more genes, blood
0475U	Hereditary prostate cancer-related disorders, genomic sequence analysis panel using next-generation sequencing (NGS), Sanger sequencing, multiplex ligation-dependent probe amplification (MLPA), and array comparative genomic hybridization (CGH), evaluation of 23 genes and duplications/deletions when indicated, pathologic mutations reported with a genetic risk score for prostate cancer

## Background

Genetic tests are laboratory studies of human deoxyribonucleic acid (DNA), chromosomes, genes or gene products to diagnose the presence of a genetic variation associated with a high risk of having or transmitting a specific genetic disorder.

According to the American College of Medical Genetics (ACMG), an important issue in genetic testing is defining the scope of informed consent. The obligation to counsel and obtain consent is inherent in the clinician-patient and investigator-subject relationships. In the case of most genetic tests, the patient or subject should be informed that the test might yield information regarding a carrier or disease state that requires difficult choices regarding their current or future health, insurance coverage, career, marriage, or reproductive options. The objective of informed consent is to preserve the individual's right to decide whether to have a genetic test. This right includes the right of refusal should the individual decide the potential harm (stigmatization or undesired choices) outweighs the potential benefits.

DNA-based mutation analysis is not covered for routine carrier testing for the diagnosis of Tay-Sachs and Sandhoff disease. Under accepted guidelines, diagnosis is primarily accomplished through biochemical assessment of serum, leukocyte, or platelet hexosaminidase A and B levels. The literature states that mutation analysis is appropriate for individuals with persistently inconclusive enzyme-based results and to exclude pseudo-deficiency (non-disease related) mutations in carrier couples.

Testing of a member who is at substantial familial risk *for* being a heterozygote (carrier) for a particular detectable mutation that is recognized to be attributable to a specific genetic disorder is only covered for the purpose of prenatal counseling under plans with this benefit (see [CPB 0189 - Genetic Counseling \(0189.html\)](#)).

Confirmation by molecular analysis of inborn errors of metabolism by traditional screening methodologies (e.g., Guthrie microbiologic assays) is covered. Rigorous clinical evaluation should precede diagnostic molecular testing.

In many instances, reliable mutation analysis requires accurate determination of specific allelic variations in a proband (affected individual in a family) before subsequent carrier testing in other at-risk family members can be accurately performed. Coverage of testing for individuals who are not Aetna members is not provided, except under the limited circumstances outlined in the policy section above.

#### Androgen Insensitivity Syndrome (AIS)

Androgen insensitivity syndrome (AIS) is an inherited X-linked condition that is characterized by evidence of feminization (i.e., undermasculization) of the external genitalia at birth, abnormal secondary sexual development in puberty, and infertility in individuals with a 46,XY karyotype. AIS is caused by mutations in the AR (androgen receptor) gene which cause partial or complete inability of the cell to respond to androgens. Individuals with this condition are genetically male, with one X chromosome and one Y chromosome in each cell; however, because their bodies are unable to respond to the male sex hormones, androgens, individuals may exhibit female external sex characteristics or signs of both male and female sexual development. Complete androgen insensitivity syndrome affects 2 to 5 per 100,000 people who are genetically male. Partial androgen insensitivity is at least as common as complete androgen insensitivity; however, mild androgen insensitivity has been found to be much less common (Gottlieb and Trifiro, 2017; Genetics Home Reference, 2019).

The diagnosis of AIS is established in persons with a 46,XY karyotype who have signs/symptoms (i.e., undermasculinization of the external genitalia, impaired spermatogenesis with otherwise normal testes, absent or rudimentary müllerian structures), have evidence of normal or increased synthesis of testosterone and its normal conversion to dihydrotestosterone, normal or increased luteinizing hormone (LH) production by the pituitary gland and/or by the identification of a hemizygous pathogenic variant in AR (Gottlieb and Trifiro, 2017).

#### Angelman and Prader-Willi Syndromes

Angelman syndrome (AS) is a neurogenetic disorder characterized by developmental delay, lack of speech, seizures and walking and balance disorders. Prader-Willi syndrome (PWS) is a genetic disorder characterized by short stature, intellectual delay, weak muscle tone (hypotonia), hypogonadism and an uncontrolled appetite that leads to life-threatening obesity.

The diagnosis of AS or PWS can be established through a variety of biochemical and genetic tests including DNA methylation analysis, deletion/duplication analysis, fluorescent in situ hybridization (FISH), chromosomal microarray (CMA), uniparental disomy (UPD) and imprinting defect (ID) studies.

DNA Methylation is a biochemical process in which a strand of DNA is modified after it is replicated. Deletion/Duplication Analysis is laboratory testing that identifies an absence of a segment of DNA (deletion) and/or the presence of an extra segment of DNA (duplication) in a coding region.

Fluorescence in Situ Hybridization (FISH) is a laboratory technique used to detect small deletions or rearrangements in chromosomes. FISH may be used in the diagnosis and prognosis of cancer and it is also utilized for the evaluation of microdeletion syndromes, such as Angelman syndrome, Prader-Willi syndrome and velocardiofacial syndrome.

Uniparental Disomy (UPD) refers to the situation in which both members of a chromosome pair or segments of a chromosome pair are inherited from one parent and neither is inherited from the other parent. UPD can result in an abnormal phenotype in some cases.

## Arrhythmogenic Right Ventricular Dysplasia/Cardiomyopathy (ARVD/C)

Arrhythmogenic right ventricular dysplasia/cardiomyopathy is a condition characterized by progressive fibro-fatty replacement of the myocardium that predisposes individuals to ventricular tachycardia and sudden death. The prevalence of ARVD/C is estimated to be 1 case per 10,000 population. Familial occurrence with an autosomal dominant pattern of inheritance and variable penetrance has been demonstrated. Recessive variants have been reported. It is estimated that half of the individuals have a family history of ARVD/C and the remaining cases are new mutations.

Genetic testing has not been demonstrated to be necessary to establish the diagnosis of ARVD/C or determine its prognosis. Twelve-lead ECG and echocardiography can be used to identify affected relatives.

The genetic abnormalities that cause ARVD/C are heterogeneous. The genes frequently associated with ARVD/C are PKP2 (plakophilin-2), DSG2 (desmoglein-2), and DSP (desmoplakin). A significant proportion of ARVD/C cases have been reported with no linkage to known chromosomal loci; in one report, 50 % of families undergoing clinical and genetic screening did not show linkage with any known genetic loci (Corrado et al, 2000).

Most affected individuals live a normal lifestyle. Management of individuals with ARVD/C is complicated by incomplete information on the natural history of the disease and the variability of disease expression even within families. High-risk individuals with signs and symptoms of ARVD/C are treated with anti-arrhythmic medications and those at highest risk who have been resuscitated or who are unresponsive to or intolerant of anti-arrhythmic therapy may be considered for an ICD.

According to the Heart Failure Society of America's Practice Guideline on the genetic evaluation of cardiomyopathy (2009), the clinical utility for all genetic testing of cardiomyopathies remains to be defined. The guideline stated, "[b]ecause the genetic knowledge base of cardiomyopathy is still emerging, practitioners caring for patients and families with genetic cardiomyopathy are encouraged to consider research participation." The Multidisciplinary Study of Right Ventricular Dysplasia (North American registry) is a 5-year study funded by the National Institutes of Health to determine how the genes responsible for ARVD/C affect the onset, course, and severity of the disease. Enrollment in the study was completed in May 2008 and the study is currently in the follow-up period.

The Heart Rhythm Society (HRS) released a 2019 consensus statement on the evaluation, risk stratification, and management of arrhythmogenic cardiomyopathy (ACM). The HRS recommends genetic testing in individuals with confirmed or suspected diagnosis of ACM. Per the HRS, "when evaluating patients suspected of an ACM, it is critical that the genetic tests conducted as part of the evaluation and the interpretation of the genetic test results be conducted by comprehensive teams with expertise in these disorders" (Towbin et al., 2019).

## BreastNext

BreastNext utilizes next generation sequencing to offer a comprehensive testing panel for hereditary breast and/or ovarian cancer and targets detection of mutations in 14 genes (ATM, BARD1, BRIP1, CDH1, CHEK2, MRE11A, MUTYH, NBN, PALB2, PTEN, RAD50, RAD51C, STK11 and TP53), excluding BRCA1 and BRCA2 (Raman, et al., 2013). Gross deletion/duplication analysis is performed for all 14 genes. Mutations in BRCA1 and BRCA2 explain hereditary breast cancer occurrence ~25–50% of the time, additional genes associated with hereditary breast cancer are emerging. Studies suggest that mutations in the genes on the BreastNext™ panel may confer an estimated 25–70% lifetime risk for breast cancer.

An assessment prepared for the Australian Health Policy Advisory Committee on Technology (HealthPACT) (Mundy, 2013) noted that many of the genes included in BreastNext are not just associated with breast cancer and therefore a mutation in one of these genes may indicate an elevated risk of a number of different cancers, making a specific management and surveillance strategy difficult. Several of the genes included in the BreastNext panel are involved in DNA repair: CHEK2, ATM, BRIP1 and PALB2. These genes are associated with an estimated two-fold risk of breast cancer in women and have been shown by numerous studies to be rare in the population. The assessment noted that there appears to be a reluctance by Australian clinicians to use this technology due to potential difficulties in interpreting the significance of mutations in some of the genes included in the panel.

The assessment noted that mutations detected in some of the genes included in the BreastNext panel may be considered amorphous in that they represent an increased risk, but there is little that may be done about that risk. In addition, the risk is not specific to one cancer, for example, a mutation in the Tp53 gene may represent an increased risk of cancer of the breast, colorectum or brain, making surveillance difficult. A positive test may therefore result in undue worry and stress on the patient.

The review identified no peer-reviewed studies in the literature that describes the use of the BreastNext panel in women considered to have a predisposition to breast cancer. Ambry Genetics have a .number of conference proceedings published on their website, The first 400 BRCA1 and BRCA2-negative women to receive testing with BreastNext were reported to the 2013 conference of the U.S. National Consortium of Breast Centers (NCBC). The NCBC is an "organisation of breast professionals, breast centres, providers of service to care providers, and corporations that supply equipment and pharmaceuticals to care providers." Of the 400 women, 41 (10%) were found to have a mutation in the following genes: PALB2 (n=9), ATM (n=9), CHEK2 (n=8), MUTYH (n=4), BARD1 (n=3), RAD50 (n=3) and one each in pTEN, RAD51C, Tp53, MRE11A and NBN (level IV diagnostic evidence). However, the significance of these results and the implications for patients of these positive results were not discussed. The HealthPACT assessment noted that positive tests may be difficult to interpret if the genetic variant is not the known founder mutation. In addition, mutations may occur in genes of known importance, but whether these mutations result in functional changes resulting in consequences for the health of the individual may remain unknown.

The HealthPACT assessment of BreastNext found that 21-gene and 14-gene panels detected a number of mutations in candidate genes, which may be of significance in women considered to be at a genetic predisposition to breast or ovarian cancer. The impact of these findings on patient outcomes was not discussed in any of the included papers. It may be assumed that these women and their first-degree relatives would then enter a surveillance programme. The HealthPACT assessment noted that, in Australia, first-degree relatives would be eligible to enter into such a programme even if testing for BRCA1 and BRCA2 was negative, therefore it is difficult to gauge the usefulness of tests such as BreastNext and OvaNext.

The HealthPACT assessment concluded that, although some of the genes included in the BreastNext panel may be associated with breast cancer, there is a paucity of evidence linking all of the included mutations with the disease. There were no peer-reviewed studies identified that could demonstrate the clinical utility of this product and therefore the impact this product may have on clinical decision making cannot be determined. Of concern is that these tests may be accessed by women who may not require testing, and that this may have consequences for the public health system. Therefore it is recommended that no further research on behalf of HealthPACT is warranted at this time.

## Brugada Syndrome

Brugada syndrome is an inherited condition comprising a specific EKG abnormality and an associated risk of ventricular fibrillation and sudden death in the setting of a structurally normal heart. Brugada syndrome is characterized by ST-segment abnormalities on EKG and a high risk of ventricular arrhythmias and sudden death. Brugada syndrome presents primarily during adulthood but age at diagnosis ranges from 2 days to 85 years. Clinical presentations may also include sudden infant death syndrome and sudden unexpected nocturnal death syndrome, a typical presentation in individuals from Southeast Asia.

Brugada et al (2005) reported that Brugada syndrome and LQTS are both due to mutations in genes encoding ion channels and that the genetic abnormalities causing Brugada syndrome have been linked to mutations in the ion channel gene SCN5A. Brugada stated that the syndrome has been identified only recently but an analysis of data from published studies indicates that the disease is responsible for 4 to 12 % of unexpected sudden deaths, and up to 50 % of all sudden death in patients with an apparently normal heart. Brugada explained that Brugada syndrome is a clinical diagnosis based on syncopal or sudden death episodes in patients with a structurally normal heart and a characteristic ECG pattern. The ECG shows ST segment elevation in the primordial leads V1-V3, with a morphology of the QRS complex resembling a right bundle branch block; this pattern may also be caused by J point elevation. When ST elevation is the most prominent feature, the pattern is called "coved-type". When the most prominent feature is J point elevation, without ST elevation the pattern is called "saddle-type". Brugada pointed out that it is important to exclude other causes of ST segment elevation before making the diagnosis of Brugada syndrome. Brugada syndrome is inherited in an autonomic dominant manner with variable penetrance. Most individuals diagnosed with Brugada syndrome have an affected parent. The proportion of cases caused by de novo mutations is estimated at 1 %. Each child of an individual with Brugada syndrome has a 50 % chance of inheriting the mutation. According to Brugada, antiarrhythmic drugs do not prevent sudden death in symptomatic or asymptomatic individuals with Brugada syndrome and that implantation of an automatic cardioverter-defibrillator is the only currently proven effective therapy.

To date the great majority of identified disease-causing mutations have been located in the SCN5A gene encoding the a subunit of the human cardiac voltage-gated sodium channel but such mutations can be identified in, at most, 30 % of affected people. Moreover, a positive genetic test adds little or nothing to the clinical management of such a person (HRUK, 2007). The identification of an SCN5A mutation does, of course, allow screening of family members but the usefulness of genetic screening may be less than for other familial syndromes, however, given that the routine 12-lead EKG (with or without provocative drug testing) appears to be a relatively effective method of screening for the condition.

## CADASIL

CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy) is a rare, genetically inherited, congenital vascular disease of the brain that causes strokes, subcortical dementia, migraine-like headaches, and psychiatric disturbances. CADASIL is very debilitating and symptoms usually surface around the age of 45. Although CADASIL can be treated with surgery to repair the defective blood vessels, patients often die by the age of 65. The exact incidence of CADASIL in the United States is unknown.

DNA testing for CADASIL is appropriate for symptomatic patients who have a family history consistent with an autosomal dominant pattern of inheritance of this condition. Clinical signs and symptoms of CADASIL include stroke, cognitive defects and/or dementia, migraine, and psychiatric disturbances. DNA testing is also indicated for pre-symptomatic patients where there is a family history consistent

with an autosomal dominant pattern of inheritance and there is a known mutation in an affected member of the family. This policy is consistent with guidelines on CADASIL genetic testing from the European Federation of Neurological Societies.

#### CancerNext

CancerNext™ (Ambry Genetics) utilizes next generation sequencing to offer a comprehensive testing panel for hereditary colon cancer and targets detection of mutations in 22 genes (APC, BMPR1A, CDH1, CHEK2, EPCAM, MLH1, MSH2, MSH6, MUTYH, PMS2, PTEN, SMAD4, STK11, and TP53) (Raman, et al., 2013). Gross deletion/duplication analysis is performed for all 22 genes. CancerNext™ is a next-generation cancer panel that simultaneously analyzes selected genes associated with a wide range of cancers. While mutations in each gene on this panel may be individually rare, they may collectively account for a significant amount of hereditary cancer susceptibility.

#### Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT)

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a highly lethal form of inherited arrhythmogenic disease characterized by adrenergically mediated polymorphic ventricular tachycardia (Liu et al, 2007). Mutations in the cardiac ryanodine receptor (RyR2) gene and the cardiac calsequestrin (CASQ2) gene are responsible for the autosomal dominant and recessive variants of CPVT, respectively. The clinical presentation encompasses exercise- or emotion-induced syncopal events and a distinctive pattern of reproducible, stress-related, bi-directional ventricular tachycardia in the absence of both structural heart disease and a prolonged QT interval.

CPVT typically begins in childhood or adolescence. The mortality rate in untreated individuals is 30 to 50 % by age 40 years. Clinical evaluation by exercise stress testing and Holter monitoring and genetic screening can facilitate early diagnosis. Beta-blockers are the most effective drugs for controlling arrhythmias in CPVT patients, yet about 30 % of patients with CPVT still experience cardiac arrhythmias on beta-blockers and eventually require an implantable cardioverter defibrillator. Liu et al (2008) stated that molecular genetic screening of the genes encoding the cardiac RyR2 and CASQ2 is critical to confirm uncertain diagnosis of CPVT.

Katz et al (2009) noted that CPVT is a primary electrical myocardial disease characterized by exercise- and stress-related ventricular tachycardia manifested as syncope and sudden death. The disease has a heterogeneous genetic basis, with mutations in the cardiac RyR2 gene accounting for an autosomal-dominant form (CPVT1) in approximately 50 % and mutations in the cardiac CASQ2 gene accounting for an autosomal-recessive form (CPVT2) in up to 2 % of CPVT cases. Both RyR2 and calsequestrin are important participants in the cardiac cellular calcium homeostasis. These researchers reviewed the physiology of the cardiac calcium homeostasis, including the cardiac excitation contraction coupling and myocyte calcium cycling.

Although the clinical presentation of CPVT is similar in many respects to the LQTS, there are important differences that are relevant to genetic testing. CPVT appears to be a more malignant condition, as many people are asymptomatic before the index lethal event and the majority of cardiac events occur before 20 years of age. Affected people are advised to avoid exercise-related triggers and start prophylactic beta-blockers with dose titration guided by treadmill testing.

Genetic testing has been recommended in individuals with clinical features considered typical of CPVT following expert clinical assessment (HRUK, 2008). Clinically the condition is difficult to diagnose in asymptomatic family members as the ECG and echocardiogram are completely normal at rest. Exercise stress testing has been advised in family members in order to identify exercise-induced

ventricular arrhythmias, but the sensitivity of this clinical test is unknown. Although the diagnostic yield from genetic testing is less than that for the LQTS (about 50 %) in patients with typical clinical features, a positive genetic test may be of value for the individual patient (given the prognostic implications) and for screening family members (given the difficulties in clinical screening methods) (HRUK, 2008). The RyR2 gene is large and a “targeted” approach is usually undertaken, in which only exons that have been previously implicated are examined.

The 2006 guidelines from the American College of Cardiology on management of patients with ventricular arrhythmias and the prevention of sudden cardiac death (Zipes et al, 2006) included the following recommendations for patients with CPVT:

- There is evidence and/or general agreement supporting the use of beta blockers for patients clinically diagnosed on the basis of spontaneous or documented stress-induced ventricular arrhythmias.
- There is evidence and/or general agreement supporting the use of an implantable ICD in combination with beta blockers for survivors of cardiac arrest who have a reasonable expectation of survival with a good functional capacity for more than 1 year.
- The weight of evidence and/or opinion supports the use of beta blockers in patients without clinical manifestations who are diagnosed in childhood based upon genetic analysis.
- The weight of evidence and/or opinion supports the use of an ICD in combination with beta blockers for patients with a history of syncope and/or sustained ventricular tachycardia while receiving beta blockers who have a reasonable expectation of survival with a good functional capacity for more than 1 year.
- The usefulness and/or efficacy of beta blockers is less well established in patients without clinical evidence of arrhythmias who are diagnosed in adulthood based upon genetic analysis.

Priori et al. (2015) state that CPVT is diagnosed in patients who are carriers of a pathogenic mutation(s) in the gene RYR2 or CASQ2 (Class of recommendation: I, Level of evidence: C). The authors state that mutations in other genes such as KCNJ2, Ank2, TRDN and CALM1 have been identified in patients with clinical features similar to CPVT; however, it is not clear whether they are phenocopies of CPVT.

CPVT occurs in the absence of structural heart disease or known associated syndromes. Affected patients may have a family history of juvenile sudden death or stress-induced syncope. Per a review in UpToDate on "Catecholaminergic polymorphic ventricular tachycardia" (Buxton, 2022), the clinical presentation of CPVT is variable, including some patients who are asymptomatic and identified as part of familial screening. Affected patients who are symptomatic typically present with syncope or cardiac arrest due to ventricular tachycardia or ventricular fibrillation precipitated by emotional or physical stress. Thus, the author recommends genetic testing in patients with "clinical presentation or pedigree that is suggestive for CPVT", in which a genetic screening panel may help support the diagnosis. "The genetic panel should include the following genes: RYR2, CASQ2, TRDN, TECRL, CALM1, CALM2, and CALM3". Furthermore, if a patient is found to have a pathogenic variant for CPVT, the authors recommend genetic screening in first-degree relatives.

#### CDH1 Mutations

According to the NCCN Guidelines Version 3.2024 Hereditary Diffuse Gastric Cancer, the genetic testing criteria for CDH1 mutations for hereditary diffuse gastric cancer include the following:

Genetic testing for CDH1 mutations should be considered when any of the following criteria is met:

- Individual with a known CDH1 pathogenic variant (PV) in the family; or
- An individual with diffuse gastric cancer (DGC) at any age; or
- Family history of ≥ first-degree or second-degree relatives with gastric cancer with at least one diagnosed at age ≤ 50 years or at least one confirmed to be DGC at any age.

#### Celiac Disease

Celiac disease is an immune disorder in which an individual is unable to tolerate gluten, a protein found in wheat, rye and barley and sometimes in products such as vitamin supplements and some medications. The diagnosis of celiac disease is based on the biopsy and histopathologic examination of the small intestine. Blood tests may be used to choose individuals for biopsy and to aid in diagnosis. Genetic testing, which may also be referred to as human leukocyte antigen (HLA) typing for celiac disease, may be ordered if results from these tests are inconclusive.

#### Charcot-Marie Tooth Disease

Charcot-Marie-Tooth (CMT) hereditary neuropathy refers to a group of disorders characterized by a chronic motor and sensory polyneuropathy, also known as hereditary motor and sensory neuropathy (HMSN). It is the most common inherited disorder that involves the peripheral nerves, affecting an estimated 150,000 people in the United States. It occurs in populations worldwide with a prevalence of about 1 in 3,300 individuals. The clinical manifestations can vary greatly in severity and age of onset. The clinical features may be so mild that they may be undetectable by patients, their families and physicians.

Charcot-Marie-Tooth disease is usually diagnosed by an extensive physical examination. Symptoms include foot drop or clubfoot, paresthesia in legs, sloping gait, later weakness and atrophy of hands, then arms, absence or reduction of deep tendon reflexes, and occasionally mild sensory loss. The clinical diagnosis is then confirmed by electromyogram and nerve conduction velocity tests, and sometimes by biopsy of muscle and of sural cutaneous nerve. Genetic testing can aid in confirming the diagnosis after EMG. Since CMT is a hereditary disease, family history can also help to confirm the diagnosis and it may be appropriate to skip EMG and go directly to genetic testing in a patient with a strong family history of confirmed CMT, especially when a relative has a known mutation. Prenatal testing for pregnancies at increased risk is possible for some types of CMT if the disease-causing mutation in the family is already known.

CMT is genetically and clinically heterogeneous. It is usually inherited in an autosomal dominant manner, and occasionally in an autosomal recessive manner. The major categories of CMT are CMT types 1 through 7 as well as an X-linked category, CMTX. In the X-linked recessive patterns, only males develop the disease, although females who inherit the defective gene can pass the disease onto their sons. In the X-linked dominant pattern, an affected mother can pass on the disorder to both sons and daughters, while an affected father can only pass it onto his daughters. Within each type, a specific disease associated with a particular gene is assigned a letter (eg, CMT1A, CMT1B, etc). The majority of cases fall within type 1 (autosomal dominant inheritance, demyelinating physiology) and type 2 (autosomal dominant inheritance, axonal physiology), with an estimated prevalence of 40 per 100,000. Charcot Marie Tooth Type I disease is a demyelinating neuropathy with hypertrophic changes in peripheral nerves, and has its onset usually during late childhood. On the other hand, CMT Type II is an axonal (non-demyelinating) neuronal disorder without hypertrophic changes, and has its onset generally during adolescence. Both CMT Types I and II are characterized by a slow degeneration of peripheral nerves and roots, resulting in distal muscle atrophy commencing in the lower extremities, and affecting the upper extremities several years later.

More than 30 genes have been identified to date, with the vast majority of cases attributed to mutations in these four genes: PMP22, MPZ, GJB1, and MFN2. The association of different mutations within the same gene with various clinical phenotypes is a common finding in the HMSN/CMT group of peripheral neuropathies. This variability suggests that these disorders represent a spectrum of related phenotypes caused by an underlying defect in peripheral nervous system myelination and/or axonal function. The most common type of CMT1, CMT1A, is caused by a duplication of the PMP22 gene, accounting for 70 to 80 percent of CMT1 cases. This gene encodes a peripheral myelin protein with an apparent molecular weight of 22,000 or a DNA duplication of a specific region 5 megabases) including the PMP22 gene in the proximal short arm of chromosome 17 (band 17p11.2-p12). Thus, patients with CMT Type IA represent approximately 50 % of all CMT cases. Therefore, single-gene testing for PMP22 duplication/deletion is recommended as the first test for CMT.

Charcot Marie Tooth disease is not a fatal disorder. It does not shorten the normal life expectancy of patients, and it does not affect them mentally. As stated earlier, there is a wide range of variation in the clinical manifestations of CMT – the degree of severity can vary considerably from patient to patient, even among affected family members within the same generation. The condition can range from having no problems to having major difficulties in ambulation in early adult life, however, the latter is unusual. Most patients are able to ambulate and have gainful employment until old age. Currently, there is no specific disease-modifying treatment for this disease. Management of the majority of patients with CMT disease consists of supportive care with emphasis on proper bracing, foot care, physical therapy and occupational counseling. For example, the legs and shoes can be fitted with light braces and springs, respectively, to overcome foot drop. If foot drop is severe and the disease has become stationary, the ankle can be stabilized by arthrodeses.

In summary, the conventional means of diagnosis of CMT is through physical examination, family history, electromyography (EMG) and nerve conduction velocity studies. Thus, the value of genetic testing for CMT is to confirm the diagnosis and to distinguish this from other causes of neuropathy.

#### Choroidal Neovascularization

RetnaGene AMD (Sequenom Center for Molecular Medicine) is a laboratory developed genetic test to assess the risk of developing choroidal neovascularization (CNV), the wet form of age-related macular degeneration (AMD), a common eye disorder of the elderly that can lead to blindness. The test identifies at-risk Caucasians, age 60 and older. A report of the American Academy of Ophthalmology (Stone, et al., 2012) recommends avoidance of routine genetic testing for genetically complex disorders like age-related macular degeneration and late-onset primary open-angle glaucoma until specific treatment or surveillance strategies have been shown in one or more published clinical trials to be of benefit to individuals with specific disease-associated genotypes. The report recommends that, in the meantime, genotyping of such patients should be confined to research studies. The report stated that complex disorders (e.g., age-related macular degeneration and glaucoma) tend to be more common in the population than monogenic diseases, and the presence of any one of the disease-associated variants is not highly predictive of the development of disease. The report stated that, in many cases, standard clinical diagnostic methods like biomicroscopy, ophthalmoscopy, tonography, and perimetry will be more accurate for assessing a patient's risk of vision loss from a complex disease than the assessment of a small number of genetic loci. The report said that genetic testing for complex diseases will become relevant to the routine practice of medicine as soon as clinical trials can demonstrate that patients with specific genotypes benefit from specific types of

therapy or surveillance. The report concluded that, until such benefit can be demonstrated, the routine genetic testing of patients with complex eye diseases, or unaffected patients with a family history of such diseases, is not warranted.

#### ColoNext

ColoNext™ (Ambry Genetics) utilizes next generation sequencing to offer a comprehensive testing panel for hereditary colon cancer and targets detection of mutations in 14 genes (APC, BMPR1A, CDH1, CHEK2, EPCAM, MLH1, MSH2, MSH6, MUTYH, PMS2, PTEN, SMAD4, STK11, and TP53) (Raman, et al., 2013). Gross deletion/duplication analysis is performed for all 14 genes. ColoNext™ is a next-generation cancer panel that simultaneously analyzes selected genes associated with a wide range of cancers. While mutations in each gene on this panel may be individually rare, they may collectively account for a significant amount of hereditary cancer susceptibility.

#### Coloseq

ColoSeq™ (University of Washington Laboratory Medicine Genetics Lab) is a comprehensive genetic test for hereditary colon cancer that uses next-generation sequencing to detect mutations in multiple genes associated with Lynch syndrome (hereditary non-polyposis colorectal cancer, HNPCC), familial adenomatous polyposis (FAP), MUTYH-associated polyposis (MAP), hereditary diffuse gastric cancer (HDGC), Cowden syndrome, Li-Fraumeni syndrome, Peutz-Jeghers syndrome, Muir-Torre syndrome, Turcot syndrome, and Juvenile Polyposis syndrome (Raman, et al., 2013). The assay sequences all exons, introns, and flanking sequences of the 13 genes. Large deletions, duplications, and mosaicism are also detected by the assay and reported.

#### Congenital Myotonia

Monteagudo et al (2015) stated that congenital myotonia (CM) is characterized by a delay in muscular relaxation after sudden contractions. In a recent outbreak of ovine CM affecting 1 % of new-born lambs in a Spanish flock of Rasa Aragonesa sheep, a comparative pathology approach was taken: because a mutation in the muscle chloride channel gene (CLCN1) was identified as responsible for CM in goats, the same gene was sequenced in the affected lambs. A non-synonymous single nucleotide variation (SNV) in the second exon of CLCN1 was associated with this pathology. Rams carrying this SNV heterozygously were thereafter identified and replaced by wild-type homozygous young males. No additional CM cases were detected in subsequent lambing seasons.

Miryounesi et al (2016) noted that congenital recessive myotonia is a rare genetic disorder caused by mutations in CLCN1, which codes for the main skeletal muscle chloride channel CIC-1. More than 120 mutations have been found in this gene. The main feature of this disorder is muscle membrane hyper-excitability. These investigators reported the case of a 59-year old male patient suffering from CM. He had transient generalized myotonia, which started in early childhood. These investigators analyzed CLCN1 sequence in this patient and other members of his family. They found a new missense mutation in CLCN1 gene (c.1886T>C, p.Leu629Pro). Co-segregation of this mutation with the disease was demonstrated by direct sequencing of the fragment in affected as well as unaffected members of this family. In addition, in silico analyses predicted that this nucleotide change would impair the protein function. The authors concluded that this new nucleotide variation can be used for prenatal diagnosis in this family.

An UpToDate review on “Myotonic dystrophy: Etiology, clinical features, and diagnosis” (Darras and Chad, 2016) states the following:

“Current understanding of the pathophysiology of this disease posits what is known

as a "trans" effect, in which the repeat expansions exert a dominant toxic effect on other genes not localized to either the DM1 or DM2 loci. This effect is mediated by two RNA-binding protein families: (i) Muscleblind-like (MBNL), and (ii) CUG-BP- and ETR-3-like-factors (CELF). According to this theory, for which there is some experimental support, the CUG and CCUG RNA expansions fold into a hairpin structure, and these mutant RNAs accumulate in the nucleus. In DM1, the mutant CUG repeat containing RNA sequesters MBNL1 and leads to loss of its function. The mutant RNAs alter RNA binding protein activity, which in turn results in aberrant splicing and abnormal function of several genes, including the bridging integrator 1 gene (BIN1), the skeletal muscle chloride channel, the insulin receptor, and cardiac troponin T. Muscle weakness may result from sequestration of MBNL1 by expanded CUG or CCUG repeats, leading to alternative splicing of BIN1 and skipping of muscle-specific exon 11 of BIN1 messenger RNA, which in turn causes disorganized T tubules and impairs excitation-contraction coupling. Skeletal muscle chloride channel dysfunction is responsible for the myotonia and is related to increased inclusion of exon 7A during misregulated alternative splicing of the CLCN1 gene. Mutant RNA appears to induce expression of the cardiac transcription factor NKX2-5, which may account for the cardiac conduction disturbances associated with DM1. Dysregulation of alpha-dystrobrevin splicing may also play a role in the muscle weakness and wasting found with DM1".

#### Congenital Stationary Night Blindness

According to Orphanet (a portal for rare diseases and orphan drugs), congenital stationary night blindness (CSNB) is an inherited retinal disorder that predominates on rods. It is a rare disease; and 3 types of transmission can be found: (i) utosomal dominant, (ii) recessive, and (iii) X-linked recessive. The affection is heterogeneous. The only symptom is hemeralopia with a moderate loss of visual acuity. Both the funduscopy and visual field are normal. In recessive forms, the "b" wave on the electrotoretinogram/electroretinography (ERG) is not found in the scotoscopic study, while the "a" wave is normal and increases with light intensity. In dominant forms, the "b" wave is seen. Levels of rhodopsine are normal and regenerate normally. Signal transmission may be the affected function. There is no specific treatment for CSNB. According to Genetic Home Reference, X-linked CSNB is a disorder of the retina. People with this condition typically have difficulty seeing in low light (night blindness). They also have other vision problems, including reduced acuity, high myopia, nystagmus, and strabismus. Color vision is typically not affected by this disorder. The visual problems associated with this condition are congenital. They tend to remain stable (stationary) over time. Researchers have identified 2 major types of X-linked CSNB: (i) the complete form, and (ii) the incomplete form. The types have very similar signs and symptoms. However, everyone with the complete form has night blindness, while not all people with the incomplete form have night blindness. The types are distinguished by their genetic cause and by the results of ERG.

In general, the diagnosis of X-linked CSNB can be made by ophthalmologic examination (including ERG) and family history consistent with X-linked inheritance (Boycott et al, 2012).

According to a Medscape review on "The Genetics of Hereditary Retinopathies and Optic Neuropathies" (Iannaccone, 2005), CSNB can be inherited according to all Mendelian inheritance patterns; 2 X-linked and 2 autosomal dominant genes have been cloned. In all types of CSNB, night vision is congenitally but non-progressively impaired and the retinal examination is normal. Most CSNB patients also have congenital nystagmus as the presenting sign, which can create a differential diagnostic challenge with Leber congenital amaurosis. Typically, patients with complete X-linked CSNB are also moderate-to-high myopes. The X-linked CSNB forms, which are the most common ones, all share an electronegative electrotoretinogram response similar to that seen in X-linked retinoschisis, and are

distinguished in CSNB type 1 (also known as complete CSNB) and CSNB type 2 (incomplete CSNB) based on additional electroretinogram features, a distinction that has been confirmed at the genetic level".

Price et al (1988) reported that 7 of 8 patients presented initially or were followed for decreased acuity and nystagmus without complaints of night blindness. The diagnosis of CSNB was established with ERG and dark adaptation testing. They stated that careful electrodiagnostic testing is needed to provide accurate genetic counseling. Two patients showed pupillary constriction to darkness, which is a sign of retinal disease in young patients.

Lorenz et al (1996) presented the clinical data of 2 families with X-linked incomplete CSNB previously undiagnosed; ERG recordings in both families were suggestive of CSNB. The ERG of the obligate carrier was normal. In an attempt to distinguish between the complete and the incomplete type, and to identify further carrier signs, scotopic perimetry and dark adaptation were performed in both affected males and carriers. Scotopic perimetry tested the rod-mediated visual pathway in its spatial distribution. In affected males with non-recordable ERGs, scotopic perimetry and dark adaptation disclosed residual rod function indicating an incomplete type. In carriers, there was a sensitivity loss at 600 nm, which may be a new carrier sign. The authors concluded that correct diagnosis of the different forms of CSNB together with the identification of carriers is important for (i) genetic counseling, and (ii) linkage studies to identify the gene(s) for CSNB.

Kim et al (2012) evaluated the frequency of negative waveform ERGs in a tertiary referral center. All patients who had an ERG performed at the electrophysiology clinic at Emory University from January 1999 through March 2008 were included in the study. Patients with b-wave amplitude less than or equal to a-wave amplitude during the dark-adapted bright flash recording, in at least 1 eye, were identified as having a "negative ERG". Clinical information, such as age, gender, symptoms, best corrected visual acuity, and diagnoses were recorded for these patients when available. A total of 1,837 patients underwent ERG testing during the study period. Of those, 73 patients had a negative ERG, for a frequency of 4.0 %. Within the adult (greater than or equal to 18 years of age) and pediatric populations, the frequencies of a negative ERG were 2.5 and 7.2 %, respectively. Among the 73 cases, negative ERGs were more common among male than female patients, 6.7 % versus 1.8 % ( $p < 0.0001$ ). Negative ERGs were most common among male children and least common among female adults, 9.6 % versus 1.1 %, respectively, ( $p < 0.0001$ ). Overall in this group of patients, the most common diagnoses associated with a negative ERG were CSNB (n = 29) and X-linked retinoschisis (XLRS, n = 7). The authors concluded that the overall frequency of negative ERGs in this large retrospective review was 4.0 %. Negative ERGs were most common among male children and least common among female adults. Despite the growing number of new diagnoses associated with negative ERGs, CSNB, and XLRS appear to be the most likely diagnoses for a pediatric patient who presents with a negative ERG.

It is also interesting to note that in a recently completed clinical trial (last verified June 2012) of "Treatment of Congenital Stationary Night Blindness with an Alga Containing High Dose of Beta Carotene", the selection criteria for participants of this trial do not include genetic testing. They included the following: (i) isolated rod response markedly reduced (less than 20 % of normal) after 20 mins dark adaptation and improved by 50 % after 2 hrs, (ii) negative maximal response ("a" wave to "b" wave ratio less than 2), and (iii) retinal mid-peripheral white dots (more than 3,000 dots).

Kumar et al (2009) noted that many independent prognostic markers have been identified for predicting survival and helping in the management of lung cancer cases. p53 protein over-expression and mutation have been the topic of numerous such publications. However, little is known about the role of anti-p53 antibodies as a prognostic marker in lung cancer. These investigators searched the MEDLINE database and the bibliographies of the retrieved manuscripts and reviews. The

retrieved studies are grouped according to the cohort studied. Out of 179 citations retrieved, 17 met selection criteria. A total of 7 studies used only non-small-cell lung cancer (NSCLC); 4 studies used only small-cell lung cancer; and 6 studies used the mixed cohort of both types of lung cancer. The studies varied in the concept design, cohort studied and the methodology. The prognostic role of anti-p53 antibodies in lung cancer remained contradictory and as some studies showed an association with poor prognosis, others showed a favorable association and still others showing no association what so ever. The frequency of detection of anti-p53 antibody was very low, highly specific with result being independent of the cohort studied. The authors concluded that adequate clinical trials, with optimized cohort, antigen and assay validation, are needed to address patients and physician's concerns regarding these associations.

Ciancio et al (2011) stated that over-expression of the tumor suppressor gene p53 and the marker for cellular proliferation Ki67 in open lung biopsies are indicated as predictor factors of survival of patients with lung cancer. However, the prognostic value of p53 and Ki67 in fiberoptic bronchial biopsies (FBB) has not been fully investigated. These researchers evaluated p53 and Ki67 immunostaining in FBB from 19 with NSCLC (12 adenocarcinomas, 5 squamous cell carcinomas and 2 NSCLC-NOS). Fiberoptic bronchial biopsy specimens were fixed in formalin, embedded in paraffin, and immunostained using anti-p53 and anti-Ki67 antibodies. Slides were reviewed by 2 independent observers and classified as positive (+ve) when the number of cells with stained nuclei exceeded 15 % for p53 or when greater than 25 % positive cells were observed throughout each section for Ki67. Positive (+ve) immunostaining was found in 9 patients for p53 (47.37 %) and 8 patients for Ki67 (42.10 %). These investigators examined overall survival (OS) curves of the patients with Mantel's log-rank test, both p53 -ve and Ki67 -ve patients had significantly higher survival rates than p53 + ve ( $p < 0.005$ ) and Ki67 + ve ( $p < 0.0001$ ), respectively. The authors concluded that the findings of this study suggested that negative immunostaining of fiberoptic bronchial biopsies for p53 and Ki67 could represent a better prognostic factor for patients with NSCLC.

Mattioni et al (2013) noted that TP53 gene mutations can lead to the expression of a dysfunctional protein that in turn may enable genetically unstable cells to survive and change into malignant cells. Mutant p53 accumulates early in cells and can precociously induce circulating anti-p53 antibodies (p53Abs); in fact, p53 over-expression has been observed in pre-neoplastic lesions, such as bronchial dysplasia, and p53Abs have been found in patients with chronic obstructive pulmonary disease, before the diagnosis of lung and other tobacco-related tumors. These researchers performed a large prospective study, enrolling non-smokers, ex-smokers and smokers with or without the impairment of lung function, to analyze the incidence of serum p53Abs and the correlation with clinicopathologic features, in particular smoking habits and impairment of lung function, in order to investigate their possible role as early markers of the onset of lung cancer or other cancers. The p53Ab levels were evaluated by a specific ELISA in 675 subjects. Data showed that significant levels of serum p53Abs were present in 35 subjects (5.2 %); no difference was observed in the presence of p53Abs with regard to age and gender, while p53Abs correlated with the number of cigarettes smoked per day and packs-year. Furthermore, serum p53Abs were associated with the worst lung function impairment. The median p53Ab level in positive subjects was 3.5 units/ml (range of 1.2 to 65.3 units/ml). Only 15 positive subjects participated in the follow-up, again resulting positive for serum p53Abs, and no evidence of cancer was found in these patients. The authors concluded that the presence of serum p53Abs was found to be associated with smoking level and lung function impairment, both risk factors of cancer development. However, in this study these researchers did not observe the occurrence of lung cancer or other cancers in the follow-up of positive subjects, therefore they cannot directly correlate the presence of serum p53Abs with cancer risk.

Lei et al (2013) stated that the diagnosis of lung cancer remains a clinical challenge. Many studies have assessed the diagnostic potential of anti-p53 antibody in lung cancer patients but with controversial results. These researchers summarized the overall diagnostic performance of anti-p53 antibody in lung cancer. Based on a comprehensive search of the PubMed and Embase, these investigators identified outcome data from all articles estimating diagnostic accuracy of anti-p53 antibody for lung cancer. A summary estimation for sensitivity, specificity, and other diagnostic indexes were pooled using a bivariate model. The overall measure of accuracy was calculated using summary receiver operating characteristic curve and the area under curve (AUC) was calculated. According to the inclusion criteria, a total of 16 studies with 4,414 subjects (2,249 lung cancers, 2,165 controls) were included. The summary estimates were: sensitivity 0.20 (95 % confidence interval [CI]: 0.15 to 0.27), specificity 0.97 (95 % CI: 0.95 to 0.98), positive likelihood ratio 6.64 (95 % CI: 4.34 to 10.17), negative likelihood ratio 0.83 (95 % CI: 0.77 to 0.89), diagnostic odds ratio 8.04 (95 % CI: 5.05 to 12.79), the AUC was 0.84. Subgroup analysis suggested that anti-p53 antibody had a better diagnostic performance for small cell lung cancer than non-small cell lung cancer. The authors concluded that anti-p53 antibody can be an assistant marker in diagnosing lung cancer, but the low sensitivity limits its use as a screening tool for lung cancer. Moreover, they stated that further studies should be performed to confirm these findings.

UpToDate reviews on "Overview of the initial evaluation, treatment and prognosis of lung cancer" (Midthun, 2014a) and "Overview of the risk factors, pathology, and clinical manifestations of lung cancer" (Midthun, 2014b) do not mention anti-p53 and anti-MAPKAPK3 as biomarkers.

Also, an UpToDate review on "Screening for lung cancer" (Deffebach and, Humphrey, 2014) does not mention anti-MAPKAPK3 as a biomarker. Moreover, it lists "Immunostaining or molecular analysis of sputum for tumor markers. As examples, p16 ink4a promoter hypermethylation and p53 mutations have been shown to occur in chronic smokers before there is clinical evidence of neoplasia" as one of the technologies under investigation.

Furthermore, National Comprehensive Cancer Network's clinical practice guideline on "Non-small cell lung cancer" (Version 4.2014) does not mention anti-p53 and anti-MAPKAPK3 as biomarkers.

SLCO1B1 testing has been proposed to predict risk of statin-induced myopathy. Talameh and, Kitzmiller (2014) noted that statins are the most commonly prescribed drugs in the United States and are extremely effective in reducing major cardiovascular events in the millions of Americans with hyperlipidemia. However, many patients (up to 25 %) cannot tolerate or discontinue statin therapy due to statin-induced myopathy (SIM). Patients will continue to experience SIM at unacceptably high rates or experience unnecessary cardiovascular events (as a result of discontinuing or decreasing their statin therapy) until strategies for predicting or mitigating SIM are identified. A promising strategy for predicting or mitigating SIM is pharmacogenetic testing, particularly of pharmacokinetic genetic variants as SIM is related to statin exposure. Data are emerging on the association between pharmacokinetic genetic variants and SIM. A current, critical evaluation of the literature on pharmacokinetic genetic variants and SIM for potential translation to clinical practice is lacking. This review focused specifically on pharmacokinetic genetic variants and their association with SIM clinical outcomes. These investigators also discussed future directions, specific to the research on pharmacokinetic genetic variants, which could speed the translation into clinical practice. For simvastatin, these researchers did not find sufficient evidence to support the clinical translation of pharmacokinetic genetic variants other than SLCO1B1. However, SLCO1B1 may also be clinically relevant for pravastatin- and pitavastatin-induced myopathy, but additional studies assessing SIM clinical

outcome are needed. CYP2D6\*4 may be clinically relevant for atorvastatin-induced myopathy, but mechanistic studies are needed. The authors concluded that future research efforts need to incorporate statin-specific analyses, multi-variant analyses, and a standard definition of SIM. As the use of statins is extremely common and SIM continues to occur in a significant number of patients, future research investments in pharmacokinetic genetic variants have the potential to make a profound impact on public health.

Kuhlenbaumer and colleagues (2014) provided a comprehensive meta-analysis and review of the clinical and molecular genetics of essential tremor (ET). Studies were reviewed from the literature. Linkage studies were analyzed applying criteria used for monogenic disorders. For association studies, allele counts were extracted and allelic association calculated whenever possible. A meta-analysis was performed for genetic markers investigated in more than 3 studies. Linkage studies have shown conclusive results in a single family only for the locus ETM2 (essential tremor monogenetic locus 2, logarithm of odds score [lod] greater than 3.3). None of the 3 ETM loci had been confirmed independently with a lod score greater than 2.0 in a single family. A mutation in the FUS gene (fused in sarcoma) was found in one ET family by exome sequencing. Two genome-wide association studies demonstrated association between variants in the LINGO1 gene (leucine-rich repeat and Ig domain containing 1) and the SLC1A2 gene (solute carrier family 1 member 2) and ET, respectively. This meta-analysis confirmed the association of rs9652490 in LINGO1 with ET. Candidate gene mutation analysis and association studies have not identified reproducible associations. The authors concluded that problems of genetic studies of ET are caused by the lack of stringent diagnostic criteria, small sample sizes, lack of biomarkers, a high phenocopy rate, evidence for non-Mendelian inheritance, and high locus heterogeneity in presumably monogenic ET. They stated that these issues could be resolved by better worldwide cooperation and the use of novel genetic techniques.

Taylor et al (2014) noted that mitochondrial disorders have emerged as a common cause of inherited disease, but their diagnosis remains challenging. Multiple respiratory chain complex defects are particularly difficult to diagnose at the molecular level because of the massive number of nuclear genes potentially involved in intra-mitochondrial protein synthesis, with many not yet linked to human disease. These researchers determine the molecular basis of multiple respiratory chain complex deficiencies. They studied 53 patients referred to 2 national centers in the United Kingdom and Germany between 2005 and 2012. All had biochemical evidence of multiple respiratory chain complex defects but no primary pathogenic mitochondrial DNA mutation. Whole-exome sequencing was performed using 62-Mb exome enrichment, followed by variant prioritization using bioinformatic prediction tools, variant validation by Sanger sequencing, and segregation of the variant with the disease phenotype in the family. Presumptive causal variants were identified in 28 patients (53 %; 95 % CI: 39 % to 67 %) and possible causal variants were identified in 4 (8 %; 95 % CI: 2 % to 18 %). Together these accounted for 32 patients (60 % 95 % CI: 46 % to 74 %) and involved 18 different genes. These included recurrent mutations in RMND1, AARS2, and MTO1, each on a haplotype background consistent with a shared founder allele, and potential novel mutations in 4 possible mitochondrial disease genes (VARS2, GARS, FLAD1, and PTCD1). Distinguishing clinical features included deafness and renal involvement associated with RMND1 and cardiomyopathy with AARS2 and MTO1. However, atypical clinical features were present in some patients, including normal liver function and Leigh syndrome (subacute necrotizing encephalomyopathy) seen in association with TRMU mutations and no cardiomyopathy with founder SCO2 mutations. It was not possible to confidently identify the underlying genetic basis in 21 patients (40 %; 95 % CI: 26 % to 54 %). The authors concluded that exome sequencing enhanced the ability to identify potential nuclear gene mutations in patients with biochemically defined defects affecting multiple

mitochondrial respiratory chain complexes. Moreover, they stated that additional study is needed in independent patient populations to determine the utility of this approach in comparison with traditional diagnostic methods.

UpToDate reviews on "Diagnostic evaluation of women with suspected breast cancer" (Esserman and Joe, 2014a), "Clinical features, diagnosis, and staging of newly diagnosed breast cancer" (Esserman and Joe, 2014b), and "Clinical manifestations and diagnosis of a palpable breast mass" (Sabel, 2014) do not mention RAD51C gene testing.

Furthermore, NCCN's clinical practice guidelines on "Breast cancer" (Version 3.2014) and "Ovarian cancer including fallopian tube cancer and primary peritoneal cancer" (Version 3.2014) do not mention RAD51C gene testing.

Yang et al (2103) stated that osteoporosis is characterized by low bone mineral density (BMD), a highly heritable trait that is determined, in part, by the actions and interactions of multiple genes. Although an increasing number of genes have been identified to have independent effects on BMD, few studies have been performed to identify genes that interact with one another to affect BMD.

Kim et al (2013) noted that BMD loci were reported in Caucasian genome-wide association studies (GWAS). These researchers investigated the association between 59 known BMD loci (+200 suggestive SNPs) and DXA-derived BMD in East Asian population with respect to sex and site specificity. They also identified 4 novel BMD candidate loci from the suggestive SNPs. A total of 2,729 unrelated Korean individuals from a population-based cohort were analyzed. The authors selected 747 single-nucleotide polymorphisms (SNPs). These markers included 547 SNPs from 59 loci with genome-wide significance (GWS, p value less than  $5 \times 10(-8)$ ) levels and 200 suggestive SNPs that showed weaker BMD association with p value less than  $5 \times 10(-5)$ . After quality control, 535 GWS SNPs and 182 suggestive SNPs were included in the replication analysis. Of the 535 GWS SNPs, 276 from 25 loci were replicated ( $p < 0.05$ ) in the Korean population with 51.6 % replication rate. Of the 182 suggestive variants, 16 were replicated ( $p < 0.05$ , 8.8 % of replication rate), and 5 reached a significant combined p value (less than  $7.0 \times 10(-5)$ , 0.05/717 SNPs, corrected for multiple testing). Two markers (rs11711157, rs3732477) are for the same signal near the gene CPN2 (carboxypeptidase N, polypeptide 2). The other variants, rs6436440 and rs2291296, were located in the genes AP1S3 (adaptor-related protein complex 1, sigma 3 subunit) and RARB (retinoic acid receptor, beta). The authors concluded that these results illustrated ethnic differences in BMD susceptibility genes and underscored the need for further genetic studies in each ethnic group. The authors were also able to replicate some SNPs with suggestive associations. These SNPs may be BMD-related genetic markers and should be further investigated.

The Institute for Clinical Systems Improvement's clinical guideline on "Diagnosis and treatment of osteoporosis" (Florence et al, 2013) did not mention the use of genetic testing.

Furthermore, an UpToDate review on "Pathogenesis of osteoporosis" (Manolagas, 2104) states that "Genetics - A portion of the variation in BMD among humans has a genetic basis. Genome-wide association studies have so far identified approximately 80 genetic loci that influence BMD. A remarkable number of these loci are involved in some aspect of Wnt/ of Wnt/beta-catenin signaling, the receptor activator of nuclear factor kappa-B (RANK)/RANK ligand (RANKL)/osteoprotegerin (OPG) axis, or in mesenchymal cell differentiation. The contribution of individual genetic variants, however, is small, and of the total variance in BMD only a small percentage is explained by variants of genes identified. To date, there are no

genome-wide association studies on fracture or BMD loss. Therefore, it remains unclear whether the same genes that determine BMD also affect the rate of bone loss with advancing age or the risk of fractures".

Copy Number Variation Detection Tools (e.g., SMASH [Short Multiply Aggregated Sequence Homologie], Marvel Genomics)

Short Multiply Aggregated Sequence Homologies (SMASH) is being advocated to have the potential to provide a drastic improvement in detection of copy number variants (CNV) with much greater resolution than current detection methods at a much lower cost than currently available technologies. The SMASH technology has the potential to be applied in other research areas including cancer, schizophrenia and other neuro-psychiatric disorders.

Wang and colleagues (2016) stated that CNVs underlie a significant amount of genetic diversity and disease. CNVs can be detected by a number of means, including chromosomal microarray analysis (CMA) and whole-genome sequencing (WGS), but these approaches suffer from either limited resolution (CMA) or are highly expensive for routine screening (both CMA and WGS). As an alternative, these researchers developed a NGS-based method for CNV analysis termed SMASH, for short multiply aggregated sequence homologies. SMASH utilizes random fragmentation of input genomic DNA to create chimeric sequence reads, from which multiple mappable tags can be parsed using maximal almost-unique matches (MAMs). The SMASH tags are then binned and segmented, generating a profile of genomic copy number at the desired resolution. Because fewer reads are needed relative to WGS to give accurate CNV data, SMASH libraries can be highly multiplexed, allowing large numbers of individuals to be analyzed at low cost. Increased genomic resolution can be achieved by sequencing to higher depth.

Zare and associates (2017) noted that CNV has gained considerable interest as a type of genomic/genetic variation that plays an important role in disease susceptibility. Advances in sequencing technology have created an opportunity for detecting CNVs more accurately. Recently whole exome sequencing (WES) has become primary strategy for sequencing patient samples and study their genomics aberrations. However, compared to WGS, WES introduces more biases and noise that make CNV detection very challenging. Additionally, tumors' complexity makes the detection of cancer specific CNVs even more difficult. Although many CNV detection tools have been developed since introducing NGS data, there are few tools for somatic CNV detection for WES data in cancer. These researchers examined the performance of the most recent and commonly used CNV detection tools for WES data in cancer to address their limitations and provide guidelines for developing new ones. They focused on the tools that have been designed or have the ability to detect cancer somatic aberrations. These investigators compared the performance of the tools in terms of sensitivity and false discovery rate (FDR) using real data and simulated data. Comparative analysis of the results of the tools showed that there is a low consensus among the tools in calling CNVs. Using real data, tools showed moderate sensitivity (approximately [~] 50 % - ~ 80 %), fair specificity (~ 70 % - ~ 94 %) and poor FDRs (~ 27 % to ~ 60 %). Also, using simulated data these researchers observed that increasing the coverage more than 10x in exonic regions did not improve the detection power of the tools significantly. The authors concluded that the limited performance of the current CNV detection tools for WES data in cancer indicated the need for developing more efficient and precise CNV detection methods. Due to the complexity of tumors and high level of noise and biases in WES data, employing advanced novel segmentation, normalization and de-noising techniques that are designed specifically for cancer data is needed. Furthermore, CNV detection development suffered from the lack of a gold standard for performance evaluation. Finally, developing tools with user-friendly user interfaces and visualization features could enhance CNV studies for a broader range of users.

## Corneal Dystrophy

Corneal dystrophies are a group of genetic eye disorders in which abnormal material builds up in the cornea. There are more than 20 different types of corneal dystrophies which are typically grouped into three categories (anterior, stromal and posterior). Symptoms can vary depending on the type of corneal dystrophy. Some individuals are asymptomatic, whereas some will experience blurred vision or vision loss. Most corneal dystrophies affect both eyes and progress slowly; however, some forms are progressive and may cause significant vision impairment. Signs and symptoms can appear at any age, and men are equally affected as women, except for Fuchs' dystrophy which affects women about four times as often as men (Boyd, 2018; Corneal Dystrophy Foundation, 2019).

Most cases of corneal dystrophy are inherited as an autosomal dominant trait with variable expressivity. Investigators have determined that several corneal dystrophies occur due to mutations of the transforming growth factor beta-induced (TGFB1) gene located on the long arm (q) of chromosome 5 (5q31). Diagnosis may be confirmed by a thorough clinical evaluation, patient history and testing, such as a slit lamp examination. Some specific corneal dystrophies can be diagnosed with molecular genetic tests. Because some individuals do not have symptoms, determining the true frequency of these disorders in the general population is difficult. The American Academy of Ophthalmology (AAO) Task Force state that all genetic testing should be done under the direction of a physician or genetic counselor through a CLIA certified lab (Boyd, 2018; Corneal Dystrophy Foundation, 2019).

The clinical utility of genetic testing for corneal dystrophy may "assist physicians to diagnose corneal dystrophies that may not be clear and present on slit lamp examination and guide decision making. (Corcoran Consulting Group, 2019). It is suggested that "refractory surgery candidates, for example, should have genetic testing when the clinical findings, personal medical history, and family medical history indicate an increased risk of [granular corneal dystrophy type II] GCD2", since "laser in situ keratomileusis, photorefractive keratectomy, and laser assisted in situ epithelial keratomileusis are strongly contraindicated in this dystrophy" (Corcoran Consulting Group, 2019; Weiss, et al., 2015).

## Cystic Fibrosis

Cystic fibrosis is the most common potentially fatal autosomal recessive disease in the United States. CF is characterized by the production of abnormally viscous mucus produced by the affected glands and usually causes respiratory infections and impaired pancreatic functions. CF produces chronic progressive disease of the respiratory system, malabsorption due to pancreatic insufficiency, increased loss of sodium and chloride in sweat, and male infertility as a consequence of atresia of the vas deferens. Pulmonary disease is the most common cause of mortality and morbidity in individuals with CF. The incidence of this disease ranges from 1:500 in Amish (Ohio) to 1:90,000 in Hawaiian Orientals, and is estimated to be 1:2,500 newborns of European ancestry. It occurs less frequently in people with other ethnic and racial backgrounds. About 1:25 persons of European ancestry is a carrier (or heterozygote), possessing one normal and one abnormal CF gene. Because of recent advances in clinical management of CF, babies born today are expected to live well into middle age.

Currently, the most frequently employed test for CF is the quantitative pilocarpine iontophoresis sweat test. Sweat chloride is more reliable than sweat sodium for diagnostic purposes with a sensitivity of 98 % and a specificity of 83 %. However, this test can not detect CF carriers because the electrolyte content of sweat is normal in heterozygotes (Wallach, 1991).

Genetic testing is used to diagnose CF in individuals with signs and symptoms of the disease. It is also used for carrier screening of potential parents to identify genetic mutations for which they are at risk of passing along to their children. Carriers may be unaffected but are at risk for producing children who are affected. Preferably carrier screening takes place before pregnancy, but can take place during the early stages of pregnancy.

The gene for CF (cystic fibrosis trans-membrane conductance regulator, CFTR) was cloned, and the principal mutant gene in white people (DF508) was characterized in 1989. This mutation is due to a 3-base-pair deletion that results in the loss of a phenylalanine at position 508 from the 1,480-amino acid coding region (Riordan et al, 1989). This mutation is found in approximately 70 % of carriers of European ancestry, but the relative frequency varies from 30 % in Ashkenazi Jews to 88 % in Danes (Cutting et al, 1992). Available evidence indicates that CFTR functions as a chloride channel, although it may also serve other functions. Since then, more than 200 CF mutations have been described. Five of the most common mutations (DF508, G542X, F551D, R553X, N1303K) constitute approximately 85 % of the alleles in the United States (Elias et al, 1991). Thus, screening procedures that test for these 5 mutations will detect approximately 85 % of CF carriers. The genetic screening test for CF is usually based on mouthwash samples collected by agitating sucrose or saline in the mouth. The DNA of these cells are amplified, digested, and subjected to separation techniques that identify 3 to 5 common mutations.

A National Institutes of Health consensus panel (1997) recommended that genetic testing for CF should be offered to adults with a positive family history of CF, to partners of people with the disease, to couples currently planning a pregnancy, and to couples seeking prenatal testing. However, the panel did not recommend genetic testing of CF to the general public or to newborn infants.

The American College of Obstetricians and Gynecologists (2001) has issued similar recommendations on genetic carrier testing for CF. ACOG recommends that obstetricians should offer CF screening to:

- Couples in whom one or both members are white and who are planning a pregnancy or seeking prenatal care;
- Individuals with a family history of CF; and
- Reproductive partners of people who have CF.

ACOG also recommends that screening should be made available to couples in other racial and ethnic groups. To date, over 900 mutations in the CF gene have been identified. As it is impractical to test for every known mutation, the ACMG Accreditation of Genetic Services Committee has compiled a standard screening panel of 25 CF mutations, which represents the standard panel that ACMG recommends for screening in the U.S. population (Grody et al, 2001). This 25-mutation panel incorporates all CF-causing mutations with an allele frequency of greater than or equal to 0.1 % in the general U.S. population, including mutation subsets shown to be sufficiently predominant in certain ethnic groups, such as Ashkenazi Jews and African Americans. This standard panel of mutations is intended to provide the greatest pan-ethnic detectability that can practically be performed.

The ACOG's update on carrier screening for CF (2011) provided the following recommendations.

- If a patient has been screened previously, CF screening results should be documented but the test should not be repeated.
- Complete analysis of the CFTR gene by DNA sequencing is not appropriate for routine carrier screening.

ACOG guidelines (2017) state that "[c]ystic fibrosis carrier screening should be offered to all women who are considering pregnancy or are currently pregnant."

Farrell and colleagues (2017) noted that CF, caused by mutations in the CF transmembrane conductance regulator (CFTR) gene, continues to present diagnostic challenges. Newborn screening and an evolving understanding of CF genetics have prompted a reconsideration of the diagnosis criteria. To improve diagnosis and achieve standardized definitions worldwide, the CF Foundation convened a committee of 32 experts in CF diagnosis from 9 countries to develop clear and actionable consensus guidelines on the diagnosis of CF and to clarify diagnostic criteria and terminology for other disorders associated with CFTR mutations. An a priori threshold of greater than or equal to 80 % affirmative votes was needed for acceptance of each recommendation statement. After reviewing relevant literature, the committee convened to review evidence and cases.

Following the conference, consensus statements were developed by an executive subcommittee. The entire consensus committee voted and approved 27 of 28 statements, 7 of which needed revisions and a 2nd round of voting. It was recommended that diagnoses associated with CFTR mutations in all individuals, from newborn to adult, be established by evaluation of CFTR function with a sweat chloride test. The latest mutation classifications annotated in the Clinical and Functional Translation of CFTR project should be used to aid in diagnosis. Newborns with a high immunoreactive trypsinogen level and inconclusive CFTR functional and genetic testing may be designated CFTR-related metabolic syndrome or CF screen positive, inconclusive diagnosis; these terms are now merged; and equivalent, and CFTR-related metabolic syndrome/CF screen positive, inconclusive diagnosis may be used. International Statistical Classification of Diseases and Related Health Problems, 10th Revision codes for use in diagnoses associated with CFTR mutations were included. One of the recommendations was that for individuals presenting with a positive newborn screen, symptoms of CF, or a positive family history, and sweat chloride values in the intermediate range (30 to 59 mmol/L) on 2 separate occasions may have CF. They should be considered for extended CFTR gene analysis and/or CFTR functional analysis (90 % vote; 0 abstain).

Edmondson and associates (2018) stated that newborn babies positively screened for CF (high serum immunoreactive trypsin (IRT) with DNA analysis) are referred for a diagnostic sweat test, which may be normal (sweat chloride less than 30 mmol/L). Unless 2 gene mutations are identified during newborn screening (NBS), the babies are discharged from follow-up. These investigators wished to check that none had subsequently developed symptoms suggestive of CF. They retrospectively reviewed patient notes and contacted general practitioners of all babies with a negative sweat test, conducted in 1 of the 4 pediatric specialist CF centers in London, over the first 6 years of screening in South East England. Of 511 babies referred, 95 (19 %) had a normal sweat test; 5 (5 %) had CF diagnosed genetically, 2 of them on extended genome sequencing after clinical suspicion; 11 (12 %) were designated as CF screen positive inconclusive diagnosis (CFSPID); 1 of the 5 CF children was originally designated as CFSPID; 79 (83 %) were assumed to be false-positive cases and discharged; follow-up data were available for 51/79 (65 %); 32/51 (63 %) had no health issues, 19/51 (37 %) had other significant non-CF pathology. These results were reassuring in that within the limitations of those lost to follow-up, CF symptoms have not emerged in the discharged children. The high non-CF morbidity in these children may relate to known causes of high IRT at birth. Clinicians need to be aware that a child can have CF despite a normal sweat test following NBS, and if symptoms suggest the diagnosis, further testing, including extended genome sequencing, is needed.

Furthermore, an UpToDate review on "Cystic fibrosis: Clinical manifestations and diagnosis" (Katkin, 2021) states that "Genetic screening panels -- Screening of newborn infants is typically performed with panels of CFTR gene mutations. The mutations included in the panel vary among states, depending on the ethnic

diversity of their populations. Most states screen for at least 23 of the most common mutations, using a panel developed for population screening by the American College of Medical Genetics (ACMG). The ACMG panel identifies approximately 90 % of CF-causing mutations in the general population (and 97 % of mutations in families of Ashkenazi Jewish ancestry). However, the panel may be less sensitive for other ethnic groups that have genetic diversity and a wider range of CF-causing mutations; in one study, it detected only 68.5 % of CF-causing mutations in a Hispanic population. Several state screening programs have therefore expanded or modified the ACMG panel to suit multiethnic populations.

For example, in the state of California, a panel of 40 mutations is employed to include CFTR gene mutations found to be more prevalent in non-European ethnicities".

#### Dilated Cardiomyopathy

Roncarati et al (2013) stated that familial dilated cardiomyopathy (DCM) is a heterogeneous disease; although 30 disease genes have been discovered, they explain only no more than 50 % of all cases; in addition, the causes of intra-familial variability in DCM have remained largely unknown. These researchers exploited the use of whole-exome sequencing (WES) to investigate the causes of clinical variability in an extended family with 14 affected subjects, 4 of whom showed particular severe manifestations of cardiomyopathy requiring heart transplantation in early adulthood. This analysis, followed by confirmative conventional sequencing, identified the mutation p.K219T in the lamin A/C gene in all 14 affected patients. An additional variant in the gene for titin (TTN), p.L4855F, was identified in the severely affected patients. The age for heart transplantation was substantially less for LMNA:p.K219T/TTN:p.L4855F double heterozygotes than that for LMNA:p.K219T single heterozygotes. Myocardial specimens of doubly heterozygote individuals showed increased nuclear length, sarcomeric disorganization, and myonuclear clustering compared with samples from single heterozygotes. The authors concluded that these findings showed that WES can be used for the identification of causal and modifier variants in families with variable manifestations of DCM. In addition, they not only indicated that LMNA and TTN mutational status may be useful in this family for risk stratification in individuals at risk for DCM, but also suggested TTN as a modifier for DCM.

Begay and colleagues (2015) noted that the TTN encodes the largest human protein, which plays a central role in sarcomere organization and passive myocyte stiffness; and TTN truncating mutations cause DCM. However, the role of TTN missense variants in DCM has been difficult to elucidate because of the presence of background TTN variation. A cohort of 147 DCM index subjects underwent DNA sequencing for 313 TTN exons covering the N2B and N2BA cardiac isoforms of TTN. Of the 348 missense variants, these researchers identified 44 "severe" rare variants by using a bioinformatic filtering process in 37 probands. Of these, 5 probands were double heterozygotes (additional variant in another DCM gene) and 7 were compound heterozygotes (2 TTN "severe" variants). Segregation analysis allowed the classification of the "severe" variants into 5 "likely" (co-segregating), 5 "unlikely" (non-co-segregating), and 34 "possibly" (where family structure precluded segregation analysis) disease-causing variants. Patients with DCM carrying "likely" or "possibly" pathogenic TTN "severe" variants did not show a different outcome compared with "unlikely" and non-carriers of a "severe" TTN variant. However, the "likely" and "possibly" disease-causing variants were overrepresented in the C-zone of the A-band region of the sarcomere. The authors concluded that TTN missense variants are common and present a challenge for bioinformatic classification, especially when informative families are not available. The authors concluded that although DCM patients carrying bioinformatically "severe" TTN variants do not appear to have a worse clinical course than non-carriers, the non-random distribution of "likely" and "possibly" disease-causing variants suggested a potential biological role for some TTN missense variants.

UpToDate reviews on "Familial dilated cardiomyopathy: Prevalence, diagnosis and treatment" (Hershberger, 2023a), "Hypertrophic cardiomyopathy: Clinical manifestations, diagnosis, and evaluation" (Maron, 2022), and "Clinical manifestations and diagnosis of arrhythmogenic right ventricular cardiomyopathy" (McKenna, 2016) do not mention genetic testing of titin (TTN) as a management tool.

Furthermore, an UpToDate review on "Genetics of dilated cardiomyopathy" (Hershberger, 2023b) states that "Mutations in the TTN gene encoding titin, the largest human protein and a key component of sarcomeric force generation, are the most common known cause of DCM. In a study restricted to identification of TTN truncating mutations (mostly nonsense or "stop" mutations, or other mutations that plausibly caused the interruption of production of the full titin protein), such mutations were observed in approximately 20 % of referred populations of idiopathic DCM (25 % of familial cases and 18 % of sporadic cases). No unique clinical characteristics have been identified for these truncating mutations but adverse events may occur earlier in men than in women. The role of missense mutations, common among all patient groups, was not addressed in the study. Furthermore, approximately 3 % of control DNAs also carried TTN truncating variants, raising the question of pathogenicity of even truncating variants. A subsequent exome sequencing study of 17 DCM families identified seven with segregating TTN truncating variants. However, two TTN truncating variants were identified that did not segregate, emphasizing this latter concern".

The Heart Failure Society of America (HFSA) issued 2018 guidelines regarding genetic evaluation of cardiomyopathy. For patients with cardiomyopathy, the HFSA recommends genetic testing to determine if a pathogenic variant can be identified to facilitate patient management and family screening, such as in persons at risk of sudden death, which may be the first presentation of at-risk family members. Hershberger et al. (2018) state that in dilated cardiomyopathy (DCM), there is evidence for prognostication value of genetic testing and management implications for specific genetic findings, such as consideration of ICD placement for primary prevention in carriers of LMNA pathogenic variants. LMNA commonly presents with "nonsyndromic cardiomyopathy" in adult cardiology practice and is well known for progressive conduction system disease (1st-, 2nd-, or 3rd-degree heart block), usually with supraventricular and/or ventricular arrhythmias before, during, or soon thereafter". Since ICDs are not recommended unless the left ventricular ejection fraction (LVEF) falls below 35%, the HFSA issued a guideline to include ICD consideration for patients with cardiomyopathy and significant arrhythmia, or known risk of arrhythmia, before the LVEF falls below 35% (Level of Evidence [LOE] = C).

A 2020 scientific statement from the American Heart Association was issued regarding genetic testing for inherited cardiovascular diseases. The authors state that with respect to specific genes that should be tested in patients with cardiomyopathy, the National Institutes of Health-funded Clinical Genome Resource Consortium (ClinGen) does not include 2019 curation reports or variant interpretation frameworks relevant to DCM.

Jordan and colleagues (2021) state that complexity of DCM genetic architecture presents challenges to clinical genetic testing and the interpretation of genetic variants in individuals with DCM. The authors report on the systematic evidence-based appraisal of genes associated with DCM and the implication of the findings. "An international panel with clinical and scientific expertise in DCM genetics evaluated evidence supporting monogenic relationships of genes with idiopathic DCM. The panel used the Clinical Genome Resource semiquantitative gene-disease clinical validity classification framework with modifications for DCM genetics to classify genes into categories on the basis of the strength of currently available evidence." More than 250 genes spanning over 10 gene ontologies have been

implicated in DCM. Of 51 genes with human genetic evidence that were curated, 12 genes (23%) were classified as having definitive (BAG3, DES, FLNC, LMNA, MYH7, PLN, RBM20, SCN5A, TNNT1, TNNT2, TTN) or strong (DSP) evidence, and 7 genes (14%; ACTC1, ACTN2, JPH2, NEXN, TNNI3, TPM1, VCL) were classified as moderate evidence. Of these 19 genes, 6 were similarly classified for hypertrophic cardiomyopathy (HCM) and 3 for arrhythmogenic right ventricular cardiomyopathy (ARVC). Of the remaining 32 genes (63%), 25 (49%) had limited evidence, 4 (8%) were disputed, 2 (4%) had no disease relationship, and 1 (2%) was supported by animal model data only. Of the 16 evaluated clinical genetic testing panels, most definitive genes were included, but panels also included numerous genes with minimal human evidence. Based on their findings, the authors recommend that high-evidence DCM genes be used for clinical practice and that caution should be exercised in the interpretation of variants in variable-evidence DCM genes. In addition, rare variants from genes without moderate, strong, or definitive evidence should not be used in clinical practice to predict DCM risk for at-risk family members.

The European Heart Rhythm Association (EHRA)/Heart Rhythm Society (HRS)/Asia Pacific Heart Rhythm Society (APHRS)/Latin American Heart Rhythm Society (LAHRS) issued an expert consensus statement on genetic testing for cardiac diseases. The EHRA/HRS/APHRS/LAHRS state that genetic testing analyzing genes with definite or strong evidence supporting disease causation is recommended, per strong observational evidence and authors' consensus, in patients with a clear specific phenotype (Wilde et al., 2022).

The 2023 European Society of Cardiology (ESC) issued guidelines for the management of cardiomyopathies. The ESC recommends genetic testing in individuals with cardiomyopathy (known as confirmatory or diagnostic testing): (i) to confirm the diagnosis; (ii) where it may inform prognosis; (iii) where it may inform treatment selection; or (iv) where it may inform their reproductive management (Class of Recommendation [COR]: I; LOE: B). The most common gene (greater than 10% tested cases) considered definitive/strong evidence of DCM is TTN. Common genes (1-10% of tested cases) considered definitive/strong evidence of DCM include BAG3, DSP, FLNC, LMNA, MYH7, RBM20, and TNNT2. Less common genes (less than 1% of tested cases) considered definitive/strong evidence of DCM include DES, DMD (associated with syndromic presentation that can include cardiomyopathy as a feature), PLN, SCN5A, and TNNT1. Less common genes (less than 1% of tested cases) considered of moderate evidence for DCM include: ACTC1, ACTN2, JPH2, NEXN, TNNI3, TPM1, and VCL. In families in whom a disease-causing genetic variant has been identified, cascade genetic testing should be offered. However, cascade testing is not indicated when a variant of uncertain significance is identified in the proband. The ESC recommends cascade genetic testing, with pre- and post-test counselling, to adult at-risk relatives if a confident genetic diagnosis (i.e. a pathogenic/likely pathogenic variant) has been established in an individual with cardiomyopathy in the family (starting with first-degree relatives if available, and cascading out sequentially) (COR: I; LOE: B) (Arbelo et al., 2023).

The 2023 ESC guidelines state that "a family history of SCD [sudden cardiac death] is usually considered clinically significant when one or more first-degree relatives have died suddenly aged <40 years with or without a diagnosis of HCM, or when SCD has occurred in a first-degree relative at any age with an established diagnosis of HCM. Family history of SCD does not appear to be an independent risk factor for SCD in childhood HCM. This may be due to a higher prevalence of de novo variants in childhood HCM, the inclusion of non-sarcomeric disease, and/or under-reporting of family history in paediatric cohorts". "In the absence of conclusive genetic information in a family, DCM is considered familial if: (i) one or more first- or

second-degree relatives have DCM; or (ii) when an otherwise unexplained SCD has occurred in a first-degree relative at any age with an established diagnosis of DCM." (Arbelo et al., 2023).

#### Epigenetic Methylation Assay (e.g., EpiSign)

EpiSign (Greenwood Genetic Center; Greenwood, SC) is a genome-wide methylation assay designed to identify disease-specific methylation patterns, or epigenetic signatures, involving multiple loci across the genome in over 90 conditions. EpiSign is offered as two different tests, the EpiSign Complete and EpiSign Variant. Both require a blood specimen.

The EpiSign Complete is a comprehensive analysis that assesses for over 90 genes in the work-up of patients with developmental delay or other clinical features suggestive of a condition with an epigenetic signature and conditions with methylation defects (imprinting disorders). Abnormalities detected using this initial screen may require additional targeted testing to confirm and further characterize the underlying genomic abnormality.

The EpiSign Variant is a targeted review of the methylation data based on a strong clinical suspicion and/or a previously identified variant of uncertain significance (VUS). Pathogenic variants in genes associated with these conditions have an established unique signature. When present, this unique signature is purported to provide evidence for variant pathogenicity. This blood test evaluates some conditions/genes that have been classified as having a more moderate signature based on signature strength, small cohort size, or types of mutations tested. Females tested for X-linked conditions may have a moderate signature or a potentially false negative result. Also, this test will not detect females with Fragile X (FMR1) expansions. As with many clinical tests, uncertain results are possible. A normal result does not rule out the possibility that the patient is affected with one of the evaluated conditions. In some cases follow-up testing may be suggested to further characterize the underlying genomic abnormality and to confirm or rule out a diagnosis.

Sadikovic and colleagues (2021) described the clinical implementation of genome-wide DNA methylation analysis in rare Mendelian disorders across the EpiSign diagnostic laboratory network and the assessment of results and clinical impact in the first subjects tested. The authors outlined the logistics and data flow between an integrated network of clinical diagnostics laboratories in Europe, the United States, and Canada. They described the clinical validation of EpiSign using 211 specimens and assess the test performance and diagnostic yield in the first 207 subjects tested involving two patient subgroups: the targeted cohort (subjects with previous ambiguous/inconclusive genetic findings including genetic variants of unknown clinical significance) and the screening cohort (subjects with clinical findings consistent with hereditary neurodevelopmental syndromes and no previous conclusive genetic findings). The authors found that among the 207 subjects tested, 57 (27.6%) were positive for a diagnostic episignature including 48/136 (35.3%) in the targeted cohort and 8/71 (11.3%) in the screening cohort, with 4/207 (1.9%) remaining inconclusive after EpiSign analysis. The authors acknowledged limitations in their findings. Unlike DNA sequencing, DNA methylation analysis is limited to peripheral blood where large reference databases are available. Other factors including age, sex, and environmental exposures can also impact the analysis and need to be accounted for in analytical processes. Further, DNA methylation episignatures can be susceptible to technical variation such as sample processing data batch effects, as well as biological parameters such as mosaicism. The validation cohort included a number of low-level mosaic imprinting disorders that may not be readily detectable by the EpiSign assay. Moreover, an overarching challenge with this technology is the rarity of Mendelian disorders. While the population prevalence of rare diseases is 3.5-5.9%, equating to 263 to 446 million

persons affected globally, given that this number encompasses over 5,000 diseases, the prevalence of rare disorders ranges between 1 to 5 per 10,000 and less than 1/1,000, 000.<sup>40</sup> Generation of EpiSigns requires cohorts of subjects with gene-specific pathogenic variants, which is currently possible for the more prevalent disorders. Also, as many of the episignatures are mild in scale, the size of the reference cohort is directly correlated to the level of sensitivity of the assay. The authors concluded that their study describes the implementation of diagnostic clinical genomic DNA methylation testing in patients with rare disorders, and that it provides strong evidence of clinical utility of EpiSign analysis, including the ability to provide conclusive findings in the majority of subjects tested, despite study limitations.

Haghshenas et al (2023) states in an abstract, "The challenges and ambiguities in providing an accurate diagnosis for patients with neurodevelopmental disorders have led researchers to apply epigenetics as a technique to validate the diagnosis provided based on the clinical examination and genetic testing results. Genome-wide DNA methylation analysis has recently been adapted for clinical testing of patients with genetic neurodevelopmental disorders. In this paper, preliminary data demonstrating a DNA methylation signature for Renpenning syndrome (RENS1 - OMIM 309500), which is an X-linked recessive neurodevelopmental disorder caused by variants in polyglutamine-binding protein 1 (PQBP1) is reported. The identified episignature was then utilized to construct a highly sensitive and specific binary classification model. Besides providing evidence for the existence of a DNA methylation episignature for Renpenning syndrome, this study increases the knowledge of the molecular mechanisms related to the disease. Moreover, the availability of more subjects in future may facilitate the establishment of an episignature that can be utilized for diagnosis in a clinical setting and for reclassification of variants of unknown clinical significance".

Karimi et al (2024) conducted a study to assess clinical features and genome-wide DNA methylation profiles in individuals affected by intellectual developmental disorder, autosomal dominant 21 (IDD21) syndrome, caused by variants in the CCCTC-binding factor (CTCF) gene. DNA samples were extracted from peripheral blood of 16 individuals with clinical features and genetic findings consistent with IDD21. DNA methylation analysis was performed using the Illumina Infinium Methylation EPIC Bead Chip microarrays. The methylation levels were fitted in a multivariate linear regression model to identify the differentially methylated probes. A binary support vector machine classification model was constructed to differentiate IDD21 samples from controls. The authors report identifying a highly specific, reproducible, and sensitive episignature associated with CTCF variants. Six variants of uncertain significance were tested, of which 2 mapped to the IDD21 episignature and clustered alongside IDD21 cases in both heatmap and multidimensional scaling plots. Comparison of the genomic DNA methylation profile of IDD21 with that of 56 other neurodevelopmental disorders provided insights into the underlying molecular pathophysiology of this disorder. The authors concluded that the robust and specific CTCF/IDD21 episignature expands the growing list of neurodevelopmental disorders with distinct DNA methylation profiles, which can be applied as supporting evidence in variant classification.

Kerkhof and colleagues (2024) aimed to assess the diagnostic utility and provide reporting recommendations for clinical DNA methylation episignature testing based on the cohort of patients tested through the EpiSign Clinical Testing Network. The EpiSign assay utilized unsupervised clustering techniques and a support vector machine-based classification algorithm to compare each patient's genome-wide DNA methylation profile with the EpiSign Knowledge Database, yielding the result that was reported. An international working group, representing distinct EpiSign Clinical Testing Network health jurisdictions, collaborated to establish recommendations for interpretation and reporting of episignature testing. Among 2399 cases analyzed, 1667 cases underwent a comprehensive screen of validated

episignatures, imprinting, and promoter regions, resulting in 18.7% (312/1667) positive reports. The remaining 732 referrals underwent targeted episignature analysis for assessment of sequence or copy-number variants (CNVs) of uncertain significance or for assessment of clinical diagnoses without confirmed molecular findings, and 32.4% (237/732) were positive. Cases with detailed clinical information were highlighted to describe various utility scenarios for episignature testing. The authors acknowledged some limitations to their study, such as, episignature analysis is limited to peripheral blood DNA, and a number of rare diseases studied thus far do not show changes in peripheral blood. Moreover, to define an episignature, a clinically evaluated reference patient cohort with confirmed pathogenic variants is required, which can make identifying the cohort challenging because of the low prevalence of rare disorders, and often requires global collaboration. Nonetheless, the authors concluded that the clinical DNA methylation testing including episignatures, imprinting, and promoter analysis provided by an integrated network of clinical laboratories enables test standardization and demonstrates significant diagnostic yield and clinical utility beyond DNA sequence analysis in rare diseases. The authors report that, although limitations persist, the potential of DNA methylation episignature testing to enhance and potentially transform diagnostic pathways in rare disease holds "tremendous" promise.

#### Epsilon-Sarcoglycan Gene (SCGE) Deletion Analysis

Myoclonus-dystonia (M-D), an autosomal dominant inherited movement disorder, has been associated with mutations in the epsilon-sarcoglycan gene (SCGE) on 7q21. Raymond et al (2008) noted that M-D due to SGCE mutations is characterized by early onset myoclonic jerks, often associated with dystonia. Penetrance is influenced by parental sex, but other sex effects have not been established. In 42 affected individuals from 11 families with identified mutations, these researchers found that sex was highly associated with age at onset regardless of mutation type; the median age onset for girls was 5 years versus 8 years for boys ( $p < 0.0097$ ). Moreover, the authors found no association between mutation type and phenotype.

Ritz et al (2009) stated that various mutations within the SGCE gene have been associated with M-D, but mutations are detected in only about 30 % of patients. The lack of stringent clinical inclusion criteria and limitations of mutation screens by direct sequencing might explain this observation. Eighty-six M-D index patients from the Dutch national referral center for M-D underwent neurological examination and were classified according to previously published criteria into definite, probable and possible M-D. Sequence analysis of the SGCE gene and screening for copy number variations were performed. In addition, screening was carried out for the 3 bp deletion in exon 5 of the DYT1 gene. Based on clinical examination, 24 definite, 23 probable and 39 possible M-D patients were detected. Thirteen of the 86 M-D index patients carried a SGCE mutation: 7 nonsense mutations, 2 splice site mutations, 3 missense mutations (2 within 1 patient) and 1 multi-exonic deletion. In the definite M-D group, 50 % carried an SGCE mutation and 1 single patient in the probable group (4 %). One possible M-D patient showed a 4 bp deletion in the DYT1 gene (c.934\_937delAGAG). The authors concluded that mutation carriers were mainly identified in the definite M-D group. However, in 50 % of definite M-D cases, no mutation could be identified.

#### Familial Adenomatous Polyposis (FAP)

Familial adenomatous polyposis (FAP) is caused by mutation of the adenomatous polyposis coli (APC) gene. According to guidelines from the American Gastroenterological Association (AGA, 2001), adenomatous polyposis coli gene testing is indicated to confirm the diagnosis of familial adenomatous polyposis, provide pre-symptomatic testing for at-risk members (1st degree relatives 10 years

or older of an affected patient), confirm the diagnosis of attenuated familial adenomatous polyposis in those with more than 20 adenomas, and test those 10 years or older at risk for attenuated FAP.

The AGA guidelines state that germline testing should first be performed on an affected member of the family to establish a detectable mutation in the pedigree. If a mutation is found in an affected family member, then genetic testing of at-risk members will provide true positive or negative results. The AGA guidelines state that, if a pedigree mutation is not identified, further testing of at-risk relatives should be suspended because the gene test will not be conclusive: a negative result could be a false negative because testing is not capable of detecting a mutation even if present. When an affected family member is not available for evaluation, starting the test process with at-risk family members can provide only positive or inconclusive results. In this circumstance, a true negative test result for an at-risk individual can only be obtained if another at-risk family member tests positive for a mutation.

#### Familial Cold Autoinflammatory Syndrome

Familial cold autoinflammatory syndrome (FCAS), also known as familial cold urticaria (FCU), is an autosomal dominant condition characterized by rash, conjunctivitis, fever/chills and arthralgias elicited by exposure to cold - sometimes temperatures below 22° C (72° F). It is rare and is estimated as having a prevalence of 1 per million people and mainly affects Americans and Europeans. Familial cold autoinflammatory syndrome is one of the cryopyrin-associated periodic syndromes (CAPS) caused by mutations in the CIAS1/NALP3 (also known as NLRP3) gene at location 1q44. Familial cold autoinflammatory syndrome shares symptoms, and should not be confused, with acquired cold urticaria, a more common condition mediated by different mechanisms that usually develop later in life and are rarely inherited. There is insufficient evidence to support the use of genetic testing in the management of patients with FCAS/FCU. UpToDate reviews on "Cold urticaria" (Maurer, 2011) and "Cryopyrin-associated periodic syndromes and related disorders" (Nigrovic, 2011) do not mention the use of genetic testing.

Santome Collazo et al (2010) noted that congenital adrenal hyperplasia (CAH) is not an infrequent genetic disorder for which mutation-based analysis for CYP21A2 gene is a useful tool. An UpToDate review on "Diagnosis of classic congenital adrenal hyperplasia due to 21-hydroxylase deficiency" (Merke, 2011) states that "[g]enetic testing also can be used to evaluate borderline cases. Genetic testing detects approximately 95 percent of mutant alleles". Furthermore, the Endocrine Society's clinical practice guideline on congenital adrenal hyperplasia (Speiser et al, 2010) suggested genotyping only when results of the adrenocortical profile following cosyntropin stimulation test are equivocal or for purposes of genetic counseling. The Task Force recommends that genetic counseling be given to parents at birth of a CAH child, and to adolescents at the transition to adult care.

#### Familial Nephrotic Syndrome (NPHS1, NPHS2)

Nephrotic syndrome comes in 2 variants: (i) Those sensitive to treatment with immunosuppressants (steroid-sensitive), and (ii) those resistant to immunosuppressants (steroid-resistant). Familial forms of nephrotic syndrome are steroid resistant (Niaudet, 2007). Mutations in two genes, NPHS1 and NPHS2, have been associated with a familial nephrotic syndrome. Mutations in the gene for podocin, called NPHS2, also known as familial focal glomerulosclerosis, are observed in patients with both familial and sporadic steroid-resistant nephrotic syndrome (SRNS). Identifying children with nephrotic syndrome due to NPHS2 mutations can avoid unnecessary exposure to immunosuppressive therapy, because immunosuppressive therapy has not been shown to be effective in treating these children (Niaudet, 2007). Thus, authorities have recommended testing for such mutations in those

with a familial history of steroid resistant nephrotic syndrome and children with steroid-resistant disease.

Some have suggested that, to avoid unnecessary exposure to steroid therapy, all children with a first episode of the nephrotic syndrome should be screened for NPHS2 mutations (Niaudet, 2007). However, given that over 85 % of children with idiopathic nephrotic syndrome are steroid-sensitive and only approximately 20 % of steroid-resistant patients have NPHS2 mutations, screening for abnormalities at this genetic locus would identify less than 5 % of all cases. However, screening a child with a first episode of the nephrotic syndrome with a familial history of steroid-resistant nephrotic syndrome has been recommended because they are at increased risk for having a NPHS2 gene mutation.

Mutations in the gene for nephrin, called NPHS1, cause the congenital nephrotic syndrome of Finnish type (CNF) (Niaudet, 2007). CNF is inherited as an autosomal recessive trait, with both sexes being involved equally. There are no manifestations of the disease in heterozygous individuals. Most infants with the CNF are born prematurely (35 to 38 weeks), with a low birth weight for gestational age. Edema is present at birth or appears during the first week of life in 50 % of cases. Severe nephrotic syndrome with marked ascites is always present by 3 months. End-stage renal failure usually occurs between 3 and 8 years of age. Prolonged survival is possible with aggressive supportive treatment, including dialysis and renal transplantation.

The nephrotic syndrome in CNF is always resistant to corticosteroids and immunosuppressive drugs, since this is not an immunologic disease (Niaudet, 2007). Furthermore these drugs may be harmful due to affected individuals' already high susceptibility to infection.

The CNF becomes manifest during early fetal life, beginning at the gestation age of 15 to 16 weeks. The initial symptom is fetal proteinuria, which leads to a more than 10-fold increase in the amniotic fluid alpha-fetoprotein (AFP) concentration (Niaudet, 2007). A parallel, but less important increase in the maternal plasma AFP level is observed. These changes are not specific, but they may permit the antenatal diagnosis of CNF in high risk families in which termination of the pregnancy might be considered. However, false positive results do occur, often leading to abortion of healthy fetuses.

Genetic linkage and haplotype analyses may diminish the risk of false positive results in informative families (Niaudet, 2007). The 4 major haplotypes, which cover 90 % of the CNF alleles in Finland, have been identified, resulting in a test with up to 95 % accuracy.

Authorities do not recommend screening for NPHS1 mutations for all children with the first episode of nephrotic syndrome, for the reasons noted above regarding NPHS2 mutation screening. However, genetic testing may be indicated for infants with congenital nephrotic syndrome (i.e., appearing within the first months of life) who are of Finnish descent and/or who have a family history that suggests a familial cause of congenital nephrotic syndrome. The primary purpose of this testing is for pregnancy planning. Detection of an NPHS1 mutation also has therapeutic implications, as such nephrotic syndrome is steroid resistant.

#### Fragile X Syndrome

Fragile X syndrome is the most common cause of inherited developmental delay/intellectually disability, seen in approximately one in 1,200 males and one in 2,500 females. Phenotypic abnormalities associated with Fragile X syndrome include developmental delay/intellectually disability, autistic behaviors, characteristic narrow face with large jaw, and speech and language disorders.

Fragile X syndrome was originally thought to be transmitted in an X-linked recessive manner; however, the inheritance pattern of fragile X syndrome has been shown to be much more complex.

Standard chromosomal analysis does not consistently demonstrate the cytogenetic abnormality in patients with fragile X syndrome, and molecular diagnostic techniques (DNA testing) have become the diagnostic procedure of choice for fragile X syndrome.

Aetna's policy on coverage of fragile X genetic testing is based on guidelines from the ACMG (1994) and the ACOG (1995).

#### Frizzled Class Receptor 6 (FZD6) Gene Sequencing for Nail Dystrophy

Nail dystrophy, specifically nonsyndromic congenital nail-10 (NDNC10), is a condition that affects the fingernails and toenails. NDNC10 is characterized by onychauxis (thick nails), hyponychia (missing part of the nail), onycholysis (nail separation), and/or claw-shaped fingernails (in some individuals). NDNC10 is an autosomal recessive disorder that can be caused by homozygous mutation in the FZD6 gene. Individuals with nonsyndromic congenital nail disorder 10 do not have any other health problems related to the condition (NIH, 2017).

There is no data demonstrating that identifying this causative mutation in persons with nail dystrophy influences clinical management. Furthermore, an UpToDate review on "Overview of nail disorders" (Rich, 2018) does not mention "FZD6" or "Frizzled class receptor 6".

#### Genesys Carrier Screening Panel

Genesys Diagnostics, Inc. offers the Genesys Carrier Panel which is a comprehensive carrier screening panel that evaluates 145 genes via next-generation sequencing obtained from a buccal swab. The panel is used to assist in identifying various genetic conditions that may be passed on to off-spring. This test is marketed for any prospective mothers and partners who want more information about their reproductive risks, and chances of having children with a genetic disorder. Testing can be performed either before or during pregnancy. Results, reported as carrier positive or negative, are expected within 7-14 days.

There is no published peer-reviewed literature on the efficacy of this test.

#### Genetic Testing for Epilepsy

Recent guidelines for Specialized Epilepsy Centers recommend genetic testing for individuals at a higher risk for having a genetic disorder as it may influence surgical decisions, therapy, diet and other management strategies. This includes those with intractable seizures, early onset, those with developmental epileptic encephalopathy or neurodevelopmental disabilities. However, no optimal genetic testing approach was noted (Lado et al, 2024). Expert consensus groups, including the Genetics Commission of the International League Against Epilepsy (ILAE) recommend single gene testing or targeted panel for those where the clinical presentation is highly suggestive of a specific disorder, and utilize whole exome sequencing where the etiology is unknown (Krey et al, 2022; Wilfong et al, 2024)

Sheidley et al (2022) stated that numerous genetic testing options for individuals with epilepsy have emerged over the last 10 years without clear guidelines regarding optimal testing strategies. In a systematic evidence review (SER) and meta-analysis, these investigators examined diagnostic yield of genetic tests commonly utilized for patients with epilepsy. They also evaluated non-yield outcomes (NYOs) such as changes in treatment and/or management, prognostic information, recurrence risk determination, and genetic counseling. The SER was

carried out in accordance with PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement, using PubMed, Embase, CINAHL, and Cochrane Central through December of 2020. These researchers included studies that employed genome sequencing (GS), exome sequencing (ES), multi-gene panel (MGP), and/or genome-wide CGH/CMA in cohorts (n of 10 or more) ascertained for epilepsy. Quality assessment was undertaken using ROBINS-I (Risk of Bias in Non-Randomized Studies of Interventions). They estimated diagnostic yields and 95 % CIs with random effects meta-analyses and narratively synthesized NYOs. From 5,985 non-duplicated articles published through 2020, 154 met inclusion criteria and were included in meta-analyses of diagnostic yield; 43 of those were included in the NYO synthesis. The overall diagnostic yield across all test modalities was 17 %, with the highest yield for GS (48 %), followed by ES (24 %), MGP (19 %), and CGH/CMA (9 %). The only phenotypic factors that were significantly associated with increased yield were the presence of developmental and epileptic encephalopathy and/or the presence of neurodevelopmental co-morbidities. Studies reporting NYOs addressed clinical and personal utility of testing. The authors concluded that this comprehensive SER, focused specifically on the literature regarding patients with epilepsy, provided a comparative evaluation of the yield of clinically available tests, which would aid in shaping clinician decision-making and policy regarding insurance coverage for genetic testing. Moreover, these researchers stated that this review highlighted the need for prospective evaluation of the clinical and personal use of genetic testing for patients with epilepsy and for standardization in reporting patient characteristics.

#### Genetic Testing for Familial Amyotrophic Lateral Sclerosis

Vajda and colleagues (2017) determined the degree of consensus among clinicians on the clinical use of genetic testing in amyotrophic lateral sclerosis (ALS) and the factors that determine decision-making. In this survey, ALS researchers worldwide were invited to participate in a detailed online survey to determine their attitudes and practices relating to genetic testing. Responses from 167 clinicians from 21 different countries were analyzed. The majority of respondents (73.3 %) do not consider that there is a consensus definition of familial ALS (FALS); 57 % consider a family history of fronto-temporal dementia and 48.5 % the presence of a known ALS genetic mutation as sufficient for a diagnosis of FALS. Most respondents (90.2 %) offered genetic testing to patients they define as having FALS and 49.4 % to patients with sporadic ALS; 4 main genes (SOD1, C9orf72, TARDBP, and FUS) were commonly tested. A total of 55.2 % of respondents would seek genetic testing if they had personally received a diagnosis of ALS; 42 % never offered pre-symptomatic testing to family members of patients with FALS. Responses varied between ALS specialists and non-specialists and based on the number of new patients seen per year. The authors concluded that there is a lack of consensus among clinicians as to the definition of FALS. Substantial variation exists in attitude and practices related to genetic testing of patients and pre-symptomatic testing of their relatives across geographic regions and between experienced specialists in ALS and non-specialists. These investigators stated that these data suggested that the clinical application of genetic testing in symptomatic patients is not always evidence-based, and that genetic counseling of patients and their families does not occur routinely as a standard of care in all instances. Pre-symptomatic testing may sometimes occur with limited recognition of the presence of genetic pleiotropy and oligogenic inheritance. They noted that these findings suggested the need for evidence-based and consensus guidelines as to the most appropriate utilization of diagnostic and pre-symptomatic genetic testing in routine clinical management of patients with ALS and their extended families.

#### Genetic Testing for Pulmonary Fibrosis

On behalf of the Pulmonary Fibrosis Foundation Genetic Testing Work Group, Newton et al (2022) examined the role of genetic testing in pulmonary fibrosis. These investigators stated that although genetic testing may provide high-yield

results in the appropriate clinical setting, widespread genetic testing is not yet justified for all patients with pulmonary fibrosis because the relevance of such results remains unclear in many settings. Genetic testing generally is not recommended for patients with sporadic pulmonary fibrosis unless they or their family members display features suggestive of a genetic syndrome. In addition, genetic testing is not currently recommended for unaffected relatives if the affected proband either has not undergone genetic testing or has shown negative results for genetic sequencing. In the genetics professions, it is considered best practice to initiate genetic testing in the individual most likely to be informative, ideally a family member with a diagnosis of the disease of interest.

#### Genetic Testing in von Willebrand Disease (VWD)

In an UpToDate review, Rick (2021) noted genetic testing for von Willebrand disease (VWD) may be useful in settings that include: diagnosis or confirmation of type 2N, distinguishing type 2N from mild hemophilia A in males, distinguishing type 2N from hemophilia A carrier status in female carriers of hemophilia A, diagnosis or confirmation of type 2M, distinguishing type 2B from platelet-type (pseudo) WD, and prenatal testing for type 3 VWD.

In a discussion by Sharma and Haberichter (2019), the utility of genetic testing was noted the following situations: 1. may help confirm the molecular diagnosis and specific subtype in type 2 VWD thus adding disease management, 2. may help to differentiate individuals with type 2N VWD vs mild hemophilia A, 3. may help differentiate platelet-type VWD from type 2B, 4. serve as confirmatory diagnosis of type 2M and type 2A VWD, and 5. may be helpful in accurately diagnosing type 3 VWD.

James and colleagues (2021) suggested targeted genetic testing over low-dose ristocetin-induced platelet agglutination (RIPA) to diagnose type 2B VWD for patients suspected of type 2A or 2B in need of additional testing. Furthermore, desmopressin carries a relative contraindication in individuals with type 2B VWD because this subtype is associated with more severe bleeding which can be exacerbated by desmopressin. The authors also suggested using either VWF:FVIII binding assay or targeted genetic testing (when available) for patients with suspected type 2N VWD in need of additional testing.

#### Genetic Testing of Age-Related Macular Degeneration

Stone (2015) stated that age-related macular degeneration (ARMD) is a very common condition that is caused by a complex interplay of genetic and environmental factors. It is likely that, in the future, genetic testing will allow physicians to achieve better clinical outcomes by administering specific treatments to patients based on their genotypes. However, improved outcomes for genotyped patients have not yet been shown in a prospective clinical trial, and as a result, the costs and risks of routine genetic testing currently out-weigh the benefits for patients with ARMD.

Seddon and colleagues (2016) stated that the Age-Related Eye Disease Study (AREDS) reported the beneficial impact of antioxidant and zinc supplements on the risk of progression to advanced stages of ARMD. These researchers evaluated the role of genetic variants in modifying the relationship between supplementation and progression to advanced ARMD. The authors concluded that the effectiveness of antioxidant and zinc supplementation appeared to differ by genotype. They stated that further study is needed to determine the biological basis for this interaction.

Hong et al (2016) examined if the complement factor H (CFH) polymorphism rs1061170/Y402H is associated with responsiveness to anti-vascular endothelial growth factor (VEGF) agents in ARMD. These investigators reviewed the English literature to examine the association between the polymorphism rs1061170/Y402H

of the CFH gene and responsiveness to treatment with anti-VEGF drugs in AMD patients. A meta-analysis of eligible studies was also performed. Pooled ORs and 95 % CIs were estimated using Stata V.12.0. Statistical heterogeneity was measured using Q-statistic testing. A total of 14 relevant studies including a total of 2,963 ARMD patients were eligible. In ARMD patients without a treatment history, individuals carrying the rs1061170/Y402H TT genotype were more likely to achieve a better outcome (OR = 1.932, 95 % CI: 1.125 to 3.317, p = 0.017) than those carrying the CC genotype. The authors concluded that the polymorphism rs1061170/Y402H might be a genetic predictor of treatment response to anti-VEGF therapy in ARMD patients. They stated that further prospective research including a larger number of patients is needed to validate this finding.

An UpToDate review on “Age-related macular degeneration: Clinical presentation, etiology, and diagnosis” (Arroyo, 2017) states that “Genetic factors - Age-related macular degeneration (AMD) has become the paradigm for a condition in which specific genotypic polymorphisms predispose to disease, and interact with modifiable and non-modifiable risk factors. Genetic polymorphisms in several genes have been identified that could account for more than half of all cases of AMD. While the strongest association is with genes involved in complement pathways, other associated genetic factors are involved in immune processes, lipid metabolism, collagen and extracellular matrix mechanisms, and angiogenesis pathways. Genetic factors influence the onset and progression of AMD, as well as differential response to treatment options ... The genetic basis for AMD may have implications for treatment. As an example, there is preliminary evidence that treatment response to vitamin and zinc supplementation may be influenced by the presence of a specific CFH genotype, although this will need further confirmation. Treatment response to VEGF inhibitors may also be related to the presence of identified polymorphisms in the CFH and other AMD-associated genes”.

Chew (2017) noted that the AREDS and AREDS2 provided evidence for treating persons with ARMD with anti-oxidant vitamins and minerals to reduce the risk of development of late ARMD. The AREDS2 data suggested that the beta-carotene in the original AREDS supplements be replaced by lutein and zeaxanthin, providing a safer drug for those who are smokers or former smokers. Even though consuming fish reduced the risk of ARMD in observational studies, the AREDS2 results showed that omega-3 long-chain polyunsaturated fatty acids (docosahexaenoic acid/eicosapentaenoic acid) had no beneficial effect on ARMD. Despite the major progress in the discovery of gene variants associated with ARMD, the use of genetic testing to predict disease has not been clinically useful. The author concluded that the use of genetic testing prior to ARMD therapies such as administering AREDS supplements is not recommended by the American Academy of Ophthalmology and other organizations.

#### Genetic Testing of HADHB for Breast Cancer

Zhou et al (2012a) stated that it is known that estrogen receptors (ERs) can function as nuclear receptors and transcription factors in the nucleus and as signaling molecules in the plasma membrane. In addition, the localization of the receptors in mitochondria suggested that they may play important roles in mitochondria. In order to identify novel proteins that are involved in ER-alpha (ER $\alpha$ )-mediated actions of estrogens, these researchers used a proteomic method that integrated affinity purification, 2-D gel electrophoresis, and mass spectrometry to isolate and identify cellular proteins that interact with ER $\alpha$ . One of the proteins identified was tri-functional protein  $\beta$ -subunit (HADHB), a mitochondrial protein that is required for  $\beta$ -oxidation of fatty acids in mitochondria. These investigators have verified the interaction between ER $\alpha$  and HADHB by co-immuno-precipitation and established that ER $\alpha$  directly binds to HADHB by performing an in-vitro binding assay. In addition, these researchers have shown that ER $\alpha$  co-localized with HADHB in the mitochondria by confocal microscopy, and the 2 proteins interact with each

other within mitochondria by performing co-immuno-precipitation using purified mitochondria as starting materials. These investigators had demonstrated that the expression of ER $\alpha$  affects HADHB activity, and a combination of 17 $\beta$ -estradiol and tamoxifen affects the activity of HADHB prepared from human breast cancer cells that express ER $\alpha$  but not from the cells that are ER $\alpha$  deficient. Furthermore, these researchers had demonstrated that 17 $\beta$ -estradiol plus tamoxifen affects the association of ER $\alpha$  with HADHB in human cell extract. The authors concluded that these findings suggested that HADHB is a functional molecular target of ER $\alpha$  in the mitochondria, and the interaction may play an important role in the estrogen-mediated lipid metabolism in animals and humans.

Zhou et al (2012b) noted that ERs are localized in mitochondria, but their functions in this organelle remain unclear. These researchers previously found that ER $\alpha$  interacted with mitochondrial protein HADHB and affected the thiolytic cleavage activity of HADHB in  $\beta$ -oxidation. It is known that ER-beta (ER $\beta$ ) binds to ER $\alpha$ . In addition, ER $\beta$  is predominately located in mitochondria. These facts led these researchers to speculate that ER $\beta$  may also be associated with HADHB in mitochondria. In order to test this hypothesis, these investigators performed co-immuno-precipitation and confocal microscopy analyses with human breast cancer MCF7 cells. The results demonstrated that ER $\beta$  was indeed associated and co-localized with HADHB within mitochondria. Interestingly, in contrast to the stimulatory effect of ER $\alpha$  on HADHB enzyme activity observed in the previous study, silencing of ER $\beta$  enhanced the enzyme activity of HADHB in the present study, suggesting that ER $\beta$  plays an inhibitory role in HADHB enzyme activity in the breast cancer cells. The authors concluded that these findings implied that ER $\alpha$  and ER $\beta$  may differentially affect cellular oxidative stress through influencing the rate of  $\beta$ -oxidation of fatty acids in breast cancer cells.

UpToDate reviews on "Screening for breast cancer: Strategies and recommendations" (Elmore, 2017) and "Genetic counseling and testing for hereditary breast and ovarian cancer" (Peshkin and Isaacs, 2017) do not mention HADHB.

Furthermore, National Comprehensive Cancer Network's clinical practice guideline on "Breast cancer" (Version 3.2017) does not mention HADHB.

#### Genetic Testing of Hereditary Hearing Loss of Unclear Etiology

Hearing loss may be classified as either syndromic or nonsyndromic. Nonsyndromic hearing loss is defined by the absence of malformations of the external ear or other medical problems in the affected individual. With the syndromic hearing loss, malformations of the external ear and/or other medical problems are present. Approximately 50% of nonsyndromic hearing loss can be attributed to a genetic cause, and may be inherited in an autosomal recessive (70% of patients), autosomal dominant (20% of patients), with mitochondrial, X-linked and other genetic causes making up the remainder of patients.

Genetic testing for hereditary hearing loss is most effective when a single gene or small number of genes are known to be associated with a specific phenotypic subtype (Li et al, 2022; Shearer et al, 2023). When hereditary hearing loss is non-syndromic and no subtype can be identified, a targeted panel representing genes curated by the ClinGen Hearing Loss Expert panel for non-syndromic hereditary hearing loss may be beneficial in those with bilateral hearing loss. If no cause is identified, exome or genome sequencing may be of value (DiStefano et al, 2020; Li et al, 2022). The etiology of non-syndromic unilateral sensorineural hearing loss is usually environmental and not genetic, with genetic testing diagnostic yields of 1-5% (Kocharyan et al, 2023; Smith et al, 2024)

Sequence variants in approximately 60 genes and some micro-RNAs have been associated with causing nonsyndromic hearing loss. Micro-RNAs are post-transcriptional regulators that consist of 20-25 nucleotides. Usher and Pendred syndromes are the most common forms of the approximately 400 forms of syndromic hearing loss. Both have autosomal recessive inheritance. Usher syndrome is characterized by sensorineural hearing loss and later development of retinitis pigmentosa. Usher syndrome has three forms that vary by the profundity of hearing loss and whether vestibular dysfunction is present. The three types of Usher syndrome have been associated with sequence variants in 9 different genes.

Pendred syndrome is characterized by congenital hearing loss and euthyroid goiter that develops in the second or third decade of life. Pendred syndrome is associated with sequence variants in the SLC26A4 gene. Some of the genes associated with Usher and Pendred syndromes may also be associated with nonsyndromic hearing loss.

The OtoGenome Test is a next-generation sequencing (NGS) assay that evaluates 110 genes in persons with apparent nonsyndromic and syndromic (e.g., Pendred syndrome, Usher syndrome) hearing loss.

The OtoSeq Hearing Loss Panel is a NGS assay that evaluates 23 genes in persons with apparent nonsyndromic and syndromic (e.g., Pendred syndrome, Usher syndrome) hearing loss. Published evidence for the OtoSeq test panel includes an epidemiological study of the use of a component of the OtoSeq panel in identifying certain hearing loss genes in 34 Pakistani families (Shahzad et al, 2013). In addition, there is a preliminary study of the performance of the OtoSeq in 8 individuals with hearing loss, comparing the results of Next Generation Sequencing with Sanger Sequencing (Sivakumaran et al, 2013).

Genes in the OtoGenome and OtoSeq panels have been curated by ClinGen, and FDA-approved resource, which provides moderate, strong, or definitive evidence of being causative of hearing loss. The ClinGen list of genes is recommended in the American College of Medical Genetics (ACMG) practice guidelines for workup of bilateral nonsyndromic hearing loss as first tier, prior to exome sequencing (DiStefano et al, 2019; Li et al, 2022).

The OtoSCOPE test has been developed to make use of next generation sequencing (NGS) capabilities, to simultaneously test for sequence variants in 224 genes and micro-RNAs associated with nonsyndromic hearing loss as well as both Usher and Pendred syndromes. The claimed advantage of the OtoScope test is that simultaneous analysis of the 224 genes included in the test may reduce the time and cost compared with genetic testing of individual genes. The current version of the test with 224 genes has no identified peer reviewed studies and the number of genes exceeds those curated by the ClinGen Hearing Loss Expert Panel as having moderate to definitive evidence of causing disease. Prior versions of the test with 66, 133, or 158 genes have been reported. In a cohort of 459 individuals with hearing loss receiving a cochlear implant, which included a mix of children and adults with uni-lateral or bi-lateral sensorineural hearing loss, the diagnostic yield was 28%. The OtoSCOPE genetic testing for hereditary hearing loss is considered experimental, investigational, or unproven because there is inadequate evidence in the peer-reviewed published clinical literature regarding its current effectiveness.

#### GeneticsNow Comprehensive

GeneticsNow Comprehensive (GoPath Global, Inc) is hereditary pan-cancer panel that uses next-generation sequencing to evaluate 88 genes associated with genetic disorders, including hereditary cancers (e.g., hereditary sarcomas, hereditary endocrine tumors, hereditary neuroendocrine tumors, hereditary cutaneous melanoma). The test uses samples obtained from peripheral blood or preserved saliva. The assay is purported to detect single- and multi-nucleotide substitutions

and small insertions and deletions in coding regions and exon-intron junctions, as well as large genomic rearrangements of the target genes. Results are reported as positive or negative for germline variants for each gene.

There is insufficient evidence in published peer-reviewed literature to support the efficacy of this test.

#### Hemoglobinopathies in Pregnancy

The American College of Obstetricians and Gynecologists (2022), in their practice advisory on hemoglobinopathies in pregnancy, "recommends offering universal hemoglobinopathy testing to persons planning pregnancy or at the initial prenatal visit if no prior testing results are available for interpretation. This helps ensure that at-risk individuals receive counseling about genetic risks; learn their reproductive options, which include preimplantation genetic testing and prenatal diagnosis; and make informed decisions. Hemoglobinopathy testing may be performed using hemoglobin electrophoresis or molecular genetic testing (eg, expanded carrier screening that includes sickle cell disease [SCD] and other hemoglobinopathies). The use of noninvasive prenatal diagnosis for SCD with cell-free fetal DNA is still experimental and currently not recommended".

#### Hereditary Ataxia Panel

Jayadev and Bird (2013) state that hereditary ataxias are considered a highly heterogeneous group of disorders characterized by gait ataxia, incoordination of eye movements, speech, and hand movements, and are usually associated with atrophy of the cerebellum. The "differential diagnosis of hereditary ataxia includes acquired, nongenetic causes of ataxia, such as alcoholism, vitamin deficiencies, multiple sclerosis, vascular disease, primary or metastatic tumors, and paraneoplastic diseases associated with occult carcinoma of the ovary, breast, or lung, and the idiopathic degenerative disease multiple system atrophy (spinal muscular atrophy). The possibility of an acquired cause of ataxia needs to be considered in each individual with ataxia because a specific treatment may be available." The authors note that there are more than 35 autosomal dominant types frequently termed spinocerebellar ataxia (SCA) and typically having adult onset.

The most common subtypes are SCA 1 (ATXN1), 2 (ATXN2), 3 (ATXN3), 6 (CACNA1A), and 7 (ATXN7), all of which are nucleotide repeat expansion disorders.

Autosomal recessive ataxias usually have onset in childhood, with the most common subtypes being Friedreich, ataxia-telangiectasia, ataxia with oculomotor apraxia type 1, and ataxia with oculomotor apraxia type 2. Four autosomal recessive types have dietary or biochemical treatment modalities (ataxia with vitamin E deficiency, cerebrotendinous xanthomatosis, Refsum, and coenzyme Q10 deficiency), whereas there are no specific treatments for other ataxias. The authors state that diagnostic genetic testing is complicated because of the large number of relatively uncommon subtypes with extensive phenotypic overlap. However, the best testing strategy is based on assessing relative frequencies, ethnic predilections, and recognition of associated phenotypic features such as seizures, visual loss, or associated movement abnormalities. Moreover, ""Although the probability of a positive result from molecular genetic testing is low in an individual with ataxia who has no family history of ataxia, such testing is usually justified to establish a specific diagnosis for the individual's medical evaluation and for genetic counseling".

de Silva et al (2019) discussed guidelines aimed to assist healthcare professionals when caring for patients with progressive ataxia, indicate evidence-based (where it exists) and best practice, and act overall as a useful resource for clinicians involved in managing ataxic patients. The authors recommend "genetic tests for FRDA, SCA 1, 2, 3, 6,7 (12, 17) and FXTAS" in adults; however, "genetic testing of asymptomatic 'at-risk' minors is not generally recommended, but should be considered on a case-by-case basis".

The most common inherited cause of hereditary ataxia results from nucleotide repeat expansions. The following include the most commonly involved genes (not an all-inclusive list): ATN1, ATXN1, ATXN2, ATXN3, ATXN7, ATXN8OS, ATXN10, CACNA1A, FGF14, FXN, RFC1, and TBP. The following are the most common genes associated with non-nucleotide repeat hereditary ataxias (not an all-inclusive list) AFG3L2, ANO10, APTX, ATM, PRKCG, SACS, SETX, SPTBN2, and SYNE1 (Perlman, 2023).

Radmard and colleagues (2023) state "Genetic forms of cerebellar ataxia are categorized based on inheritance pattern: autosomal dominant, autosomal recessive, X-linked, and mitochondrial inheritance. A detailed family history can reveal an inheritance pattern that directs genetic testing. Notably, autosomal dominant cerebellar ataxia could still be found in 5% of cases without a family history. Determining the underlying genetic diagnosis is prudent for counseling on disease progression, family planning, and/or enrollment in clinical trials for disease-modifying therapies". "The predominant autosomal dominant, autosomal recessive, and X-linked cerebellar ataxias are repeat expansions and thus are not commonly detected by whole exome sequencing. Thus, the first step of genetic evaluation for cerebellar ataxias is repeat expansion panels, which should include both coding and noncoding repeat expansions."

In an UpToDate review "Overview of cerebellar ataxia in adults", Todd and Shakkottai (2024) state that a genetic evaluation is indicated if the initial workup is negative for obvious and/or reversible causes of ataxia and the condition is most consistent with a progressive degenerative condition of the cerebellum and brainstem.

#### Hereditary Hemochromatosis

Hemochromatosis, a condition involving excess accumulation of iron, can lead to iron overload, which in turn can result in complications such as cirrhosis, diabetes, cardiomyopathy, and arthritis (Burke 1992; Hanson et al, 2001).

Hereditary hemochromatosis (HHC) is characterized by inappropriately increased iron absorption from the duodenum and upper intestine, with consequent deposition in various parenchymal organs, notably the liver, pancreas, joints, heart, pituitary gland and skin, with resultant end-organ damage (Lindi and Crampton, 2004). Clinical features may be non-specific and include lethargy and malaise, or reflect target organ damage and present with abnormal liver tests, cirrhosis, diabetes mellitus, arthropathy, cardiomyopathy, skin pigmentation and gonadal failure. Early recognition and treatment (phlebotomy) is essential to prevent irreversible complications such as cirrhosis and hepatocellular carcinoma.

HHC is an autosomal recessive condition associated with mutations of the HFE gene. Two of the 37 allelic variants of the HFE gene, C282Y and H63D, are significantly correlated with HHC. C282Y is the more severe mutation, and homozygosity for the C282Y genotype accounts for the majority of clinically penetrant cases. Hanson et al (2001) reported that homozygosity for the C282Y mutation has been found in 52 to 100 % of previous studies on clinically diagnosed index cases. Five percent of HHC probands were found by Hanson et al to be compound heterozygotes (C282Y/H63D), and 1.5 % were homozygous for the H63D mutation; 3.6 % were C282Y heterozygotes, and 5.2 % were H63D heterozygotes. In 7 % of cases, C282Y and H63D mutations were not present. In the general population, the frequency of the C282Y/C282Y genotype is 0.4 %.

HHC is a very common genetic defect in the Caucasian population. C282Y heterozygosity ranges from 9.2 % in Europeans to nil in Asian, Indian subcontinent, African, Middle Eastern, Australian and Asian populations (Hanson et al, 2001). The H63D carrier frequency is 22 % in European populations.

Accurate data on the penetrance of the different HFE genotypes are not available. But current data suggest that clinical disease does not develop in a substantial proportion of people with this genotype. Available data suggest that up to 38 % to 50 % of C282Y homozygotes may develop iron overload, with up to 10 % to 33 % eventually developing hemochromatosis-associated morbidity (Whitlock et al, 2006). A pooled analysis found that patients with the HFE genotypes C282Y/H63D and H63D/H63D are also at increased risk for iron overload, yet overall, disease is likely to develop in fewer than 1 % of people with these genotypes (Burke, 1992). Thus, DNA-based tests for hemochromatosis identify a genetic risk rather than the disease itself.

Environmental factors such as diet and exposure to alcohol or other hepatotoxins may modify the clinical outcome in patients with hemochromatosis, and variations in other genes affecting iron metabolism may also be a factor. As a result, the clinical condition of iron overload is most reliably diagnosed on the basis of biochemical evidence of excess body iron (Burke, 1992).

Whether it is beneficial to screen asymptomatic people for a genetic risk of iron overload is a matter of debate. To date, population screening for HHC is not recommended because of uncertainties about optimal screening strategies, optimal care for susceptible persons, laboratory standardization, and the potential for stigmatization or discrimination (Hanson et al, 2001; Whitlock et al, 2006). A systematic evidence review prepared for the U.S. Preventive Services Task Force concluded: "Research addressing genetic screening for hereditary hemochromatosis remains insufficient to confidently project the impact of, or estimate the benefit from, widespread or high-risk genetic screening for hereditary hemochromatosis" (Whitlock et al, 2006).

#### Hereditary Hemorrhagic Telangiectasia

Hereditary hemorrhagic telangiectasia (HHT), also called Osler-Weber-Rendu syndrome, is an autosomal dominant trait disorder that results in the development of multiple abnormalities in the blood vessels. Some arterial vessels flow directly into veins rather than into the capillaries resulting in arteriovenous malformations. When they occur in vessels near the surface of the skin, where they are visible as red markings, they are known as telangiectases (the singular is telangiectasia). Nosebleeds are very common in people with HHT, and more serious problems may arise from hemorrhages in the brain, liver, lungs, or other organs. Forms of HHT include type 1, type 2, type 3, and juvenile polyposis/hereditary hemorrhagic telangiectasia syndrome. People with type 1 tend to develop symptoms earlier than those with type 2, and are more likely to have blood vessel malformations in the lungs and brain. Type 2 and type 3 may be associated with a higher risk of liver involvement. Women are more likely than men to develop blood vessel malformations in the lungs with type 1, and are also at higher risk of liver involvement with both type 1 and type 2. Individuals with any form of hereditary hemorrhagic telangiectasia, however, can have any of these problems.

Genetic testing utilizes a blood test to determine whether or not an at risk individual carries the genes responsible for the development of disease. Mutations in two genes, endoglin and ALK-1, have been shown to be responsible for pure HHT, with the disease subtypes designated HHT1 and HHT2. Mutations in Smad4 result in a juvenile polyposis-HHT overlap syndrome.

In 2010, Shah and group wrote that hereditary hemorrhagic telangiectasia (HHT) is an autosomal dominant disorder with age-dependent penetrance characterized by recurrent epistaxis, mucocutaneous telangiectasias, and visceral arteriovenous malformations (AVMs). AVMs can occur in multiple organs, including brain, liver, and lungs, and are associated with a large portion of disease morbidity. Pulmonary AVMs (PAVMs) can be asymptomatic or manifest as dyspnea and hypoxemia

secondary to shunting. The presence of untreated PAVMs can also lead to transient ischemic attacks, stroke, hemothorax, and systemic infection, including cerebral abscesses. Definitive diagnosis is made when three or more clinical findings are present, which include the features mentioned above and a first-degree relative diagnosed with HHT. Diagnosis is suspected when two findings are present. Genetic testing can help confirm diagnosis. Mutations in three genes are known to cause disease: ENG, ACVRL1, and SMAD4. Genetic testing involves sequence and duplication/deletion analysis and identifies a mutation in roughly 80% of patients with clinical disease.

The textbook *Flint: Cummings Otolaryngology: Head & Neck Surgery* (2010) states that genetic testing is available for prenatal diagnosis of hereditary hemorrhagic telangiectasia. This is important, because catastrophic hemorrhage can occur in children with clinically silent disease, thus screening imaging for cerebral and pulmonary arteriovenous malformations is indicated in children who have a family history.

According to the textbook of *Feldman: Sleisenger and Fordtran's Gastrointestinal and Liver Disease* (2010), genetic testing to detect mutations in the ENG, ALK-1, or MAHD4 genes may be helpful in selected cases. Patients suspected of having HHT should be screened for cerebral and pulmonary arteriovenous malformations (AVMs), and family members of the patient should consider genetic testing.

The textbook *Cassidy: Management of Genetic Syndromes* (2005), reports that, to date, mutation testing has not been widely used in the diagnosis of HHT. However, mutations in either ALK1 or endoglin have been demonstrated in over 70% of unrelated, affected individuals tested using direct gene sequencing of genomic DNA. Genetic testing for HHT will have an important role in both the testing of individuals for whom the diagnosis is uncertain and in presymptomatic testing of young adults at risk of HHT.

In 2006, Bossler and group describe the results of mutation analysis on a consecutive series of 200 individuals undergoing clinical genetic testing for HHT. The observed sensitivity of mutation detection was similar to that in other series with strict ascertainment criteria. A total of 127 probands were found, with sequence changes consisting of 103 unique alterations, 68 of which were novel. In addition, eight intragenic rearrangements in the ENG gene and two in the ACVRL1 gene were identified in a subset of coding sequence mutation-negative individuals. Most individuals tested could be categorized by the number of HHT diagnostic criteria present. Surprisingly, almost 50% of the cases with a single symptom were found to have a significant sequence alteration; three of these reported only nosebleeds. The authors concluded, "genetic testing can confirm the clinical diagnosis in individuals and identify presymptomatic mutation carriers. As many of the complications of HHT disease can be prevented, a confirmed molecular diagnosis provides an opportunity for early detection of AVMs and management of the disease."

A Scientific Statement for Healthcare Professionals from the American Heart Association/American Stroke Association (Derdeyn et al., 2017) states that 10 to 25% of people with HHT will have at least 1 brain arteriovenous malformation (bAVM). The authors state that "The hereditary hemorrhagic telangiectasia bAVM population is an interesting subgroup as it relates to predicting ICH [intracranial hemorrhage] risk in part because of the characteristic angioarchitectural manifestations (small bAVM size, cortical location, and multiplicity) but also the genotypes (ENG, ALK1, or SMAD4) involved. The capillary malformation-AVM syndrome (RASA1) is interesting for the same reasons. Although there is no evidence that any of the genotypes confer higher ICH risk or a particular bAVM appearance, the concept of combining imaging and genetic information to assess risk will grow as biomarker investigation expands".

Second International Guidelines for the diagnosis and management of HHT were developed using the AGREE II (Appraisal of Guidelines for Research and Evaluation II) framework and GRADE (Grading of Recommendations Assessment, Development and Evaluation) methodology. The expert panel included physicians (clinical and genetic) from 15 countries who were considered "experts" in HHT. A systematic literature search was done in June 2019, and articles meeting a priori criteria were included to generate evidence tables. The authors state that In 97% of patients with a definite clinical diagnosis of HHT, a causative mutation is identified in one of the following genes: endoglin (ENG, HHT type 1), activin receptor-like kinase-1 (ACVRL1, HHT type 2), and mothers against decapentaplegic homolog 4 (SMAD4, juvenile polyposis-HHT overlap). Recommendation from the expert panel advises that diagnostic genetic testing be offered for asymptomatic children of a parent with HHT (Quality of evidence: high [agreement, 96%]). An affected family member should be tested first to determine the causative mutation before testing an asymptomatic child who does not meet the clinical diagnostic criteria for HHT (Faughman et al, 2020).

HHT may be diagnosed clinically (using three or more Curaçao Criteria or by documentation of a pathogenic or likely pathogenic variant in an HHT gene. International consensus diagnostic criteria (the Curacao diagnostic criteria) are based upon four findings. Three or four of these criteria are considered "definite". Two criteria are considered "suspected", and zero or one criterion is considered "unlikely" for HHT (Shovlin, 2023):

- Spontaneous and recurrent epistaxis
- Multiple mucocutaneous telangiectasia at characteristic sites (e.g., skin of the hands, lips, face, or inside of the nose or mouth)
- Visceral involvement (e.g., gastrointestinal telangiectasia; pulmonary, cerebral, or hepatic arteriovenous malformations [AVMs])
- A first-degree relative (i.e., parent, sibling, child) with HHT.

An UpToDate review on "Clinical manifestations and diagnosis of hereditary hemorrhagic telangiectasia (Osler-Weber-Rendu syndrome)" (Shovlin, 2023) state that the diagnosis of HHT may be established (using 3 or more Curacao Criteria) or by genetic testing showing a pathogenic sequence variant in and HHT gene (ENG, ACVRL1, SMAD4, or GDF2). Although genetic testing is not required to make a diagnosis of HHT, the Second International Guidelines suggest genetic testing for all individuals with HHT, as it "may facilitate family testing and additional evaluations (e.g., screening colonoscopies for individuals with pathogenic variants in the SMAD4 gene)".

#### Hereditary Non-Polyposis Colon Cancer

Hereditary non-polyposis colon cancer ([HNPCC], Lynch syndrome) is one of the most common cancer predisposition syndromes affecting 1 in 200 individuals and accounting for 13 to 15 % of all colon cancer. HNPCC is defined clinically by early-onset colon carcinoma and by the presence of other cancers such as endometrial, gastric, urinary tract and ovarian found in at least 3 first-degree relatives. Two genes have been identified as being primary responsible for this syndrome: hMLH1 at chromosome band 3p21 accounts for 30 % of HNPCC2,3 and hMLH2 or FCC at chromosome band 2p22 which together with hMLH1 accounts for 90 % of HNPCC.

Unlike other genetic disorders that are easily diagnosed, the diagnosis of HNPCC relies on a very strongly positive family history of colon cancer. Specifically, several organizations have defined criteria that must be met to make the diagnosis of HNPCC.

Although HNPCC lacks strict clinical distinctions that can be used to make the diagnosis, and therefore diagnosis is based on the strong family history, genetic testing is now available to study patient's DNA for mutations to one of the mismatch repair genes. A mutation to one of these genes is a characteristic feature and confirms the diagnosis of HNPCC. Identifying individuals with this disease and performing screening colonoscopies on affected persons may help reduce colon cancer mortality.

Microsatellite instability (MSI) is found in the colorectal cancer DNA (but not in the adjacent normal colorectal mucosa) of most individuals with germline mismatch repair gene mutations. In combination with immunohistochemistry for MSH2 and MLH1, MSI testing using the Bethesda markers should be performed on the tumor tissue of individuals putatively affected with HNPCC. A result of MSI-high in tumor DNA usually leads to consideration of germline testing for mutations in the MSH2 and MLH1 genes. Individuals with MSI-low or microsatellite stable (MSS) results are unlikely to harbor mismatch repair gene mutations, and further genetic testing is usually not pursued.

HNPCC is caused by germline mutation of the DNA mismatch repair genes. Over 95 % of HNPCC patients have mutations in either MLH1 or MSH2. As a result, sequencing for mismatch repair gene mutations in suspected HNPCC families is usually limited to MLH1 and MSH2 and sometimes MSH6 and PMS2. In general, MSH6 and PMS2 sequence analysis is performed in persons meeting aforementioned criteria for genetic testing for HNPCC, and who do not have mutations in either the MLH1 or MSH2 genes. In addition, single site MSH6 or PMS2 testing may be appropriate for testing family members of persons with HNPCC with an identified MSH6 or PMS2 gene mutation.

HNPCC is a relatively rare disease, which makes screening the entire populace burdensome and ineffective. The incidence of this disease, even among the families of patients with colon cancer, is too small to make screening effective. See also [CPB\\_0189 - Genetic Counseling \(0189.html\)](#) and [CPB\\_0227 - BRCA Testing, Prophylactic Mastectomy, and Prophylactic Oophorectomy \(..//200\\_299/0227.html\)](#).

#### Heterotaxy

Bedard et al (2011) noted that patients with heterotaxy have characteristic cardiovascular malformations, abnormal arrangement of their visceral organs, and midline patterning defects that result from abnormal left-right patterning during embryogenesis. Loss of function of the transcription factor ZIC3 causes X-linked heterotaxy and isolated congenital heart malformations and represents one of the few known monogenic causes of congenital heart disease. The birth incidence of heterotaxy-spectrum malformations is significantly higher in males, but the authors' previous work indicated that mutations within ZIC3 did not account for the male over-representation. Therefore, cross species comparative sequence alignment was used to identify a putative novel fourth exon, and the existence of a novel alternatively spliced transcript was confirmed by amplification from murine embryonic RNA and subsequent sequencing. This transcript, termed Zic3-B, encompasses exons 1, 2, and 4 whereas Zic3-A encompasses exons 1, 2, and 3. The resulting protein isoforms are 466 and 456 amino acid residues respectively, sharing the first 407 residues. Importantly, the last 2 amino acids in the 5th zinc finger DNA binding domain are altered in the Zic3-B isoform, indicating a potential functional difference that was further evaluated by expression, subcellular localization, and transactivation analyses. The temporo-spatial expression pattern of Zic3-B overlaps with Zic3-A in-vivo, and both isoforms are localized to the nucleus in-vitro. Both isoforms can transcriptionally activate a Gli binding site reporter, but only ZIC3-A synergistically activates upon co-transfection with Gli3, suggesting that the isoforms are functionally distinct. The authors concluded that

screening 109 familial and sporadic male heterotaxy cases did not identify pathogenic mutations in the newly identified fourth exon and larger studies are necessary to establish the importance of the novel isoform in human disease.

Tariq et al (2011) heterotaxy-spectrum cardiovascular disorders are challenging for traditional genetic analyses because of clinical and genetic heterogeneity, variable expressivity, and non-penetrance. In this study, high-resolution single nucleotide polymorphisms (SNPs) genotyping and exon-targeted array comparative genomic hybridization (CGH) platforms were coupled to whole-exome sequencing to identify a novel disease candidate gene. SNP genotyping identified absence-of-heterozygosity regions in the heterotaxy proband on chromosomes 1, 4, 7, 13, 15, 18, consistent with parental consanguinity. Subsequently, whole-exome sequencing of the proband identified 26,065 coding variants, including 18 non-synonymous homozygous changes not present in dbSNP132 or 1000 Genomes. Of these 18, only 4 - 1 each in CXCL2, SHROOM3, CTSO, RXFP1 - were mapped to the absence-of-heterozygosity regions, each of which was flanked by more than 50 homozygous SNPs, confirming recessive segregation of mutant alleles. Sanger sequencing confirmed the SHROOM3 homozygous missense mutation and it was predicted as pathogenic by 4 bio-informatic tools. SHROOM3 has been identified as a central regulator of morphogenetic cell shape changes necessary for organogenesis and can physically bind ROCK2, a rho kinase protein required for left-right patterning. Screening 96 sporadic heterotaxy patients identified 4 additional patients with rare variants in SHROOM3. The authors concluded that using whole exome sequencing, the authors identify a recessive missense mutation in SHROOM3 associated with heterotaxy syndrome and identify rare variants in subsequent screening of a heterotaxy cohort, suggesting SHROOM3 as a novel target for the control of left-right patterning. This study revealed the value of SNP genotyping coupled with high-throughput sequencing for identification of high yield candidates for rare disorders with genetic and phenotypic heterogeneity.

Also, UpToDate reviews on "Clinical manifestations, pathophysiology, and diagnosis of atrioventricular (AV) canal defects" (Fleishman and Tugertimur, 2013) and "Congenital heart disease (CHD) in the newborn: Presentation and screening for critical CHD" (Altman, 2013) do not mention the use of genetic testing as a management tool.

#### Home Genetic Tests

Walker (2010) stated that according to an undercover investigation by the Government Accountability Office (GAO), home genetic tests often provide incomplete or misleading information to consumers. For the GAO investigation, investigators purchased 10 tests each from 4 different direct-to-consumer genetic tests companies: 23andMe, deCode Genetics, Navigenics, and Pathway Genomics. Five saliva donors each sent 2 DNA samples to each company. In one sample, the donor used his or her real personal and medical information, and for the second sample, they developed faux identifying and medical information. The results, according to the GAO, were far from precise. For example, a donor was told by a company that he had a "below average" risk of developing hypertension, but a second company rated his risk as "average", while a third company, using DNA from the same donor, said the sample revealed an "above average" risk for hypertension. In some cases, the results conflicted with the donor's real medical condition. None of the genetic tests currently offered to consumers has undergone FDA pre-market review.

#### Hypertrophic Cardiomyopathy

Hypertrophic cardiomyopathy (HCM) is a disease of the myocardium in which a portion of the myocardium is hypertrophied without any obvious cause; it is among the most common genetically transmitted cardiovascular diseases. In HCM, the

heart muscle is so strong that it does not relax enough to fill with the heart with blood and therefore has reduced pumping ability.

The genetic abnormalities that cause HCM are heterogeneous. Hypertrophic cardiomyopathy is most commonly due to a mutation in one of 9 genes that results in a mutated protein in the sarcomere. Some of the genes responsible for HCM have not yet been identified, and among those genes that have been identified, the spectrum of possible disease-causing mutations is incomplete. As a result, a thorough evaluation of known genes requires extensive DNA sequencing, which is onerous for routine clinical testing. Less rigorous methods (such as selective sequencing) reduces the likelihood of identifying the responsible mutation.

Population studies have demonstrated that some patients are compound heterozygotes (inheriting 2 different mutations within a single HCM gene), double heterozygotes (inheriting mutations in 2 HCM genes), or homozygotes (inheriting the same mutation from both parents). To be certain of detecting such genotypes, sequencing of candidate genes would need to continue in a given patient even after a single mutation was identified.

In many persons with HCM mutations, the disease can be mild and the symptoms absent or minimal. In addition, phenotypic expression of HCM can be influenced by factors other than the basic genetic defect, and the clinical consequences of the genetic defect can vary. There is sufficient heterogeneity in the clinical manifestations of a given gene mutation that, even when a patient's mutation is known, his or her clinical course can not be predicted with any degree of certainty.

In addition, the prognostic impact of a given mutation may relate to a particular family and not to the population at large. Many families have their own "private" mutations and thus knowledge of the gene abnormalities can not be linked to experience from other families.

Family members with echocardiography evidence of HCM should be managed like other patients with HCM. In general, genetically affected but phenotypically normal family members should not be subjected to the same activity restriction as patients with HCM.

Bos and colleagues (2009) stated that over the past 20 years, the pathogenic basis for HCM, the most common heritable cardiovascular disease, has been studied extensively. Affecting about 1 in 500 persons, HCM is the most common cause of sudden cardiac death (SCD) among young athletes. In recent years, genomic medicine has been moving from the bench to the bedside throughout all medical disciplines including cardiology. Now, genomic medicine has entered clinical practice as it pertains to the evaluation and management of patients with HCM. The continuous research and discoveries of new HCM susceptibility genes, the growing amount of data from genotype-phenotype correlation studies, and the introduction of commercially available genetic tests for HCM make it essential that cardiologists understand the diagnostic, prognostic, and therapeutic implications of HCM genetic testing.

Hudecova et al (2009) noted that the clinical symptoms of HCM are partly dependent on mutations in affected sarcomere genes. Different mutations in the same gene can present as malignant with a high-risk of SCD, while other mutations can be benign. The clinical symptomatology can also be influenced by other factors such as the presence of polymorphisms in other genes. Currently, the objective of intensive clinical research is to assess the contribution of molecular genetic methods in HCM diagnostics as well as in risk stratification of SCD. It is expected that genetic analyses will have an important consequence in the screening of the relatives of HCM patients and also in the prenatal diagnostics and genetic counseling.

Shephard and Semsarian (2009) stated that genetic heart disorders are an important cause of SCD in the young. While pharmacotherapies have made some impact on the prevention of SCD, the introduction of implantable cardioverter-defibrillator (ICD) therapy has been the single major advance in the prevention of SCD in the young. In addition, the awareness that most causes of SCD in the young are inherited, means family screening of relatives of young SCD victims allows identification of previously unrecognized at-risk individuals, thereby enabling prevention of SCD in relatives. The role of genetic testing, both in living affected individuals as well as in the setting of a "molecular autopsy", is emerging as a key factor in early diagnosis of an underlying cardiovascular genetic disorder.

The Heart Failure Society of America's practice guideline on "Genetic evaluation of cardiomyopathy" (Hershberger et al, 2009) stated that genetic testing is primarily indicated for risk assessment in at-risk relatives who have little or no clinical evidence of cardiovascular disease. Genetic testing for HCM should be considered for the one most clearly affected person in a family to facilitate family screening and management. Specific genes available for testing for HCM include MYH7, MYBPC3, TNNT2, TNN13, TPM1, ACTC, MYL2, and MYL3. MYH7 and MYBPC each accounts for 30 % to 40 % of mutations; TNNT2 for 10 % to 20 %. Genetic cause can be identified in 35 % to 45 % overall; up to 60 % to 65 % when the family history is positive.

The BlueCross BlueShield Association Technology Evaluation Center (TEC)'s assessment on genetic testing for predisposition to inherited HCM (2010) concluded that the use of genetic testing for inherited HCM meets the TEC criteria for individuals who are at-risk for development of HCM, defined as having a close relative with established HCM, when there is a known pathogenic gene mutation present in an affected relative. In order to inform and direct genetic testing for at-risk individuals, genetic testing should be initially performed in at least 1 close relative with definite HCM (index case) if possible. This testing is intended to document whether a known pathologic mutation is present in the family, and optimize the predictive value of predisposition testing for at-risk relatives. Due to the complexity of genetic testing for HCM and the potential for misinterpretation of results, the decision to test and the interpretation of test results should be performed by, or in consultation with an expert in the area of medical genetics and/or HCM.

The TEC assessment also concluded that genetic testing for inherited HCM does not meet the TEC criteria for predisposition testing in individuals who are at-risk for development of HCM, defined as having a close relative with established HCM, when there is no known pathogenic gene mutation present in an affected relative. This includes: (i) patients with a family history of HCM, with unknown genetic status of affected relatives; and (ii) patients with a family history of HCM, when a pathogenic mutation has not been identified in affected relatives.

Hershberger and colleagues (2019) discussed the clinical practice resource from the American College of Medical Genetics and Genomics (ACMG) on genetic evaluation of cardiomyopathy. The authors note that "no large randomized, placebo-controlled studies meeting usual criteria for highest levels of evidence are available in the genetic cardiomyopathy literature". Thus, each recommendation was assigned to teams of individuals by expertise, literature was reviewed, and recommendations were decided by consensus of the writing group. The recommendations include genetic testing on a confirmed affected individual. The timing for ordering genetic testing in a patient with cardiomyopathy has not been studied. Because results may guide management, the authors recommend genetic testing at the time a new cardiomyopathy diagnosis is made; however, it can be conducted at any time following diagnosis. The level of evidence for testing in HCM is based on studies showing a high diagnostic yield of genetic testing in children and adults and prognostic value of genotype status. The diagnostic yield of HCM testing is

approximately 30 to 60%. The authors state that the yield of testing is higher in individuals who have a known family history of HCM. Pathogenic variants in MYH7 and MYBPC3 account for approximately 80% of all cases for which a molecular diagnosis is achieved. Beyond sarcomeric genes, core genes to screen in patients with HCM include GLA, PRKAG2, and LAMP2. If a pathogenic or likely pathogenic variant is identified in the index patient initially tested, opportunities emerge for the predictive testing of at-risk family members; however, variants of uncertain significance (VUS) are not useful to conduct predictive genetic testing. The authors do note that a distinct limitation is that they were "unaware of published outcomes data to support, validate, or refute the above guidance, which can only be considered as expert opinion. This emphasizes the need for well-designed rigorous studies examining outcomes of phenotyping and family studies following secondary or incidental findings of variants relevant for the cardiomyopathies".

The AHA/ACC issued 2020 guidelines for the diagnosis and treatment of patients with hypertrophic cardiomyopathy (HCM). The AHA/ACC states that genetic testing in patients with HCM is beneficial to elucidate the genetic basis to facilitate the identification of family members at risk for developing HCM (cascade testing) (COR: 1; LOE: B-NR [nonrandomized]). In patients with an atypical clinical presentation of HCM or when another genetic condition is suspected to be the cause, a work-up including genetic testing for HCM and other genetic causes of unexplained cardiac hypertrophy is recommended (COR: 1; LOE: B-NR). Additional recommendations include when performing genetic testing in an HCM proband, the initial tier of genes tested should include genes with strong evidence to be disease-causing in HCM (COR: 1; LOE: B-NR) (Ommen et al., 2020).

The EHRA/HRS/APHRS/LAHRs issued a 2022 consensus which provides the following recommendations supported by "strong observational evidence and authors' consensus" (Wilde et al., 2022):

- For genetic testing in a proband with HCM, the initial tier of genes tested should include genes with definitive or strong evidence of pathogenicity (currently MYH7, MYBPC3, TNNI3, TPM1, MYL2, MYL3, ACTC1, and TNNT2)
- In patients with HCM, genetic testing is recommended for identification of family members at risk of developing HCM.
- In patients with atypical clinical presentation of HCM, or when another genetic condition associated with unexplained hypertrophy is suspected (e.g. HCM phenocopy) genetic testing is recommended
- Predictive genetic testing in related children is recommended in those aged greater than 10 to 12 years.

An UpToDate review on "Hypertrophic cardiomyopathy: Clinical manifestations, diagnosis, and evaluation" (Maron, 2022a) state that "routine genetic testing in isolation (i.e., without concurrent clinical evaluation with ECG, imaging, etc) is not recommended for the diagnosis of HCM. However, targeted genetic testing may be useful (in conjunction with clinical evaluation) in two specific scenarios:

- When family history or clinical evaluation raises suspicion for another genetic condition known to cause LVH (ie, Fabry disease, lysosomal storage diseases, etc); or
- In first degree family members of a proband with HCM and a definitively identified gene-causing mutation.

An UpToDate review on "Hypertrophic cardiomyopathy: Gene mutations and clinical genetic testing" (Maron, 2022b) state, "While some experts recommend genetic testing in first-degree relatives for the pathogenic mutation identified in proband prior to performing clinical evaluation with ECG and echocardiography, UpToDate experts recommend only offering cascade genetic testing of family members after initial comprehensive clinical evaluation to determine if HCM is present". Furthermore, the authors state that "Although there was a tremendous amount of

optimism that genetic testing could be used to help stratify patients for risk of sudden death, this hope has generally been unrealized. Indeed, given the substantial amount of genetic heterogeneity responsible for HCM, it has not been possible to classify mutations as being definitively "benign" or "malignant". Therefore, a patient's clinical course cannot be predicted with any degree of certainty based on the type of mutation. As a result, management decisions such as ICD therapy for primary prevention should not be predicated solely on the presence of a specific type of sarcomere mutation".

Gartzonikas and colleagues (2023) state that "HCM is a diagnosis of exclusion since secondary causes of left ventricular hypertrophy should first be ruled out. These include hypertension, aortic stenosis, infiltrative disease, metabolic and endocrine disorders, mitochondrial cardiomyopathies, neuromuscular disorders, malformation syndromes and some chronic drug use". The authors state that the two most frequently mutated genes, which encode sarcomeric proteins, are MYBPC3 and MYH7. Screening for a larger number of genes entails a much greater chance of variants of unknown significance (VUS) being involved in the final report, which may complicate the explanation of the results. Variants responsible for HCM can be found in various genes, but the majority is detected in the genes encoding sarcomeric proteins. In up to 70% of variant-positive patients, either MYBPC3 or MYH7 are identified, while other genes such as TNNI3, TNNT2, TPM1, MYL2, MYL3, and ACTC1 each account for a much smaller proportion of patients (1-5%). The authors state that the main reason behind genetic testing is to identify relatives who are carriers of the pathogenic variant and those who are not. They state that this is a "cost-efficient way that enables the clinicians to determine who should be further screened and continue follow-up and who should be released from further follow-up or clinical testing and evaluation". Furthermore, genetic testing can also be useful to identify nonsarcomeric causes of LVH, which may benefit from etiological treatment.

The 2023 European Society of Cardiology (ESC) issued guidelines for the management of cardiomyopathies. The ESC recommends genetic testing in individuals with cardiomyopathy (known as confirmatory or diagnostic testing): (i) to confirm the diagnosis; (ii) where it may inform prognosis; (iii) where it may inform treatment selection; or (iv) where it may inform their reproductive management (Class of Recommendation [COR]: I; LOE: B). The most common gene (greater than 10% tested cases) considered definitive/strong evidence of HCM include MYBPC3, MYH7, and TNNT2. Common genes (1-10% of tested cases) considered definitive/strong evidence of HCM include MYL2, MYL3, and TNNI3. In families in whom a disease-causing genetic variant has been identified, cascade genetic testing should be offered. However, cascade testing is not indicated when a variant of uncertain significance is identified in the proband. The ESC recommends cascade genetic testing, with pre- and post-test counselling, to adult at-risk relatives if a confident genetic diagnosis (i.e. a pathogenic/likely pathogenic variant) has been established in an individual with cardiomyopathy in the family (starting with first-degree relatives if available, and cascading out sequentially) (COR: I; LOE: B) (Arbelo et al., 2023).

The 2023 ESC guidelines state that "a family history of SCD [sudden cardiac death] is usually considered clinically significant when one or more first-degree relatives have died suddenly aged <40 years with or without a diagnosis of HCM, or when SCD has occurred in a first-degree relative at any age with an established diagnosis of HCM. Family history of SCD does not appear to be an independent risk factor for SCD in childhood HCM. This may be due to a higher prevalence of de novo variants in childhood HCM, the inclusion of non-sarcomeric disease, and/or under-reporting of family history in paediatric cohorts" (Arbelo et al., 2023).

Mornet and Nunes (2016) noted that "hypophosphatasia is characterized by defective mineralization of bone and/or teeth in the presence of low activity of serum and bone alkaline phosphatase. Clinical features range from still-birth without mineralized bone at the severe end to pathologic fractures of the lower extremities in later adulthood at the mild end. Although the disease spectrum is a continuum, 6 clinical forms are usually recognized based on age at diagnosis and severity of features ... Except in prenatal context where genetic diagnosis is essential, hypophosphatasia can be often diagnosed by routine clinical, biochemical, and radiographic means. The diagnosis is confirmed in a proband with identification of bi-allelic pathogenic variants or a heterozygous pathogenic variant in ALPL on molecular genetic testing".

#### Inherited Thrombophilias

Thrombophilia is a disorder of blood coagulation that increases the risk for blood clots (thrombosis) in veins or arteries. Thrombophilia can be acquired or inherited. The most common acquired thrombophilias occur as a result of injury, surgery or a medical condition. The most common hereditary thrombophilias are factor V leiden (FVL), due to a mutation in the F5 gene and prothrombin G20210A, as a result of a mutation in the F2 gene.

Factor V Leiden mutation is the most common hereditary blood coagulation disorder in the United States. It is present in 5 % of the Caucasian population and 1.2 % of the African-American population. Factor V Leiden increases the risk of venous thrombosis 3 to 8 fold for heterozygous individuals and 30 to 140 fold for homozygous individuals. Factor V Leiden mutation has been associated with the following complications:

- Cerebrovascular accident and myocardial infarction
- Deep venous thrombosis
- Gallbladder dysfunction
- Preeclampsia and/or eclampsia
- Pulmonary embolism
- Unexplained miscarriage
- Venous thrombosis.

According to the American College of Medical Genetics, Factor V Leiden genetic testing is indicated in the following patients:

- Age less than 50, any venous thrombosis; or
- Myocardial infarction in female smokers under age 50; or
- Recurrent venous thrombosis; or
- Relatives of individuals with venous thrombosis under age 50; or
- Venous thrombosis and a strong family history of thrombotic disease; or
- Venous thrombosis in pregnant women or women taking oral contraceptives; or
- Venous thrombosis in unusual sites (such as hepatic, mesenteric, and cerebral veins).

The ACMG does not recommend random screening of the general population for factor V Leiden. Routine testing is also not recommended for patients with a personal or family history of arterial thrombotic disorders (e.g., acute coronary syndromes or stroke) except for the special situation of myocardial infarction in young female smokers. According to the ACMG, testing may be worthwhile for young patients (less than 50 years of age) who develop acute arterial thrombosis in the absence of other risk factors for atherosclerotic arterial occlusive disease. The ACMG does not recommend prenatal testing or routine newborn screening for factor V Leiden mutation.

The ACMG does not recommend general screening for factor V Leiden mutation before administration of oral contraceptives. The ACMG recommends targeted testing prior to oral contraceptive use in women with a personal or family history of venous thrombosis.

Factor V Leiden screening of asymptomatic individuals with other recognized environmental risk factors, such as surgery, trauma, paralysis, and malignancy is not necessary or recommended by the ACMG, since all such individuals should receive appropriate medical prophylaxis for thrombosis regardless of carrier status. When Factor V Leiden testing is indicated, the ACMG recommends either direct DNA-based genotyping or factor V Leiden-specific functional assay (e.g., activated protein C (APC) resistance). Patients who test positive by a functional assay should then be further studied with the DNA test for confirmation and to distinguish heterozygotes from homozygotes. According to the ACMG, patients testing positive for factor V Leiden or APC resistance should be considered for molecular genetic testing for prothrombin 20210A, the most common thrombophilia with overlapping phenotype for which testing is easily and readily available. The prothrombin 20210A mutation is the second most common inherited clotting abnormality, occurring in 2 % of the general population. It is only a mild risk factor for thrombosis, but may potentiate other risk factors (such as Factor V Leiden, oral contraceptives, surgery, trauma, etc.).

A factor V gene haplotype (HR2) defined by the R2 polymorphism (A4070G) may confer mild APC resistance and interact with the factor V Leiden mutation to produce a more severe APC resistance phenotype (Bernardi et al, 1997; de Visser et al, 2000; Migozzi et al, 2003). In one study, co-inheritance of the HR2 haplotype increased the risk of venous thromboembolism associated with factor V Leiden by approximately 3-fold (Faioni et al, 1999). However, double heterozygosity for factor V Leiden and the R2 polymorphism was not associated with a significantly higher risk of early or late pregnancy loss than a heterozygous factor V Leiden mutation alone (Zammiti et al, 2006). Whether the HR2 haplotype alone is an independent thrombotic risk factor is still unclear. Several studies have suggested that the HR2 haplotype is associated with a 2-fold increase in risk of venous thromboembolism (Alhenc-Gelas et al, 1999; Jadaon and Dashti, 2005). In contrast, other studies (de Visser 2000; Luddington et al, 2000; Dindagur et al, 2006) found no significant increase in thrombotic risk (GeneTests, University of Washington, Seattle, 2007).

Plasminogen activator inhibitor-1 (PAI-1) is an inhibitor of fibrinolysis, the clot dissolving portion of the coagulation process. PAI-1 is under investigation as a risk factor for conditions such as cardiovascular disease, thrombophilia and pregnancy-related complications. The PAI-1 test is an antibody-based enzyme assay.

#### Interstitial Lung Disease

Devine and Garcia (2012) stated that the interstitial lung diseases (ILDs), or diffuse parenchymal lung diseases, are a heterogeneous collection of more than 100 different pulmonary disorders that affect the tissue and spaces surrounding the alveoli. Patients affected by ILD usually present with shortness of breath or cough; for many, there is evidence of pulmonary restriction, decreased diffusion capacity, and radiographical appearance of alveolar and/or reticulo-nodular infiltrates. The authors reviewed the inherited ILDs, with a focus on the diseases that may be seen by pulmonologists caring for adult patients. They concluded by briefly discussing the utility of genetic testing in this population.

Kitazawa et al (2013) stated that mutations in genes critical for surfactant metabolism, including surfactant protein C (SP-C) and ABCA3, are well-recognized causes of ILD. Recessive mutations in ABCA3 were first attributed to fatal respiratory failure in full-term neonates, but they are also increasingly being recognized as a cause of respiratory disorders with less severe phenotypes in older

children and also adults. These investigators reported the case of a 20-month old boy with ILD caused by 2 distinct ABCA3 mutations. Initial treatment with methylprednisolone was unsuccessful, but the additional administration of hydroxychloroquine was effective. The family history revealed that the patient's older brother had died of idiopathic ILD at 6 months of age, suggesting a genetic etiology of the disease. Sequence analyses of SP-C and ABCA3 genes were performed using DNA samples from the patient himself, his parents, and his brother. These analyses revealed novel compound heterozygous mutations in the coding exons of ABCA3 in both the patient and his brother: c.2741A > G, of paternal origin, and c.3715\_3716insGGGGGG, of maternal origin. The authors concluded that since ABCA3 mutations appeared to be a heterogeneous entity with various phenotypes, they recommended genetic testing for mutations in SP-C and ABCA3 genes to be considered in children with unexplained ILD.

Campo et al (2014) noted that ILD occurring in children is a condition characterized by high frequency of cases due to genetic aberrations of pulmonary surfactant homeostasis, that are also believed to be responsible of a fraction of familial pulmonary fibrosis. To the authors' knowledge, ABCA3 gene was not previously reported as causative agent of fibrosis affecting both children and adults in the same kindred. These researchers investigated a large kindred in which 2 members, a girl whose ILD was first recognized at age of 13, and an adult, showed a diffuse pulmonary fibrosis with marked differences in terms of morphology and imaging. An additional, asymptomatic family member was detected by genetic analysis. Surfactant abnormalities were investigated at biochemical, and genetic level, as well as by cell transfection experiments. Broncho-alveolar lavage fluid analysis of the patients revealed absence of surfactant protein C, whereas the gene sequence was normal. By contrast, sequence of the ABCA3 gene showed a novel homozygous G > A transition at nucleotide 2891, localized within exon 21, resulting in a glycine to aspartic acid change at codon 964. Interestingly, the lung specimens from the girl displayed a morphologic usual interstitial pneumonitis-like pattern, whereas the specimens from 1 of the 2 adult patients showed rather a non-specific interstitial pneumonitis-like pattern. The authors concluded that they had detected a large kindred with a novel ABCA3 mutation likely causing interstitial lung fibrosis affecting either young and adult family members. They suggested that ABCA3 gene should be considered in genetic testing in the occurrence of familial pulmonary fibrosis.

An UpToDate review on "Approach to the adult with interstitial lung disease: Clinical evaluation" (King, 2016a) states that "An autosomal dominant pattern of inheritance has been reported for idiopathic pulmonary fibrosis (IPF), tuberous sclerosis, and neurofibromatosis. In a study of 111 families with 2 or more cases of pulmonary fibrosis, 20 pedigrees demonstrated vertical transmission, consistent with autosomal dominant inheritance, and 45 demonstrated phenotypic heterogeneity. Having ever smoked cigarettes was strongly associated with the development of pulmonary fibrosis, suggesting that an interaction between environmental exposure and gene or gene(s) may contribute to the pathogenesis of this disease". Furthermore, an UpToDate review on "Approach to the adult with interstitial lung disease: Diagnostic testing" (King, 2016b) does not mention genetic testing.

On the other hand, an UpToDate review on "Approach to the infant and child with interstitial lung disease" (Young, 2016) states that "Diagnostic Approach – The overall approach to the diagnostic evaluation is guided by an assessment of the clinical context and disease severity, taking into consideration factors such as the presence of hypoxemia, pulmonary hypertension, failure to thrive, immunocompetence, family history, and trend toward worsening or improvement. Diagnostic studies are used to evaluate for predisposing disorders, to assess the extent and severity of disease, and to identify the primary ILD disease, if possible. The most useful approach to diagnosis is obtaining a history and physical

examination, followed by non-invasive tests and invasive studies if needed. Genetic testing may obviate the need for more invasive studies such as lung biopsy, and thus should be considered early in the evaluation in the appropriate clinical context .... Genetic testing – Analysis of blood can identify the common genetic mutations that cause surfactant dysfunction and thus avoid the need for more invasive evaluations such as lung biopsy. This testing is suggested for infants presenting with acute respiratory failure in the absence of other explanations, or in older children with chronic presentation or family history of ILD, especially if the diagnostic imaging is consistent with ILD ". The authors recommended genetic testing for selected patients (e.g., neonates presenting with respiratory failure, or children with symptoms of ILD and a family history of a similarly affected sibling, or based on radiographic patterns or bronchoscopic findings).

#### Kennedy Disease (Spinal and Bulbar Muscular Atrophy (SBMA))

Kennedy disease, or SBMA, is a rare X-linked slowly progressive neuromuscular condition that affects mainly males and typically occurs in adulthood between the ages of 20 and 50 years old. SBMA is caused by a mutation in the AR (androgen receptor) gene which encodes androgen receptor on the X chromosome. SBMA is characterized by degeneration of lower motor neurons which result in muscle weakness, muscle atrophy, and fasciculations. In addition, affected individuals often show gynecomastia, testicular atrophy, and reduced fertility as a result of mild androgen insensitivity. Kennedy disease affects fewer than 1 in 350,000 males. Males who inherit this condition will be affected; however, females that inherit it will be carriers and will typically not be affected because they are protected by their low levels of circulating testosterone (NORD, 2018; Spada, 2017).

Diagnosis of SBMA can be confirmed by molecular genetic testing on a blood sample for CAG trinucleotide repeat expansion in the AR gene. Individuals with greater than 36 CAG trinucleotide repeats in the AR gene are diagnosed with the condition (NORD, 2018; Spada, 2017).

Treatment is symptomatic and supportive, and life expectancy is normal; however, approximately 10% succumb to the disease in their 60's or 70's due to aspiration pneumonia or asphyxiation resulting from the bulbar weakness (NORD, 2018).

#### Lactose Intolerance

Lactase-phlorizin hydrolase, which hydrolyzes lactose, the major carbohydrate in milk, plays a critical role in the nutrition of the mammalian neonate (Montgomery et al, 1991). Lactose intolerance in adult humans is common, usually due to low levels of small intestinal lactase. Low lactase levels result from either intestinal injury or (in the majority of the world's adult population) alterations in the genetic expression of lactase. Although the mechanism of decreased lactase levels has been the subject of intensive investigation, no consensus has yet emerged.

The LactoTYPE Test (Prometheus Laboratories) is a blood test that is intended to identify patients with genetic-based lactose intolerance. According to the manufacturer, this test provides a more definitive diagnosis and scientific explanation for patients with persistent symptoms.

There is insufficient evidence that the assessment of the genetic etiology of lactose intolerance would affect the management of patients such that clinical outcomes are improved. Current guidelines on the management of lactose intolerance do not indicate that genetic testing is indicated (NHS, 2005; National Public Health Service for Wales, 2005).

#### Left Ventricle Noncompaction

The Heart Failure Society of America (HFSA) issued 2018 practice guidelines on genetic evaluation of cardiomyopathy. Hershberger and colleagues state that the left ventricular noncompaction (LVNC) phenotype may be observed in conjunction with all other cardiomyopathy phenotypes, so considerations related to genetic testing should always be directed by findings of a cardiomyopathy (or other cardiovascular) phenotype. Genetic testing is not recommended when the LVNC phenotype is identified by chance in asymptomatic individuals with otherwise normal cardiovascular structure and function.

An UpToDate review on "Isolated left ventricular noncompaction in adults: Management and prognosis" (Attenhofer-Jost and Connolly, 2022) state that "given the limited specificity of criteria for LVNC, the 2018 Heart Failure Society of America (HFSA) guideline on genetic evaluation of cardiomyopathy limited application of its LVNC recommendations to only those with the most prominent disease". Thus, the authors recommend genetic testing in patients with left ventricular noncompaction (LVNC), especially in children and those with a family history of cardiomyopathy". The most common gene mutation in LVNC include MYH7, MYBPC3, and TTN.

#### Legius Syndrome

Per GeneReviews (Legius and Stevenson, 2020), Legius syndrome, also known as neurofibromatosis type 1-like syndrome, is characterized by multiple café au lait macules without neurofibromas or other tumor manifestations of neurofibromatosis type 1 (NF1). Additional clinical manifestations reported commonly include intertriginous freckling, lipomas, macrocephaly, and learning disabilities / attention-deficit/hyperactivity disorder (ADHD) / developmental delays. Legius syndrome is inherited in an autosomal dominant manner. The diagnosis of Legius syndrome is established in a proband with suggestive findings and a heterozygous pathogenic variant in SPRED1 identified by molecular genetic testing.

The diagnostic criteria for mosaic NF1 (signs are limited to a particular area of the body) are met if any of the following is present (Legius et al, 2021):

- A pathogenic heterozygous NF1 variant with a variant allele fraction of significantly less than 50% in apparently normal tissue such as white blood cells and one other NF1 diagnostic criterion (except a parent fulfilling diagnostic criteria for NF1);
- An identical pathogenic heterozygous NF1 variant in two anatomically independent affected tissues (in the absence of a pathogenic NF1 variant in unaffected tissue);
- A clearly segmental distribution of café au lait macules or cutaneous neurofibromas and
  - another NF1 diagnostic criterion (except a parent fulfilling diagnostic criteria for NF1); or
  - child fulfilling diagnostic criteria for NF1);
- Only one NF1 diagnostic criterion from the following list: freckling in the axillary and inguinal region, optic pathway glioma, two or more Lisch nodules or two or more choroidal abnormalities, distinctive osseous lesion typical for NF1, two or more neurofibromas or one plexiform neurofibroma and a child fulfilling the criteria for NF1.

The diagnostic criteria for mosaic Legius syndrome are met if any of the following is present (Legius et al, 2021):

- A heterozygous pathogenic SPRED1 variant allele fraction of significantly less than 50% in apparently normal tissue such as white blood cells and six or more café au lait macules;
- An identical pathogenic heterozygous SPRED1 variant in two independent affected tissues (in the absence of a pathogenic SPRED1 variant in unaffected tissue);
- A clearly segmental distribution of café au lait macules and a child fulfilling the criteria

for Legius syndrome.

In an UpToDate review of "Neurofibromatosis type 1 (NF1): Pathogenesis, clinical features, and diagnosis", Korf et al (2024) state that the clinical features of Legius syndrome include a subset of those of neurofibromatosis type 1 (NF1), such as multiple café-au-lait macules, axillary freckling, and macrocephaly. However, the syndrome lacks neurofibromas and central nervous system (CNS) tumors. Thus, genetic testing can be performed to confirm diagnosis in questionable cases and help direct screening of family members. If the NF1 mutation testing is negative, then the diagnostic laboratory may perform sprouty-related EVH1 domain-containing 1 (SPRED1) mutation testing to evaluate for Legius syndrome. A positive genetic test may shorten the period of diagnostic uncertainty.

#### Long QT Syndrome

Long QT Syndrome (LQTS) is a disorder of the heart's electrical system that predisposes individuals to irregular heartbeats, fainting spells and sudden death. The irregular heartbeats are typically brought on by stress or vigorous activity. There are multiple genetic forms of LQTS including Anderson-Tawil syndrome, Jervell and Lange-Nielsen syndrome, Romano-Ward syndrome and Timothy syndrome.

Voltage-gated sodium channels are transmembrane proteins that produce the ionic current responsible for the rising phase of the cardiac action potential and play an important role in the initiation, propagation, and maintenance of normal cardiac rhythm. Inherited mutations in the sodium channel alpha-subunit gene (SCN5A), the gene encoding the pore-forming subunit of the cardiac sodium channel, have been associated with distinct cardiac rhythm syndromes such as the congenital long QT3 syndrome (LQT3), Brugada syndrome, isolated conduction disease, sudden unexpected nocturnal death syndrome (SUNDS), and sudden infant death syndrome (SIDS). Electrophysiological characterization of heterologously expressed mutant sodium channels have revealed gating defects that, in many cases, can explain the distinct phenotype associated with the rhythm disorder.

The long QT syndrome (LQTS) is a familial disease characterized by an abnormally prolonged QT interval and, usually, by stress-mediated life-threatening ventricular arrhythmias (Priori et al, 2001). Characteristically, the first clinical manifestations of LQTS tend to appear during childhood or in teenagers. Two variants of LQTS have been described: a rare recessive form with congenital deafness (Jervell and Lange-Nielsen syndrome, J-LN), and a more frequent autosomal dominant form (Romano-Ward syndrome, RW). Five genes encoding subunits of cardiac ion channels have been associated to LQTS and genotype-phenotype correlation has been identified. Of the 5 genetic variants of LQTS currently identified, LQT1 and LQT2 subtypes involve 2 genes, KCNQ1 and HERG, which encode major potassium currents. LQT3 involves SCN5A, the gene encoding the cardiac sodium current. LQT5 and LQT6 are rare subtypes also involving the major potassium currents.

The principal diagnostic and phenotypic hallmark of LQTS is abnormal prolongation of ventricular repolarization, measured as lengthening of the QT interval on the 12-lead ECG (Maron et al, 1998). This is usually most easily identified in lead II or V1, V3, or V5, but all 12 leads should be examined and the longest QT interval used; care should also be taken to exclude the U wave from the QT measurement.

LQT3 appears to be the most malignant variant and may be the one less effectively managed by beta blockers. LQT1 and LQT2 have a higher frequency of syncopal events but their lethality is lower and the protection afforded by beta-blockers, particularly in LQT1, is much higher. The Jervell and Lange-Nielsen recessive variant is associated with very early clinical manifestations and a poorer prognosis

than the Romano-Ward autosomal dominant form. The presence of syndactyly seems to represent a different genetic variant of LQTS also associated with a poor prognosis.

Guidelines on sudden cardiac death from the European College of Cardiology (Priori et al, 2001) state that identification of specific genetic variants of LQTS are useful in risk stratification. The clinical variants presenting association of the cardiac phenotype with syndactyly or with deafness (Jervell and Lange-Nielsen syndrome) have a more severe prognosis. Genetic defects on the cardiac sodium channel gene (SCN5A) are also associated with higher risk of sudden cardiac death. In addition, identification of specific genetic variants may help in suggesting behavioral changes likely to reduce risk. LQT1 patients are at very high risk during exercise, particularly swimming. LQT2 patients are quite sensitive to loud noises, especially when they are asleep or resting.

#### Common External Causes of Prolongation of QTc Interval

- Bradycardia
- Heart disease (heart failure, ischemia)
- Hypocalcemia
- Hypomagnesemia
- Hypokalemia
- Hypothyroidism
- Antiarrhythmic medications (quinidine, procainamide, amiodarone, sotalol, and dofetilide)
- Tricyclic and tetracyclic antidepressants (e.g., amitriptyline)
- Erythromycin
- Cisapride
- Pimozide
- Thioridazine

Genetic testing for long QT syndrome has not been evaluated in patients who present with a borderline QT interval, suspicious symptoms (e.g., syncope), and no relevant family history (Roden, 2008). In these patients, the incidence of false positive and false negative results and their implications for management remain unknown.

Genetic testing may also be necessary in person with long QT syndrome in sudden death close relatives.

An UpToDate review on "Congenital long QT syndrome: Diagnosis" (Schwartz and Ackerman, 2019) state that diagnostic strategy includes "careful evaluation of the QTc, exclusion of secondary causes of QT prolongation, ambulatory ECG monitoring (with 12-lead ECG if at all possible), exercise testing, and calculation of the LQTS diagnostic score (the "Schwartz score")". Calculating the QTc on the ECG is the most useful diagnostic and prognostic parameter for LQTS. In addition, the authors assess for exercise-associated arrhythmias, changes in T wave morphology, and the presence of maladaptive QT response during the recovery phase after exercise. The response of QTc during exercise recovery may be helpful in identifying LQTS in asymptomatic relatives of individuals with LQTS, as well as in identifying probands. Findings of QTc at rest are highly probable of LQTS when  $\geq 470$  milliseconds is seen in males or  $\geq 480$  milliseconds in females with personal or family history suspicious for LQTS. The LQTS diagnostic score (Schwartz score) may be a useful tool in evaluating individuals with suspected LQTS; however, it is not adequate for screening of family members of affected individuals. The authors provide the following recommendations for genetic testing:

- Patients with a high clinical suspicion of congenital LQTS based on history, family history, ECG findings, and results of any additional testing, such as a high Schwartz

score  $\geq 3.5$  (Class I recommendation).

- Patients with an intermediate clinical suspicion of congenital LQTS based on history, family history, ECG findings, and results of any additional testing, such as an intermediate Schwartz score of 1.5 to 3 (Class II recommendation).
- Asymptomatic patients without a family history of congenital LQTS but who have serial ECGs with QTc  $\geq 480$  milliseconds before puberty or  $\geq 500$  milliseconds post-puberty (Class I recommendation).
- Asymptomatic patients without a family history of congenital LQTS but who have serial ECGs with QTc  $\geq 460$  milliseconds before puberty or  $\geq 480$  milliseconds post-puberty (Class II recommendation).
- Cascade/variant-specific testing of all appropriate relatives when the disease-causative variant has been identified in the proband (Class I recommendation).

The EHRA/HRS/APHRS/LAQRS issued a 2022 consensus which provides the following recommendations supported by "strong observational evidence and authors' consensus" (Wilde et al., 2022):

- Molecular genetic testing for definitive disease associated genes (currently KCNQ1, KCNH2, SCN5A, CALM1, CALM2, and CALM3) should be offered to all index patients with a high probability diagnosis of LQTS, based on examination of the patient's clinical history, family history, and ECG characteristics obtained at baseline, during ECG Holter recording and exercise stress test (Schwartz Score  $\geq 3.5$ )
- Analysis of specific genes should be offered to patients with a specific diagnosis as follows: KCNQ1 and KCNE1 in patients with Jervell and Lange-Nielsen syndrome, CACNA1C in Timothy syndrome, KCNJ2 in Andersen-Tawil syndrome, and TRDN in patients suspected to have triadin knockout syndrome
- Variant-specific genetic testing is recommended for family members and appropriate relatives following the identification of the disease-causing variant.

"In patients with an intermediate probability of LQTS (e.g. prolonged QTc with a Schwartz score 1.5–3.0), testing of genes with limited, disputed and refuted evidence should not be performed, while testing of the established genes may be considered, mostly to help rule out the diagnosis after extensive phenotypic investigation" (Wilde et al., 2022).

Romano-Ward syndrome (RWS) is a form of long QT syndrome that are typically associated with mutations in the KCNQ1, KCNH2, and SCN5A genes. The genes CALM1, CALM2 and TRDN have also undisputable evidence as LQTS-causative genes in RWS (Orphanet, 2023).

The European Society of Cardiology (ESC) issued their 2022 guidelines for managing patients with ventricular arrhythmias and prevention of sudden cardiac death (SCD). The authors state that undisputed genes that are known to cause LQT1, LQT2 and LQTS3 include KCNQ1, KCNH2 and SCN5A, respectively, with gene-specific triggers being exercise (LQTS1), emotional stress (LQTS2) and sleep (LQTS3). Genetic screening identifies a mutation in 75% of LQTS cases and three main genes account for 90% of positively genotyped cases. The authors also identified subtypes of LQTS, such as Andersen-Tawil syndrome, Timothy syndrome and Jervell and Lange-Nielsen syndrome. The ESC panel recommends that LQTS is diagnosed with either QTc  $\geq 480$  ms in repeated 12-lead ECGs with or without symptoms or LQTS diagnostic score greater than 3 (COR: I; LOE: C). In patients with clinically diagnosed LQTS, genetic testing and genetic counselling are recommended (COR: I; LOE: C). In the presence of arrhythmic syncope or cardiac arrest, the panel agrees that a QTc  $\geq 460$  ms is sufficient to consider a diagnosis of LQTS (Zeppenfeld et al., 2022).

Wappler (2010) stated that malignant hyperthermia (MH)-susceptible patients have an increased risk during anesthesia. The aim of this review was to present current knowledge about pathophysiology and triggers of MH as well as concepts for safe anesthesiological management of these patients. Trigger substances and mechanisms have been well-defined to date. Anesthesia can be safely performed with i.v. anesthetics, nitrous oxide, non-depolarizing muscle relaxants, local anesthetics as well as xenon. Attention must be directed to the preparation of the anesthetic machine because modern work-stations need longer cleansing times than their predecessors. Alternatively, activated charcoal might be beneficial for elimination of volatile anesthetics. Day case surgery can be performed in MH-susceptible patients, if all safety aspects are regarded. Whether there is an association between MH susceptibility and other disorders is still a matter of debate. The authors concluded that the incidence of MH is low, but the prevalence can be estimated as up to 1: 3,000. Because MH is potentially lethal, it is relevant to establish management concepts for peri-operative care in susceptible patients. This includes pre-operative genetic and in-vitro muscle contracture test (IVCT), preparation of the anesthetic work-station, use of non-triggering anesthetics, adequate monitoring, availability of sufficient quantities of dantrolene and appropriate post-operative care. Taking these items into account, anesthesia can be safely performed in susceptible patients.

Moreover, an UpToDate review on "Susceptibility to malignant hyperthermia" (Litman, 2011) states that "the contracture test is performed at specific centers around the world (four in the United States). Following testing, the referring physician receives a report indicating whether testing was positive, negative, or equivocal. Positive or equivocal results should be followed-up with genetic testing. Referral information can be found on the Malignant Hyperthermia Association of the United States (MHAUS) website". Genetic testing for MH is indicated in the following groups: (i) patients with a positive or equivocal contracture test to determine the presence of a specific mutation, (ii) individuals with a positive genetic test for MH in a family member, and (iii) patients with a clinical history suspicious for MH (acute MH episode, masseter muscle rigidity, post-operative myoglobinuria, heat or exercise induce rhabdomyolysis) who are unable or unwilling to undergo contracture testing.

#### Malignant Hyperthermia Susceptibility and Central Core Disease

Central core disease (CCD) also known as central core myopathy and Shy-Magee syndrome, is an inherited neuromuscular disorder characterized by central cores on muscle biopsy and clinical features of a congenital myopathy. Prevalence is unknown but the condition is probably more common than other congenital myopathies. CCD typically presents in infancy with hypotonia and motor developmental delay and is characterized by predominantly proximal weakness pronounced in the hip girdle; orthopedic complications are common and malignant hyperthermia susceptibility (MHS) is a frequent complication.

Malignant hyperthermia (MH) or malignant hyperpyrexia is a rare but severe pharmacogenetic disorder that occurs when patients undergoing anesthesia experience a hyperthermic reaction when exposed to certain anesthetic agents. Anesthetic agents that may trigger MH are desflurane, enflurane, halothane, isoflurane, sevoflurane, and suxamethonium chloride. MH usually occurs in the operating theater, but can occur at anytime during anesthesia and up to an hour after discontinuation.

CCD and MHS are allelic conditions both due to (predominantly dominant) mutations in the skeletal muscle ryanodine receptor (RYR1) gene, encoding the principal skeletal muscle sarcoplasmic reticulum calcium release channel (RyR1). Altered excitability and/or changes in calcium homeostasis within muscle cells due to mutation-induced conformational changes of the RyR protein are considered the main pathogenetic mechanism(s).

The diagnosis of CCD is based on the presence of suggestive clinical features and central cores on muscle biopsy; muscle MRI may show a characteristic pattern of selective muscle involvement and aid the diagnosis in cases with equivocal histopathological findings. Mutational analysis of the RYR1 gene may provide genetic confirmation of the diagnosis. Further evaluation of the underlying molecular mechanisms may provide the basis for future rational pharmacological treatment.

The reference standard test for establishing a clinical diagnosis of MHS is the caffeine halothane contracture test (CHCT) in the United States, and the in vitro contracture test (IVCT) in Europe and Australasia. The CHCT and IVCT are similar and measure the muscle contracture in the presence of the anesthetic halothane and caffeine. Both tests categorize patients as being MHS, MH equivocal (MHE), or MH negative (MHN). These tests are invasive and must be performed using a skeletal muscle biopsy that is less than 5 hours old. Sequence variants in the ryanodine receptor 1 (skeletal) (RYR1) gene have been shown to be associated with MH susceptibility (MHS) and are found in up to 80% of patients with confirmed MH, usually with an autosomal dominant pattern of inheritance. Although additional genetic loci have been associated with MH, the contribution of these other loci to MH is low.

Genetic testing for RYR1 sequence variants from commercial providers is performed by polymerase chain reaction (PCR) followed by direct sequencing. Genetic tests for RYR1 sequence variants can be performed to either identify sequence variants in genetic hot spots of the RYR1 gene that cover all exons on which causative MH variants can be found, or for screening of sequence variants across the entire 106 exons of the RYR1 gene.

Examples of commercially available tests are: Malignant Hyperthermia/Central Core Disease (570-572) RYR1 Sequencing (Prevention Genetics); Malignant hyperthermia (RYR1 gene sequenced analysis, partial) (University of Pittsburgh Medical Center, Division of Molecular Diagnostics [UPMC Molecular Diagnostics]).

#### Malignant Melanoma

An estimated 8 to 12 % of persons with melanoma have a family history of the disease, but not all of these individuals have hereditary melanoma (Tsao and Haluska, 2007). In some cases, the apparent familial inheritance pattern may be due to clustering of sporadic cases in families with common heavy sun exposure and susceptible skin type.

Approximately 10 percent of melanomas are familial. 62 CDKN2A/p16 (also known as MTS1, INK4, MLM, p16INK4A) (eg, MELARIS) is the major gene associated with melanoma. A subset of CDKN2A mutations carrier families also displays an increased risk of pancreatic cancer; however, at this time, detecting a CDKN2A mutation does not affect the clinical management of an affected patient or at-risk family members. Other genes commonly associated with hereditary pancreatic cancer include BRCA2 and PALB2 (eg, PANEXIA). Regardless of the results of genetic testing, close dermatologic surveillance is recommended for individuals at risk for familial melanoma based on family history, and the efficacy of screening for pancreatic cancer is uncertain.

A melanoma susceptibility locus has been identified on chromosome 9p21; this has been designated CDKN2A (also known as MTS1 (multiple tumor suppressor 1)) (Tsao and Haluska, 2007). There is a variable rate of CDKN2A mutations in patients with hereditary melanoma. The risk of CDKN2A mutation varies from approximately 10 % for families with at least 2 relatives having melanoma, to more than 40 % for families having multiple affected 1st degree relatives spanning several generations.

Persons at increased risk of melanoma are managed with close clinical surveillance and education in risk-reduction behavior (e.g., sun avoidance, sunscreen use). It is unclear how CDKN2A genetic test information would alter clinical recommendations (Tsao and Haluska, 2007). The negative predictive value of a negative test for a CDKN2A mutation is also not established since many familial cases occur in the absence of CDKN2A mutations. It is estimated that the prevalence of CDKN2A mutation carriers is less than 1 % in high incidence populations. Thus, no mutations will be identifiable in the majority of families presenting to clinical geneticists.

The American Society of Clinical Oncology (ASCO) has issued a consensus report on the utility of genetic testing for cancer susceptibility (ASCO, 1996), and recommendations for the process of genetic testing were updated in 2003 (ASCO, 2003). The report notes that the sensitivity and specificity of the commercially available test for CDKN2A mutations are not fully known. Because of the difficulties with interpretation of the genetic tests, and because test results do not alter patient or family member management, ASCO recommends that CDKN2A testing be performed only in the context of a clinical trial.

The Scottish Intercollegiate Guidelines Network (SIGN, 2003) protocols on management of cutaneous melanoma reached similar conclusions, stating that "[g]enetic testing in familial or sporadic melanoma is not appropriate in a routine clinical setting and should only be undertaken in the context of appropriate research studies."

The Melanoma Genetics Consortium recommends that genetic testing for melanoma susceptibility should not be offered outside of a research setting (Kefford et al, 2002). They state that "[u]ntil further data become available, however, clinical evaluation of risk remains the gold standard for preventing melanoma. First-degree relatives of individuals at high risk should be engaged in the same programmes of melanoma prevention and surveillance irrespective of the results of any genetic testing."

#### Maturity Onset Diabetes of the Young (MODY)

In a review on "Personalized medicine in diabetes mellitus", Kleinberger and Pollin (2015) stated that "... there are some 40 genes implicated in the complex etiology of type 1 diabetes, with currently unknown practical clinical implications ... MODY3 is the most common form of MODY, comprising 52 % of cases in the well-characterized United Kingdom, though prevalence varies by ethnicity and geographic region. It is caused by a mutation in HNF1A, which encodes the transcription factor hepatic nuclear factor 1- $\alpha$  (HNF1- $\alpha$ ), which promotes transcription of multiple genes related to glucose metabolism, insulin secretion, and insulin production. HNF1- $\alpha$  has 55 % amino acid similarity with hepatic nuclear factor 4- $\alpha$  (HNF4- $\alpha$ ), which is mutated in MODY1. MODY1 makes up about 10 % of MODY cases in the United Kingdom ... The most well-established treatment changes that can result from a genetic diagnosis are high-dose sulfonylureas rather than insulin for KCNJ11/ABCC8-related diabetes (usually neonatal), low-dose sulfonylureas rather than insulin (especially at early stages) for MODY1 (HNF4A) and MODY3 (HNF1A), and no treatment for MODY2 (GCK)".

Furthermore, an UpToDate review on "Classification of diabetes mellitus and genetic diabetic syndromes" (McCulloch, 2019) notes the glucokinase gene (GCK) (frequency 15 % to 31 %), hepatic nuclear factor 1- $\alpha$  (HNF1- $\alpha$ ) (frequency 52 % to 65 %), and hepatic nuclear factor 4- $\alpha$  (HNF4- $\alpha$ ) (frequency less than 10 %) as commonly identified gene mutations in MODY.

Strasser et al (2012) stated that Alport syndrome (ATS) is a type-IV collagen inherited disorder, caused by mutations in COL4A3 and COL4A4 (autosomal recessive) or COL4A5 (X-linked). Clinical symptoms include progressive renal disease, eye abnormalities and high-tone sensori-neural deafness. A renal histology very similar to ATS is observed in a subset of patients affected by mutations in MYH9, encoding non-muscle-myosin Type Ila – a cytoskeletal contractile protein. MYH9-associated disorders (May-Hegglin anomaly, Epstein and Fechtner syndrome, and others) are inherited in an autosomal dominant manner and characterized by defects in different organs (including eyes, ears, kidneys and thrombocytes). These researchers described here a 6-year old girl with hematuria, proteinuria, and early sensori-neural hearing loss. The father of the patient is affected by ATS, the mother by isolated inner ear deafness. Genetic testing revealed a pathogenic mutation in COL4A5 (c.2605G>A) in the girl and her father and a heterozygous mutation in MYH9 (c.4952T>G) in the girl and her mother. The paternal COL4A5 mutation seems to account for the complete phenotype of ATS in the father and the maternal mutation in MYH9 for the inner ear deafness in the mother. It has been discussed that the interaction of both mutations could be responsible for both the unexpected severity of ATS symptoms and the very early onset of inner ear deafness in the girl.

An UpToDate review on “Congenital and acquired disorders of platelet function” (Coutre, 2013) states that “Giant platelet disorders – Inherited platelet disorders with giant platelets are quite rare (picture 2 and algorithm 1 and table 4). These include platelet glycoprotein abnormalities (e.g., Bernard-Soulier syndrome), deficiency of platelet alpha granules (e.g., gray platelet syndrome), the May-Hegglin anomaly, which also involves the presence of abnormal neutrophil inclusions (i.e., Dohle-like bodies), and some kindreds with type 2B von Willebrand disease (the Montreal platelet syndrome)”. This review does not mention the use of genetic testing as a management tool for giant platelet disorders.

UpToDate reviews on “Inborn errors of metabolism: Epidemiology, pathogenesis, and clinical features” (Sutton, 2013a) and “Inborn errors of metabolism: Classification” (Sutton, 2013b) do not mention the use of genetic testing as a management tool.

#### Menkes Disease

Kaler (2010) stated that for children with low serum copper (0 to 55 µg/dL) and low serum ceruloplasmin (10 to 160 mg/L) concentrations, direct sequence analysis of the ATP7A coding region and flanking intron sequences detects about 80 % of mutations, and deletion/duplication analysis can be used to detect deletion of an ATP7A exon, multiple exons, or the whole gene in about 15 % of affected patients.

#### Migrainous Vertigo

Migrainous vertigo is a term used to describe episodic vertigo in patients with a history of migraines or with other clinical features of migraine. Approximately 20 to 33 % of migraine patients experience episodic vertigo. The underlying cause of migrainous vertigo is not very well understood. There are no confirmatory diagnostic tests or susceptible genes associated with migrainous vertigo. Other conditions, specifically Meniere's disease and structural and vascular brainstem disease, must be excluded (Black, 2006).

#### Mitochondrial Genome Sequencing

Mitochondria are tiny organelles housed in nearly every cell in the body and are responsible for creating cellular energy. Mitochondrial disorders are chronic, genetic conditions that can be inherited and occur when mitochondria fail to produce sufficient energy for the body to function. Mitochondrial disorders can be caused by mutations in DNA (nuclear DNA) or in DNA contained in the mitochondria (mitochondrial DNA [mtDNA]).

Mitochondrial diseases are a clinically heterogeneous group of disorders that arise as a result of dysfunction of the mitochondrial respiratory chain (Chinnery, 2014). They can be caused by mutation of genes encoded by either nuclear DNA or mitochondrial DNA (mtDNA). Mitochondrial disorders may be caused by mutation of an mtDNA gene or mutation of a nuclear gene. Mitochondrial DNA variants are transmitted by maternal inheritance (mitochondrial inheritance). Nuclear gene variants may be inherited in an autosomal recessive, autosomal dominant, or X-linked manner.

Mitochondrial disorders can occur at any age. Symptoms can involve one or more organs with varying degrees of severity. Some individuals display features that fall into distinct syndromes such as chronic progressive external ophthalmoplegia (CPEO), Kearns-Sayre syndrome (KSS), Leber hereditary optic neuropathy (LHON), Leigh syndrome (LS), mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS), myoclonic epilepsy with ragged-red fibers (MERRF) and neurogenic weakness with ataxia and retinitis pigmentosa (NARP). Individuals may also present with overlapping range of disease, such as mitochondrial recessive atrophy syndrome (MIRAS). Often affected individuals do not fit into a specific category. Common symptoms include ataxia, cardiomyopathy, diabetes mellitus, exercise intolerance, external ophthalmoplegia, fluctuating encephalopathy, myopathy, optic atrophy, pigmentary retinopathy, ptosis, seizures, sensorineural deafness and spasticity. Treatment of mitochondrial disorders is largely supportive.

Molecular genetic testing may be carried out on genomic DNA extracted from blood (suspected nuclear DNA mutations and some mtDNA mutations) or on genomic DNA extracted from muscle (suspected mtDNA mutations) (Chinnery, 2014). Studies for mtDNA mutations are usually carried out on skeletal muscle DNA because a pathogenic mtDNA variant may not be detected in DNA extracted from blood.

Genetic testing panels have been proposed to aid in the diagnosis of individuals with suspected mitochondrial disorders and may involve point mutations analysis, Approaches to molecular genetic testing of a proband to consider are serial testing of single genes, deletion/duplication analysis, multi-gene panel testing (simultaneous testing of multiple genes), and genomic testing (e.g., sequencing the entire mitochondrial genome; whole-exome sequencing or whole-genome sequencing to identify mutation of a nuclear gene) (Chinnery, 2014) (eg, Combined Mito Genome Plus Mito Nuclear Gene Panel, Comprehensive Mitochondrial Nuclear Gene Panel, MitoMet Plus aCGH Analysis).

In contrast to genomic testing, serial testing of single genes and multi-gene panel testing rely on the clinician developing a hypothesis about which specific gene or set of genes to test (Chinnery, 2014). Hypotheses may be based on (i) mode of inheritance, (ii) distinguishing clinical features, and/or (iii) other discriminating features. The potential role of genomic testing is where single-gene testing (and/or use of a multi-gene panel) has not confirmed a diagnosis in an individual with features of a mitochondrial disorder. Such testing includes whole-exome sequencing, whole-genome sequencing, and whole mitochondrial sequencing. False negative rates vary by genomic region; therefore, genomic testing may not be as accurate as targeted single gene testing or multi-gene molecular genetic testing panels (Chinnery, 2014). Most laboratories confirm positive results using a second, well-established method. Certain DNA variants may not be detectable through genomic testing, such as large deletions or duplications (>8-10 bp in length), triplet repeat expansions, and epigenetic alterations.

Exome sequencing has shown promise in defining the genetic basis of mitochondrial disorders caused by mutation of nuclear genes. To determine the molecular basis of multiple respiratory chain complex deficiencies, Taylor, et al. (2014) studied 53 patients referred to 2 national centers in the United Kingdom and Germany between 2005 and 2012. All subjects had evidence of histochemical and/or

biochemical diagnosis of mitochondrial disease in a clinically affected tissue (skeletal muscle, liver, or heart) confirming decreased activities of multiple respiratory chain complexes based on published criteria. Subjects had no large-scale mtDNA rearrangements, mtDNA depletion, and mtDNA point mutations, in persons in whom decreased levels of mtDNA were confirmed in muscle (mtDNA depletion). In those with congenital structural abnormalities, major nuclear gene rearrangements were excluded by comparative genomic hybridization arrays.

Whole-exome sequencing was performed using 62-Mb exome enrichment, followed by variant prioritization using bioinformatics prediction tools, variant validation by Sanger sequencing, and segregation of the variant with the disease phenotype in the family. Presumptive causal variants were identified in 28 patients (53%; 95% CI, 39%-67%) and possible causal variants were identified in 4 (8%; 95% CI, 2%-18%). Together these accounted for 32 patients (60% 95% CI, 46%-74%) and involved 18 different genes. These included recurrent mutations in RMND1, AARS2, and MTO1, each on a haplotype background consistent with a shared founder allele, and potential novel mutations in 4 possible mitochondrial disease genes (VARS2, GARS, FLAD1, and PTCD1). Distinguishing clinical features included deafness and renal involvement associated with RMND1 and cardiomyopathy with AARS2 and MTO1. However, atypical clinical features were present in some patients, including normal liver function and Leigh syndrome (subacute necrotizing encephalomyopathy) seen in association with TRMU mutations and no cardiomyopathy with founder SCO2 mutations. It was not possible to confidently identify the underlying genetic basis in 21 patients (40%; 95% CI, 26%-54%). The authors stated that additional study is required in independent patient populations to determine the utility of this approach in comparison with traditional diagnostic methods.

#### Mitochondrial Recessive Ataxia Syndrome

Lee et al (2007) stated that spino-cerebellar ataxia (SCA) is a heterogeneous group of neurodegenerative disorders with common features of adult-onset cerebellar ataxia. Many patients with clinically suspected SCA are subsequently diagnosed with common SCA gene mutations. Previous reports suggested some common mitochondrial DNA (mtDNA) point mutations and mitochondrial DNA polymerase gene (POLG1) mutations might be additional underlying genetic causes of cerebellar ataxia. These researchers tested whether mtDNA point mutations A3243G, A8344G, T8993G, and T8993C, or POLG1 mutations W748S and A467T are found in patients with adult-onset ataxia who did not have common SCA mutations. A total of 476 unrelated patients with suspected SCA underwent genetic testing for SCA 1, 2, 3, 6, 7, 8, 10, 12, 17, and DRPLA gene mutations. After excluding these SCA mutations and patients with paternal transmission history, 265 patients were tested for mtDNA mutations A3243G, A8344G, T8993G, T8993C, and POLG1 W748S and A467T mutations. No mtDNA A3243G, A8344G, T8993G, T8993C, or POLG1 W748S and A467T mutation was detected in any of the 265 ataxia patients, suggesting that the upper limit of the 95 % confidence interval (CI) for the prevalence of these mitochondrial mutations in Chinese patients with adult-onset non-SCA ataxia is no higher than 1.1 %. The authors concluded that the mtDNA mutations A3243G, A8344G, T8993G, T8993C, or POLG1 W748S and A467T are very rare causes of adult-onset ataxia in Taiwan. Routine screening for these mutations in ataxia patients with Chinese origin is of limited clinical value.

Gramstad et al (2009) noted that mutations in the catalytic subunit of polymerase gamma (POLG1) produce a wide variety of neurological disorders including a progressive ataxic syndrome with epilepsy: mitochondrial SCA and epilepsy (MSCAE). The authors' earlier studies of patients with this syndrome raised the possibility of more prominent right than left hemisphere dysfunction. To investigate this in more detail, 8 patients (6 women, 2 men; mean age of 22.3 years) were studied. All completed an intelligence test (Wechsler Adult Intelligence Scale; WAIS), and 4 were also given memory tests and a comprehensive neuropsychological test battery. Patients with MSCAE showed significant cognitive

dysfunction. Mean Verbal IQ (84.3) was significantly better than Performance IQ (71.8) ( $t = 5.23$ ,  $p = 0.001$ ), but memory testing and neuropsychological testing failed to detect a consistent unilateral dysfunction. The authors concluded that further studies are needed to define the profile and development of cognitive symptoms in this disorder.

Isohanni et al (2011) stated that mitochondrial DNA polymerase  $\gamma$  (POLG1) mutations in children often manifest as Alpers syndrome, whereas in adults, a common manifestation is mitochondrial recessive ataxia syndrome (MIRAS) with severe epilepsy. Because some patients with MIRAS have presented with ataxia or epilepsy already in childhood, these investigators searched for POLG1 mutations in neurologic manifestations in childhood. They investigated POLG1 in 136 children, all clinically suspected to have mitochondrial disease, with one or more of the following: ataxia, axonal neuropathy, severe epilepsy without known epilepsy syndrome, epileptic encephalopathy, encephalohepatopathy, or neuropathologically verified Alpers syndrome. A total of 7 patients had POLG1 mutations, and all of them had severe encephalopathy with intractable epilepsy. Four patients had died after exposure to sodium valproate. Brain MRI showed parieto-occipital or thalamic hyper-intense lesions, white matter abnormality, and atrophy. Muscle histology and mitochondrial biochemistry results were normal in all. The authors concluded that POLG1 analysis should belong to the first-line DNA diagnostic tests for children with an encephalitis-like presentation evolving into epileptic encephalopathy with liver involvement (Alpers syndrome), even if brain MRI and morphology, respiratory chain activities, and the amount of mitochondrial DNA in the skeletal muscle are normal. POLG1 analysis should precede valproate therapy in pediatric patients with a typical phenotype. However, POLG1 is not a common cause of isolated epilepsy or ataxia in childhood.

Tang et al (2012) determined the prevalence of MNGIE-like phenotype in patients with recessive POLG1 mutations. Mutations in the POLG1 gene, which encodes for the catalytic subunit of the mitochondrial DNA polymerase gamma essential for mitochondrial DNA replication, cause a wide spectrum of mitochondrial disorders. Common phenotypes associated with POLG1 mutations include Alpers syndrome, ataxia-neuropathy syndrome, and progressive external ophthalmoplegia (PEO). Mitochondrial neuro-gastro-intestinal encephalomyopathy (MNGIE) is an autosomal recessive disorder characterized by severe gastrointestinal dysmotility, cachexia, PEO and/or ptosis, peripheral neuropathy, and leukoencephalopathy. MNGIE is caused by TYMP mutations. Rare cases of MNGIE-like phenotype have been linked to RRM2B mutations. Recently, POLG1 mutations were identified in a family with clinical features of MNGIE but no leukoencephalopathy. The coding regions and exon-intron boundaries of POLG1 were sequence analyzed in patients suspected of POLG1-related disorders. Clinical features of 92 unrelated patients with 2 pathogenic POLG1 alleles were carefully reviewed. Three patients, accounting for 3.3 % of all patients with 2 pathogenic POLG1 mutations, were found to have clinical features consistent with MNGIE but no leukoencephalopathy. Patient 1 carries p.W748S and p.R953C; patient 2 is homozygous for p.W748S, and patient 3 is homozygous for p.A467T. In addition, patient 2 has a similarly affected sibling with the same POLG1 genotype. POLG1 mutations may cause MNGIE-like syndrome, but the lack of leukoencephalopathy and the normal plasma thymidine favor POLG1 mutations as responsible molecular defect.

Furthermore, UpToDate reviews on "Overview of the hereditary ataxias" (Opal and Zoghbi, 2013a) and "The spinocerebellar ataxias" (Opal and Zoghbi, 2013b) do not mention the use of POLG1 genetic testing.

Karam and colleagues (2019) noted that performing DNA genetic testing (DGT) for hereditary cancer genes is now a well-accepted clinical practice; however, the interpretation of DNA variation remains a challenge for laboratories and clinicians.

Adding RNA genetic testing (RGT) enhances DGT by clarifying the clinical actionability of hereditary cancer gene variants, thus improving clinicians' ability to accurately apply strategies for cancer risk reduction and treatment. These researchers examined if RGT is associated with improvement in the diagnostic outcome of DGT and in the delivery of personalized cancer risk management for patients with hereditary cancer predisposition. Diagnostic study in which patients and/or families with inconclusive variants detected by DGT in genes associated with hereditary breast and ovarian cancer, Lynch syndrome, and hereditary diffuse gastric cancer sent blood samples for RGT from March 2016 to April 2018.

Clinicians who ordered genetic testing and received a re-classification report for these variants were surveyed to examine if RGT-related variant re-classifications changed clinical management of these patients. To quantify the potential number of tested individuals who could benefit from RGT, a cohort of 307,812 patients who underwent DGT for hereditary cancer were separately queried to identify variants predicted to affect splicing. Data analysis was conducted from March 2016 and September 2018. Main outcome measures included variant re-classification outcomes following RGT, clinical management changes associated with RGT-related variant re-classifications, and the proportion of patients who would likely be affected by a concurrent DGT and RGT multi-gene panel testing approach. In total, 93 of 909 eligible families (10.2 %) submitted samples for RGT. Evidence from RGT clarified the interpretation of 49 of 56 inconclusive cases (88 %) studied; 26 (47 %) were re-classified as clinically actionable and 23 (41 %) were clarified as benign.

Variant re-classifications based on RGT results changed clinical management recommendations for 8 of 18 patients (44 %) and 14 of 18 families (78 %), based on responses from 18 of 45 clinicians (40 %) surveyed. A total of 7,265 of 307,812 patients who underwent DGT had likely pathogenic variants or variants of uncertain significance potentially affecting splicing, indicating that approximately 1 in 43 (2.3 %) individuals could benefit from RGT. The authors concluded that in this diagnostic study, conducting RNA testing resolved a substantial proportion of variants of uncertain significance in a cohort of individuals previously tested for cancer predisposition by DGT. Performing RGT might change the diagnostic outcome of at least 1 in 43 (2.3 %) patients if performed in all individuals undergoing genetic evaluation for hereditary cancer.

The authors stated that this study had several drawbacks. First, the selection of variants in this study was limited by patient availability to submit an additional blood sample for RGT. Therefore, studies designed to include RGT concurrently with DNA analysis are needed to fully assess the impact of RGT if available to all patients. Medical management data were also limited because of survey participation; however, based on current practice guidelines, these investigators would expect results to remain consistent across the remaining cases. Results from this study may have also under-estimated the clinical impact of variant re-classification, which extend well beyond the index family. More than 400 patients received amended reports as a result of re-classifications occurring in this study alone. Because these variants will continue to be observed in future patients tested, these re-classifications have a down-stream impact as well. Further, as part of routine data sharing with ClinVar, these results would be expected to also affect patients with the same variants identified through other clinical laboratories, also not quantified in this study. It is clear that RGT contributes to a decrease in VUS rates; however, there are substantial limitations to performing RGT after DGT has been conducted. The process of re-contacting patients retrospectively places a significant time burden on clinicians and laboratory staff. In addition, RGT requires an additional blood sample, which can be logistically challenging to obtain after the fact. As shown in this study, samples were received for only 10 % of index cases recruited for RGT. Performing RGT after DGT also imposed an inherent delay in the re-classification of VUS, which limited the clinical utility of the DGT results. Based

on the frequency of splicing alterations in this multi-gene DGT cohort, these researchers expected that RGT results could potentially lead to variant re-classifications for at least 1 of 43 (2.3 %) patients undergoing genetic testing. As a comparator, gross deletion and/or duplication analysis, which is routinely included as a component of DGT, identified pathogenic variants in 1 of 142 patients tested. Thus, if RGT were routinely performed alongside DGT, the potential consequences would exceed that of gross deletion and/or duplication analysis. This estimation also under-estimates the overall number of individuals, since RGT may also detect intronic splicing variants outside the analytical range of clinical DGT that are currently not accounted for. It will be important to evaluate the use of concurrent DGT and RGT in a prospective cohort to assess the ability of RNA analysis to decrease VUS rates and increase the identification of clinically actionable variants in an un-biased fashion.

#### MTHFR Genetic Testing for Risk Assessment of Hereditary Thrombophilia

Variations in the MTHFR gene have been studied as risk factors for numerous conditions, including cardiovascular disease, thrombophilia, stroke, hypertension and pregnancy-related complications; however, its role remains unclear.

Coriu and colleagues (2014) noted that pregnancy is a normal physiological state that predisposes to thrombosis, determined by hormonal changes in the body. These changes occur in the blood flow (venous stasis), changes in the vascular wall (hypotonia, endothelial lesion) and changes in the coagulation factors (increased levels of factor VII, factor VIII, factor X, von Willebrand factor) and decreased activity levels of natural anti-coagulants (protein C, protein S). In this retrospective study, these researchers examined a possible association between thrombosis and inherited thrombophilia in pregnant women. A total of 151 pregnant women with a history of complicated pregnancy: maternal thrombosis and placental vascular pathology (intra-uterine growth restriction, pre-eclampsia, recurrent pregnancy loss), who were admitted in the authors' hospital during the period January 2010 to July 2014 were included in this study. These investigators performed genetic analyses to detect the factor V Leiden mutation, the G20210A mutation in the prothrombin gene, the C677T mutation and the A1298C mutation in methylenetetrahydrofolate reductase (MTHFR) gene. The risk of thrombosis in patients with factor V Leiden is 2.66 times higher than the patients negative for this mutation (odds ratio [OR] 2.66 95 % CI: 0.96 to 7.37 p = 0.059). The authors did not find any statistical association with mutations in the MTHFR gene. Pregnant women with a family history of thrombosis presented a 2.18-fold higher risk of thrombosis (OR 2.18, CI: 0.9 to 5.26 p = 0.085). Of 151 pregnant women, thrombotic events occurred in 24 patients: deep vein thrombosis, pulmonary embolism, cerebral venous sinus thrombosis and ischemic stroke. The occurrence of thrombotic events was identified in the last trimester of pregnancy, but especially post-partum.

An UpToDate review on "Screening for inherited thrombophilia in asymptomatic individuals" (Bauer, 2015) states that "Homocysteine and the MTHFR variant – There is no clinical rationale for measurement of fasting plasma homocysteine levels or for assaying for presence of the MTHFR 677C→T variant in asymptomatic individuals, and we never order this testing to evaluate venous or arterial thrombosis".

An UpToDate review on "Screening for inherited thrombophilia in children" (Raffini, 2015) states that "The strength of the association between each IT and the development of VTE varies, and they are often classified into "weak" or "strong" risk factors. Although there are numerous other inherited defects that have been described, none have gained widespread acceptance. Testing for polymorphisms in

the methylene tetrahydrofolate reductase (MTHFR) gene is not indicated, because these extremely common polymorphisms do not frequently cause hyperhomocysteinemia, and they are not, by themselves, associated with VTE".

Furthermore, the American College of Medical Genetics and Genomics (2015) noted the following:

"Don't order MTHFR genetic testing for the risk assessment of hereditary thrombophilia. The common MTHFR gene variants, 677C>T and 1298A>G, are prevalent in the general population. Recent meta-analyses have disproven an association between the presence of these variants and venous thromboembolism".

#### MTHFR Testing for Essential Hypertension

Natekar et al (2014) provided an updated review on current genetic aspects possibly affecting essential hypertension (EH), and further elucidated their role in EH. These investigators searched for genetic and epigenetic factors in major studies associated with EH between Jan 2008 to Oct 2013 using PubMed. They limited their search to reviews that discussed mostly human studies, and were accessible through the university online resource. They found 11 genome wide association studies (GWAS), as well as 5 methylation and 3 miRNA studies that fit the search criteria. A distinction was not made between genes with protective effects or negative effects, as this article was only meant to be a summary of genes associated with any aspect of EH. These researchers found 130 genes from the studies that met the inclusion/exclusion criteria. Of note, genes with multiple study references included: STK39, CYP17A1, methylenetetrahydrofolate reductase (MTHFR)-NPPA, MTHFR-NPPB, ATP2B1, CSK, ZNF652, UMOD, CACNB2, PLEKHA7, SH2B3, TBX3-TBX5, ULK4, CSK-ULK3, CYP1A2, NT5C2, CYP171A, PLCD3, SH2B3, ATXN2, CACNB2, PLEKHA7, SH2B3, TBX3-TBX5, ULK4, and HFE. The following genes overlapped between the genetic studies and epigenetic studies: WNK4 and BDKRB2. Several of the identified genes were found to have functions associated with EH. Many epigenetic factors were also correlated with EH. Of the epigenetic factors, there were no articles discussing siRNA and its effects on EH that met the search criteria, thus the topic was not included in this review. Among the miRNA targets found to be associated with EH, many of the genes involved were also identified in the GWAS studies. The authors concluded that genetic hypertension risk algorithms could be developed in the future but may be of limited benefit due to the multi-factorial nature of EH. With emerging technologies, like next-generation sequencing, more direct causal relationships between genetic and epigenetic factors affecting EH will likely be discovered creating a tremendous potential for personalized medicine using pharmacogenomics.

Yang et al (2014) stated that the MTHFR C677T gene polymorphism has been suggested to be associated with the risk of EH, however, results remain inconclusive. To investigate this association, the present meta-analysis of 27 studies including 5,418 cases and 4,997 controls was performed. The pooled odds ratio (OR) and its corresponding 95 % confidence interval (CI) were calculated using the random-effects model. A significant association between the MTHFR C677T gene polymorphism and EH was found under the allelic (OR, 1.32; 95 % CI: 1.20 to 1.45; p = 0.000), dominant (OR, 1.39; 95 % CI: 1.25 to 1.55; p = 0.000), recessive (OR, 1.38; 95 % CI: 1.18 to 1.62; p = 0.000), homozygote (OR, 1.59; 95 % CI: 1.32 to 1.92; p = 0.000), and heterozygote (OR, 1.32; 95 % CI: 1.20 to 1.45; p = 0.000) genetic models. A strong association was also revealed in subgroups, including Asian, Caucasian and Chinese. The Japanese subgroup did not show any significant association under all models. Meta-regression analyses suggested that the study design was a potential source of heterogeneity, whereas the subgroup analysis additionally indicated that the population origin may also be an explanation. Another subgroup analysis revealed that hospital-based studies have a stronger association than population-based studies, however, the former suffered a greater

heterogeneity. Funnel plot and Egger's test manifested no evidence of publication bias. The authors concluded that the present study supported the evidence for the association between the MTHFR C677T gene polymorphism and EH in the whole population, as well as in subgroups, such as Asian, Caucasian and Chinese. The carriers of the 677T allele are susceptible to EH.

Wu et al (2014) stated that many studies have investigated the role of 5,10- MTHFR C677T/A1298C polymorphisms in EH, but results are inconclusive. The purpose of this meta-analysis was to clarify the effects of MTHFR C677T/A1298C polymorphisms on the risk of EH. Electronic databases were searched to identify relevant studies published until January 2014. Data were extracted by 2 independent authors; ORs with 95 % CIs were used to assess the association between MTHFR C677T/A1298C polymorphisms and the risk of EH using random effect models or fixed effect models. A total of 30 studies with 5,207 cases and 5,383 controls were included for C677T polymorphism and 6 studies with 1,009 cases and 994 controls were included for A1298C polymorphism. Meta-analysis results indicated that MTHFR C677T polymorphism contributed to an increased risk of EH (for T versus C: OR = 1.30, 95 % CI: 1.18 to 1.43; for TT+CT versus CC: OR = 1.34, 95 % CI: 1.24 to 1.46; for TT versus CC: OR = 1.62, 95 % CI: 1.32 to 1.99; for TT versus CT+CC: OR = 1.41, 95 % CI: 1.26 to 1.59). However, no significant association was detected between MTHFR A1298C polymorphism and the risk of EH. The authors concluded that the findings of this meta-analysis supported that MTHFR C677T polymorphism plays a role in developing EH. MTHFR A1298C polymorphism may not be associated with an increased risk of EH. Moreover, they stated that further large and well-designed studies are needed to confirm these findings.

Perez-Razo et al (2015) stated that MTHFR have been associated with diastolic blood pressure, hypertension, and other cardiovascular diseases; however, results of these studies are still controversial. In this study, these researchers examined if 2 functional variants (rs1801133 and rs13306560) within the MTHFR are associated with hypertension in Mexican-Mestizos. They performed a case-control study with 1,214 subjects including adults and children to test for the association of both single nucleotide polymorphisms with essential hypertension. The adult group included 764 participants (372 patients and 391 controls) and the group of children included 418 participants (209 patients and 209 controls). rs13306560 was associated with essential hypertension in adults (OR, 4.281; 95 % CI: 1.841 to 9.955; p = 0.0003) with a statistical power greater than 0.8. In children, none of the polymorphisms was associated with EH. In addition, these investigators assessed the effect of the rs13306560 polymorphism on the MTHFR promoter region by means of luciferase reporter gene assays using human umbilical vein endothelial cells. Cells transfected with the pMTHFRaLUC construct showed an approximately 25 % reduction in luciferase activity (p = 0.003). Furthermore, the promoter activity was reduced considerably by in-vitro methylation of CpG sequences. The authors concluded that these findings suggested that the rs13306560 polymorphism of the MTHFR may be part of the observed hypertension process in Mexican-Mestizo populations, but further studies are needed.

Amrani-Midoun et al (2016) stated that many studies have investigated the role of 5,10- MTHFR C677T gene polymorphism in EH, but with conflicting results. These researchers determined the eventual association between 5,10- MTHFR C677T gene polymorphism and hypertension in a sample of Algerian population from the Oran city. A case-control study has been performed in 154 subjects including 82 hypertensives defined as subjects with elevated systolic blood pressure (SBP) greater than or equal to 140 mmHg and or sustained diastolic blood pressure (DBP) greater than or equal to 90mmHg, and 72 normotensive subjects. Polymerase chain reaction (PCR) combined with restrictive fragment length polymorphism (RFLP) was used to detect the MTHFR C677T variant. The authors observed no significant differences between allelic and genotypic frequencies between cases

and controls for C677T polymorphism (OR = 1.51, 95 % CI: 0.89 to 2.56, p = 0.13). Analyses adjusted for age, sex and body mass index improved the association level, though the association was still not significant (30 % versus 22 %, OR = 1.75, 95 % CI: 0.95 to 3.24, p = 0.07). The authors concluded that the findings of this study showed that genetic polymorphism related to the MTHFR gene (C677T) is not associated with the risk of hypertension in this sample of Algerian population. Moreover, they stated that larger case-control samples are needed to clearly assess the role of this genetic variant in EH.

#### Muscular Dystrophy

Genetic testing may be used to analyze DNA to detect gene mutations to assist in diagnosing a genetic disorder in individuals who exhibit disease signs and symptoms. It may also be used to determine if an asymptomatic individual may be at risk for developing a genetic disorder since an individual's risk might be higher if genes are inherited that cause or increase susceptibility to a disorder. Genetic testing for disease risk, also referred to as predictive, presymptomatic or predispositional genetic testing, may be offered to asymptomatic individuals with a family history of the genetic disorder or if a disease-causing, or pathogenic, mutation has been identified in an affected relative. Testing may be offered for conditions such as Duchenne muscular dystrophy (DMD), Becker muscular dystrophy (BMD), myotonic dystrophy or spinal muscular atrophy (SMA).

Becker Muscular Dystrophy (BMD) is an inherited disorder that involves slowly progressing muscle weakness of the legs and pelvis. BMD is similar to DMD, though it is less common and progresses at a slower rate.

Duchenne Muscular Dystrophy (DMD) is the most severe form of muscular dystrophy, DMD usually affects young boys and causes progressive muscle weakness, usually beginning in the legs.

Facioscapulohumeral Muscular Dystrophy (FSHD) is a disorder characterized by muscle weakness and atrophy. The areas of the body most often affected include the muscles of the face, shoulder blades and upper arms.

Myotonic Dystrophy is an inherited neuromuscular disorder characterized by progressive muscle weakness and wasting.

#### Myelodysplastic Syndromes

National Comprehensive Cancer Network's clinical practice guideline on "Myelodysplastic syndromes" (2014) stated that further evaluations are necessary to establish the role of these genetic lesions on risk stratification systems in myelodysplastic syndrome. The guidelines stated that mutations in TET2 are among the most common mutations reported in patients with myelodysplastic syndromes (about 20 % of cases). Mutations in SF3B1 are one of several common molecular abnormalities involving the RNA splicing machinery, occurring in 14.5 to 16.0 % of MDS cases.

#### MYH-Associated Polyposis

MYH is a DNA repair gene that corrects DNA base pair mismatch errors in the genetic code before replication. Mutation of the MYH gene may result in colon cancer. In this regard, the MYH gene has been found to be significantly involved in colon cancer, both in cases where there is a clear family history of the disease, as well as in cases without any sign of a hereditary cause.

The National Comprehensive Cancer Network (NCCN)'s practice guidelines on colorectal cancer screening (2006) recommended testing for MYH mutations for individuals with personal history of adenomatous polyposis (more than 10

adenomas, or more than 15 cumulative adenomas in 10 years) either consistent with recessive inheritance or with adenomatous polyposis with negative adenomatous polyposis coli (APC) mutation testing. The guideline noted that when polyposis is present in a single person with negative family history, de novo APC mutation should be tested; if negative, testing for MYH should follow. When family history is positive only for a sibling, recessive inheritance should be considered and MYH testing should be done first. In a polyposis family with clear autosomal dominant inheritance, and absence of APC mutation, MYH testing is unlikely to be informative. Members in such family are treated according to the polyposis phenotype, including classical or attenuated FAP.

#### MyPhenome Hungry Gut

MyPhenome Hungry Gut (Phenomix Sciences) is a saliva test that uses machine learning to predict how an individual will respond to different weight loss interventions based on their obesity phenotype.

MyPhenome Hungry Gut is a physician-ordered test that is purported to evaluate a patient's response to GLP-1 medications (e.g., Wegovy, Ozempic [Novo Nordisk]) by "looking for close to 6,000 changes across 22 genes involved in the brain-to-gut signaling pathway", which signals when to stop/start eating. Machine learning algorithms assign a score after this analysis as either "Hungry Gut positive" or "Hungry Gut negative", meaning that the hormone response is either normal or abnormal (Hollowell, 2024).

Acosta et al (2022) conducted a pragmatic trial to evaluate weight loss outcomes from selection of antiobesity medications (AOM) based on obesity phenotypes. Participants were recruited from the community by standard advertising, and all tests were performed at the Mayo Clinic Clinical Research Trials Unit after an 8-hour fasting period. The trial included 450 obese participants whose body composition, resting energy expenditure (REE), satiety, satiation, eating behavior, affect, and physical activity were measured by "validated" studies and questionnaires. These variables were used to classify obesity phenotypes. The four obesity-related phenotypes are defined as: (i) "hungry brain", characterized by excessive calories consumed to terminate meal; (ii) "emotional hunger", characterized by negative mood, emotional eating, cravings, and reward-seeking behaviors, despite having normal homeostatic eating behavior; (iii) "hungry gut", characterized by reduced duration of fullness, quantified objectively by rapid gastric emptying; and (iv) "slow burn", characterized by reduced REE, reduced reported physical activity and exercise, and with lower muscle mass. The prevalence of each phenotype was hungry brain 32% (n = 143/450), emotional hunger 21% (n = 96/450), hungry gut 32% (n = 144/450), slow burn 21% (n = 82/400), and no identified phenotype 15% (n = 68/450). Of those, 312 were randomly assigned to phenotype-guided treatment or non-phenotype-guided treatment with AOM: phentermine, phentermine/topiramate, bupropion/naltrexone, lorcaserin, and liraglutide. The primary outcome was the percentage of total body weight loss at 12 months. The authors reported the outcomes of 84 patients who received phenotype-guided treatment compared with 228 patients who received standard of care weight loss treatment with medications not based on phenotype characterization. Inclusion criteria were: (i) patients with  $BMI \geq 27 \text{ kg/m}^2$  with adiposity-related comorbidities or patients with  $BMI \geq 30 \text{ kg/m}^2$  with or without adiposity-related comorbidities; (ii) patients prescribed FDA-approved AOMs; (iii) follow-up of at least 3 months; and (iv) 2 or more face-to-face visits with one of the physicians at the Mayo Clinic Weight Management Program. The authors excluded all patients who (i) had prior major gastrointestinal surgery, (ii) had prior endoscopic weight loss intervention, (iii) did not fill the medication prescription, and (iv) were taking FDA-approved AOMs prior to the first visit. All the information was collected from physician's documentation, including outcomes and adverse events. The authors found that the 4 phenotypes of obesity were identified in 383 of 450 participants (85%). In 15% of participants,

no phenotype was identified. Two or more phenotypes were identified in 27% of patients. Further, the phenotype-guided approach was associated with 1.75-fold greater weight loss after 12 months with mean weight loss of 15.9% compared with 9.0% in the non-phenotype-guided group (difference -6.9% [95% CI -9.4% to -4.5%],  $P < 0.001$ ), and the proportion of patients who lost more than 10% at 12 months was 79% in the phenotype-guided group compared with 34% with non-phenotype-guided treatment group. Although actionable phenotypes of obesity were identified, the authors acknowledged that the outcomes of their study require replication and validation in larger, more racially and metabolically diverse cohorts, preferably in multicenter, randomized studies.

Cifuentes and colleagues (2023) conducted a single-center, non-randomized, proof-of-concept study to compare the outcome of a standard lifestyle intervention (SLI) to phenotype-tailored lifestyle interventions (PLI) on weight loss, cardiometabolic risk factors and physiologic variables contributing to obesity. The 12-week trial included men and women aged 18-65 years with a body mass index (BMI) greater than 30 without history of any bariatric procedure and current use of any medication known to affect weight. Participants lived anywhere in the United States, and underwent in-person testing in Rochester, MN at a teaching hospital. All participants completed in-person phenotype testing at baseline and after 12 weeks. Participants were assigned to an intervention based on their period of enrollment. In the first phase, participants were assigned to SLI with a low-calorie diet (LCD), moderate physical activity, and weekly behavioral therapy sessions. In the second phase, other participants were assigned to PLI according to phenotype: abnormal satiation (time-restricted volumetric LCD); abnormal postprandial satiety (LCD with pre-meal protein supplementation); emotional eating (LCD with intensive behavioral therapy); and abnormal resting energy expenditure (LCD with post-workout protein supplementation and high-intensity interval training). The primary outcome was total body weight loss in kg at 12 weeks using multiple imputation for missing data. Linear models estimated the association of study group allocation and study endpoints adjusting for age, sex, and baseline weight. Between July 2020 and August 2021, 211 participants were screened, and 165 were assigned to one of the two treatments in the two phases: 81 SLI (mean [SD] age 42.9 [12] years; 79% women; BMI 38.0 [6.0]) and 84 PLI (age 44.8 [12.2] years; 83% women; BMI 38.7 [6.9]); 146 completed the 12-week programs. The weight loss was -7.4 kg (95%CI, -8.8, -6.0) with PLI vs. -4.3 kg (95%CI, -5.8, -2.7) with SLI (difference, -3.1 kg [95%CI, -5.1 to -1.1];  $P = 0.004$ ). No adverse events were reported in any group. The authors concluded that phenotype-tailored lifestyle interventions may result in significant weight loss; however, a randomized controlled trial is required to confirm causality.

#### Next-Generation Sequencing for the Diagnosis of Learning Disabilities in Children

Beale and colleagues (2015) stated that learning disability (LD) is a serious and lifelong condition characterized by the impairment of cognitive and adaptive skills. Some cases of LD with unidentified causes may be linked to genetic factors. Next-generation sequencing (NGS) techniques are new approaches to genetic testing that are expected to increase diagnostic yield. These investigators described current pathways that involve the use of genetic testing; collected stakeholder views on the changes in service provision that would need to be put in place before NGS could be used in clinical practice; described the new systems and safeguards that would need to be put in place before NGS could be used in clinical practice; and explored the cost-effectiveness of using NGS compared with conventional genetic testing. A research advisory group was established. This group provided ongoing support by e-mail and telephone through the lifetime of the study and also contributed face-to-face through a workshop. A detailed review of published studies and reports was undertaken. In addition, information was collected through 33 semi-structured interviews with key stakeholders. Next-generation sequencing techniques consist of targeted gene sequencing, whole-exome sequencing (WES)

and whole-genome sequencing (WGS). Targeted gene panels, which are the least complex, are in their infancy in clinical settings. Some interviewees thought that during the next 3 to 5 years targeted gene panels would be superseded by WES. If NGS technologies were to be fully introduced into clinical practice in the future a number of factors would need to be overcome. The main resource-related issues pertaining to service provision are the need for additional computing capacity, more bioinformaticians, more genetic counsellors and also genetics-related training for the public and a wide range of staff. It is also considered that, as the number of children undergoing genetic testing increases, there will be an increase in demand for information and support for families. The main issues relating to systems and safeguards are giving informed consent, sharing unanticipated findings, developing ethical and other frameworks, equity of access, data protection, data storage and data sharing. There is little published evidence on the cost-effectiveness of NGS technologies. The major barriers to determining cost-effectiveness are the uncertainty around diagnostic yield, the heterogeneity of diagnostic pathways and the lack of information on the impact of a diagnosis on health care, social care, educational support needs and the wider family. Furthermore, as NGS techniques are currently being used only in research, costs and benefits to the National Health Services (NHS) are unclear. The authors concluded that NGS technologies are at an early stage of development and it is too soon to say whether they can offer value for money to the NHS as part of the LD diagnostic process. They stated that substantial organizational changes, as well as new systems and safeguards, would be needed if NGS technologies were to be introduced into NHS clinical practice; and considerable further research is needed to establish whether using NGS technologies to diagnose learning disabilities is clinically effective and cost-effective.

#### Noonan Syndrome

Noonan is diagnosed on clinical grounds by observation of key features.

#### Oculopharyngeal Muscular Dystrophy (OPMD)

Oculopharyngeal muscular dystrophy (OPMD) is a rare genetic muscle disorder that is characterized by slowly progressive myopathy which include ptosis and swallowing difficulties. The onset usually occurs during adulthood between 40 and 60 years old, with the mean age of onset of ptosis around 48 years old, and dysphagia, 50 years old. Swallowing difficulties determine prognosis, and increase the risk for potentially life-threatening aspiration pneumonia and poor nutrition. Other signs/symptoms include tongue weakness, proximal lower and upper extremity weakness, pooling of saliva, facial muscle weakness, and limitation of upward gaze. Some individuals with severe involvement may eventually need a wheelchair (NORD, 2012; Trollet et al., 2014).

OPMD is inherited in either an autosomal dominant or an autosomal recessive manner. Diagnosis is suspected based on clinical evaluation, detailed patient history and characteristic findings. Diagnosis is confirmed by molecular genetics testing of PABPN1. Confirmatory diagnosis depends on detection of an expansion of a GCN trinucleotide repeat in the first exon of PABPN1. Normal alleles contain ten GCN trinucleotide repeats. Autosomal dominant alleles range in size from 12 to 17 GCN repeats; autosomal recessive alleles comprise 11 GCN repeats. Muscle biopsy is warranted only in individuals with suspected OPMD who have two normal PABPN1 alleles (Trollet et al., 2014).

The treatment of OPMD is directed toward the specific symptoms that are apparent in each individual. Ptosis may be treated cautiously with plastic surgery on the eyelids (blepharoptosis repair). In cases severe dysphagia, a surgical procedure known as cricopharyngeal myotomy may be used. Orthopedic devices such as canes, leg braces, or walkers can assist individuals who have difficulty walking. Other treatment is symptomatic and supportive (NORD, 2012; Trollet et al., 2014).

Optical genome mapping, a DNA-based method, entails analysis of high molecular weight genomic DNA by means of Augusta/Bionano Optical Genome Mapping technologies from blood, frozen tissue and cell lines. This diagnostic test evaluates the entire nuclear genome. Report contains annotated structural variants believed to be associated with patient's diseases/disorders (e.g., acute lymphoblastic leukemia, autism spectrum disorder, developmental delay, dysmorphic features, facioscapulohumeral muscular dystrophy, fragile X and other congenital anomalies, intellectual disability, multiple congenital anomalies, and solid tumors).

Goldrich et al (2021) noted that genomic structural variants comprise a significant fraction of somatic mutations driving cancer onset and progression; however, such variants are not readily revealed by standard NGS. Optical genome mapping (OGM) surpasses short-read sequencing in detecting large (greater than 500 bp) and complex structural variants (SVs) but requires isolation of ultra-high-molecular-weight DNA from the tissue of interest. These researchers have successfully applied a protocol involving a paramagnetic nano-bind disc to a wide range of solid tumors. Using as little as 6.5 mg of input tumor tissue, these investigators showed successful extraction of high-molecular-weight genomic DNA that provided a high genomic map rate and effective coverage by optical mapping. They demonstrated the system's utility in identifying somatic SVs affecting functional and cancer-related genes for each sample. Duplicate/triplicate analysis of select samples showed intra-sample reliability but also intra-sample heterogeneity. These researchers also showed that simply filtering SVs based on a GRCh38 human control database provided high positive and negative predictive values (PPVs and NPVs) for true somatic variants. The authors concluded that the findings of this study indicated that the solid tissue DNA extraction protocol, OGM and SV analysis could be applied to a wide variety of solid tumors to capture SVs across the entire genome with functional importance in cancer prognosis and treatment.

The authors stated that the ability to isolate DNA from up to 8 simultaneous samples using the current protocol amplified throughput and reduced tissue-to-data processing time, increasing both laboratory convenience as well as expanding potential for clinical utility where rapid data turnaround is paramount. Furthermore, the strong inter-sample SV correspondence demonstrated by most tissue types in duplicate/triplicate sample analysis showed the reproducibility of this technique; intra-sample heterogeneity of select samples may be attributed to non-tumor normal tissue within some tissue fragments, or attributed to specific cancer subtype, and merits further investigation. These researchers stated that although the isolation protocol described in this study afforded many advantages, there are some limitations to this protocol. While high-quality DNA isolation and OGM SV analysis was obtained for a wide variety of tumor types that were tested, it may not be generalizable to every additional untested solid tumor type. These investigators stated that future directions include continuing to validate this protocol in additional tissue types, and evaluating additional tumor samples to examine broader trends in the role of specific OGM-identified SVs in individual cancer subtypes.

Cope et al (2021) noted that currently available SV detection methods do not span the complete spectrum of disease-causing SVs. In this study, OGM, an emerging technology with the potential to resolve diagnostic dilemmas, was carried out to examine clinically-relevant SVs in a 4-year-old boy with an epileptic encephalopathy of undiagnosed molecular origin. OGM was employed to image long, megabase-size DNA molecules, fluorescently labeled at specific sequence motifs throughout the genome with high sensitivity for detection of SVs greater than 500 bp in size. OGM results were confirmed in a CLIA-certified laboratory via mate-pair sequencing. OGM identified a mosaic, de-novo 90 kb deletion and inversion on the X chromosome disrupting the CDKL5 gene. Detection of the mosaic deletion, which had been previously undetected by chromosomal microarray, an infantile epilepsy

panel including exon-level microarray for CDKL5, exome sequencing as well as genome sequencing, resulted in a diagnosis of X-linked dominant early infantile epileptic encephalopathy-2. The authors concluded that OGM afforded an effective technology for the detection of SVs, especially those that were mosaic, since these remain difficult to detect with current NGS technologies and with conventional chromosomal microarrays. Moreover, these researchers stated that while OGM is an emerging technology that requires further validation before wide-spread use in the clinical realm, it has the potential to be a valuable diagnostic tool. The development of OGM represents a new opportunity to resolve undiagnosed diseases and further research into the use of OGM in undiagnosed populations is needed.

Lestringant et al (2021) stated that acute lymphoblastic leukemias (ALL) are characterized by a large number of cytogenetic abnormalities of clinical interest that require the use of several complementary techniques; OGM is based on analysis of ultra-high molecular weight DNA molecules that provides a high-resolution genome-wide analysis highlighting copy number and structural anomalies, including balanced translocations. These researchers compared OGM to standard techniques (karyotyping, FISH, single nucleotide polymorphism (SNP)-array and reverse transcription multiplex ligation-dependent probe amplification) in 10 selected B-ALL or T-ALL. A total of 80 abnormalities were found using standard techniques of which 72 (90 %) were correctly detected using OGM; 8 discrepancies were identified, while 12 additional anomalies were found by OGM. Among the discrepancies, 4 were detected in raw data but not retained because of filtering issues. However, 4 were truly missed, either because of a low variant allele frequency or because of a low coverage of some regions. Of the additional anomalies revealed by OGM, 7 were confirmed by another technique, some of which were recurrent in ALL such as LMO2-TRA and MYC-TRB fusions. Despite false positive anomalies due to background noise and a case of inter-sample contamination secondarily identified, the OGM technology was relatively simple to use with little practice. The authors concluded that OGM represents a promising alternative to cytogenetic techniques currently carried out for ALL characterization. It enables a timely and cost-effective analysis allowing identification of complex cytogenetic events, including those currently inaccessible to standard techniques.

Mantere et al (2021) noted that chromosomal aberrations including SVs are a major cause of human genetic diseases. Their detection in clinical routine still relies on standard cytogenetics. Limitations of these tests are a very low resolution (karyotyping) and the inability to detect balanced SVs or indicate the genomic localization and orientation of duplicated segments or insertions (copy number variant [CNV] microarrays). These investigators examined the ability of OGM to detect known constitutional chromosomal aberrations. Ultra-high-molecular-weight DNA was isolated from 85 blood or cultured cells and processed via OGM. A de-novo genome assembly was carried out followed by SV and CNV calling and annotation, and results were compared to known aberrations from standard-of-care (SOC) tests (karyotype, FISH, and/or CNV microarray). In total, these researchers analyzed 99 chromosomal aberrations, including 7 aneuploidies, 19 deletions, 20 duplications, 34 translocations, 6 inversions, 2 insertions, six isochromosomes, one ring chromosome, and four complex rearrangements. Several of these variants encompass complex regions of the human genome involved in repeat-mediated microdeletion/microduplication syndromes. High-resolution OGM reached 100 % concordance compared to standard assays for all aberrations with non-centromeric breakpoints. This proof-of-principle study demonstrated the ability of OGM to detect nearly all types of chromosomal aberrations. They also suggested suited filtering strategies to prioritize clinically relevant aberrations and discussed future improvements. The authors concluded that these findings highlighted the potential for OGM to provide a cost-effective and easy-to-use alternative that would allow comprehensive detection of chromosomal aberrations and SVs, which could give rise to an era of "next-generation cytogenetics".

Luhmann et al (2021) noted that acute lymphoblastic leukemia (ALL) is the most prevalent type of cancer occurring in children; it is characterized by structural and numeric genomic aberrations that strongly correlate with prognosis and clinical outcome. Usually, a combination of cyto- and molecular genetic methods (karyotyping, array-CGH, FISH, RT-PCR, RNA-Seq) is needed to identify all aberrations relevant for risk stratification. These investigators examined the feasibility of OGM to detect these aberrations in an all-in-one approach. As proof of principle, 12 pediatric ALL samples were analyzed by OGM, and results were validated by comparing OGM data to results obtained from routine diagnostics. All genomic aberrations including translocations (e.g., dic(9;12)), aneuploidies (e.g., high hyper-diploidy) and CNVs (e.g., IKZF1, PAX5) known from other techniques were also detected by OGM. Moreover, OGM was superior to well-established techniques for resolution of the more complex structure of a translocation t(12;21) and had a higher sensitivity for detection of copy number alterations. More importantly, a new and unknown gene fusion of JAK2 and NPAT due to a translocation t(9;11) was detected. The authors showed the feasibility of OGM to detect well-established as well as new putative prognostic markers in an all-in-one approach in ALL. These researchers hope that these preliminary findings will be confirmed with testing of more samples in the future.

The authors stated that a drawback of OGM is the detection of whole arm translocations (Robertsonian translocations) and SVs located in telomeric regions due to the absence of labels in these regions. Depending on the amount of data generated (up to 4,000 Gb), detection of SVs with allele frequencies below 10 % may be possible. Similar to observations by Lestringant et al (2021), these investigators were unable to detect CRLF2/P2RY8 fusions in patients #2 and #5 because of sub-clonality (#2) and the repetitive nature of the PAR region at Xp22.33. To integrate OGM into the routine diagnostic workflow and on a larger scale, further validation and optimization of the workflow (e.g., increasing computing capacity and automated sample preparation to reduce processing time) is needed.

#### OvaNext

OvaNext (Ambry Genetics) is a next generation (next-gen) sequencing panel that simultaneously analyses 19 genes that contribute to increased risk for breast, ovarian, and/or uterine cancers (Raman, et al., 2013). The test is intended to determine if a woman has an increase chance of developing breast, ovarian, and/or uterus cancer.

#### Panexia

PANEXIA® (Myriad Genetics) detects mutations in genes that result in an increased risk of pancreatic cancer, offering insight about the risk of future hereditary cancers for patients and their families (Raman, et al., 2013). PANEXIA, via a blood test, analyzes the PALB2 and BRCA2 genes, the two genes most commonly identified in families with hereditary pancreatic cancer. The PANEXIA test results provide information for patients and their family members about the inherited risks of pancreatic cancer as well as breast, ovarian, and other cancers. This knowledge may allow at-risk family members the opportunity to lower their risks for some of these cancers through surveillance, preventative options, or lifestyle choices. The test is intended to determine if a person has an increase risk of developing pancreatic and/or breast cancer. The test determines the presence of the PALB2 and BRCA2 genes. The results of the test are intended to enable the development of a patient-specific medical management plan to reduce the risk of cancer.

#### Polycystic Liver Disease

In a review on "Diagnosis and management of polycystic liver disease", Gevers and Drenth (2013) stated that "[m]utation analysis for PCLD (PRKCSH and SEC63) is rarely performed in routine clinical practice, as it is not needed for clinical decision-making for these patients".

Crossen et al (2016) stated that isolated autosomal dominant polycystic liver disease (ADPLD) is a Mendelian disorder. Heterozygous PRKCSH (where PRKCSH is protein kinase C substrate 80K-H (80 kDa protein, heavy chain; MIM\*177060) mutations are the most frequent cause. Routine molecular testing using Sanger sequencing identifies pathogenic variants in the PRKCSH (15 %) and SEC63 (where SEC63 is *Saccharomyces cerevisiae* homolog 63 (MIM\*608648); 6 %) genes, but about approximately 80 % of patients meeting the clinical ADPLD criteria carry no PRKCSH or SEC63 mutation. Cyst tissue often shows somatic deletions with loss of heterozygosity that was recently recognized as a general mechanism in ADPLD. These researchers hypothesized that germline deletions in the PRKCSH gene may be responsible for hepatic cystogenesis in a significant number of mutation-negative ADPLD patients. In this study, these investigators designed a multiplex ligation-dependent probe amplification (MLPA) assay to screen for deletions of PRKCSH exons. Genomic DNA from 60 patients with an ADPLD phenotype was included; MLPA analysis detected no exon deletions in mutation-negative ADPLD patients. The authors concluded that large copy number variations on germline level were not present in patients with a clinical diagnosis of ADPLD. They stated that MLPA analysis of the PRKCSH gene should not be considered as a diagnostic method to explain hepatic cystogenesis.

Furthermore, an UpToDate review on "Diagnosis and management of cystic lesions of the liver" (Regev and Reddy, 2015) does not mention genetic testing as a management tool.

#### Prediction of Genitourinary Toxicity After Prostate Cancer Radiation

The PROSTOX assays (i.e., PROSTOX ultra and PROSTOX CFRT) (MiraDX, Inc., Los Angeles, CA) offers germline DNA analysis to determine if an individual has a genetically higher risk of late grade  $\geq 2$  genitourinary (GU) toxicity following radiation therapy (RT). The PROSTOX ultra assay predicts toxicity from stereotactic body radiotherapy (SBRT) ( $> 7$  Gy per fraction over 7 or less sessions) for the management of an individual's prostate cancer.

Kishan et al. (2022) conducted a study to determine whether single nucleotide polymorphisms (SNPs) disrupting microRNA targets (mirSNPs) can serve as predictive biomarkers for toxicity following radiotherapy for prostate cancer and whether these may be differentially predictive depending on radiation fractionation.

The investigators identified 201 men treated with two forms of definitive radiotherapy for prostate cancer at two institutions: conventionally-fractionated radiotherapy (CF-RT) ( $n = 108$ ) and stereotactic body of radiotherapy (SBRT) ( $n = 93$ ). Germline DNA was assessed for the presence of functional mirSNPs. Random forest, boosted trees and elastic net models were created to predict late grade  $\geq 2$  genitourinary (GU) toxicity by the Radiation Therapy Oncology Group (RTOG) scale.

The crude incidence of late grade  $\geq 2$  GU toxicity was 16% after CF-RT and 15% after SBRT. An elastic net model based on 22 mirSNPs differentiated CF-RT patients at high risk (71.5%) versus low risk (7.5%) for toxicity, with an area under the curve (AUC) values of 0.76–0.81. An elastic net model based on 32 mirSNPs differentiated SBRT patients at high risk (64.7%) versus low risk (3.9%) for toxicity, with an area under the curve (AUC) values of 0.81–0.87.

Study limitations included the following: retrospective in nature(i.e., post-hoc analysis of subset of retrospectively treated patients) and selection biases; CF-RT cohort had a significantly larger area of tissue radiation; presence of other underlying differences between patients treated with CF-RT and SBRT; physician-scored toxicity in place of patient-reported outcomes; other important factors (e.g., smoking status, dosimetry, medical comorbidities such as diabetes) could have influenced the development of late grade  $\geq 2$  GU toxicity; and follow-up time was unequal with far greater follow-up in the CF-RT cohort.

The investigators noted that the findings in this study and their significance on patient decision-making warrant validation in a prospective clinical trial.

#### Primary Dystonia (DYT-1)

Dystonia consists of repetitive, patterned, twisting, and sustained movements that may be either slow or rapid. Dystonic states are classified as primary, secondary, or psychogenic depending upon the cause (Jankovic, 2007). By definition, primary dystonia is associated with no other neurologic impairment, such as intellectual, pyramidal, cerebellar, or sensory deficits. Cerebral palsy is the most common cause of secondary dystonia.

Primary dystonia may be sporadic or inherited (Jankovic, 2007). Cases with onset in childhood usually are inherited in an autosomal dominant pattern. Many patients with hereditary dystonia have a mutation in the TOR1A (DYT1) gene that encodes the protein torsinA, an ATP-binding protein in the 9q34 locus. The role of torsinA in the pathogenesis of primary dystonia is unknown. DNA testing for the abnormal TOR1A gene can be performed on individuals with dystonia. The purpose of such testing is to help rule out secondary or psychogenic causes of dystonia, and for family planning purposes.

#### Prostate Cancer / UroSeq Hereditary Gene Panel

The UroSeq Hereditary Gene Panel is used for diagnosis, prognosis, recurrence/risk assessment, as well as therapeutic management of patients with prostate cancer. Of the 12 genes listed under the UroSeq Genetic Panel for prostate cancer, all but 2 (EPCAM and HOXB13) are listed under National Comprehensive Cancer Network's Biomarkers Compendium (2020) menu of "Prostate cancer" (Category 2A).

#### ProstateNow

ProstateNow (GoPath Global, Inc.) is a germline test that uses next-generation sequencing to analyze 24 genes, obtained from a blood or saliva sample, to calculate a genetic risk score on a patient's risk of developing prostate cancer compared to other patients in the general population. ProstateNow is used to screen for pathogenic mutations in DNA repair genes and identify patients who may respond better to targeted treatments, such as PARP inhibitors and platinum-based chemotherapies.

The National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines in Oncology on "Prostate cancer early detection" (Version 2.2024) and NCCN Biomarkers Compendium for "Prostate Cancer Early Detection" do not make a recommendation for testing all 24 genes. Moreover, NCCN Biomarkers Compendium does not provide a recommendation for use of a saliva specimen, nor includes a recommendation via blood specimen, for the biomarkers profiled in the ProstateNow panel.

ProstateNow also includes genotyping of HSD3B1 1245C, a biomarker for predicting response to androgen deprivation therapy (ADT). NCCN guidelines and compendia for prostate cancer do not include a recommendation for HSD3B1 testing.

GeneReviews' webpage on "RFC1 CANVAS / spectrum disorder" (Cortese et al, 2020) stated that the diagnosis of RFC1 CANVAS / spectrum disorder is established in a proband with suggestive findings and bi-allelic intronic AAGGG pentanucleotide expansions in RFC1 identified by molecular genetic testing that is targeted to detect these expansions. The authors stated that pathogenic AAGGG repeat expansions in RFC1 cannot be detected by sequence-based multi-gene panels or exome sequencing; however, they can be suspected based on genome sequencing.

Costales et al (2022) noted that the bi-allelic inheritance of an expanded intronic pentamer (AAGGG)exp in the gene encoding replication factor C subunit 1 (RFC1) has been found to be a cause of cerebellar ataxia, neuropathy, and vestibular areflexia syndrome (CANVAS). In a retrospective, descriptive study, these investigators described clinical and genetic features of patients with clinical suspicion of the syndrome. This study employed an ataxia database comprising 500 patients. Specific genetic testing for CANVAS was carried out in 13 patients with clinical suspicion of complete or incomplete syndrome. The clinical diagnosis was supported by quantitative vestibular hypofunction, cerebellar atrophy, and abnormal sensory nerve conduction testing. A total of 9 of 13 (69 %) patients met clinical diagnostic criteria for definite CANVAS disease. The 1st manifestation of the syndrome was lower limb dysesthesia in 8 of 13 patients and gait imbalance in 5 of 13; and 11 of 13 (85 %) patients were carriers of the bi-allelic (AAGGG)exp in RFC1. The authors concluded that genetic cause of CANVAS has recently been discovered. These researchers proposed genetic screening for bi-allelic expansions of the AAGGG pentamer of RFC1 in all patients with clinical suspicion of CANVAS, since accurate early diagnosis could improve the quality of life (QOL) of these patients.

In a retrospective study, Borsche et al (2022) examined the association between disease duration and the severity of bilateral vestibulopathy in individuals with complete or incomplete CANVAS and bi-allelic RFC1 repeat expansions. These researchers analyzed clinical data and the vestibulo-ocular reflex (VOR) quantified by the video head impulse test in 20 patients with confirmed bi-allelic RFC1 repeat expansions. VOR gain at 1st admittance  $6.9 \pm 5.0$  years after disease onset was 0.16 [0.15 to 0.31] (median [inter-quartile range (IQR)]). Cross-sectional analysis revealed that gain reduction was associated with disease duration. Follow-up measurements were available for 10 individuals: 8 of them exhibited a progressive decrease of the VOR gain over time. At the 1st visit, 6 of the 20 patients (30 %) did not show clinical signs of cerebellar ataxia. The authors concluded that these findings suggested a pathological horizontal head impulse test, which could easily be obtained in many outpatient clinics, as a sign of bilateral vestibulopathy in genetically confirmed CANVAS that could precede clinically accessible cerebellar ataxia at least in a subset of patients. The presumably continuous decline over time possibly reflects the neurodegenerative character of the disease. Therefore, genetic testing for RFC1 mutations in (isolated) bilateral vestibulopathy might allow disease detection before the onset of cerebellar signs.

Ronco et al (2023) stated that CANVAS is an autosomal recessive neurodegenerative disease characterized by adult-onset and slowly progressive sensory neuropathy, cerebellar dysfunction, and vestibular impairment. In most cases, the disease is caused by bi-allelic (AAGGG)n repeat expansions in the 2nd intron of the replication factor complex subunit 1 (RFC1). However, a small number of cases with typical CANVAS do not carry the common bi-allelic repeat expansion. These researchers expanded the genotypic spectrum of CANVAS by identifying sequence variants in RFC1-coding region associated with this condition. A total of 15 individuals diagnosed with CANVAS and carrying only 1 heterozygous (AAGGG)n expansion in RFC1 underwent whole-genome sequencing or WES to test for the presence of a 2nd variant in RFC1 or other unrelated gene. To examine the effect of truncating variants on RFC1 expression, these investigators tested the level of

RFC1 transcript and protein on patients' derived cell lines. These researchers identified 7 patients from 5 unrelated families with clinically defined CANVAS carrying a heterozygous (AAGGG)n expansion together with a 2nd truncating variant in trans in RFC1, which included the following: c.1267C>T (p.Arg423Ter), c.1739\_1740del (p.Lys580SerfsTer9), c.2191del (p.Gly731GlufsTer6), and c.2876del (p.Pro959GlnfsTer24). Patient fibroblasts containing the c.1267C>T (p.Arg423Ter) or c.2876del (p.Pro959GlnfsTer24) variants demonstrated nonsense-mediated mRNA decay and reduced RFC1 transcript and protein. The authors concluded that this study expanded the genotype spectrum of RFC1 disease. Full RFC1 sequencing is recommended in cases affected by typical CANVAS and carrying mono-allelic (AAGGG)n expansions. Furthermore, this trial shed further light on the pathogenesis of RFC1 CANVAS because it supported the existence of a loss-of-function mechanism underlying this complex neurodegenerative condition.

Furthermore, an UpToDate review on "Overview of cerebellar ataxia in adults" (Todd and Shakkottai, 2024) states that "Cerebellar ataxia, neuropathy, and vestibular areflexia syndrome (CANVAS) - CANVAS is an adult-onset disorder characterized by the association of bilateral vestibulopathy with cerebellar ataxia and sensory neuronopathy. The phenotypic spectrum also includes late-onset ataxia without vestibulopathy or neuropathy and ataxia with chronic cough. Most familial and sporadic forms are caused by homozygous AAGGG repeat expansion within intron 2 of the RFC1 gene, which encodes a large subunit of replication factor C and is involved in DNA synthesis and repair. Interestingly, this repeat is not only expanded but is a different sequence from the major alleles (AAAAG or AAAGG). As the allele frequency of the AAGGG repeat variant is quite common (approximately 0.7 to 4 %), RFC1 is likely an underrecognized cause of ataxia. In rare patients with a typical CANVAS phenotype and only monoallelic repeat expansion of RFC1, sequencing has identified truncating pathogenic variants in the coding region of the other RFC1 allele. Clinical features of CANVAS include gait imbalance in all, and variable presence of dysesthesia, oscillopsia, impaired vibratory sensation, and loss of ankle reflexes. Up to half of patients have motor system involvement with upper and/or lower motor neuron signs or, less commonly, parkinsonism. All patients have impairment of the oculocephalic reflex (doll's eyes or visually enhanced vestibulo-ocular reflex), which is tested in conscious patients by turning the head quickly from side to side while the patient fixates on an immobile target; the abnormal response is that the eyes fall short of the target due to a hypoactive vestibulo-ocular reflex and make a compensatory saccade ("catch-up" saccade to reach the target). In addition, symptoms of autonomic dysfunction are common. Brain MRI frequently reveals atrophy of the anterior and dorsal cerebellar vermis. Electrodiagnostic testing shows loss of sensory nerve action potentials with or without motor neuron involvement. Neuropathologic changes involve a dorsal root ganglionopathy with secondary spinal cord degeneration in the posterior columns, atrophy of cranial sensory ganglia, and cerebellar atrophy with loss of Purkinje cells, mainly in the vermis".

#### Selected Ciliopathies

Simms et al (2011) wrote that nephronophthisis (NPHP) is an autosomal recessive cystic kidney disease and a leading genetic cause of established renal failure in children and young adults; early presenting symptoms in children with NPHP include polyuria, nocturia, or secondary enuresis, pointing to a urinary concentrating defect. The authors further note that NPHP is associated with extra renal manifestations in 10-15% of patients. The most frequent extrarenal association is retinal degeneration, leading to blindness. Increasingly, molecular genetic testing is being utilized to diagnose NPHP and avoid the need for a renal biopsy.

Barisic et al (2015) stated that Meckel-Gruber syndrome is a rare autosomal recessive lethal ciliopathy characterized by the triad of cystic renal dysplasia, occipital encephalocele and postaxial polydactyly. The authors conducted the

largest population-based epidemiological study to date using data provided by the European Surveillance of Congenital Anomalies network using a study population of 191 cases identified between January 1990 and December 2011 in 34 European registries. The mean prevalence was 2.6 per 100,000 births in a subset of registries. The investigators found that there were 145 (75.9%) terminations of pregnancy after prenatal diagnosis, 13 (6.8%) fetal deaths, and 33 (17.3%) live births. In addition to cystic kidneys (97.7%), encephalocele (83.8%) and polydactyly (87.3%), frequent features include other central nervous system anomalies (51.4%), fibrotic/cystic changes of the liver (65.5% of cases with post mortem examination) and orofacial clefts (31.8%). Most cases (90.2%) are diagnosed prenatally at 14.3 ± 2.6 (range 11-36) gestational weeks and pregnancies are mainly terminated, reducing the number of live births to one-fifth of the total prevalence rate. Barisic et al concluded that early diagnosis is important for timely counseling of affected couples regarding the option of pregnancy termination and prenatal genetic testing in future pregnancies.

Ece Solmaz et al (2015) stated that Bardet-Biedl syndrome (BBS) is characterized by obesity, rod-cone dystrophy, postaxial polydactyly, renal abnormalities, genital abnormalities and learning difficulties; mutations in 21 different genes have been described as being responsible for BBS. Recently, sequential gene sequencing has been replaced by NGS applications. The investigators conducted a study in which 15 patients with clinically diagnosed BBS were investigated using an NGS panel including 17 known BBS causing genes (BBS1, BBS2, ARL6, BBS4, BBS5, MKKS, BBS7, TTC8, BBS9, BBS10, TRIM32, BBS12, MKS1, NPHP6, WDPCP, SDCCAG8, NPHP1). A genetic diagnosis was achieved in 13 patients (86.6%) and involved 9 novel and 3 previously described pathogenic variants in 6 of 17 BBS causing genes; also, three of the 13 patients had an affected sibling. The authors concluded that although limited association between certain genes and phenotypic features was observed in this study, additional studies are needed to better characterize the genotype-phenotype correlation of BBS. Nevertheless, the results demonstrate that NGS panels are a feasible and effective method for providing high diagnostic yields in the diseases caused by multiple genes such as BBS.

Roosing et al (2015) reported that effective primary ciliogenesis or cilium stability forms the basis of human ciliopathies, including Joubert syndrome (JS). They evaluated patients with defective cerebellar vermis development and performed a high-content genome-wide small interfering RNA screen to identify genes regulating ciliogenesis as candidates for JS. The investigators identified 591 likely candidates; intersection of this data with whole exome results from 145 individuals with unexplained JS identified six families with predominantly compound heterozygous mutations in KIAA0586. A c.428del base deletion in 0.1% of the general population was found with a second mutation in an additional set of 9 of 163 unexplained JS patients. Thus, the investigators concluded that KIAA0586 is an orthologue of chick Talpid3, required for ciliogenesis and sonic hedgehog signaling, uncovering a relatively high frequency cause for JS and contributing a list of candidates for future gene discoveries in ciliopathies.

Suspitsin et al (2015) stated that at least 19 genes have been shown to be associated with BBS, and therefore, genetic testing is highly complicated. The authors used an Illumina MiSeq platform for WES analysis of a family with strong clinical features of BBS and found a homozygous c.1967\_1968delTAinsC (p.Leu656fsX673; RefSeq NM\_176824.2) mutation in BBS7 in both affected children, while their healthy sibling and the non-consanguineous parents were heterozygous for this allele. Therefore, genotyping of 2,832 DNA samples obtained from Russian blood donors revealed 2 additional heterozygous subjects (0.07%) with the c.1967\_1968delTAinsC mutation.

Wheway et al (2015) identified 112 candidate ciliogenesis and ciliopathy genes, including 44 components of the ubiquitin-proteasome system, 12 G-protein-coupled receptors, and 3 pre-mRNA processing factors (PRPF6, PRPF8 and PRPF31) mutated in autosomal dominant retinitis pigmentosa. Combining the screen with exome sequencing data identified recessive mutations in PIBF1, also known as CEP90, and C21orf2, also known as LRRC76, as causes of the ciliopathies Joubert and Jeune syndromes. The authors' approaches provide insights into ciliogenesis complexity and identify roles for unanticipated pathways in human genetic disease.

Suspitsin et al.(2016) stated that BBS is a rare autosomal recessive genetic disorder, characterized by heterogeneous clinical manifestations including primary features of the disease such as rod-cone dystrophy, polydactyly, obesity, genital abnormalities, renal defects, learning difficulties and secondary BBS characteristics such as developmental delay, speech deficit, brachydactyly or syndactyly, dental defects, ataxia or poor coordination, olfactory deficit, diabetes mellitus, and congenital heart disease. A minimum of 20 BBS genes have already been identified, and all of them are involved in primary cilia functioning. Genetic diagnosis of BBS is complicated due to lack of gene-specific disease symptoms, but is gradually becoming more accessible with the invention of multigene sequencing technologies. Progress in DNA testing technologies is likely to rapidly resolve all limitations in BBS diagnosis; however, much slower improvement is expected with regard to BBS treatment.

#### Sleep-Walking

Licis et al (2011) stated that sleep-walking is a common and highly heritable sleep disorder. However, inheritance patterns of sleep-walking are poorly understood and there have been no prior reports of genes or chromosomal localization of genes responsible for this disorder. These researchers described the inheritance pattern of sleep-walking in a 4-generation family and identified the chromosomal location of a gene responsible for sleep-walking in this family. A total of 9 affected and 13 unaffected family members of a single large family were interviewed and DNA samples collected. Parametric linkage analysis was performed. Sleep-walking was inherited as an autosomal dominant disorder with reduced penetrance in this family. Genome-wide multi-point parametric linkage analysis for sleep-walking revealed a maximum logarithm of the odds score of 3.44 at chromosome 20q12-q13.12 between 55.6 and 61.4 cM. The authors described the first genetic locus for sleep-walking at chromosome 20q12-q13.12; and concluded that sleep-walking may be transmitted as an autosomal dominant trait with reduced penetrance.

In an editorial that accompanied the afore-mentioned study, Dogu and Pressman (2011) noted that "[a]ccording to currently accepted evidence-based theories, the occurrence of sleepwalking requires genetic predisposition, priming factors such as severe sleep deprivation or stress, and, in addition, a proximal trigger factor such as noise or touch. These factors form the background for a "perfect storm," all of which must occur before a sleepwalking episode will occur. Hereditary factors likely play an important role, with recessive and multifactorial inheritance patterns having been reported. A recent genetic study has shown that the HLADQB1\*05 Ser74 variant is a major susceptibility factor for sleepwalking in familial cases, but this finding has yet to be replicated. Another study attempted to find a causal relationship between sleepwalking and sleep-disordered breathing in cosegregated families of both disorders. However, this study was limited by the absence of molecular data .... The current diagnosis of sleepwalking is based almost entirely on clinical history. There are no objective, independent means of confirming the diagnosis. Additionally, treatment of sleepwalking is symptomatic, aimed at suppressing arousal or reducing deep sleep. Identification of causative genes may eventually permit development of an independent test and treatments aimed at the underlying causes of this disorder".

## Spinal Muscular Atrophy

Spinal Muscular Atrophy (SMA) is a group of inherited diseases that cause muscle damage and weakness, which get worse over time and eventually lead to death. The most severe form is SMA type I, also called Werdnig-Hoffman disease. Infants with SMA type II have less severe symptoms during early infancy, but become weaker with time. SMA type III is the least severe form of the disease. Rarely, SMA may begin in adulthood and is usually a milder form of the disease.

Spinal muscular atrophy (SMA), which has an estimated prevalence of 1 in 10,000, is characterized by proximal muscle weakness resulting from the degeneration of anterior horn cells in the spinal cord. SMA type I is typically diagnosed at birth or within the first 3 to 6 months of life; affected children are unable to sit unassisted and usually die from respiratory failure within 2 years. Those with SMA type II, which is diagnosed before 18 months of age, are unable to stand or walk unaided, although they may be able to sit and may survive beyond age 4. The clinical features of SMA types III and IV are milder and manifest after 18 months of age or in adulthood, respectively. SMA is inherited in an autosomal recessive manner and is caused by alterations in the survival motor neuron 1 (SMN1) gene located on chromosome 5 at band q12.2 to q13.3. Approximately 95% of SMA patients have the condition as a result of a homozygous deletion involving at least exon 7 of SMN1. Approximately 5% are compound heterozygotes, with a deletion in 1 allele of SMN1 and a subtle intragenic variation in the other. SMN2, a gene nearly identical in sequence to SMN1, is located in the same highly repetitive region on chromosome 5. Although it does not cause SMA, it has been shown to modify the phenotype of the condition; those with the milder SMA types II or III tend to have more copies of SMN2 than those with the severe type I.

SMN1 deletions are detected by polymerase chain reaction (PCR) amplification of exon 7 of the SMN genes, followed by restriction fragment length polymorphism (RFLP) analysis. Following amplification, exon 7 of SMN2 will be cut with the restriction enzyme Dral, while exon 7 of SMN1 will remain intact. SMA patients with homozygous SMN1 deletions will show an absence of the uncut SMN1 exon 7 PCR products. To detect heterozygous SMN1 deletions in SMA carriers or compound heterozygotes, quantitative PCR (qPCR) is performed. To identify subtle intragenic variations in SMA patients found to have only 1 copy of the deletion, the SMN1 gene is typically sequenced. Candidates for diagnostic testing include infants, children, and adults with generalized hypotonia and proximal muscle weakness of unknown etiology. Carrier testing may be offered to couples considering pregnancy, including those with a family history of SMA, and prenatal diagnosis should be made available to all identified carriers.

The American College of Obstetricians and Gynecologists (ACOG, 2017) recommends that "[s]creening for spinal muscular atrophy should be offered to all women who are considering pregnancy or are currently pregnant. The guidelines state that, "in patients with a family history of spinal muscular atrophy, molecular testing reports of the affected individual and carrier testing of the related parent should be reviewed, if possible, before testing. If the reports are not available, SMN1 deletion testing should be recommended for the low-risk partner."

## Spinocerebellar Ataxia

Spinocerebellar ataxia (SCA) is an inherited progressive neurodegenerative disease. It is characterized by dysfunction of the cerebellum, the part of the brain that controls walking and balance and is manifested by progressive uncoordinated movements (ataxia). There are at least 25 different types of SCA conditions. Diagnostic genetic testing may be used for individuals with signs and symptoms of SCA. Genetic testing has also been proposed for at-risk individuals with a family history of SCA.

Blum and associates (2015) noted that the Brain Reward Cascade (BRC) is an interaction of neurotransmitters and their respective genes to control the amount of dopamine released within the brain. Any variations within this pathway, whether genetic or environmental (epigenetic), may result in addictive behaviors or reward deficiency syndrome (RDS), which was coined to define addictive behaviors and their genetic components. These investigators searched a number of important databases including: Filtered: Cochrane Systematic reviews; DARE; PubMed Central Clinical Queries; National Guideline Clearinghouse and unfiltered resources: PsychINFO; ACP PIER; PsychSage; PubMed/Medline. The major search terms included: dopamine agonist therapy for addiction; dopamine agonist therapy for reward dependence; dopamine antagonistic therapy for addiction; dopamine antagonistic therapy for reward dependence and neurogenetics of RDS. While there are many studies claiming a genetic association with RDS behavior, not all are scientifically accurate. The authors concluded that albeit their bias, this Clinical Pearl discussed the facts and fictions behind molecular genetic testing in RDS and the significance behind the development of the Genetic Addiction Risk Score (GARSPREDX™), the first test to accurately predict one's genetic risk for RDS. The clinical value of the Genetic Addiction Risk Score (GARSPREDX™) has yet to be determined.

#### The OncoVue Breast Cancer Risk Test

The OncoVue breast cancer risk test (Intergenetics, Inc., Oklahoma City, OK) is a genetic-based breast cancer risk test that incorporates both individualized genetic-based single nucleotide polymorphisms (SNPs) and personal history measures to arrive at an estimate of a woman's breast cancer risk at various stages in her life.

Cells that are collected from the inside of the cheek are analyzed using thousands of proprietary (Intergenetic, Inc.) combinations of multiple genes. The genetic information and the data from the medical history are combined to assign a numeric value that tells a woman's lifetime risk of developing breast cancer. Her OncoVue risk test will tell her if she is standard, moderate or high risk for developing breast cancer during each stage of her life.

OncoVue is based on an un-published case-controlled associative study that examined common genetic polymorphisms and medical history variables. Currently, 117 common polymorphisms (mostly SNPs) located in over 87 genes believed to alter breast cancer risk are examined. Most result in amino acid changes in the proteins encoded by the genes in which they occur. The medical history variables include answers to questions concerning women's reproductive histories, family histories of cancer and a few other questions related to general health.

There are no published controlled studies on the OncoVue breast cancer risk test in the peer-reviewed medical literature.

Gail (2009) evaluated the value of adding SNP genotypes to a breast cancer risk model. Criteria that are based on 4 clinical or public health applications were used to compare the National Cancer Institute's Breast Cancer Risk Assessment Tool (BCRAT) with BCRATplus7, which includes 7 SNPs previously associated with breast cancer. Criteria included number of expected life-threatening events for the decision to take tamoxifen, expected decision losses (in units of the loss from giving a mammogram to a woman without detectable breast cancer) for the decision to have a mammogram, rates of risk re-classification, and number of lives saved by risk-based allocation of screening mammography. For all calculations, the following assumptions were made: Hardy-Weinberg equilibrium, linkage equilibrium across SNPs, additive effects of alleles at each locus, no interactions on the logistic scale among SNPs or with factors in BCRAT, and independence of SNPs from factors in

BCRAT. Improvements in expected numbers of life-threatening events were only 0.07 % and 0.81 % for deciding whether to take tamoxifen to prevent breast cancer for women aged 50 to 59 and 40 to 49 years, respectively. For deciding whether to recommend screening mammograms to women aged 50 to 54 years, the reduction in expected losses was 0.86 % if the ideal breast cancer prevalence threshold for recommending mammography was that of women aged 50 to 54 years. Cross-classification of risks indicated that some women classified by BCRAT would have different classifications with BCRATplus7, which might be useful if BCRATplus7 was well calibrated. Improvements from BCRATplus7 were small for risk-based allocation of mammograms under costs constraints. The author reported that the gains from BCRATplus7 were small in the applications examined and that models with SNPs, such as BCRATplus7, have not been validated for calibration in independent cohort data. The author concluded that additional studies are needed to validate a model with SNPs and justify its use.

There is insufficient evidence on the effectiveness of the OncoVue breast cancer risk test in determining a woman's breast cancer risk at various stages in her life.

#### The Phosphatase and Tensin Homolog (PTEN) Gene Test

Phosphatase and tensin homolog (PTEN) hamartoma tumor syndrome is an autosomal dominant group of disorders with significant clinical overlap, most notably predisposition to hamartomatous polyposis of the gastro-intestinal tract. Laurent-Puig et al (2009) stated that the occurrence of KRAS mutation is predictive of non-response and shorter survival in patients treated by anti-epidermal growth factor receptor (anti-EGFR) antibody for metastatic colorectal cancer (mCRC), leading the European Medicine Agency to limit its use to patients with wild-type KRAS tumors. However, only 50 % of these patients will benefit from treatment, suggesting the need to identify additional biomarkers for cetuximab-based treatment efficacy. These investigators retrospectively collected tumors from 173 patients with mCRC. All but 1 patient received a cetuximab-based regimen as second-line or greater therapy. KRAS and BRAF status were assessed by allelic discrimination. EGFR amplification was assessed by chromogenic *in situ* hybridization and fluorescent *in situ* hybridization, and the expression of PTEN was assessed by immunochemistry. In patients with KRAS wild-type tumors ( $n = 116$ ), BRAF mutations ( $n = 5$ ) were weakly associated with lack of response ( $p = 0.063$ ) but were strongly associated with shorter progression-free survival ( $p < 0.001$ ) and shorter overall survival (OS;  $p < 0.001$ ). A high EGFR polysomy or an EGFR amplification was found in 17.7 % of the patients and was associated with response ( $p = 0.015$ ). PTEN null expression was found in 19.9 % of the patients and was associated with shorter OS ( $p = 0.013$ ). In multi-variate analysis, BRAF mutation and PTEN expression status were associated with OS. The authors concluded that BRAF status, EGFR amplification, and cytoplasmic expression of PTEN were associated with outcome measures in KRAS wild-type patients treated with a cetuximab-based regimen. They stated that more studies in clinical trial cohorts are needed to confirm the clinical utility of these markers.

Siena et al (2009) noted that the monoclonal antibodies panitumumab and cetuximab that target the EGFR have expanded the range of treatment options for mCRC. Initial evaluation of these agents as monotherapy in patients with EGFR-expressing chemotherapy-refractory tumors yielded response rates of approximately 10 %. The realization that detection of positive EGFR expression by immunostaining does not reliably predict clinical outcome of EGFR-targeted treatment has led to an intense search for alternative predictive biomarkers. Oncogenic activation of signaling pathways downstream of the EGFR, such as mutation of KRAS, BRAF, or PIK3CA oncogenes, or inactivation of the PTEN tumor suppressor gene is central to the progression of colorectal cancer. Tumor KRAS mutations, which may be present in 35 % to 45 % of patients with colorectal cancer, have emerged as an important predictive marker of resistance to panitumumab or

cetuximab treatment. In addition, among colorectal tumors carrying wild-type KRAS, mutation of BRAF or PIK3CA or loss of PTEN expression may be associated with resistance to EGFR-targeted monoclonal antibody treatment, although these additional biomarkers require further validation before incorporation into clinical practice. Additional knowledge of the molecular basis for sensitivity or resistance to EGFR-targeted monoclonal antibodies will allow the development of new treatment algorithms to identify patients who are most likely to respond to treatment and could also provide rationale for combining therapies to overcome primary resistance. The use of KRAS mutations as a selection biomarker for anti-EGFR monoclonal antibody (e.g., panitumumab or cetuximab) treatment is the first major step toward individualized treatment for patients with mCRC.

#### Thoracic Aortic Aneurysms and Dissections

A number of conditions are associated with aortic dysfunction and dilation, including Marfan syndrome, Loeys Dietz syndrome, Ehler Danlos syndrome type IV, Turner syndrome, and arterial tortuosity syndrome.

Ehlers-Danlos syndrome (EDS) is a group of inherited disorders affecting the connective tissues. Common characteristics of EDS include easy bruising, skin hyperelasticity or laxity, joint hypermobility and tissue weakness. EDS is categorized by type: Classic (EDS types I and II); Hypermobility (EDS type III); Vascular (EDS type IV); kyphoscoliosis (EDS type VI); Arthrochalasia (EDS types VIIA & B); Dermatosparaxis (EDS type VIIC).

Classic, hypermobility and vascular type occur more frequently than the other types. Other more rare forms include spondylocheirodysplasia EDS and musculocontractural EDS; additional rare variants of EDS have also been described.

Acrogeria refers to looseness and wrinkling of the skin of the hands and feet that is caused by loss of subcutaneous fat and collagen and gives the appearance of premature aging.

Dermatosparaxis is an inherited defect in collagen synthesis caused by a deficiency of procollagen peptidase. Results in fragility, hyperelasticity and laxity of the skin.

Musculocontractural is a rare form of EDS with the following characteristics: distinctive craniofacial dysmorphism, congenital contractures of thumbs and fingers, clubfeet, severe kyphoscoliosis, muscular hypotonia, hyperextensible thin skin with easy bruising and atrophic scarring, wrinkled palms, joint hypermobility and ocular involvement.

Spondylocheirodysplasia is a rare form of EDS with the following clinical features: postnatal growth retardation, moderate short stature, protuberant eyes with bluish sclerae, hands with finely wrinkled palms, atrophy of the thenar muscles and tapering fingers.

Ehlers-Danlos syndrome type IV (EDS type IV) is characterized by thin, translucent skin; easy bruising; characteristic facial appearance; and arterial, intestinal, and/or uterine fragility (Pepin & Byers, 2011). The diagnosis of EDS type IV is based on clinical findings and confirmed by identification of a causative mutation in COL3A1. EDS type IV is inherited in an autosomal dominant manner.

Arterial tortuosity syndrome (ATS) is characterized by severe and widespread arterial tortuosity of the aorta and middle-sized arteries (with an increased risk of aneurysms and dissections) and focal and widespread stenosis which can involve the aorta and/or pulmonary arteries (Callewaert, et al., 2014). The diagnosis of ATS

is established in a proband with generalized arterial tortuosity and biallelic (homozygous or compound heterozygous) pathogenic variants in SLC2A10. ATS is inherited in an autosomal recessive manner.

Loeys-Dietz syndrome (LDS) is an inherited connective tissue disorder characterized by aortic aneurysms and other blood vessel abnormalities. Mutations in either the TGFBR1 or TGFBR2 gene can cause LDS. LDS is characterized by vascular findings (cerebral, thoracic, and abdominal arterial aneurysms and/or dissections) and skeletal manifestations (pectus excavatum or pectus carinatum, scoliosis, joint laxity, arachnodactyly, talipes equinovarus) (Loeys & Dietz, 2014). The diagnosis of LDS is based on characteristic clinical findings in the proband and family members and molecular genetic testing of TGFBR1, TGFBR2, SMAD3, and TGFB2. LDS is inherited in an autosomal dominant manner.

Marfan syndrome is a genetic disorder in which the body's connective tissue is abnormal. The disorder affects many parts of the body; primarily, the connective tissue of the heart, blood vessels, eyes, bones, lungs and covering of the spinal cord. Marfan syndrome diagnosis relies on a set of strict major and minor criteria known as the Ghent nosology, a scoring system developed to aid in the clinical diagnosis of Marfan syndrome. Two fundamental features of the Ghent nosology are aortic root aneurysm and ectopia lentis. In the absence of a family history of Marfan syndrome, the presence of aortic root aneurysm and ectopia lentis are sufficient to diagnose Marfan syndrome. Without these two conditions, or a combination of systemic features described in the Ghent nosology, genetic testing may be required to confirm a diagnosis. Even with the availability of genetic testing, establishing a diagnosis of Marfan syndrome depends heavily upon significant clinical findings.

Marfan syndrome is a systemic disorder of connective tissue with a high degree of clinical variability (Dietz, 2014). Cardinal manifestations involve the ocular, skeletal, and cardiovascular systems. Cardiovascular manifestations include dilatation of the aorta at the level of the sinuses of Valsalva, a predisposition for aortic tear and rupture, mitral valve prolapse with or without regurgitation, tricuspid valve prolapse, and enlargement of the proximal pulmonary artery. Marfan syndrome is a clinical diagnosis based on family history and the observation of characteristic findings in multiple organ systems. Marfan syndrome is caused by mutation of FBN1. The sensitivity of molecular genetic testing of FBN1 is substantial yet incomplete for unknown reasons; it may be explained by atypical location or character of FBN1 pathogenic variants in some individuals (e.g., large deletions or promoter mutations) or to locus heterogeneity. Marfan syndrome is inherited in an autosomal dominant manner.

A variety of conditions including, but not limited to, Loeys-Dietz syndrome (LDS) and familial thoracic aortic aneurysm and dissection (TAAD) have overlapping clinical manifestations of Marfan syndrome and should be distinguished from Marfan syndrome with documentation of discriminating features, biochemical and/or genetic testing, when indicated.

Familial TAAD is an inherited disorder that causes the aorta to weaken and stretch. Mutations in any of several genes are associated with familial TAAD.

Predictive genetic testing for LDS, Marfan syndrome and TAAD may be sought for at-risk asymptomatic or presymptomatic family members to detect mutations in the genes known to cause these disorders to determine if the individual will develop the condition.

Several labs offer multigene panels, often using next-generation sequencing (NGS) for familial TAAD, LDS and Marfan syndrome that include not only the FBN1 gene but also a number of other genes associated with disorders featuring aortic aneurysms and dissections. With the introduction of NGS, laboratories can

simultaneously analyze numerous genes reportedly associated with Marfan syndrome and related conditions. Examples include: Marfan Syndrome and Aortic Aneurysms (Marfan/AA) Test; Marfan Syndrome/Thoracic Aortic Aneurysm and Dissection (TAAD) and Related Disorders Test; and TAADNEXT.

Guidelines from the American College of Cardiology (Hiratzka, et al., 2010) state, if a mutant gene (FBN1, TGFB1, TGFB2, COL3A1, ACTA2, MYH11) associated with aortic aneurysm and/or dissection is identified in a patient, first-degree relatives should undergo counseling and testing. Then, only the relatives with the genetic mutation should undergo aortic imaging.

Clinical laboratories may offer a multi-gene Marfan syndrome/Loeys-Dietz syndrome/familial thoracic aortic aneurysms and dissections panel that includes FBN1 as well as a number of other genes associated with disorders that include aortic aneurysms and dissections (Dietz, 2014). These panels vary by methods used and genes included; thus, the ability of a panel to detect a pathogenic variant or pathogenic variants in any given individual also varies. In most circumstances a comprehensive clinical evaluation and imaging studies will point to a specific diagnosis (or subset of diagnoses) that has the highest probability, and thus should be pursued first for molecular confirmation. In the absence of such hypothesis-driven testing, there is an increased risk of erroneous interpretation of variants of uncertain significance when multi-gene panels are applied, especially if the physician requesting testing is not familiar with the specific diagnoses and/or genes under consideration.

Guidelines from the American College of Cardiology and American Heart Association (ACC/AHA) (Isselbacher et al., 2022) recommend a multigene panel comprising of genes suspected to cause heritable thoracic aortic disease (HTAD) for patients with aortic root/ascending aortic aneurysm or aortic dissection and have any of the following risk factors of HTAD:

- Thoracic aortic disease (TAD) and syndromic features of Marfan syndrome, Loeys-Dietz syndrome, or vascular Ehlers-Danlos syndrome
- TAD presenting at age less than 60 years
- A family history of either TAD or peripheral/intracranial aneurysms in a first- or second-degree relative
- A history of unexplained sudden death at a relatively young age in a first- or second-degree relative.

Isselbacher et al. (2022) state that HTAD genetic testing panels include 11 genes (FBN1, LOX, COL3A1, TGFB1, TGFB2, SMAD3, TGFB2, ACTA2, MYH11, MYLK, and PRKG1) that have a highly penetrant risk for TAD. The authors also note that mutations in ACTA2, MYH11, MYLK, LOX, and PRKG1 have been confirmed to cause HTAD in the absence of significant features of Marfan syndrome or Loeys-Dietz syndrome. For first-degree relatives of all persons with TAD, the ACC/AHA recommend thoracic aortic imaging to detect asymptomatic aneurysms.

#### Tuberous Sclerosis Complex

Northrop and colleagues (2015) noted that tuberous sclerosis complex (TSC) involves abnormalities of the skin (hypo-melanotic macules, facial angiofibromas, shagreen patches, cephalic plaques, ungual fibromas); brain (cortical dysplasias, sub-ependymal nodules and sub-ependymal giant cell astrocytomas [SEGAs], seizures, intellectual disability/developmental delay, psychiatric illness); kidney (angiomyolipomas, cysts, renal cell carcinomas); heart (rhabdomyomas, arrhythmias); and lungs (lymphangioleiomyomatosis [LAM]). Tumors of the central nervous system (CNS) are the leading cause of morbidity and mortality; renal disease is the 2nd leading cause of early death. The diagnosis of TSC is based on clinical findings. Heterozygous pathogenic variants can be identified in 75 % to 90

% of individuals who meet the clinical diagnostic criteria for TSC. Among those in whom a pathogenic variant can be identified, pathogenic variants in TSC1 are found in 31 % and pathogenic variants in TSC2 in 69 %. Treatment of manifestations: For enlarging SEGAs: mTOR inhibitors; neurosurgery when size causes life-threatening neurologic symptoms. For seizures: Vigabatrin and other anti-epileptic drugs, and on occasion, epilepsy surgery. For renal angiomyolipomas greater than 4 cm, or greater than 3 cm and growing rapidly: mTOR inhibitors are the recommended 1st line of therapy with secondary therapy options being embolization or renal sparing surgery. For LAM: mTOR inhibitors. For facial angiofibromas: Topical mTOR inhibitors. For symptomatic cardiac rhabdomyomas: Surgical intervention or consideration of mTOR inhibitor therapy.

#### Type 2 Diabetes

Available evidence has shown that screening for a panel of gene variants associated with type 2 diabetes does not substantially improve prediction of risk for the disease than an assessment based on traditional risk factors. Available evidence suggests that both genetic and environmental factors play a role in the development of type 2 diabetes. Recent genetic studies have identified 18 gene variants that appear to increase the risk for type 2 diabetes.

A study reported in the New England Journal of Medicine evaluated the potential utility of genetic screening in predicting future risk of type 2 diabetes (Meigs et al, 2008). The investigators analyzed records from the Framingham Offspring Study, which follows a group of adult children of participants of the original Framingham Heart study, to evaluate risk factors for the development of cardiovascular disease, including diabetes. Full genotype results for the 18 gene variants as well as clinical outcomes were available for 2,377 participants, 255 of whom developed type 2 diabetes during 28 years of follow-up. Each participant was assigned a genotype score, based on the number of risk-associated gene copies inherited. The investigators compared the predictive value of the genotype score to that of family history alone or of physiological risk factors. Overall, the genetic score was 17.7 among those who developed diabetes and 17.1 among those who did not. The investigators found that, while the genetic score did help predict who would develop diabetes, once other known risk factors were taken into consideration, it offered little additional predictive power. The investigators concluded that: "[t]he genotype score resulted in the appropriate risk reclassification of, at most, 4 % of the subjects, compared with risk estimates based on age, sex, blood lipids, body mass index, family history, and other standard risk factors." The investigators reported that "[o]ur findings underscore the view that identification of adverse phenotypic characteristics remains the cornerstone of approaches to predicting the risk of type 2 diabetes," the authors said.

A similar study among Swedish and Finnish patients, published in the same issue of the New England Journal of Medicine, also found only a small improvement in risk estimates when genetic factors were added to traditional risk factors (Lyssenko et al, 2008).

#### Unverricht-Lundborg Disease (EPM1)

Unverricht-Lundborg disease (EPM1) is an inherited neurodegenerative disorder characterized by stimulus-sensitive myoclonus, and tonic-clonic epileptic seizures, which has typical onset from 6 to 15 years of age and worsens over time. Episodes of myoclonus may be brought on by exercise, stress, light, or other stimuli. Symptoms that occur, typically years after the onset, include ataxia, incoordination, intentional tremor, and dysarthria. Individuals with EPM1 are mentally alert but show emotional lability, depression, and mild decline in intellectual performance over time (Lehesjoki and Kalviainen, 2014; NIH/GARD, 2018).

Unverricht-Lundborg disease (EPM1) is an autosomal recessive condition caused by mutations in the CSTB gene. The diagnosis can be confirmed by identifying the common dodecamer repeat expansion or other pathogenic variants in CSTB. Carrier testing for at-risk relatives and prenatal testing for pregnancies at increased risk are possible if both CSTB pathogenic variants in a family are known. Pharmacologic and rehabilitative management, including psychosocial support, are the mainstay of care. Lifelong clinical follow up includes evaluation of drug treatment and rehabilitation (Lehesjoki and Kalviainen, 2014; NIH/GARD, 2018).

#### Versiti Panels

##### Versiti aHUS Genetic Evaluation Panel

The Versiti aHUS Genetic Evaluation Panel entails genomic sequence analysis in blood, buccal swab, or amniotic fluid of 14 genes (ADAMTS13, C3, C4BPA, C4BPB, CFB, CFH, CFHR1, CFHR3, CFHR4, CFHR5, CFI, DGKE, MCP [CD46], THBD) for detection of genetic variants related to aHUS and membranoproliferative glomerulonephritis type II (also known as dense deposit disease [DDD]).

In a consensus report of South Korean experts regarding “Diagnosis and management of atypical hemolytic uremic syndrome”, Lee et al (2020) noted that “genes associated with aHUS include CFH, CD46 or MCP, C3, CFI, CFB, THBD, PLG, and DGKE. Genetic information supports diagnosis, clarity regarding the underlying pathophysiology, and guides treatment decisions; moreover, it is predictive of prognosis. However, the absence of variants of a given set of genes does not preclude the diagnosis of aHUS”.

Piras and associates (2020) stated that atypical hemolytic uremic syndrome (aHUS) is an ultra-rare disease characterized by microangiopathic hemolysis, thrombocytopenia, and renal impairment and is associated with dysregulation of the alternative complement pathway on the microvascular endothelium. Outcomes have greatly improved with pharmacologic complement C5 blockade.

Abnormalities in complement genes (CFH, CD46, CFI, CFB, C3, and THBD), CFH-CFHR genomic rearrangements, and anti-FH antibodies have been reported in 40 % to 60 % of cases. The penetrance of aHUS is incomplete in carriers of complement gene abnormalities; and multiple hits, including the CFH-H3 and CD46 GGAAC risk haplotypes and the CFHR1 \* B risk allele, as well as environmental factors, contribute to disease development. These investigators examined the determinants of penetrance of aHUS associated with CD46 genetic abnormalities.

They studied 485 aHUS patients and found CD46 rare variants (RVs) in about 10 %. The c.286+2T>G RV was the most prevalent (13/485) and was associated with less than 30 % penetrance. These researchers carried out an in-depth study of a large pedigree including a proband who was heterozygous for the c.286+2T>G RV, who experienced a severe form of aHUS and developed end-stage renal failure. The father and paternal uncle with the same variant in homozygosity and 6 heterozygous relatives were unaffected. Flow cytometry analysis showed about 50 % reduction of CD46 expression on blood mononuclear cells from the heterozygous proband and over 90 % reduction in cells from the proband's unaffected homozygous father and aunt. Further genetic studies did not reveal RVs in known aHUS-associated genes or common genetic modifiers that segregated with the disease. More importantly, a specific ex-vivo test showed excessive complement deposition on endothelial cells exposed to sera from the proband, and also from his mother and maternal uncle, who do not carry the c.286+2T>G RV, indicating that they share a circulating defect that resulted in complement dysregulation on the endothelium. The authors concluded that these findings highlighted the complexity of the genetics of aHUS and indicated that CD46 deficiency may not be enough to induce aHUS. These researchers hypothesized that the proband inherited from his

mother a genetic abnormality in a complement circulating factor that has not been identified yet, which synergized with the CD46 RV in predisposing him to the aHUS phenotype.

Ardissino and co-workers (2021) noted that aHUS is mainly due to complement regulatory gene abnormalities with a dominant pattern but incomplete penetrance; therefore, healthy carriers can be identified in any family of aHUS patients, but it is unpredictable if they will eventually develop aHUS. In a cohort study, these researchers examined the risk of aHUS among family members of patients carrying complement regulatory gene abnormality. Patients are screened for 10 complement regulatory gene abnormalities and once a genetic alteration is identified, the search is extended to at-risk family members. This trial included 257 subjects from 71 families: 99 aHUS patients (71 index cases + 28 affected family members) and 158 healthy relatives with a documented complement gene abnormality. A total of 14 families (19.7 %) experienced multiple cases. Over a cumulative observation period of 7,595 person-years, only 28 family members carrying gene mutations experienced aHUS (overall penetrance of 20 %), leading to a disease rate of 3.69 events for 1,000 person-years. The disease rate was 7.47 per 1,000 person-years among siblings, 6.29 among offspring, 2.01 among parents, 1.84 among carriers of variants of uncertain significance, and 4.43 among carriers of causative variants. The authors concluded that the risk of developing the disease in any given relative of a patient carrying complement regulatory gene mutations responsible for aHUS in a family member can be estimated to be 20 %, thus lower than the reported 50 %. This information, which is missing in the current literature, can be very important for patient's relatives who wonder if they or their children may also develop this severe life-threatening disease. Although the penetrance is not as high as previously reported, in the authors' opinion the severity of the condition may justify the screening of relatives for the specific mutation responsible for the disease in their family. They tend to screen subjects based on their willingness to be aware of their specific risk (particularly in case of mutations involving C3 or CFH genes in the index case, multiple cases in the same family, young brothers and siblings of the index case, and no clear trigger in the index case). These investigators stated that the awareness of the risk can be very important in specific settings (peri-partum or in case of severe triggering diseases or major surgeries) where the timing of treatment may be crucial for a better outcome or even for surviving this life-threatening disease.

The GeneReviews' webpage on "Genetic atypical hemolytic-uremic syndrome" (Noris et al, 2021) states that "The diagnosis of genetic aHUS is established in a proband with aHUS by identification of a pathogenic variant(s) in one or more of the genes known to be associated with genetic aHUS. The genes associated with genetic aHUS include C3, CD46 (MCP), CFB, CFH, CFHR1, CFHR3, CFHR4, CFHR5, CFI, DGKE, THBD, and VTN".

#### Versiti Autosomal Dominant Thrombocytopenia Panel

The Versiti Autosomal Dominant Thrombocytopenia Panel entails genomic sequence analysis in blood, bone marrow, buccal swab, or amniotic fluid of 22 genes (ACTB, ACTN1, ANKRD26, CDC42, CYCS, DIAPH1, ETV6, FLI1, GFI1B, GP1BA, GP1BB, GP9, HOXA11, ITGA2B, ITGB3, MECOM, MYH9, RUNX1, SLFN14, SRC, STIM1, TUBB1) associated with thrombocytopenia, specifically inherited in an autosomal dominant manner.

Chojnowski and colleagues (2020) noted that inherited thrombocytopenias (IT) comprise a highly heterogeneous group of rare hemostatic disorders that vary in terms of degree of thrombocytopenia, platelet size, pattern of inheritance and clinical course. Due to difficult diagnosis and frequently mild clinical phenotype,

these disorders are often under-diagnosed. These investigators provided clinical and laboratory characteristics of the most important types of IT. Diagnostic management includes genetic testing with NGS method.

Palma-Barqueros et al (2021) state that inherited platelet disorders (IPDs), affecting either platelet count or platelet functions, comprise a heterogenous group of about 60 rare diseases caused by molecular anomalies in many culprit genes. Their clinical relevance is highly variable according to the specific disease and even within the same type, ranging from almost negligible to life-threatening. The authors state that unlike the progress in diagnosis, there have been no major advances in the clinical management of IPDs. Educational and preventive measures, few hemostatic drugs, platelet transfusions, thrombopoietin receptor agonists, and in life-threatening IPDs, allogeneic hematopoietic stem cell transplantation are therapeutic possibilities. Gene therapy may be a future option.

In an UpToDate review of "Approach to the child with unexplained thrombocytopenia", Neunert (2022) states that "In many cases, the cause of thrombocytopenia can be determined based on the history, physical examination, and initial laboratory testing (CBC and blood smear)". However, additional testing may be useful in determining the cause of thrombocytopenia if it remains uncertain after initial evaluation. "Patients with giant platelets and/or family history of thrombocytopenia should undergo testing for inherited platelet disorders, including MYH9-related disorder and Bernard-Soulier syndrome. Many of the inherited thrombocytopenias can be identified by genetic testing either with a targeted panel or single-gene testing if a particular diagnosis is suspected."

In an UpToDate review of "Inherited platelet function disorders (IPFDs)", Lowe and Hayward (2023) state that some IPFDs are considered autosomal dominant, meaning offspring have a 50% chance of inheriting the gene variant associated with the disease. The authors discuss various autosomal dominant conditions (e.g., Bernard-Soulier syndrome, GP9, GP1BA, or GP1BB; Gray platelet syndrome, GFI1B; MYH9-related disease; Paris-Trousseau syndrome, FLI1) for which several are considered rare. The authors also include other IPFDs associated with thrombocytopenia (e.g., ANKRD26-related, TUBB1-related), as well as some conditions that may have a normal platelet count (e.g., ETV6-related thrombocytopenia). The authors state that genetic testing can be confirmatory and can facilitate testing of first-degree relatives if symptomatic. Moreover, aggregometry and genetic testing should be ordered by a hematologist or other hemostasis expert.

#### Versiti Coagulation Disorders Panel

The Versiti Coagulation Disorders Panel entails genomic sequence analysis in blood, buccal swab, or amniotic fluid for detection of germline variants in 20 genes (F10, F11, F13A1, F13B, F2, F5, F7, F8, F9, FGA, FGB, FGG, GGCX, LMAN1, MCFD2, SERPINA1, SERPINE1, SERPINF2, VKORC1, and VWF) associated with coagulation disorders. It can be used when patient's history suggests multiple coagulation disorders, or for identification of carriers with family history of unspecified bleeding disorders to provide accurate reproductive risk assessment and genetic counseling.

Dardik and associates (2021) stated that hemophilia A (HA) is an X-linked bleeding disorder caused by factor VIII (FVIII) deficiency or dysfunction due to F8 gene mutations. HA carriers are usually asymptomatic because their FVIII levels correspond to approximately 50 % of the concentration found in healthy individuals. However, in rare cases, a carrier may exhibit symptoms of moderate-to-severe HA primarily due to skewed inactivation of her non-hemophilic X chromosome. These investigators examined X-chromosome inactivation (XCI) patterns in HA carriers, with special emphasis on 3 karyotypically normal HA carriers presenting with moderate-to-severe HA phenotype due to skewed XCI, in an

attempt to elucidate the molecular mechanism underlying skewed XCI in these symptomatic HA carriers. The study was based on the hypothesis that the presence of a pathogenic mutation on the non-hemophilic X chromosome is the cause of extreme inactivation of that X chromosome. XCI patterns were studied by PCR analysis of the CAG repeat region in the HUMARA gene. HA carriers that demonstrated skewed XCI were further studied by whole-exome sequencing (WES) followed by X chromosome-targeted bio-informatic analysis. All 3 HA carriers presenting with the moderate-to-severe HA phenotype due to skewed XCI were found to carry pathogenic mutations on their non-hemophilic X chromosomes.

Patient 1 was diagnosed with a frameshift mutation in the PGK1 gene that was associated with familial XCI skewing in 3 generations. Patient 2 was diagnosed with a missense mutation in the SYTL4 gene that was associated with familial XCI skewing in 2 generations. Patient 3 was diagnosed with a nonsense mutation in the NKAP gene that was associated with familial XCI skewing in 2 generations. The authors concluded that these findings indicated that the main reason for skewed XCI in their female HA patients was negative selection against cells with a disadvantage caused by an additional deleterious mutation on the silenced X chromosome; thereby complicating the phenotype of a monogenic X-linked disease. Based on the findings of this study, these researchers are currently offering the X inactivation test to symptomatic hemophilia carriers and plan to expand this approach to symptomatic carriers of other X-linked diseases, which can be further used in pregnancy planning.

James and colleagues (2021) noted that von Willebrand disease (VWD) is the most common inherited bleeding disorder known in humans. Accurate and timely diagnosis presents numerous challenges. These investigators presented evidence-based guidelines of the American Society of Hematology (ASH), the International Society on Thrombosis and Hemostasis (ISTH), the National Hemophelia Foundation (NHF), and the World Federation of Hemophilia (WFH) to support patients, clinicians, and other health care professionals in their decision-making regarding the diagnosis of VWD. ASH, ISTH, NHF, and WFH established a multi-disciplinary guideline panel that included 4 patient representatives and was balanced to minimize potential bias from conflicts of interest. The Outcomes and Implementation Research Unit at the University of Kansas Medical Center (KUMC) supported the guideline-development process, including performing or updating systematic evidence reviews up to January 8, 2020. The panel prioritized clinical questions and outcomes according to their importance for clinicians and patients. The panel used the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach, including GRADE Evidence-to-Decision frameworks, to evaluate evidence and make recommendations, which were subsequently subject to public comment. The panel agreed on 11 recommendations.

- For Recommendation 10, the panel suggested targeted genetic testing over low-dose ristocetin-induced platelet agglutination (RIPA) to diagnose type 2B VWD for patients suspected of type 2A or 2B in need of additional testing (conditional recommendation based on low certainty in the evidence from diagnostic accuracy studies).
- For Recommendation 11, the panel suggested using either VWF FVIII binding (VWF:FVIIIB) or targeted genetic testing (when available) for patients with suspected type 2N VWD in need of additional testing (conditional recommendation based on low certainty in the evidence from diagnostic accuracy).

An UpToDate review on "Approach to the adult with a suspected bleeding disorder" (Ma, 2021) states that "Genetic testing for known platelet function disorders (e.g., using a gene panel) is becoming more widely available and may be reasonable in certain individuals with a suspected platelet disorder of genetic origin". However, genetic testing is not listed in the "Summary and Recommendations" section of this UTD review.

Furthermore, an UpToDate review on “Approach to the child with bleeding symptoms” O’Brien, 2021) does not mention genetic testing as a diagnostic tool.

The specific genetic tests one would need would depend on which clotting factors are deficient, rather than testing for all coagulation disorders.

#### Versiti Comprehensive Bleeding Disorder Panel

The Versiti Comprehensive Bleeding Disorder Panel entails genomic sequence analysis in blood, buccal swab, or amniotic fluid of 66 genes and one targeted variant by aCGH (ACVRL1, ANO6, AP3B1, AP3D1, ARPC1B, BLOC1S3, BLOC1S6(HPS9), DTNBP1(HPS7), ENG, EPHB4, F2, F5, F7, F8, F9, F10, F11, F13A1, F13B, FERMT3, FGA, FGB, FGG, FLI1, FLNA, FYB1(FYB), GATA1, GDF2, GFI1B, GGCX, GP1BA, GP1BB, GP6, GP9, HPS1, HPS3, HPS4, HPS5, HPS6, ITGA2B, ITGB3, KDSR, LMAN1, LYST, MCFD2, NBEA, NBEAL2, P2RY12, PLA2G4A, PLAU, PRKACG, RASA1, RASGRP2, RUNX1, SERPINA1, SERPINE1, SERPINF2, SLFN14, SMAD4, SRC, STIM1, TBXA2R, TBXAS1, VIPAS39, VKORC1, VPS33B, VWF) associated with bleeding due to disorders of coagulation, platelet function, and/or hereditary hemorrhagic telangiectasia (HHT).

Rodeghiero and colleagues (2019) noted that healthy subjects frequently report minor bleedings that are frequently “background noise” of normality rather than a true disorder. Nevertheless, unexpected or unusual bleeding may be alarming; therefore, the distinction between normal and pathologic bleeding is critical. Understanding the underlying pathologic mechanism in patients with an excessive bleeding is essential for their counseling and treatment. Most of these patients with significant bleeding will result affected by non-severe inherited bleeding disorders (BD), collectively denominated mild or moderate BD for their relatively benign course. Unfortunately, practical recommendations for the management of these disorders are still lacking due to the current state of fragmented knowledge of pathophysiology and lack of a systematic diagnostic approach. To address this gap, an International Working Group (IWG) was established by the European Hematology Association (EHA) to develop consensus-based guidelines on these disorders. The IWG agreed that grouping these disorders by their clinical phenotype under the single category of mild-to-moderate bleeding disorders (MBD) reflects current clinical practice and will facilitate a systematic diagnostic approach. Based on standardized and harmonized definitions a conceptual unified framework was proposed to distinguish normal subjects from affected patients. The IWG proposed a provisional comprehensive patient-centered initial diagnostic approach that will result in classification of MBD into distinct clinical-pathological entities under the over-arching principle of clinical utility for the individual patient. The authors presented a general overview of the global management of patients with MBD, this conceptual framework will be adopted and validated in the evidence-based, disease-specific guidelines under development by the IWG. These researchers concluded that in the absence of general evidence-based outcomes on which to make operative decisions and in the lack of standardized and harmonized definitions, the IWG worked to maximize consensus by proposing clinically relevant definitions, and well-grounded objectives for the investigation of subjects suspected of having MBD. They stated that the strength of the proposal lies in the unanimous panel consensus. This construct has several purposes. First, it proposes a general diagnostic approach to distinguish normal subjects from individuals with a pathologic bleeding tendency, primarily based on a standardized quantitative assessment of the bleeding history. Second, a multi-stage laboratory approach is proposed for patients with a qualifying phenotype, from standard screening to more specific laboratory or genetic studies. This approach awaits confirmation from the analysis of the subsequent disease-specific evidence-based guidelines. However, the IWG recognizes that some individuals will ultimately not receive a specific diagnosis (bleeders of unknown cause [BUC]), despite a qualifying bleeding

phenotype and a full investigation. The IWG proposes this construct to serve as a guide for future development of guidelines specific for distinct MBDs, under the over-arching principle of clinical utility.

Almazni and associates (2020) stated that inherited bleeding disorders (IBDs) comprise an extremely heterogeneous group of diseases that reflect abnormalities of blood vessels, coagulation proteins, and platelets. Previously the UK-GAPP study has used WES in combination with deep platelet phenotyping to identify pathogenic genetic variants in both known and novel genes in approximately 40 % of the patients. To examine the remaining "unknown" cohort and improve this detection rate, these researchers employed an IBD-specific gene panel of 119 genes using the Congenica Clinical Interpretation Platform to detect both SNVs and CNVs in 126 patients. A total of 135 different heterozygous variants in genes implicated in bleeding disorders were identified. Of which, 22 were classified pathogenic, 26 likely pathogenic, and the remaining were of uncertain significance. There were marked differences in the number of reported variants in individuals between the 4 patient groups: platelet count (35), platelet function (43), combined platelet count and function (59), and normal count (17). Furthermore, these investigators reported 3 novel CNVs not previously detected. They showed that a combined SNV/CNV analysis using the Congenica platform not only improved detection rates for IBDs, suggesting that such an approach can be used to other genetic disorders where there is a high degree of heterogeneity. Moreover, these researchers stated that to their knowledge, this was one of the first studies, albeit in a research setting, to implement this software for both SNV and CNV analysis. These investigators see this as a leap forward in the ability to classify highly complex disorders with a high degree of heterogeneity within the wider scientific community providing concise and definitive diagnosis for patients.

#### Versiti Comprehensive Platelet Disorder Panel

The Versiti Comprehensive Platelet Disorder Panel is a genetic test used to detect germline variants in 63 genes (ABCG5, ABCG8, ACTB, ACTN1, ANKRD26, ANO6, AP3B1, AP3D1, ARPC1B, BLOC1S3, BLOC1S6(HPS9), CDC42, CYCS, DIAPH1, DTNBP1(HPS7), ETV6, FERMT3, FLI1, FLNA, FYB1(FYB), GATA1, GFI1B, GNE, GP1BA, GP1BB, GP6, GP9, HOXA11, HPS1, HPS3, HPS4, HPS5, HPS6, ITGA2B, ITGB3, KDSR, LYST, MECOM, MYH9, MPIG6B, MPL, NBEA, NBEAL2, P2RY12, PLA2G4A, PLAU, PRKACG, RASGRP2, RBM8A, RNU4ATAC, RUNX1, SLFN14, SRC, STIM1, STXBP2, TBXA2R, TBXAS1, THPO, TUBB1, VIPAS39, VPS33B, WAS, WIPF1) associated with platelet function disorders and/or inherited thrombocytopenia.

Thrombocytopenia is associated with a variety of conditions that cannot be classified easily or are associated with more than one pathologic mechanism. Genetic disorders can result in isolated thrombocytopenia or thrombocytopenia associated with other clinical findings (Fernandes, 2023).

Almazni et al (2019) discuss genes and genetic variant which may be associated with bleeding in persons with inherited thrombocytopenia (IT). The authors state that IT results from genetic mutations in genes implicated in megakaryocyte differentiation and/or platelet formation and clearance. The identification of the underlying causative gene of IT is challenging given the high degree of heterogeneity, but important due to the presence of various clinical presentations and prognosis, where some defects can lead to hematological malignancies. To date, 40 genes and their mutations have been implicated to cause many different forms of inherited thrombocytopenia. Nevertheless, despite this advancement in the diagnosis of IT, the molecular mechanism underlying IT in some patients remains unexplained. "Targeted NGS platforms can be efficiently applied to determine the causative genes of IT. As the molecular basis of ITs remain unknown in many patients, WGS or WES may be required which improves the knowledge of ITs at the molecular level. Several national and international consortia have

adopted these approaches to identify disease-causing genes associated with IT. The genes SLFN14, FYB, STIM1, GFI1b, and ETV6 are some examples of causative genes detected by these approaches. The results obtained by HTS [high-throughput sequencing] improves the understanding of the functional role in some causative genes, whose function in platelet production was previously unknown." The authors state that these techniques will bring substantial benefits to improve understanding of the molecular mechanisms in megakaryocyte and platelet biogenesis; however, distinguishing pathogenic variants from non-pathogenic variants often requires complex functional and cell line studies to prove causality.

#### Versiti Congenital Neutropenia Panel

The Versiti Congenital Neutropenia Panel is a genetic test used to detect germline variants in 35 genes (AK2, AP3B1, AP3D1, BTK, CLPB, CSF3R, CXCR2, CXCR4, EFL1, ELANE, G6PC3, GATA1, GATA2, GFI1, GINS1, HAX1, JAGN1, LYST, RAC2, SBDS, SLC37A4, SMARCD2, SRP19, SRP54, SRP68, SRP72, SRPRA, TAFazzin, TCIRG1, USB1, VPS13B, VPS45, WAS, WDR1, WIPF1) associated with severe congenital neutropenia (SCN), including cyclic neutropenia, non-syndromic neutropenia and syndromic neutropenia with non-hematological manifestations. Test samples include whole blood, bone marrow, buccal swab, and amniotic fluid.

Bonilla et al (2015) state that "Cyclic neutropenia is also referred to as SCN type 1 (SCN1) (elastase, neutrophil expressed [ELANE] defect) and can be cyclic or persistent. SCN2 is caused by defects of growth factor independent 1 transcription repressor (GFI1); Kostmann syndrome (also SCN3) is caused by defects in HCLS1-associated protein X-1 (HAX1); SCN4 results from defects in glucose 6 phosphatase, catalytic, 3 (G6PC3); and SCN5 arises from defects in vacuolar protein sorting 45 homolog (VPS45). Rare patients can have the WAS variant X-linked neutropenia". Additional genetic lesions have been identified in persons with various syndromes in which neutropenia is a component, such as, glycogen storage disease caused by mutations in the SLC37A4 gene, Barth syndrome due to the TAZ gene, Cohen syndrome due to the VPS13B gene, and the syndrome of poikiloderma with neutropenia, which arises from mutations in the USB1 gene. The authors state that "additional genetic lesions should be investigated in patients with clinical and laboratory features consistent with neutrophil defects who are not found to have any of the disorders listed previously".

In a 2018 GeneReviews discussion by Dale and Makaryan on ELANE-related neutropenia, the authors state that molecular genetic testing approaches can include single-gene testing or use of a multigene panel that includes ELANE and other genes of interest that are included in the differential diagnosis (e.g., Kostmann disease [HAX1], nonsyndromic SCN or SCN4 [G6PC3], GFI1-related SCN, genes associated with Shwachman-Diamond syndrome, Barth syndrome, Wiskott-Aldrich syndrome).

Furutani et al (2019) opine on the "practical guide to germline genetic testing for neutropenia". The authors state that persistent symptomatic or severe neutropenia (ANC <500 cells/ $\mu$ L) without an apparent cause, or neutropenia along physical anomalies or medical comorbidities suggestive of a genetic neutropenia disorder, a family history suggestive of a neutropenia syndrome, or a personal or family history of MDS or acute myeloid leukemia (AML) should prompt consideration of an inherited syndrome. However, there are various testing platforms in which advantages and disadvantages of each approach must be considered when selecting a testing method (i.e., single gene analysis, targeted multigene panel, whole exome sequencing, or whole genome sequencing). For example, the utility of any targeted gene panel is dependent on (i) the specific set of genes included, (ii) the coverage of coding regions and pathogenic noncoding regions, and (iii) the post-sequencing analysis. The authors acknowledge that there is a "there is a paucity of data regarding the phenotypic spectrum and natural history of genetic

neutropenias, and neutropenia registries and consortia continue to be instrumental for the study of congenital neutropenia syndromes". Moreover, "clinical utility of genetic testing and the ease of interpreting results will depend on the development of publicly accessible, regularly updated databases with expert annotation of genetic variants".

McNulty et al (2020) state that the majority of autosomal-dominant cases of severe congenital neutropenia (SCN) are attributable to mutations in ELANE gene. Many other genes have also been implicated, either alone or in combination; however, the genetic cause(s) of 30% to 50% of SCN cases remains unknown.

Lazzareschi and colleagues (2022) review the assessment and management of congenital neutropenia (CN) in children. The authors state that ELANE mutations have been found in 35-to-63% of cases with severe congenital neutropenia (SCN) (also known as Kostmann syndrome). In addition, there are other gene mutations associated with CN (e.g., HAX1, G6PC3, GFI1, WAS). The authors state that "A proband with a suggestive clinical scenery that recurs over time requires genetic testing, and diagnosis follows the identification of one heterozygous pathogenic variant in the ELANE gene. Testing a panel of genes rather than a single gene may be useful since distinct genetic disorders can be associated with variably cycling neutrophil counts. The differential diagnosis between severe congenital neutropenia and cyclic neutropenia is important, as the severity of infections may be higher in the first and because of the risk of developing potentially life-threatening diseases, such as myelodysplastic syndrome and acute myeloid leukemia. Cyclic neutropenia is usually not associated with malignant transformation to hematologic cancer, except for very few cases."

An UpToDate review on "Congenital neutropenia" (Coates, 2023) states that the "term congenital neutropenia primarily refers to severe congenital neutropenia (SCN), with the Kostmann Syndrome (HAX1 mutation) as one subtype. In a broader sense, cyclic neutropenia and Shwachman-Diamond syndrome (SDS) are also included, although these disorders are usually referred to directly by their specific names". Affected patients usually have isolated neutropenia with an ANC less than 500/microL. "Bone marrow aspiration can be helpful in the differential diagnosis of congenital neutropenia. Bone marrow examination in SCN characteristically shows normal or somewhat decreased cellularity with an early myeloid "arrest" at the promyelocyte/myelocyte stage, often with atypical nuclei and cytoplasmic vacuolization. This bone marrow morphology is seen most typically in SCN due to mutations in ELANE, HAX1, WASP, G6PC3, and G-CSF receptors." However, "Mutations in more than 20 genes, including G6PC3, GFI1, SBDS, JAGN1, SRP54, and DNAJC21 have been described, but the genetic basis is not defined in approximately one-quarter of cases. The pathogenesis of SCN from a historical and molecular basis has been reviewed, delineating the progression in our understanding of SCN in parallel with advances in understanding of the process of granulocyte differentiation." The author states that "The diagnosis of the congenital neutropenias may be guided by the presence of associated clinical features, although the final diagnosis rests on identification of the gene mutation and results of the bone marrow examination."

European guidelines on diagnosis and management of neutropenia (Fioredda et al, 2023) recommend genetic testing in patients with congenital neutropenia (CN). The authors state that following negative results of first-level investigations (e.g., CBCs, liver/kidney function), all patients with severe chronic neutropenia and recurrent infections and/or family history of severe neutropenia and typical anomalies should undergo genetic work-up either by single-gene Sanger sequencing or by multigene next generation sequencing (NGS) methods. Sanger sequencing of ELANE (mutated in ~45% of patients with severe CN) is recommended as the first approach to genetic diagnosis of typical cases. However, family history or clinical findings may suggest another specific neutropenia-associated gene to be sequenced. The

authors report that severe congenital neutropenia is associated with ELANE, CSF3R, CXCR2, and WAS genes of inheritance, and that "genetic testing and analysis of CN patients is important to confirm diagnosis, to estimate the relative risk of late complications such as MDS/AML, and to offer genetic counseling to affected patients and family members". The authors state that a "targeted NGS panel including all genes known to be mutated in CN (>30) is a reasonable first step that provides uniform sequencing coverage for all genes of interest and requires simpler bioinformatic analysis. The choice of genes within the panel should include not only all those that strictly cause neutropenia when mutated but also genes resulting in diseases in which neutropenia is a secondary feature (immunodeficiency/immune dysregulation, metabolic and nutritional deficiency, and other BMF syndromes)".

#### Versiti Fibrinolytic Disorder Panel

The Versiti Fibrinolytic Disorder Panel is a genetic test used to detect germline variants in 8 genes (F13A1, F13B, FGA, FGB, FGG, SERPINA1, SERPINE1, SERPINF2) and one targeted variant associated with delayed bleeding due to hyperfibrinolysis.

An UpToDate review in "Thrombotic and hemorrhagic disorders due to abnormal fibrinolysis" (Fay, 2024) states that bleeding phenotypes may be caused by hyperfibrinolysis. Primary hyperfibrinolytic conditions are rare and are due to excessive production of plasminogen activators or decreased production of fibrinolysis inhibitors. In persons with abnormal bleeding, initial laboratory testing should assess for defects within the coagulation system or platelets. A congenital or acquired defect of fibrinolysis should be considered if a disorder of coagulation or platelets is not found and bleeding is consistent with a fibrinolytic disorder. However, "congenital disorders of fibrinolysis are very rare. The prevalence has not been defined, as publications of these abnormalities generally involve reports of isolated cases or extended pedigrees".

#### Versiti Inherited Thrombocytopenia Panel

The Versiti Inherited Thrombocytopenia Panel entails genomic sequence analysis in blood, bone marrow, buccal swab, or amniotic fluid of 42 genes (ABCG5, ABCG8, ACTB, ACTN1, ANKRD26, ARPC1B, CDC42, CYCS, DIAPH1, ETV6, FLI1, FLNA, FYB1(FYB), GATA1, GFI1B, GNE, GP1BA, GP1BB, GP9, HOXA11, ITGA2B, ITGB3, KDSR, MECOM, MPIG6B, MPL, MYH9, NBEAL2, PRKACG, RBM8A, RNU4ATAC, RUNX1, SLFN14, SRC, STIM1, STXBP2, THPO, TUBB1, VIPAS39, VPS33B, WAS, WIPF1) associated with inherited thrombocytopenia.

UpToDate reviews on "Approach to the child with unexplained thrombocytopenia" (Despotovic, 2021a), "Causes of thrombocytopenia in children" (Despotovic, 2021b), and "Diagnostic approach to the adult with unexplained thrombocytopenia" (Arnold and Cuker, 2021) do not mention the use of comprehensive genetic panel as a management tool.

#### Versiti Platelet Function Disorder Panel

The Versiti Platelet Function Disorder Panel is a genetic test used for the detection of germline variants in 41 genes (ANO6, AP3B1, AP3D1, ARPC1B, BLOC1S3, BLOC1S6(HPS9), DTNBP1(HPS7), FERMT3, FLI1, FLNA, FYB1(FYB), GATA1, GFI1B, GP1BA, GP1BB, GP6, GP9, HPS1, HPS3, HPS4, HPS5, HPS6, ITGA2B, ITGB3, KDSR, LYST, NBEA, NBEAL2, P2RY12, PLA2G4A, PLAU, PRKACG, RASGRP2, RUNX1, SLFN14, SRC, STIM1, TBXA2R, TBXAS1, VIPAS39, VPS33B) associated with inherited platelet dysfunction.

Palma-Barqueros et al (2021) state that inherited platelet disorders (IPDs), affecting either platelet count or platelet functions, comprise a heterogenous group of about 60 rare diseases caused by molecular anomalies in many culprit genes. Their clinical relevance is highly variable according to the specific disease and even

within the same type, ranging from almost negligible to life-threatening. The authors state that unlike the progress in diagnosis, there have been no major advances in the clinical management of IPDs. Educational and preventive measures, few hemostatic drugs, platelet transfusions, thrombopoietin receptor agonists, and in life-threatening IPDs, allogeneic hematopoietic stem cell transplantation are therapeutic possibilities. Gene therapy may be a future option.

In an UpToDate review of "Inherited platelet function disorders (IPFDs)", Lowe and Hayward (2023) state that many genes have been implicated in inherited disorders of platelet function. Some IPFDs are considered extremely rare recessive disorders (with less than 50 patients worldwide), including those caused by pathogenic variants in SLFN14, P2RY12, FYB1, and EPHB2. Many of the more commonly encountered IPFDs that increase risks for bleeding have an unknown genetic cause.

#### Versiti Red Cell Genotyping Panel

The Versiti Red Cell Genotyping Panel includes 72 PCR-hybridization probes which are used in 36 polymerase chain reactions (PCR) to identify the alleles associated with 44 blood group antigens. This is considered a genetic test used to evaluate immune-mediated red cell destruction from a whole blood sample.

In an UpToDate review on "Overview of hemolytic anemias in children", DeBaun (2022) states that intrinsic hemolytic anemias include hemoglobinopathies, erythrocyte membrane defects, and enzyme deficiencies. Confirmatory testing in hemolytic anemia is based on laboratory findings, such as increased indirect bilirubin and LDH, decreased haptoglobin, reticulocytosis, and abnormalities on the peripheral smear (e.g., spherocytes, schistocytes, increased polychromasia). Further testing is guided by the clinical history, initial lab results, and peripheral smear findings. The author states that additional tests may include: direct antiglobulin test (DAT), G6PD testing if deficiency is suspected, testing for other erythrocyte enzymopathies (e.g., PK deficiency), eosin-5-maleimide (EMA) binding/RBC band 3 testing if an erythrocyte membrane defect is suspected, or hemoglobin analysis if a hemoglobinopathy is suspected.

#### Versiti Thrombosis Panel

The Versiti Thrombosis Panel is a genetic test used for the detection of germline variants in 12 genes and 2 targeted variants (ADAMTS13, F2, F5, FGA, FGB, FGG, HRG, KNG1, PLG, PROC, PROS1, SERPINC1, SERPIND1, THBD) associated with an increased risk for developing venous thromboembolism.

In an UpToDate review on "Overview of the causes of venous thrombosis", Bauer and Lip (2023) state that "Inherited thrombophilia is a genetic tendency to venous thromboembolism. The most frequent causes of an inherited (primary) hypercoagulable state are the factor V Leiden mutation and the prothrombin gene mutation, which together account for 50 to 60 percent of cases. Defects in protein S, protein C, and antithrombin (formerly known as antithrombin III) account for most of the remaining cases". The authors state that a number of other genetic variants associated with an increased risk for a first episode of venous thromboembolism (VTE) have been found by candidate and genome-wide screens with odds ratios generally less than 1.5. "Further study is required before these data can be useful clinically."

#### Very Long Chain AcylCoA Dehydrogenase Deficiency (VLCADD)

An UpToDate review on "Newborn screening" (Sielski, 2013) states that "MS-MS [tandem mass spectrometry] detects more cases of inborn errors of metabolism than clinical diagnosis. In a study from New South Wales and the Australian Capital Territory, Australia, the prevalence of 31 inborn errors of metabolism affecting the urea cycle, amino acids (excluding PKU), organic acids, and fatty acid oxidation

detected by MS-MS in 1998 to 2002 was 15.7 per 100,000 births, compared to 8.6 to 9.5 per 100,000 births in the four four-year cohorts preceding expanded screening. The increased rate of diagnosis was most apparent for the medium-chain and short-chain acyl-Co-A dehydrogenase deficiencies. Whether all children with disorders detected by MS-MS would have become symptomatic is uncertain .... The American Academy of Pediatrics has developed newborn screening fact sheets for 12 disorders, biotinidase deficiency, congenital adrenal hyperplasia, congenital hearing loss, congenital hypothyroidism, cystic fibrosis, galactosemia, homocystinuria, maple syrup urine disease, medium-chain acyl-coenzyme A dehydrogenase deficiency, PKU, sickle cell disease and other hemoglobinopathies, and tyrosinemia .... With the use of tandem mass spectrometry (MS-MS), the prevalence of a confirmed metabolic disorder detected by newborn screening is 1:4000 live births (about 12,500 diagnoses each year) in the United States. The most commonly diagnosed conditions are hearing loss, primary congenital hypothyroidism, cystic fibrosis, sickle cell disease, and medium-chain acyl-CoA dehydrogenase deficiency". This review does not mention very long chain acylCoA dehydrogenase deficiency.

#### Whole Exome Sequencing for Evaluation of a Fetus During Pregnancy

The American College of Obstetricians and Gynecologists and the Society for Maternal Fetal Medicine in their joint Committee Opinion 682: Microarrays and Next-Generation Sequencing Technology The Use of Advanced Genetic Diagnostic Tools in Obstetrics and Gynecology, reaffirmed 2023 that states: ...." the College and the Society for Maternal-Fetal Medicine currently do not recommend whole-exome sequencing for routine use in prenatal diagnosis. In select circumstances (recurrent or lethal fetal anomalies in which other approaches have been noninformative), whole-exome sequencing may be considered as a diagnostic tool, but only after other appropriate testing has been noninformative and after extensive counseling by an obstetrician-gynecologist or other health care provider with genetics expertise who is familiar with these new technologies and their limitations".

A review of the literature finds that at this time the only fetal condition that has evidence to meet the outlined standard is non-immune hydrops fetalis when chromosome microarray is non-informative. Sparks et al (2020) discuss exome sequencing for prenatal diagnosis in nonimmune hydrops fetalis (NIHF) evaluated a series of 127 consecutive unexplained cases of NIHF that were defined by the presence of fetal ascites, pleural or pericardial effusions, skin edema, cystic hygroma, increased nuchal translucency, or a combination of these conditions. Records were obtained and reviewed for medical and family history, previous genetic testing, detailed prenatal and postnatal phenotyping, and pregnancy outcomes. The primary outcome was the diagnostic yield of exome sequencing for detecting genetic variants that were classified as either pathogenic or likely pathogenic according to the criteria of the American College of Medical Genetics and Genomics. Secondary outcomes were the percentage of cases associated with specific genetic disorders and the proportion of variants that were inherited. In 37 of the 127 cases (29%), they identified diagnostic genetic variants, including those for disorders affecting the RAS-MAPK cell-signaling pathway (known as RASopathies) (30% of the genetic diagnoses); inborn errors of metabolism and musculoskeletal disorders (11% each); lymphatic, neurodevelopmental, cardiovascular, and hematologic disorders (8% each); and others. Prognoses ranged from a relatively mild outcome to death during the perinatal period. Overall, 68% of the cases (25 of 37) with diagnostic variants were autosomal dominant (of which 12% were inherited and 88% were de novo), 27% (10 of 37) were autosomal recessive (of which 95% were inherited and 5% were de novo), 1 was inherited X-linked recessive, and 1 was of uncertain inheritance. The authors identified potentially diagnostic variants in an additional 12 cases. The authors concluded that in this case series of 127 fetuses with unexplained NIHF, they identified a diagnostic genetic variant in approximately one third of the cases. However, this study is not

without limitations. Although the participants were geographically diverse, more than half identified themselves as White. Among cases with increased nuchal translucency or cystic hygroma, many later showed additional fluid collections or concurrent anomalies, and the diagnostic yield for isolated increased nuchal translucency or cystic hygroma cases was low. Further studies are warranted to determine the usefulness of exome sequencing for isolated increased nuchal translucency or cystic hygroma, since the risk of subsequent pathological conditions is unknown. There are limitations of prenatal phenotyping, especially in early gestation. Because accurate genetic variant classification relies in part on phenotypic fit, it is possible that disease-causing variants were missed or incorrectly classified. Although some copy-number variants and intronic variants were detected, exome sequencing is not designed to routinely detect these genomic changes. Future studies in which whole-genome sequencing or functional assays are used to evaluate additional genomic changes when exome sequencing shows normal results are warranted. Finally, it is possible that providers and patients motivated by the desire for genomic information were more likely to participate, potentially affecting the generalization of their results.

Zhou et al (2021) performed a retrospective analysis to review the value of exome sequencing in diagnosis and management of recurrent NIHF. The purpose of the study was to use exome sequencing (ES) to study the contribution of single-gene disorders to recurrent NIHF and retrospectively evaluate the value of genetic diagnosis on prenatal management and pregnancy outcome. From January 2012 to October 2018, a cohort of 28 fetuses with recurrent NIHF was analyzed by trio ES. Fetuses with immune hydrops, non-genetic factors (including infection, etc.), karyotype, or CNV abnormalities were excluded. Variants were interpreted based on ACMG/AMP guidelines. Fetal therapy was performed on seven fetuses. Of the 28 fetuses, 10 (36%) were found to carry causal genetic variants (pathogenic or likely pathogenic) in eight genes (GBA, GUSB, GBE1, RAPSN, FOXC2, PIEZO1, LZTR1, and FOXP3). Five (18%) fetuses had variant(s) of uncertain significance (VUS). Of the 10 fetuses with definitive molecular diagnosis, five (50%) were diagnosed with inborn errors of metabolism. Among the seven fetuses who received fetal therapy, two had definitive molecular diagnosis and resulted in neonatal death. Among the remaining five fetuses with negative results, four had newborn survival and one had intrauterine fetal death. The authors concluded that trio ES could facilitate genetic diagnosis of recurrent NIHF and improve the prenatal management and pregnancy outcome. The authors acknowledged that the turnaround time (4–6 weeks) is relatively long for a prenatal application, and the sample size in their study is too small to systematically evaluate the profile and frequency of each single-gene disorder in NIHF. A large prospective study of the NIHF cohort will be expected in the future study.

Norton et al (2022) compared the diagnostic yield of exome sequencing with the simulated application of commercial targeted gene panels in a large cohort of fetuses with nonimmune hydrops fetalis. This was a secondary analysis of a cohort study of exome sequencing for nonimmune hydrops fetalis, in which recruitment, exome sequencing, and phenotype-driven variant analysis were completed in 127 pregnancies with features of nonimmune hydrops fetalis. An Internet search was performed to identify commercial laboratories that offer targeted gene panels for the prenatal evaluation of nonimmune hydrops fetalis or for specific disorders associated with nonimmune hydrops fetalis using the terms "non-immune hydrops fetalis," "fetal non-immune hydrops," "hydrops," "cystic hygroma," "lysosomal storage disease," "metabolic disorder," "inborn error of metabolism," "RASopathy," and "Noonan." The authors primary outcome was the proportion of all genetic variants identified through exome sequencing that would have been identified if a targeted gene panel had instead been used. The secondary outcomes were the proportion of genetic variants that would have been identified by type of targeted gene panel (general nonimmune hydrops fetalis, RASopathy, or metabolic) and the percent of variants of uncertain significance that would have been identified on the

panels, assuming 100% analytical sensitivity and specificity of panels for variants in the included genes. The authors state that exome sequencing identified a pathogenic or likely pathogenic variant in 37 of 127 cases (29%) in a total of 29 genes. A variant of uncertain significance, strongly suspected to be associated with the phenotype, was identified in another 12 cases (9%). The authors identified 7 laboratories that offer 10 relevant targeted gene panels; 6 are described as RASopathy panels, 3 as nonimmune hydrops fetalis panels, and 1 as a metabolic panel. The median number of genes included on each of these panels is 22, ranging from 11 to 148. The authors state that had a nonimmune hydrops fetalis targeted gene panel been used instead of exome sequencing, 13 to 15 of the 29 genes (45%-52%) identified in their nonimmune hydrops fetalis cohort would have been sequenced, and 19 to 24 of the pathogenic variants (51%-62%) would have been detected. The yield was predicted to be the lowest with the metabolic panel (11%) and the highest with the largest nonimmune hydrops fetalis panel (62%). The largest nonimmune hydrops fetalis targeted gene panel would have had a diagnostic yield of 18% compared with 29% with exome sequencing. The exome sequencing platform used provided 30x or more coverage for all of the exons on the commercial targeted gene panels, supporting our assumption of 100% analytical sensitivity for exome sequencing. The authors concluded that the broader coverage of exome sequencing for genetically heterogeneous disorders, such as nonimmune hydrops fetalis, made it a superior alternative to targeted gene panel testing. The authors acknowledged limitations to their study. While exome sequencing was performed in all reported cases, the targeted gene panel results were modeled based on the genes listed on each laboratory's website with an assumption of 100% analytic sensitivity and specificity. It is not known with certainty that a targeted gene panel would detect all variants in a gene, even if that gene is included on a panel. Likewise, it is not certain that their exome would have detected all variants in the genes on each panel had a variant in one of these genes been present. Neither exome sequencing nor panels will detect all disease-causing variants, and copy number variants, indels, variants in non-coding regions, and other types of variants may not be identified with either test. Some commercial laboratories offering targeted gene panels will concurrently evaluate for copy number variants in the targeted list of genes, which is not routinely performed with exome sequencing, and was not considered in our calculations. The authors did not discuss use post mortem.

#### Whole Genome/Exome Sequencing and Genome-Wide Association Studies

Whole genome sequencing (WGS) is a laboratory test utilized to determine the arrangement (sequence) of an individual's entire genome at a single time. WGS allows the identification of mutations in the genome without having to target a gene or chromosome region based upon an individual's personal or family history. WGS may also be referred to as full genome sequencing, complete genome sequencing or entire genome sequencing.

Exome sequencing, also referred to as whole exome sequencing or WES, is an alternative to WGS. It is a laboratory test used to determine the sequence of the protein coding regions of the genome. The exome is the part of the genome that encodes protein, where roughly 85% of variants are known to contribute to diseases in humans. Exome sequencing has been proposed as a diagnostic method to identify these genetic variants in patients not diagnosed by traditional diagnostic and genetic testing approaches.

Genome-wide association studies (GWAS), also referred to as genome-wide analysis, is a method to identify genes involved in human disease by comparing the genome of individuals who have a disease or condition to the genome of individuals without the disease or condition. GWAS are performed using microarrays to search

the genome for small variations, called single nucleotide polymorphisms (SNPs, pronounced "snips"), that occur more often in individuals with a specific disorder than in those who do not have a disorder.

The American College of Medical Genetics and Genomics recommends whole exome or whole genome sequencing as a first-tier test in individuals with one or more congenital anomalies, developmental delay, and intellectual disability. The guidelines excluded isolated autism without intellectual disability or congenital malformations from their evidence review, indicating a low diagnostic yield and emerging clinical utility (Manickam et al, 2021).

Rapid whole exome and whole genome sequencing refers to prioritized testing with an average turn around time of 14 days, compared to the weeks or months associated with typical exome or genome testing. When utilized in acutely ill children who have a phenotype highly suspicious of a genetic disease where a rapid answer is necessary to best manage acute care, studies have demonstrated an average diagnostic yield of 37% and changed clinical management in 26% (Dimmock et al, 2021; Elliott et al, 2019; Kingsmore et al, 2022; Petrikis et al, 2015).

#### Whole Transcriptome Sequencing

Whole transcriptome sequencing (WTS), also known as total RNA-Seq, denotes the sequencing of RNA that has been depleted of ribosomal RNA (rRNA), which represents the majority of RNA molecules, both coding and non-coding. Total RNA consists of multiple RNA species (e.g., rRNA, precursor messenger RNA (pre-mRNA), mRNA), and several types of non-coding RNA (ncRNA), including transfer RNA (tRNA), microRNA (miRNA), and long ncRNA (lncRNA; transcripts longer than 200 nucleotides not translated into protein). The removal of rRNA in the total RNA-Seq procedure results in improved sequencing data that enables the characterization of these diverse non-rRNA species. Whole transcriptome sequencing entails a snapshot measurement of the complete complement of transcripts in a cell. By examining the whole transcriptome, researchers are able to determine global expression levels of each transcript, identify exons, introns and map their boundaries; thus, this approach reports transcript level and processing changes believed to be causative of a patient's condition. However, there is currently insufficient evidence to support the clinical value of WTS.

Jiang and co-workers (2015) stated that whole transcriptome analysis plays an essential role in deciphering genome structure and function, identifying genetic networks underlying cellular, physiological, biochemical and biological systems and establishing molecular biomarkers that respond to diseases, pathogens and environmental challenges. These investigators reviewed transcriptome analysis methods and technologies that have been employed to carry out whole transcriptome shotgun sequencing or whole transcriptome tag/target sequencing analyses. They focused on how adaptors/linkers were added to both 5' and 3' ends of mRNA molecules for cloning or PCR amplification before sequencing. The authors concluded that NGS platforms had accelerated releases of the large amounts of gene expression data. It is now time for the genome research community to assemble whole transcriptomes of all species and collect signature targets for each gene/transcript; thus, using known genes/transcripts to determine known transcriptomes directly in the future.

Walter and colleagues (2021) noted that considering the clinical and genetic characteristics, acute lymphoblastic leukemia (ALL) is a heterogeneous hematological neoplasm for which current standard diagnostics require various analyses encompassing morphology, immunophenotyping, cytogenetics, and molecular analysis of gene fusions and mutations. Thus, it would be desirable to rely on a technique and an analytical workflow that allows the simultaneous

analysis and identification of all the genetic alterations in a single approach. Moreover, based on the results with standard methods, many patients have no established abnormalities and therefore, could not further be stratified. These researchers carried out WTS and WGS in 279 ALL patients (B-cell: n = 211; T-cell: n = 68) to evaluate the accuracy of WTS, to detect relevant genetic markers, and to classify ALL patients. DNA- and RNA-based genotyping was performed to ensure correct WTS-WGS pairing. Gene expression analysis reliably assigned samples to the B Cell Precursor (BCP)-ALL or the T-ALL group. Sub-classification of BCP-ALL samples was carried out progressively, examining first the presence of chromosomal re-arrangements by the means of fusion detection. Compared to the standard methods, 97 % of the recurrent risk-stratifying fusions could be identified by WTS, assigning 76 samples to their respective entities. Furthermore, read-through fusions (indicative of CDKN2A and RB1 gene deletions) were recurrently detected in the cohort along with 57 putative novel fusions, with yet untouched diagnostic potentials. Next, CNVs were inferred from WTS data to identify relevant ploidy groups, classifying an additional of 31 samples. Lastly, gene expression profiling (GEP) detected a BCR-ABL1-like signature in 27 % of the remaining samples. These researchers demonstrated that typical genetic alterations can be identified with high accuracy, while at the same time the unbiased assessment of the transcriptome also allows the identification of potentially new targets in patients, where these genetic aberrations are absent. They stated that these findings further suggested that careful selection of the algorithms for each molecular type is beneficial for accurate sample classification. Moreover, these researchers stated that with the decrease in sequencing costs, the integration of WTS in routine diagnostics of ALL patients appears feasible, however, further investigations should require the definition of standardized quality parameters and data analysis workflows to enable reproducibility and comparability between laboratories.

The authors stated that estimating abnormalities involving the chromosome number, plays a major role in ALL classification and prognostication. While ALL with high hyper-diploidy is associated with a favorable prognosis, ALL with low hypo-diploidy shows a poor outcome. Due to the interplay of multiple regulating factors, inferring copy number changes from WTS data is challenging. In this study, the determination of ploidy groups had the highest error rate, missing 5 cases, compared to the ones from WGS, array CGH, and FISH. However, chromosomal banding analysis (CBA) missed 4 low hypo-diploid/near-triploid cases due to low in-vitro proliferation, which were identified based on WTS data and confirmed by WGS. The resolution of the applied algorithm was too low to identify the iAMP21 case, or to reliably detect single gene deletions. While in the case of iAMP21 the gene expression could be used for the classification, the same did not hold true for gene deletions. In this study, the analysis of isoforms and differential transcript usage might provide the needed insights; however, these analyses were out of the scope of this work. Furthermore, a larger set of iAMP21-positive cases is needed to prove the validity of CHAF1B and DYRK1A gene expression as biomarkers for the presence of iAMP21, since this cohort included only 1 such case.

Iacobucci and associates (2021) stated that ALL is the most successful paradigm of how risk-adapted therapy and detailed understanding of the genetic alterations driving leukemogenesis and therapeutic response may dramatically improve therapeutic outcomes, with cure rates now exceeding 90 % in children. However, ALL still represents a leading cause of cancer-related death in the young, and the outcome for older adolescents and young adults with ALL remains poor. In the last 10 years, NGS has enabled critical advances in the understanding of leukemogenesis. These include the identification of risk-associated ALL subtypes (e.g., those with re-arrangements of MEF2D, DUX4, NUTM1, ZNF384 and BCL11B; the PAX5 P80R and IKZF1 N159Y mutations; and genomic phenocopies such as Ph-like ALL) as well as the genomic basis of disease evolution. These advances have been complemented by the development of novel therapeutic approaches,

including those that are of mutation-specific, such as tyrosine kinase inhibitors, and those that are mutation-agnostic, including antibody and cellular immunotherapies, and protein degradation strategies such as proteolysis-targeting chimeras. These investigators reviewed the genetic taxonomy of ALL with a focus on clinical implications and the implementation of genomic diagnostic approaches. Moreover, these researchers noted that NGS approaches, especially WTS, have enabled several research groups the identification of a large number of novel genetic alterations. These include cryptic re-arrangements not identifiable by conventional approaches; novel subtypes that "phenocopy" established subtypes sharing similar gene expression profile but having different founding alterations; and subtypes defined by a single-point mutation.

An UpToDate review on "Pathobiology of Burkitt lymphoma" (Brown et al, 2021) states that "In a third study, whole genome and transcriptome sequencing of 4 BL cases identified 7 recurrently mutated genes. Further evaluation of these genes in an extension cohort demonstrated ID3 mutations in 36 of 53 (68 %) of patients with BL but only 6 of 47 (13 %) of other B cell lymphomas with Ig-MYC translocation. The Burkitt Lymphoma Genome Sequencing Project employs whole genome sequencing coupled with transcriptome analyses in an effort to comprehensively characterize the genetics of BL. This ongoing work has identified 22 genes with mutations that are deemed to be significant, as judged by their presence in multiple tumors and their likely disruptive effects on gene function. Newly identified mutated genes include DDX3X (which encodes a putative RNA helicase linked to cell cycle progression) and a number of chromatin factors, including chromatin remodeling factors (ARID1A), chromatin "writers" (KMT2D), and even histones (HIST1H1E). How perturbation of these factors contribute to BL pathobiology remains to be determined".

Furthermore, an UpToDate review on "Tools for genetics and genomics: Gene expression profiling" (Steiling and Christenson, 2021) states that "Platforms for profiling gene expression take advantage of increased knowledge of the sequence of the human genome and require smaller quantities of starting RNA. Current platforms for profiling gene expression include oligonucleotide arrays (microarrays) and transcriptome sequencing ... Gene expression profiling is emerging as a potential approach for the diagnosis and prognosis of complex human disease. However, a number of important barriers remain, including validation of these biomarkers in prospective multi-center studies to demonstrate their reproducibility and accuracy across multiple sites and operators".

#### X-Linked Intellectual Disability Panels

Intellectual disability (ID, formerly called mental retardation) is a developmental brain disorder commonly defined by an IQ below 70 and limitations in both intellectual functioning and adaptive behavior (Piton et al, 2013). ID can originate from environmental causes or genetic anomalies, and its incidence in children is estimated to be of 1 % to 2 %.

ID is more common in males than females in the population (the male-to-female ratio is 1.3-1.4 to 1), assumed to be due to mutations on the X chromosome. Impaired mental functioning occurs as an isolated feature or as part of many X-linked syndromes (McKusick et al, 2010). ID that is not associated with other distinguishing features is referred to as 'nonspecific' or 'nonsyndromic.'

X-linked intellectual disability (XLID) is a genetically heterogeneous disorder with more than 100 genes known to date (Tzchach et al, 2015). Fragile X syndrome remains the most common XLMR gene discovered so far (Raymond, 2006). FMR1 is a target of the unstable expansion mutation responsible for fragile X syndrome and accounts for about 1 % to 2 % of all ID cases.

Half of the known genes carrying mutations responsible for XLID are associated with syndromic forms (i.e., ID associated with defined clinical or metabolic manifestations), which facilitates the identification of causative mutations in the same gene because unrelated probands with comparable phenotypes can be more easily matched. The other half of known genes carrying mutations responsible for XLID appear to be associated with nonsyndromic or paucisyndromic forms.

Next-generation sequencing panels have been developed to identify mutations associated with XLID. However, little has been published on their analytic validity, clinical validity and clinical utility.

Tzschach et al (2015) performed targeted enrichment and next-generation sequencing of 107 XLID genes in a cohort of 150 male patients. One hundred patients had sporadic intellectual disability, and 50 patients had a family history suggestive of XLID. The investigators also analyzed a sporadic female patient with severe ID and epilepsy because she had strongly skewed X-inactivation. Target enrichment and high parallel sequencing allowed a diagnostic coverage of >10 reads for approximately 96% of all coding bases of the XLID genes at a mean coverage of 124 reads. The investigators reported finding 18 pathogenic variants in 13 XLID genes (AP1S2, ATRX, CUL4B, DLG3, IQSEC2, KDM5C, MED12, OPHN1, SLC9A6, SMC1A, UBE2A, UPF3B and ZDHHC9) among the 150 male patients. Thirteen pathogenic variants were present in the group of 50 familial patients (26%), and 5 pathogenic variants among the 100 sporadic patients (5%). Systematic gene dosage analysis for low coverage exons detected one pathogenic hemizygous deletion. An IQSEC2 nonsense variant was detected in the female ID patient, providing further evidence for a role of this gene in encephalopathy in females. The investigators noted that skewed X-inactivation was more frequently observed in mothers with pathogenic variants compared with those without known X-linked defects. The investigators concluded that the mutation rate in the cohort of sporadic patients corroborates previous estimates of 5-10% for X-chromosomal defects in male ID patients.

Piton et al (2013) used data from a large-scale sequencing project to question the implication of XLID in several of the genes proposed to be involved in XLID. The authors stated that mutations causing monogenic XLID have now been reported in over 100 genes, most of which are commonly included in XLID diagnostic gene panels. Nonetheless, the boundary between true mutations and rare non-disease-causing variants often remains elusive. The authors stated that sequencing of a large number of control X chromosomes, required for avoiding false-positive results, was not systematically possible in the past. Such information is now available thanks to large-scale sequencing projects such as the National Heart, Lung, and Blood (NHLBI) Exome Sequencing Project, which provides variation information on 10,563 X chromosomes from the general population. The authors used this NHLBI cohort to systematically reassess the implication of 106 genes proposed to be involved in monogenic forms of XLID. Based on this reassessment, the authors particularly questioned the implication in XLID of ten of them (AGTR2, MAGT1, ZNF674, SRPX2, ATP6AP2, ARHGEF6, NXF5, ZCCHC12, ZNF41, and ZNF81), in which truncating variants or previously published mutations are observed at a relatively high frequency within this cohort. The authors also highlighted 15 other genes (CCDC22, CLIC2, CNKSR2, FRMPD4, HCFC1, IGBP1, KIAA2022, KLF8, MAOA, NAA10, NLGN3, RPL10, SHROOM4, ZDHHC15, and ZNF261) for which replication studies are warranted. The authors proposed that similar reassessment of reported mutations (and genes) with the use of data from large-scale human exome sequencing would be relevant for a wide range of other genetic diseases.

MelanomaNext is a NGS panel that simultaneously analyzes 9 genes (BAP1, BRCA2, CDK4, CDKN2A, MITF, POT1, PTEN, RB1, and TP53) that supposedly are associated with increased risk for melanoma and other cancers.

An UpToDate review on “Melanoma: Clinical features and diagnosis” (Swetter and Geller, 2024) does not mention next generation sequencing / genetic testing panel as a management option.

#### Repeat Germline Genetic Testing

The American Academy of Family Physician’s Choosing Wisely Recommendation (AFP, 2023) stated that “Prior to ordering a genetic test for an inherited condition, the health care provider should ask a patient about prior genetic testing and review the medical record for previously performed genetic tests. Repeating a genetic test should be considered if the existing result is inconsistent with the individual’s clinical presentation or if the test methodology has changed and may yield a different result from the original report that could impact patient management”.

#### SF3B2 Genetic Testing for Evaluation of Hemi-Facial Microsomia

Hemi-facial microsomia is also known as cranio-facial microsomia (CFM), and Goldenhar syndrome. Timberlake et al (2021) stated that CFM is the second most common congenital facial anomaly; however, its genetic etiology remains unclear. These researchers carried out whole-exome or genome sequencing of 146 kindreds with sporadic ( $n = 138$ ) or familial ( $n = 8$ ) CFM, identifying a highly significant burden of loss of function variants in SF3B2 ( $p = 3.8 \times 10^{-10}$ ), a component of the U2 small nuclear ribonucleoprotein complex, in probands. They described 20 individuals from 7 kindreds harboring de-novo or transmitted haplo-insufficient variants in SF3B2. Probands display mandibular hypoplasia, microtia, facial and pre-auricular tags, epibulbar dermoids, lateral oral clefts in addition to skeletal as well as cardiac abnormalities. Targeted morpholino knockdown of SF3B2 in *Xenopus* resulted in disruption of cranial neural crest precursor formation and subsequent cranio-facial cartilage defects, supporting a link between spliceosome mutations and impaired neural crest development in congenital cranio-facial disease. The results established haplo-insufficient variants in SF3B2 as the most prevalent genetic cause of CFM, explaining approximately 3 % of sporadic and approximately 25 % of familial cases. The authors concluded that these findings defined SF3B2 as a novel gene responsible for CFM, showing dominant inheritance with inter- and intra-familial phenotypic variability. They recommended adding SF3B2 to cranio-facial genetic panels designed to screen patients with features of mandibulo-facial dysostosis.

StatPearls’ webpage on “Hemifacial microsomia” (Young and Spinner, 2023) did not discuss testing for SF3B2 specifically; it provided the following information:

- The heterogenous phenotypical appearance of HFM has been theorized to be caused by a combination of genetic and environmental factors that disrupt the vascularization and development of the 1st and 2nd pharyngeal arches, which form during the first 4 weeks of pregnancy.
- Genetic mutations and chromosomal abnormalities that are associated with HFM include trisomy 10p, 12p13.33 microdeletion, 22q11.2 microdeletion, large 5p deletion, and 10.7 cM on chromosome 14q32.
- Most cases are sporadic, with some studies suggesting both autosomal dominant and recessive inheritance patterns with incomplete penetrance.
- Chromosomal analysis and genetic counseling can also be offered for families with suspected genetic inheritance.

Furthermore, an UpToDate review on “Syndromes with craniofacial abnormalities” (Buchanan, 2024) does not mention SF3B2 genetic testing as a management option.

UpToDate reviews on "Clinical manifestations and diagnosis of Ehlers-Danlos syndromes" (Pauker, 2024), "Clinical manifestations and diagnosis of hypermobile Ehlers-Danlos syndrome and hypermobility spectrum disorder" (Hakim and Tofts, 2024), "Osteogenesis imperfecta: An overview" (Balasubramanian, 2024), and "Skeletal dysplasias: Specific disorders" (Bacino, 2024) do not mention genetic testing for GNE and VCP as a management option.

#### Unity Carrier Screen (BillionToOne)

The Unity Carrier Screen (BillionToOne, Inc.) is a noninvasive prenatal test (NIPT) that screens for genetic fetal conditions from a maternal blood sample. This test uses next-generation DNA sequencing and deletion analysis of HBA1, HBA2, HBB, CFTR, and SMN1 to diagnose carrier status for cystic fibrosis, spinal muscular atrophy, and hemoglobinopathies (alpha-thalassemia, beta-thalassemia, and sickle cell disease). Single nucleotide variants in the gene panel are identified by next-generation sequencing (NGS) of exons. Deletion analysis is performed by digital multiplex ligation-dependent probe amplification (MLPA). This test offers fetal risk assessment NIPT of these single-gene disorders when a carrier is identified from pregnant, maternal blood. NIPT is offered as a reflex test that analyzes cell-free DNA (cfDNA) in blood plasma (NLM, 2025).

#### Venous Thromboembolism Risk Assessment

The Lifetime Genomic Risk Assessment for venous thromboembolism (VTE) (GenomicMD, Chicago, IL) is a test that offers a microarray analysis of an individual's DNA to identify genome-wide single-nucleotide polymorphism variants, including F2 and F5 gene analysis, from an individual's blood or saliva sample and provides a polygenic risk score report for VTE.

Shi et al. (2023) conducted a retrospective analysis of prospectively collected data for cancer-associated thrombosis (CAT) patients from the UK Biobank (UKB), a population-based cohort ( $N = 70,406$ ). Patient study inclusion was limited to individuals who received a cancer diagnosis (e.g., cancer diagnosis based on self-report, inpatient diagnosis, and/or the UK cancer registry) following the initial study recruitment. CAT rate was estimated by cancer sites and inherited factors among cancer patients.

The investigators obtained genotypes for the Factor V Leiden F5 gene c.1601G>A mutation and F2 gene G20210A mutation as well as venous thromboembolism (VTE) risk-associated single nucleotide polymorphisms (SNPs) from the UK Biobank Axiom SNP array. A published polygenic score for VTE (PGSVTE) was calculated based on 1,092,045 SNPs.

The 12-month CAT rate following cancer diagnosis was 2.37% in general but varied considerably among cancer sites (i.e., 13.9% for pancreatic, 9.13% for stomach, 1% or lower for the other 15 sites). Of the 10 cancer sites classified as 'high-risk' of CAT by the National Comprehensive Cancer Network guidelines, 6 had CAT rate < 5%. By contrast, 5 cancer sites with an 'average-risk' guidelines classification had CAT rate > 5%.

An assessment of inherited risk factors showed that both known mutation carriers in F5 and/or F2 genes and PGSVTE were independently associated with increased CAT risk. While F5 and/or F2 gene mutations identified 6% of patients with high genetic-risk for CAT, adding PGSVTE identified 13% of patients at equivalent/higher genetic-risk to CAT than that of F5 and/or F2 gene mutations.

Several study limitations included the following: cancer characteristics (grade and stage), cancer treatment (surgery and chemotherapy), anticoagulation therapy is limited in the UKB; the vast majority of cancer patients in the the UKB are White and PGS<sub>VTE</sub> was developed in subjects of European ancestry, cautioning generalizability to other ancestry populations; and other known major genes for VTE (SERPINC1, PROC, and PROS1) were not included in this study.

The investigators concluded that use of a large populations-based prospective cohort in their study showed evidence that CAT rate differs considerably by cancer sites and differs from the classification of current clinical guidelines. Additionally, the study suggested that PGS<sub>VTE</sub> is independent of known monogenic genes for inherited risk assessment of CAT and can identify > 2-fold more patients at higher inherited risk of CAT.

## Glossary of Terms

<b>Term</b>	<b>Definition</b>
First-degree blood relative	Parent, full-sibling, child
First degree relative	Siblings, parents, and offspring
Lynch syndrome-associated cancer	Heredity non-polyposis colon cancer

## Appendix

**Note:** Reference to "see above" indicates additional criteria outlined in policy.

### Suspected Diagnoses

#### List 1: Suspected Diagnoses

Achondroplasia (FGFR3)	Huntington's disease (HTT, HD (Huntington)) (see above)
Albinism	Hypochondroplasia (FGFR3)
Alpha-1 antitrypsin deficiency (SERPINA1)	Hypertrophic cardiomyopathy (see above)
Alpha thalassemia/Hb Bart hydrops fetalis syndrome/HbH disease <sup>++</sup> (HBA1/HBA2, alpha globin 1 and alpha globulin 2) (see above)	Jackson-Weiss syndrome (FGFR2)
Androgen insensitivity syndrome (AIS) (see above)	Joubert syndrome
Angelman syndrome (GABRA, SNRPN) (see above)	Kallmann syndrome (FGFR1)
Arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C) (see above)	Kennedy disease (SBMA) (see above)
Bardet-Biedl syndrome	Leber hereditary optic neuropathy (LHON)
Beta thalassemia <sup>++</sup> (beta globin) (see above)	Leigh Syndrome and NARP (neurogenic muscle weakness, ataxia, and retinitis pigmentosa)
Bloom syndrome (BLM)	Loeys-Dietz Syndrome (LDS) (see above)&nbsp;
CADASIL (see above)	Long QT syndrome (see above)
Cadherin-1 (hereditary diffuse gastric cancer) (see above)	Limb girdle muscular dystrophy (LGMD1, LGMD2) (FKRP (Fukutin related protein))
Canavan disease (ASPA (aspartoacylase A))	Malignant hyperthermia (RYR1) (see above)
Catecholaminergic polymorphic ventricular tachycardia (CPVT) (see above)	Maple syrup urine disease (branched-chain keto acid dehydrogenase E1)
Charcot-Marie Tooth disease (PMP22)	Marfan's syndrome (TGFBR1, TGFBR2) (see above)
Classical lissencephaly	Maturity onset diabetes of the young (MODY2, MODY3) (see above)
Congenital adrenal hyperplasia/21 hydroxylase deficiency (CYP21A2) <sup>+++</sup>	McArdle's disease
	Medium chain acyl coA dehydrogenase deficiency

Congenital amegakaryocytic thrombocytopenia	(ACADM)
Congenital central hypoventilation syndrome (PHOX2B)	Medullary thyroid carcinoma
Congenital muscular dystrophy type 1C (MDC1C) (FKRP (Fukutin related protein))	MELAS (mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes) (MTTL1, tRNAleu)
Corneal dystrophy (see above)	Meckel-Gruber syndrome
Crouzon syndrome (FGFR2, FGFR3)	Mucolipidosis type IV (MCOLN1, mucolipin 1)
Cystic fibrosis (CFTR) (see above)	Mucopolysaccharidoses type 1 (MPS-1)
Dentatorubral-pallidoluysian atrophy	Muenke syndrome (FGFR3)
Dravet syndrome (SCN1A, SCN2A)	Multiple endocrine neoplasia type 1
Duchenne/Becker muscular dystrophy (dystrophin) (see above)	Muscle-Eye-Brain disease (POMGNT1)
Dysferlin myopathy	MUTYH-associated polyposis (MYH) (see above)
Ehlers-Danlos syndrome (see above)	Myoclonic epilepsy (MERRF) (MTTK (tRNALys))
Emery-Dreifuss muscular dystrophy (EDMD1, 2, and 3)	Myotonic dystrophy (DMPK, ZNF-9) (see above)
Fabry disease	Neimann-Pick disease, type A (SMPD1, sphingomyelin phosphodiesterase)
Factor V Leiden mutation (F5 (Factor V)) (see above)	Nephrotic syndrome, congenital (NPHS1, NPHS2) (see above)
Factor XIII deficiency, congenital (F13 (Factor XIII beta globulin))	Neurofibromatosis type 1 (NF1, neurofibromin) (see above)
Familial adenomatous polyposis coli (APC) (see above)	Neurofibromatosis type 2 (Merlin) (see above)
Familial dysautonomia (IKBKAP)	Neutropenia, congenital cyclic
Familial hypocalciuric hypercalcemia (see above)	Nephronophthisis
Familial Mediterranean fever (MEFV)	Oculopharyngeal muscular dystrophy (OPMD) (see above)
Fanconi anemia (FANCC, FANCD)	Osteogenesis imperfecta (see above)
Fragile X syndrome, FRAXA (FMR1) (see above)	Phenylketonuria (PAH)
Friedreich's ataxia (FRDA (frataxin))	Pfeiffer syndrome (FGFR1)
Galactosemia (GALT)	Prader-Willi-Angelman syndrome (SNRPN, GABRA5, NIPA1, UBE3A, ANCR, GABA ) (see above)
Gaucher disease (GBA (acid beta glucosidase))	Primary dystonia (TOR1A (DYT1)) (see above)
Gitelman's syndrome	Prothrombin (F2 (Factor II, 20210G> A mutation)) (see above)
Hemoglobin E thalassemia <sup>++</sup>	Pyruvate kinase deficiency (PKD)
Hemoglobin S and/or C <sup>++</sup>	Retinoblastoma (Rh)
Hemophilia A/VWF (F8 ( Factor VIII))	Rett syndrome (FOXP1, MECP2)
Hemophilia B (F9 (Factor IX))	RPE65 mutation-associated retinal dystrophy
Hereditary amyloidosis (TTR variants)	Saethre-Chotzen syndrome (TWIST, FGFR2)
Hereditary deafness (GJB2 (Connexin-26, Connexin-32))	SHOX-related short stature (see above)
Hereditary hemorrhagic telangiectasia (HHT)	Smith-Lemli-Opitz syndrome
Hereditary hemochromatosis (HFE) (see above)	Spinal muscular atrophy (SMN1, SMN2) (see above)
Hereditary leiomyomatosis and renal cell cancer (HLRCC) syndrome (fumarate hydratase (FH) gene)	Spinocerebellar ataxia (SCA types 1, 2, 3 (MJD), 6 (CACNA1A), 7, 8, 10, 17 and DRPLA) (see above)
Hereditary neuropathy with liability to pressure palsies (HNPP)	Sucrase-isomaltase deficiency (SI)
Hereditary non-polyposis colorectal cancer (HNPCC) (MLH1, MSH2, MSH6, MSI) (see above)	Tay-Sachs disease (HEXA (hexosaminidase A)) (see above)
Hereditary pancreatitis (PRSS1) (see above)	Thanatophoric dysplasia (FGFR3)
Hereditary paraganglioma (SDHD, SDHB)	Thoracic aortic aneurysms/dissections (TAAD) (see above)
Hereditary polyposis coli (APC) (see above)	Unverricht-Lundborg disease (EPM1) (see above)
Hereditary spastic paraparesis 3 (SPG3A) and 4 (SPG4, SPAST)	Von Gierke disease (G6PC, Glycogen storage disease, Type 1a)
	Von Hippel-Lindau syndrome (VHL) (see above)
	Walker-Warburg syndrome (POMGNT1)
	22q11 deletion syndromes (DCGR (CATCH-22))

<sup>++</sup> Electrophoresis is the appropriate initial laboratory test for individuals judged to be at-risk for a hemoglobin disorder.

+++ Medically necessary if results of the adrenocortical profile following cosyntropin stimulation test are equivocal or for purposes of genetic counseling.

In the absence of specific information regarding advances in the knowledge of mutation characteristics for a particular disorder, the current literature indicates that genetic tests for inherited disease need only be conducted once per lifetime of the member.

#### Amsterdam II Criteria

At least 3 relatives must have an HNPCC-related cancer§, and all of the following criteria must be present:

- At least 1 of the relatives with cancer associated with HNPCC should be diagnosed before age 50 years; and
- At least 2 successive generations must be affected; and
- FAP should be excluded in the colorectal cancer cases (if any); and
- One must be a 1st-degree relative of the other two; and
- Tumors should be verified whenever possible.

#### Revised Bethesda Criteria

Member must meet 1 or more of the following criteria:

- Colorectal cancer is diagnosed in a member with 1 or more 1st-degree relatives with an HNPCC-related cancer§, with one of the cancers diagnosed under age 50 years; or
- Colorectal cancer is diagnosed in a member with 2 or more 1st- or 2nd-degree relatives with an HNPCC-related cancer§, regardless of age; or
- Member has colorectal cancer diagnosed before age 50 years; or
- Member has colorectal cancer with microsatellite instability-high (MSI-H) histology, where cancer is diagnosed before age 60 years; or
- Member has synchronous or metachronous HNPCC-related cancers§, regardless of age.

§ Hereditary nonpolyposis colorectal cancer (HNPCC)-related cancers include colorectal, endometrial, gastric, ovarian, pancreas, ureter and renal pelvis, brain (usually glioblastoma as seen in Turcot syndrome), and small intestinal cancers, as well as sebaceous gland adenomas and keratoacanthomas in Muir-Torre syndrome.

#### Marfan Syndrome

##### List 2: Clinical Diagnostic Criteria for Marfan Syndrome

- I. In the absence of family history of Marfan syndrome, the presence of any of the following is diagnostic for Marfan syndrome:
  - A. Aortic criterion (aortic diameter Z greater than or equal to two or aortic root dissection and ectopia lentis§§; or
  - B. Aortic criterion (aortic diameter Z greater than or equal to two or aortic root dissection and a systemic score greater than or equal to seven§§ (refer to Table 2).
- II. In the presence of family history of Marfan syndrome, the presence of one of any of the following is diagnostic for Marfan syndrome:
  - A. Aortic criterion (aortic diameter Z greater than or equal to two above 20 years old, Z greater than or equal to three below 20 years old or aortic root dissection)§§; or
  - B. Ectopia lentis; or
  - C. Systemic score greater than or equal to seven points§§ (refer to Table 1)

§§ The diagnosis of Marfan syndrome can be made only in the absence of discriminating features of Shprintzen-Goldberg syndrome (SGS), LDS or vascular Ehlers-Danlos syndrome (vEDS).

Table 1: Calculation of the Systemic Score for Marfan Syndrome

Feature	Value
Wrist and thumb sign	3
Wrist or thumb sign	1
Pectus carinatum deformity	2
Pectus excavatum or chest asymmetry	1
Hindfoot deformity	2
Plain flat foot (pes planus)	1
Pneumothorax	2
Ductal ectasia	2
Protrusio acetabula	2
Reduced upper segment/lower segment AND increased arm span/height ratios and no severe scoliosis	1
Scoliosis or thoracolumbar kyphosis	1
Reduced elbow extension	1
Three of five facial features	1
Skin striae	1
Myopia	1
Mitral valve prolapse	1

Schwartz Score for Long QT Syndrome (LQTS)

Table 2: Scoring system for clinical diagnosis of LQTS

Findings			Points
<b>ECG<sup>1</sup></b>	<b>QTc<sup>2</sup></b>	≥480 ms	3
		460-479 ms	2
		450-459 ms (in males)	1
		≥480 ms during 4th minute of recovery from exercise stress test	1
		Torsade de pointes <sup>3</sup>	2
		T wave alternans	1
<b>Clinical History</b>	Syncope <sup>3</sup>	Notched T wave in 3 leads	1
		Low heart rate for age <sup>4</sup>	0.5
<b>Family History</b>		with stress	2
		without stress	1
<b>Total Score</b>		Family member(s) w/definite LQTS <sup>5</sup>	1
		Unexplained sudden cardiac death at age <30 yrs in immediate family <sup>5</sup>	0.5

Adapted from Schwartz & Crotti (2011) and Alders et al. (2018)

Scoring:

- less than or equal to 1.0 point = low probability of LQTS

- between 1.5 to 3.0 points = intermediate probability of LQTS
- greater than 3.5 points = high probability of LQTS

Notes:

1. In the absence of medications or disorders known to affect these electrocardiographic (ECG) features
2. QTc (corrected QT) calculated by Bazett's formula where  $QTc = QT/\sqrt{RR}$
3. Mutually exclusive
4. Resting heart rate below the second percentile for age
5. The same family member cannot be counted for both criteria.

#### Task Force Criteria for Diagnosis of Diagnosis of Arrhythmogenic Right Ventricular Cardiomyopathy/Dysplasia

#### 2010 Revised Task Force criteria for the diagnosis of arrhythmogenic right ventricular cardiomyopathy (ARVC)

(<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3448576/table/TU1/?report=objectonly>)

(Marcus, et al., 2010)

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