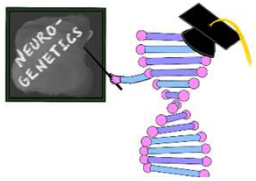
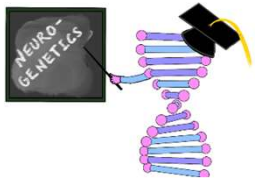
Dual Diagnoses

MODULE 13



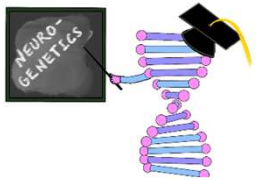
Learning Objectives

- Recognize when a patient may have two or more genetic diagnoses
- Interpret the clinical significance of genetic variants in genetic test reports
- Understand how dual or molecular diagnoses may result in intrafamilial or interfamilial variability



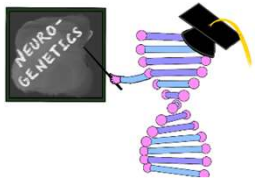
Chief Complaint

- 2-year-old female with epilepsy and delayed development



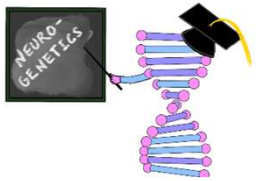
HPI

- Presented at 3 months with **drug-resistant focal seizures**
- Developed **paroxysmal hemiplegia** at 7 months
  - Alternates between sides, sometimes quadriplegia
  - Improves after sleeping
- Also has **dystonia** (opisthotonic posturing, hemi-dystonia)
- **Abnormal eye movements** outside of seizures, including mononuclear nystagmus



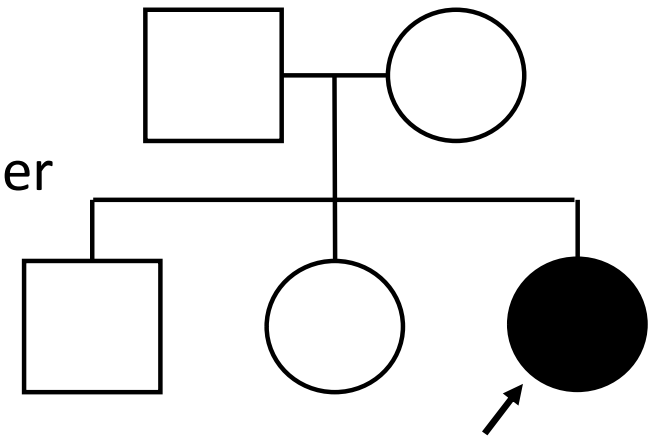
HPI Continued

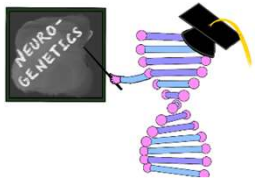
- Developmental history:
  - No babbling or words at 2-years-old
  - Passes toys past midline, can hold bottle
  - Sits without support, crawls, pulls to stand.
- **Failed hearing screen.**



Family History

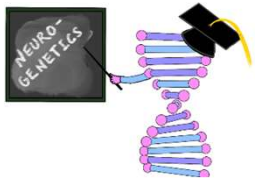
- Last of three children
- Parental age at birth: mother 30, father 33
  - Parents healthy
- Older brother (7 yo) – oppositional defiant disorder
- Older sister (6 yo) – speech delay, articulation disorder
- No family history of seizures, developmental disorders, hearing loss

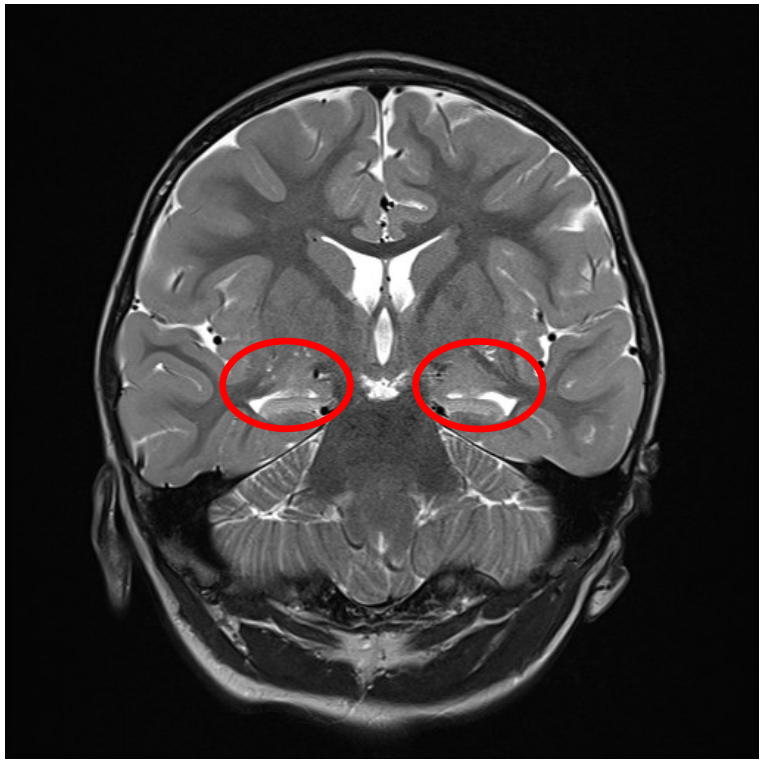




Workup

- Auditory brainstem response:
  - **Moderate sensorineural hearing loss**
- Brain MRI:
  - **Bilateral mesial temporal lobe sclerosis**
  - Focal nodular signal intensity which is T2 isointense to gray matter, minimally T2/FLAIR hyperintense, minimally T1 hypointense, located within the juxtacortical right parietal lobe
    - **Possible focal cortical dysplasia**





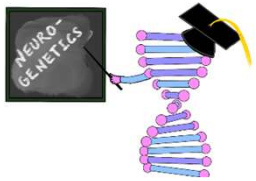
What Genetic Test Would You Order?

Single gene testing

- May be appropriate if high clinical suspicion for single gene disorder
- Genetic heterogeneity for most conditions (e.g., hereditary motor and sensory neuropathies) severely limits diagnostic utility
- May or may not include copy number analysis*

Epilepsy gene panels

- May cover most (but not all) epilepsy associated genes
- May or may not include copy number analysis*



* Read the fine print!

What Genetic Test Would You Order?

Chromosomal Microarray (CMA)

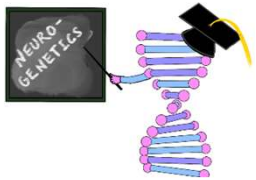
- Covers large deletions or duplications (e.g., *CDKL5* deletions)
- Does not detect single nucleotide variants (SNVs) or indels (small deletions/duplications)

Exome Sequencing (ES)

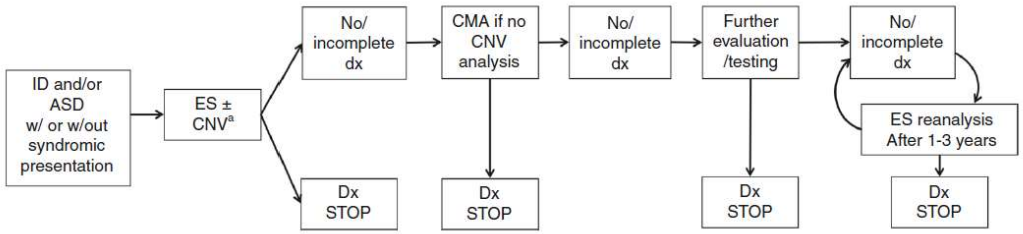
- Covers nearly all 20,000 protein coding genes
- May or may not detect small or large copy number variations (CNVs)

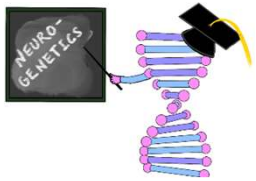
Genome Sequencing (GS)

- Can detect SNVs, indels, large and small CNVs, copy number neutral structural variants (SVs) like inversions, translocations
- Limited availability in clinical setting
- Still evolving in the clinical setting – use with caution!



Exome Sequencing is the Optimal First Test for Neurodevelopmental Disorders





SYSTEMATIC REVIEW | Genetics inMedicine

Open

Meta-analysis and multidisciplinary consensus statement: exome sequencing is a first-tier clinical diagnostic test for individuals with neurodevelopmental disorders

Siddharth Srivastava, MD[1], Jamie A. Love-Nichols, MS, MPH[1], Kira A. Dies, ScM[1], David H. Ledbetter, PhD[2], Christa L. Martin, PhD[2], Wendy K. Chung, MD, PhD[3,4], Helen V. Firth, DM, FRCP[5,6], Thomas Frazier, PhD[7], Robin L. Hansen, MD[8], Lisa Prock, MD, MPH[1,9], Han Brunner, MD[10,11,12], Ny Hoang, MS[13,14,15], Stephen W. Scherer, PhD[14,15,16,17], Mustafa Sahin, MD PhD[1], David T. Miller, MD PhD[18] and the NDD Exome Scoping Review Work Group

Purpose: For neurodevelopmental disorders (NDDs), etiological evaluation can be a diagnostic odyssey involving numerous genetic tests, underscoring the need to develop a streamlined algorithm maximizing molecular diagnostic yield for this clinical indication. Our objective was to compare the yield of exome sequencing (ES) with that of chromosomal microarray (CMA), the current first-tier test for NDDs.

Methods: We performed a PubMed scoping review and meta-analysis investigating the diagnostic yield of ES for NDDs as the basis of a consensus development conference. We defined NDD as global developmental delay, intellectual disability, and/or autism spectrum disorder. The consensus development conference included input from genetics professionals, pediatric neurologists, and developmental behavioral pediatricians.

Results: After applying strict inclusion/exclusion criteria, we identified 30 articles with data on molecular diagnostic yield in individuals with isolated NDD, or NDD plus associated conditions (such as Rett-like features). Yield of ES was 36% overall, 31% for isolated NDD, and 53% for the NDD plus associated conditions. ES yield for NDDs is markedly greater than previous studies of CMA (15–20%).

Conclusion: Our review demonstrates that ES consistently outperforms CMA for evaluation of unexplained NDDs. We propose a diagnostic algorithm placing ES at the beginning of the evaluation of unexplained NDDs.

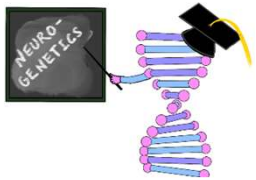
Genetics in Medicine (2019) 21:2413–2421; https://doi.org/10.1038/s41436-019-0554-6

Keywords: autism; consensus development conference; diagnostic yield; genetic testing; intellectual disability

Srivastava S, et al. Genet Med. 2019 Nov;21(11):2413-2421. PMID: 31182824

Interpretation of Genetic Test Results

- Review actual test results
- Look at disorders and modes of inheritance; discuss if they fit patient's phenotype
- Use following resources: OMIM, ClinVar, GeneReviews, PubMed



Genetic Test Results

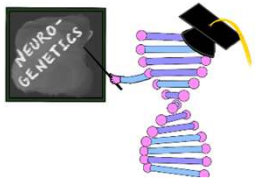
CHILD NEUROLOGY SOCIETY

Causative Variant(s) in Disease Genes Associated with Reported Phenotype:

Gene	Disease	Mode of Inheritance	Variant	Zygosity	Inherited From	Classification
ATP1A3	ATP1A3-related neurodevelopmental and movement disorder spectrum	Autosomal Dominant	c.2407 G>C p.(G803R)	Heterozygous	Unknown	Pathogenic Variant
USH2A	USH2A-related Usher syndrome	Autosomal Recessive	c.2299del p.(E767Sfs*21)	Homozygous	Mother + Unknown	Pathogenic Variant

Variant(s) in Disease Genes Possibly Associated with Reported Phenotype:

Gene	Disease	Mode of Inheritance	Variant	Zygosity	Inherited From	Classification
MTOR	MTOR-related disorder	Autosomal Dominant	c.1543 C>G p.(P515A)	Heterozygous	Unknown	Variant of Uncertain Significance



Alternating Hemiplegia of Childhood

- Delayed development
- Hemiplegia
- Dystonia
- Abnormal eye movement
- Seizures

* 182350

ATPase, Na+/K+ TRANSPORTING, ALPHA-3 POLYPEPTIDE; ATP1A3

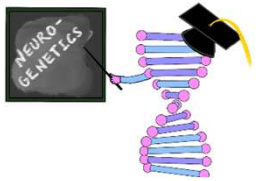
Alternative titles; symbols

SODIUM-POTASSIUM-ATPase, ALPHA-3 POLYPEPTIDE
ATPase, Na+/K+, ALPHA III

50 YEARS OMIM
Human Genetics Knowledge for the World

Gene-Phenotype Relationships

Location	Phenotype (View Clinical Synopses)	Phenotype MIM number	Inheritance	Phenotype mapping key
19q13.2	Alternating hemiplegia of childhood 2	614820	AD	3
	CAPOS syndrome	601338	AD	3
	Developmental and epileptic encephalopathy 99	619606	AD	3
	Dystonia-12	128235	AD	3



Revised AHC Criteria 2021

1. Essential criteria

✓ 1. Paroxysmal episodes of Hemiplegia that alternate between the two sides and/or of quadriplegia
✓ 2. Evidence of background abnormal neurological development

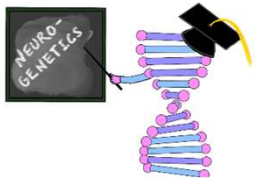
2. Major criteria

✓ 1. Onset before 18 months of age
✓ 2. Episodes of dystonia
✓ 3. Different types of episodes occur independently or together at the same time with evolution from one or more symptoms to others during that one episode.
✓ 4. Paroxysmal episodes of abnormal eye movements such as nystagmus, and especially monocular nystagmus
✓ 5. *ATP1A3* mutation
✓ 6. Plegia spells improve with sleep

3. Minor criteria

✓ 1. Epileptic seizures alone or in combination with other spells
✓ 2. Episodes of altered consciousness, not epileptic in nature, alone or in combination with other spells
✓ 3. Abnormal motor function such as tone abnormalities (in particular hypotonia or dystonia that can co-exist)21, ataxia, choreoathetosis and oral motor control
4. Episodes of autonomic dysfunction

Monocular nystagmus



Mikati MA, Panagiotakaki E, Arzimanoglou A. Eur J Paediatr Neurol. 2021 May;32:A4-A5. PMID: 33975787.

Usher Syndrome

* 608400

USHERIN; USH2A

Alternative titles; symbols

USH2A GENE
USH2; US2

▼ **Description**

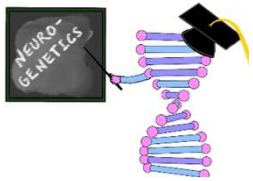
Usher syndrome is a clinically and genetically heterogeneous autosomal recessive disorder characterized by sensorineural hearing deficiencies at birth and later development of progressive retinitis pigmentosa (RP). It is the most frequent cause of combined deafness and blindness in adults and affects 3 to 6% of children born with hearing impairment. In brief, patients with Usher syndrome type II have mild hearing impairment with normal vestibular responses. Type II is the most common of the 3 Usher syndromes (Eudy et al., 1998). See 276900 for clinical characterization of Usher syndrome types I, II, and III.

HGNC Approved Gene Symbol: USH2A

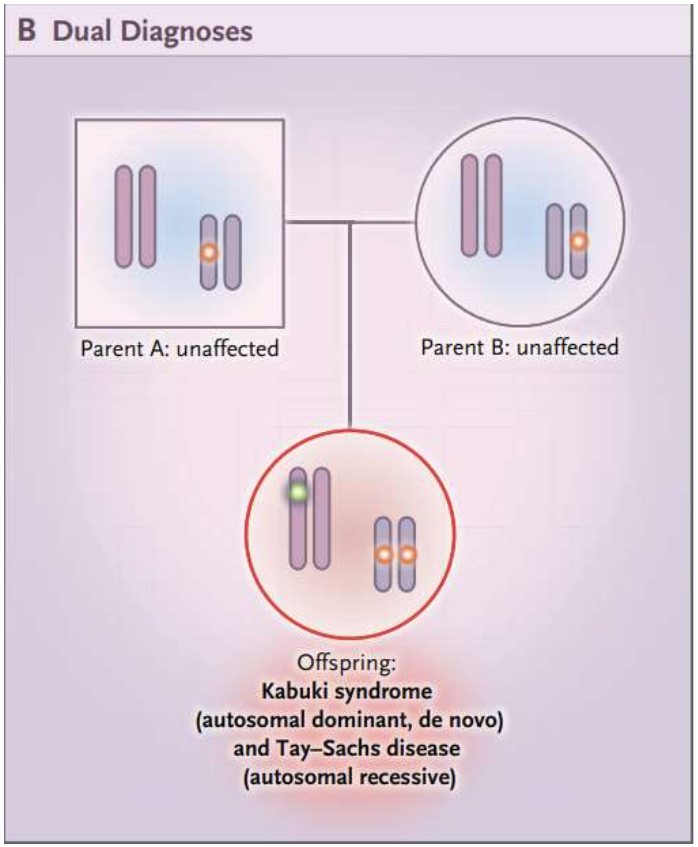
Cytogenetic location: 1q41 ***Genomic coordinates (GRCh38): 1:215,622,891-216,423,448*** (from NCBI)

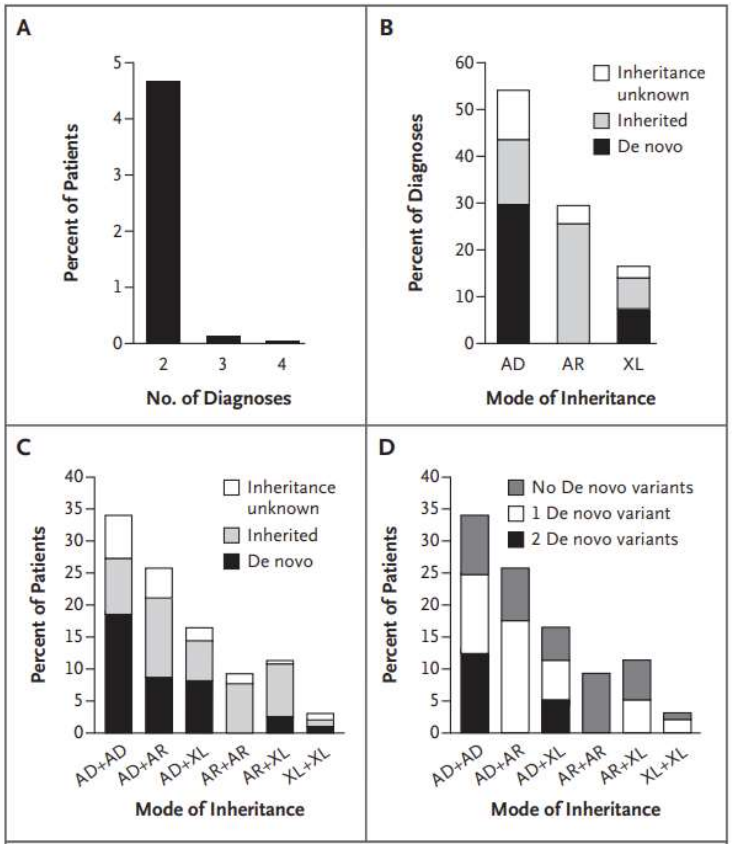
Gene-Phenotype Relationships

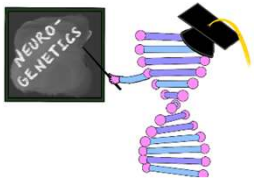
Location	Phenotype (View Clinical Synopses)	Phenotype MIM number	Inheritance	Phenotype mapping key
1q41	Retinitis pigmentosa 39	613809	AR	3
	Usher syndrome, type 2A	276901	AR	3



Dual Molecular Diagnoses

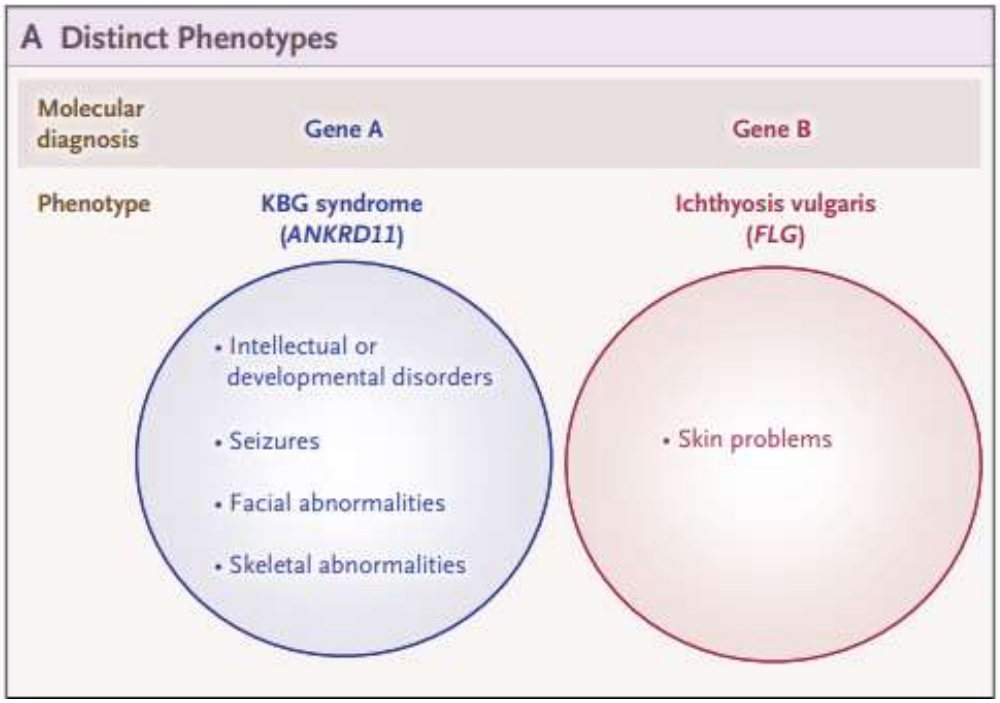






Posey JE, et al N Engl J Med. 2017 Jan 5;376(1):21-31. PMID: 27959697

Dual Molecular Diagnoses Results in Blended Phenotypes



Gene A

AHC (*ATP1A3*)

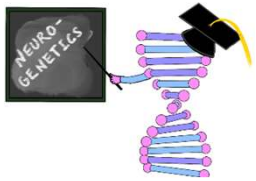
- DD/ID
- Hemiplegia
- Dystonia
- Seizures
- Abnormal eye movements

Gene B

Usher syndrome 2A (*USH2A*)

- Sensorineural hearing loss
- Retinitis pigmentosa

*Sensorineural hearing loss is seen in a different *ATP1A3* phenotype: (CAPOS: ***C****erebellar ataxia,* ***A****reflexia,* ***P****es cavus,* ***O****ptic atrophy, and* ***S****ensorineural hearing loss)*



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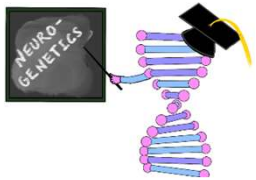


What about *MTOR*?

* 601231

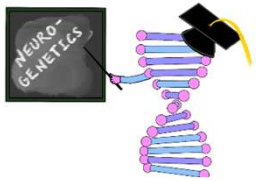
MECHANISTIC TARGET OF RAPAMYCIN; MTOR

▼ Description

Smith-Kingsmore syndrome (SKS) is a rare autosomal dominant syndromic intellectual disability syndrome characterized by macrocephaly, seizures, umbilical hernia, and facial dysmorphic features including frontal bossing, midface hypoplasia, small chin, hypertelorism with downslanting palpebral fissures, depressed nasal bridge, smooth philtrum, and thin upper lip (Smith et al., 2013; Baynam et al., 2015).

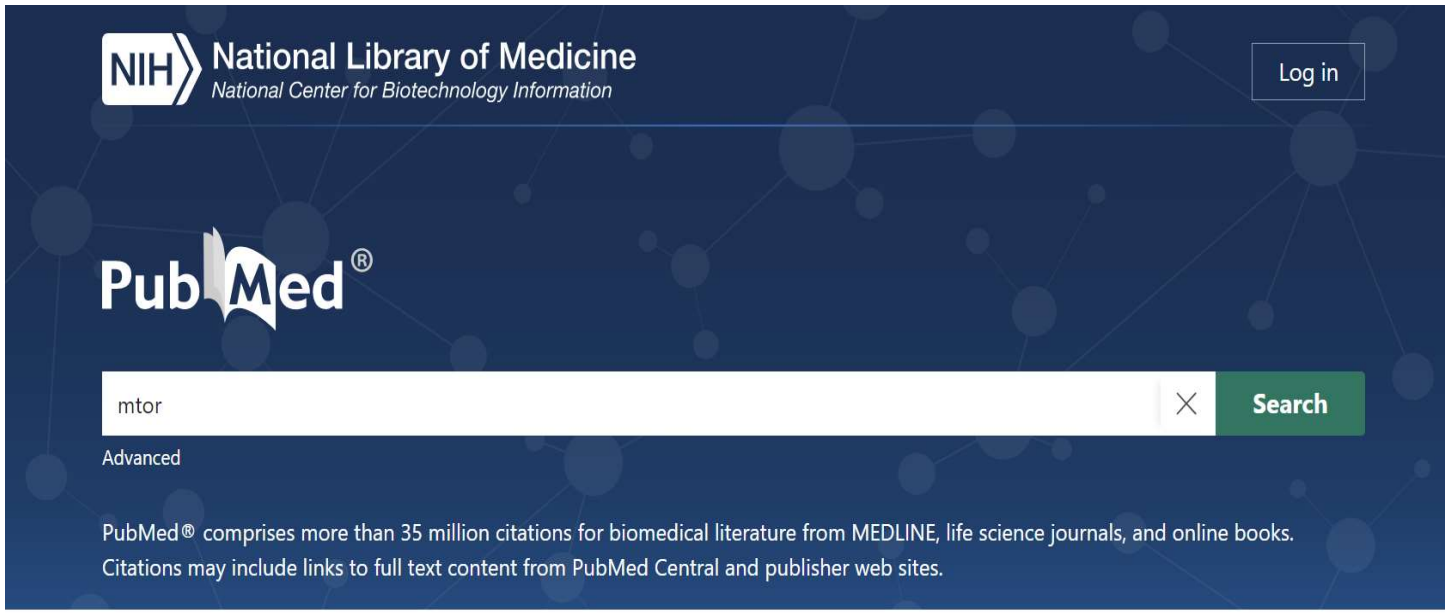
Gene-Phenotype Relationships

Location	Phenotype View Clinical Synopses	Phenotype MIM number	Inheritance	Phenotype mapping key
1p36.22	Focal cortical dysplasia, type II, somatic	607341		3
	Smith-Kingsmore syndrome	616638	AD	3



Doesn't sound like a fit, but…

Look at the Literature



› Neurol Genet. 2016 Oct 31;2(6):e118. doi: 10.1212/NXG.0000000000000118. eCollection 2016 Dec.

Germline and somatic mutations in the *MTOR* gene in focal cortical dysplasia and epilepsy

› Neurol Genet. 2016 Oct 31;2(6):e118. doi: 10.1212/

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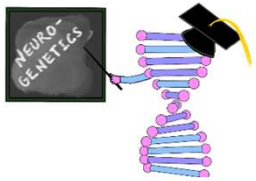
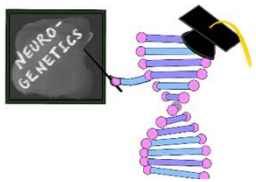


MTOR in the Literature

- **Germline *de novo* missense *MTOR* variants in 6 individuals**
- **Focal epilepsy** (less frequently generalized)
- Mild or no brain malformations
- With or without ID



Germline and somatic mutations in the *MTOR* gene in focal cortical dysplasia and epilepsy

OPEN

Rikke S. Møller, PhD*
Sarah Weckhuysen, MD, PhD*
Mathilde Chipaux, MD, PhD
Elise Marsan, MSc
Valerie Taly, PhD
E. Martina Bebin, MD, MPA
Susan M. Hiatt, PhD
Jeremy W. Prokop, PhD
Kevin M. Bowling, PhD
Davide Mei, MSc
Valerio Conti, PhD
Pierre de la Grange, PhD
Sarah Ferrand-Sorbets, MD
Georg Dorfmüller, MD
Virginie Lambrecq, MD, PhD
Line H.G. Larsen, MSc
Eric Leguern, MD, PhD
Renzo Guerrini, MD, FRCP
Guido Rubboli, MD
Gregory M. Cooper, PhD
Stéphanie Baulac, PhD

ABSTRACT

Objective: To assess the prevalence of somatic *MTOR* mutations in focal cortical dysplasia (FCD) and of germline *MTOR* mutations in a broad range of epilepsies.

Methods: We collected 20 blood-brain paired samples from patients with FCD and searched for somatic variants using deep-targeted gene panel sequencing. Germline mutations in *MTOR* were assessed in a French research cohort of 93 probands with focal epilepsies and in a diagnostic Danish cohort of 245 patients with a broad range of epilepsies. Data sharing among collaborators allowed us to ascertain additional germline variants in *MTOR*.

Results: We detected recurrent somatic variants (p.Ser2215Phe, p.Ser2215Tyr, and p.Leu1460Pro) in the *MTOR* gene in 37% of participants with FCD II and showed histologic evidence for activation of the mTORC1 signaling cascade in brain tissue. We further identified 5 novel de novo germline missense *MTOR* variants in 6 individuals with a variable phenotype from focal, and less frequently generalized, epilepsies without brain malformations, to macrocephaly, with or without moderate intellectual disability. In addition, an inherited variant was found in a mother–daughter pair with nonlesional autosomal dominant nocturnal frontal lobe epilepsy.

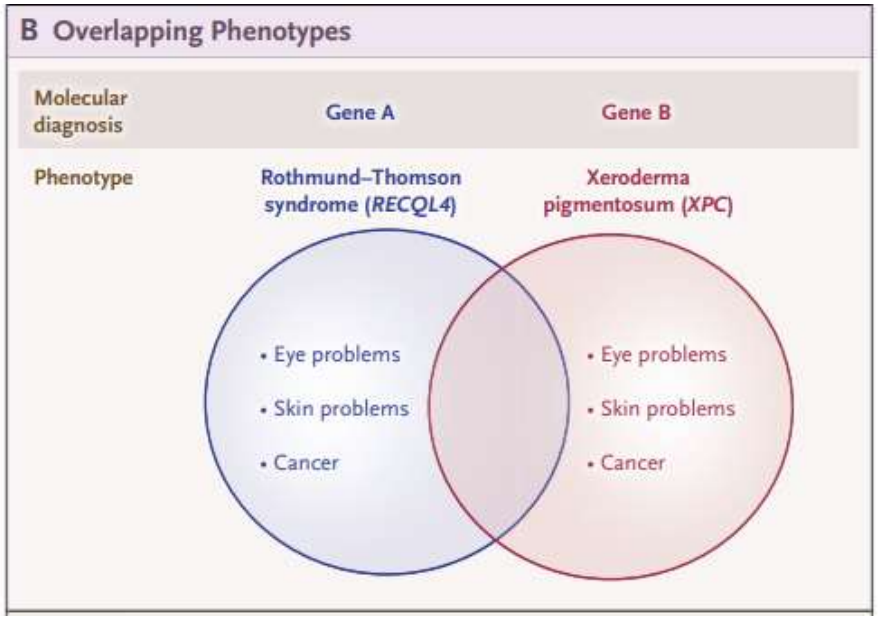
Conclusions: Our data illustrate the increasingly important role of somatic mutations of the *MTOR* gene in FCD and germline mutations in the pathogenesis of focal epilepsy syndromes with and without brain malformation or macrocephaly. ***Neurol Genet* 2016;2:e118; doi: 10.1212/NXG.0000000000000118**

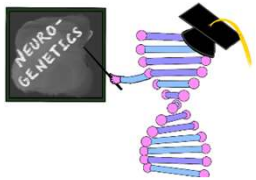
GLOSSARY

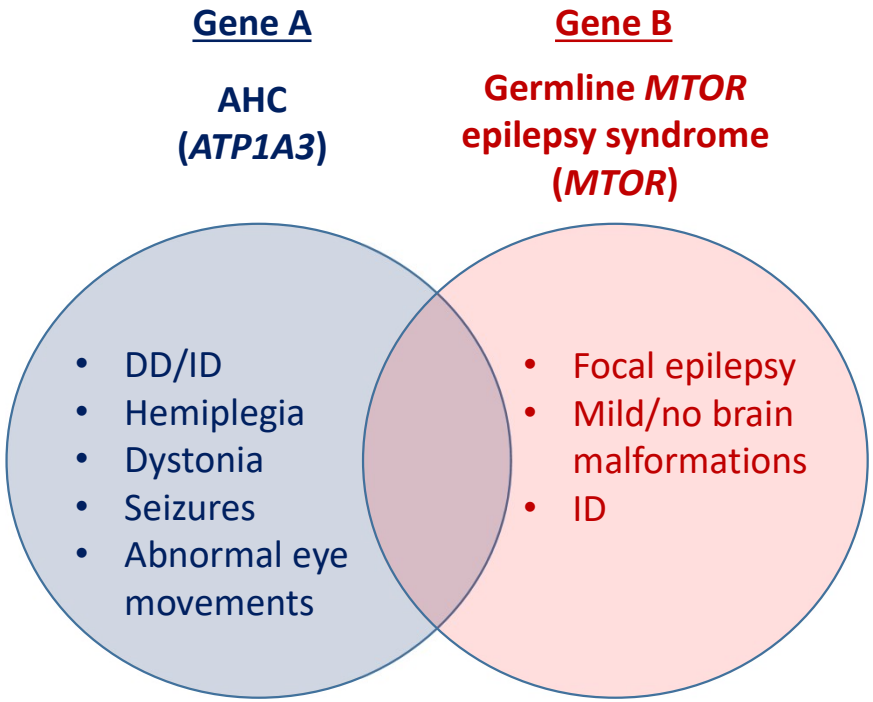
ADHD = attention-deficit/hyperactivity disorder; **ADNFLE** = autosomal dominant nocturnal frontal lobe epilepsy; **CADD** = Combined Annotation Dependent Depletion; **DEPDC5** = Dishevelled, Egl-10, and Pleckstrin domain-containing protein 5; **DEPTOR** = domain-containing mTOR-interacting protein; **dPCR** = digital PCR; **ELM** = eukaryotic linear motif; **ExAC** = Exome Aggregation Consortium; **FAT** = FRAP, ATM, TRRAP; **FCD** = focal cortical dysplasia; **GATOR1** = GTPase-activating protein activity toward Rags complex 1; **HEAT** = huntingtin, elongation factor 3, protein phosphatase 2A, and TOR1; **H&E** = hematoxylin and eosin; **mTOR** = mammalian target of rapamycin; **NPRL** = nitrogen permease regulator-like.

Møller RS, et al. Neurol Genet. 2016 Oct 31;2(6):e118. PMID: 27830187

Multi-Locus Pathogenic Variation May Modify Phenotypic Severity – Overlapping Phenotype







Posey JE, et al N Engl J Med. 2017 Jan 5;376(1):21-31. PMID: 27959697

Tools for VUS Resolutions

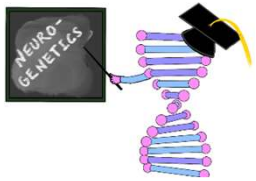
Look at inheritance pattern

Look at phenotypic match

Look at variant – present in population? Effect on Protein?

Familial Segregation Studies

Functional studies – measure enzyme or transporter activity



MTOR:c.1543C>G, p.(P515A)

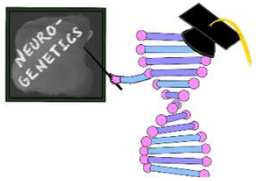
- Absent from gnomAD ✓
- Highly conserved (phylopP100 9.435) ✓
- Predicted damaging (CADD 23.5) ✓
- Paternal testing confirmed *de novo* ✓

gnomAD
Genome Aggregation Database

gnomAD (broadinstitute.org)

CADD - Combined Annotation Dependent Depletion

CADD - Combined Annotation Dependent Depletion (washington.edu)



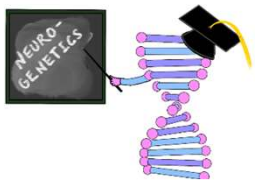
Reclassified as 'likely pathogenic'

*Could also reach out to *MTOR* experts for advice, functional evaluation of *MTOR* variant

ACMG Standards and Guidelines for the Interpretation of Sequence Variants

A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology

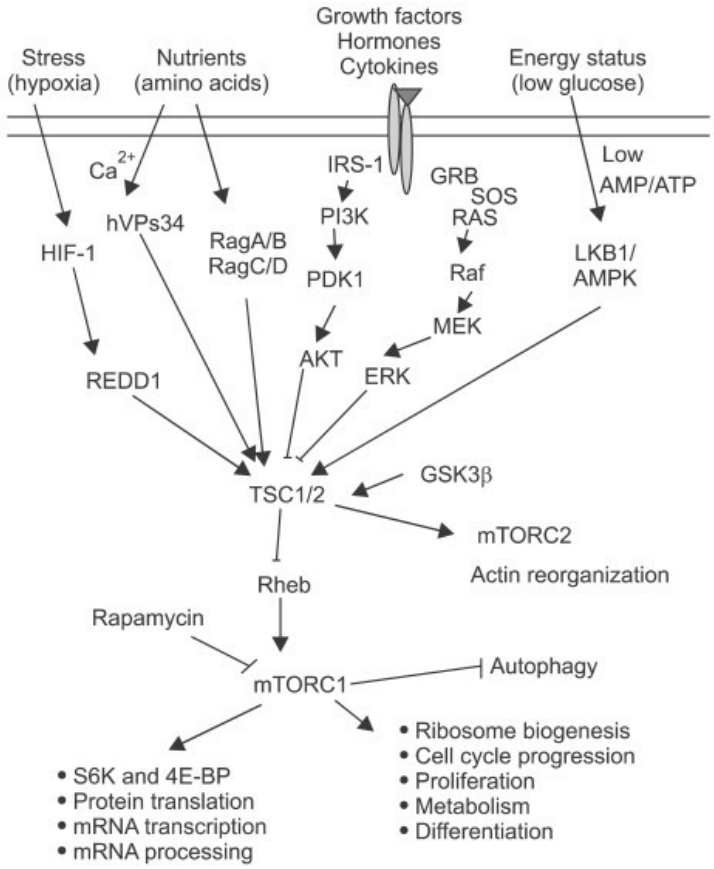
	Benign		Pathogenic			
	Strong	**Supporting**	**Supporting**	**Moderate**	**Strong**	**Very Strong**
Population Data	MAF is too high for disorder *BA1/BS1* **OR** observation in controls inconsistent with disease penetrance *BS2*			Absent in population databases *PM2*	Prevalence in affecteds statistically increased over controls *PS4*	
Computational And Predictive Data		Multiple lines of computational evidence suggest no impact on gene /gene product *BP4* Missense in gene where only truncating cause disease *BP1* Silent variant with non predicted splice impact *BP7*	Multiple lines of computational evidence support a deleterious effect on the gene /gene product *PP3*	Novel missense change at an amino acid residue where a different pathogenic missense change has been seen before *PM5* Protein length changing variant *PM4*	Same amino acid change as an established pathogenic variant *PS1*	Predicted null variant in a gene where LOF is a known mechanism of disease *PVS1*
Functional Data	Well-established functional studies show no deleterious effect *BS3*		Missense in gene with low rate of benign missense variants and path. missenses common *PP2*	Mutational hot spot or well-studied functional domain without benign variation *PM1*	Well-established functional studies show a deleterious effect *PS3*	
Segregation Data	Non-segregation with disease *BS4*		Co-segregation with disease in multiple affected family members *PP1*	Increased segregation data →		
De novo Data				*De novo* (without paternity & maternity confirmed) *PM6*	*De novo* (paternity & maternity confirmed) *PS2*	
Allelic Data		Observed in *trans* with a dominant variant *BP2* Observed in *cis* with a pathogenic variant *BP2*		For recessive disorders, detected in *trans* with a pathogenic variant *PM3*		
Other Database		Reputable source w/out shared data = benign *BP6*	Reputable source = pathogenic *PP5*			
Other Data		Found in case with an alternate cause *BP5*	Patient's phenotype or FH highly specific for gene *PP4*			

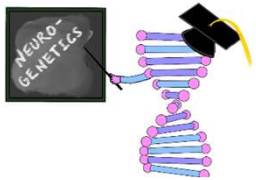


Richards et al. *Genetics in Medicine*. 2015 May; 17(5): 405-424

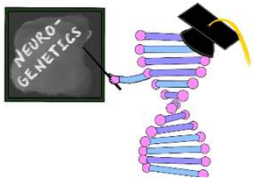
Why Pursue the *MTOR* Variant?

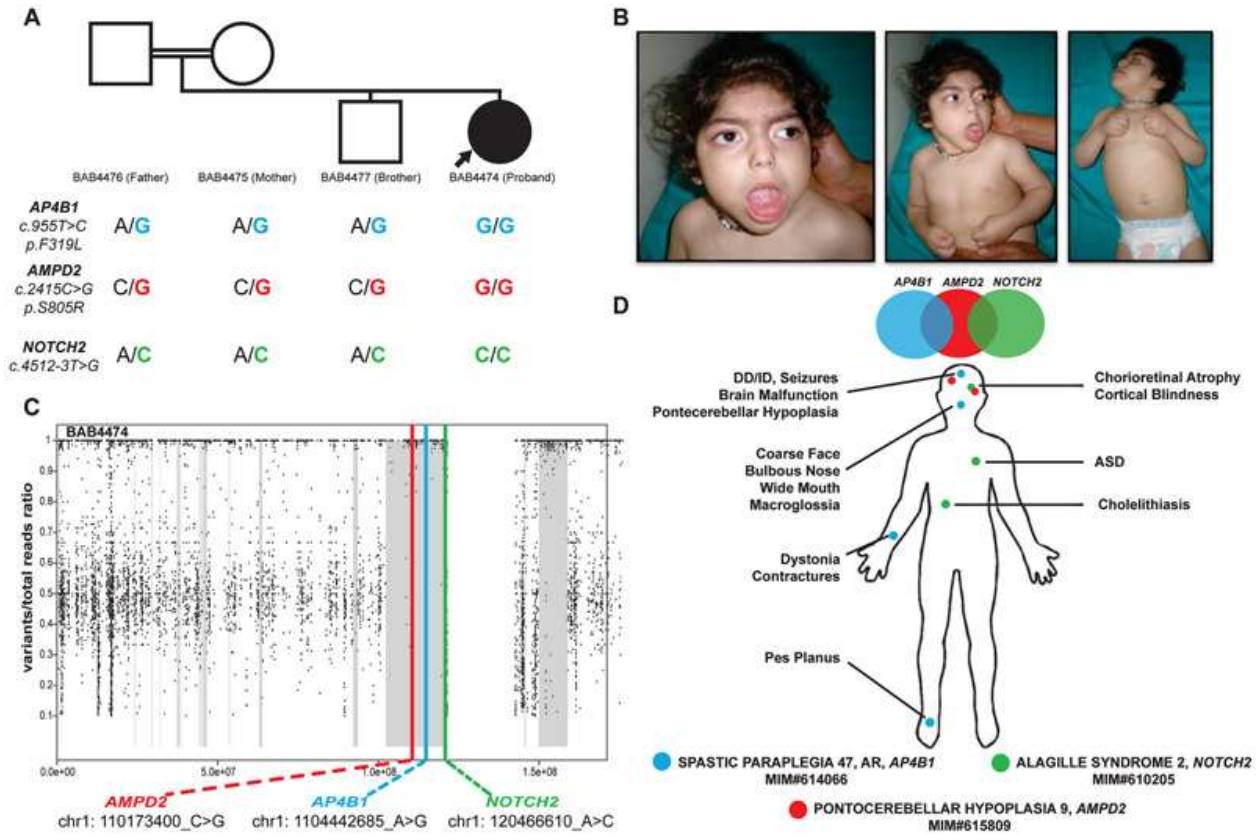
- Potentially treatable with everolimus
- May explain patient's drug-resistant epilepsy
- May have prognostic implications



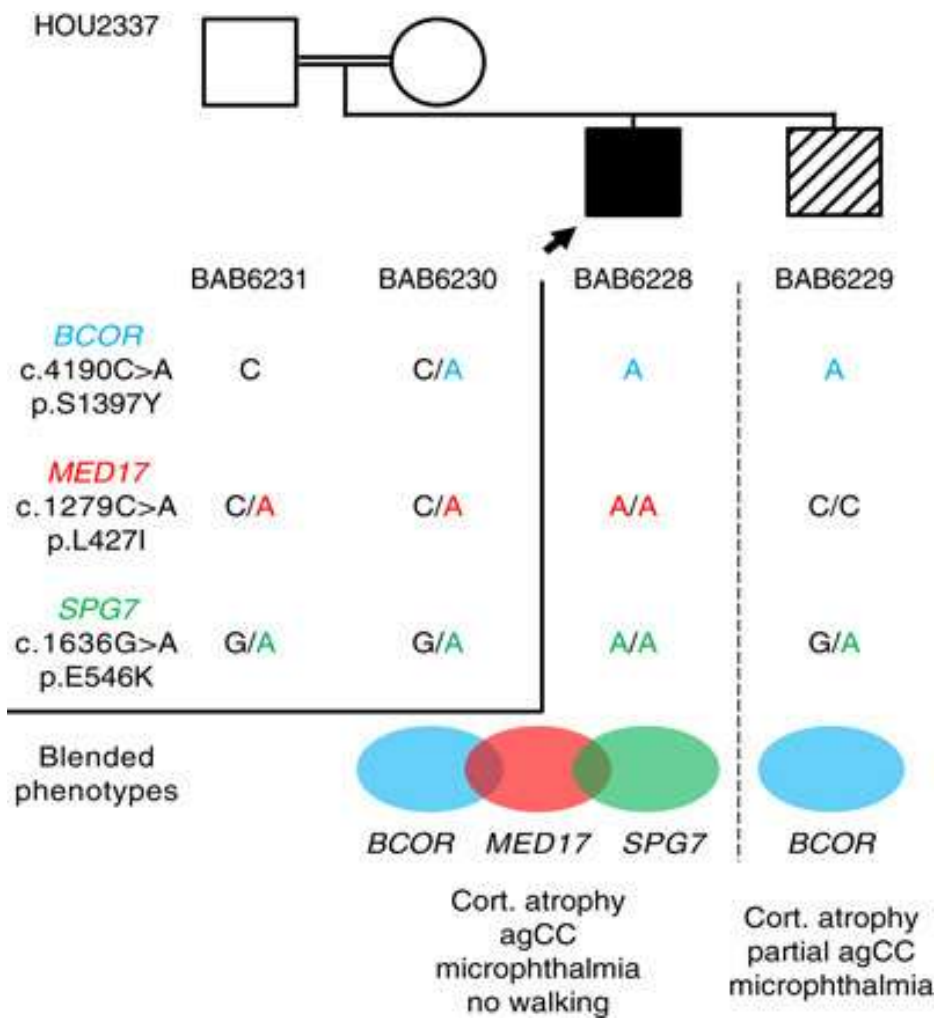


Complex Phenotypes Due to Multiple Molecular Diagnoses

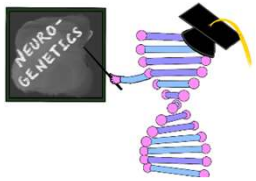




Karaca E, et al. Genet Med. 2018 Dec;20(12):1528-1537. PMID: 29790871



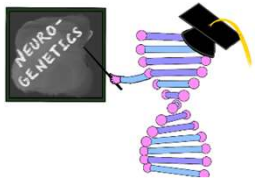
Multiple molecular diagnoses can result in intrafamilial or interfamilial variability



Karaca E, et al. Genet Med. 2018 Dec;20(12):1528-1537. PMID: 29790871

Suggested Reading

- Posey JE, et al. Resolution of Disease Phenotypes Resulting from Multilocus Genomic Variation. N Engl J Med. 2017 Jan 5;376(1):21-31. PMID: 27959697
- Posey JE. Genome sequencing and implications for rare disorders. Orphanet J Rare Dis. 2019 Jun 24;14(1):153. PMID: 31234920
- Karaca E, et al. Phenotypic expansion illuminates multilocus pathogenic variation. Genet Med. 2018 Dec;20(12):1528-1537. PMID: 29790871
- Srivastava S, et al. Meta-analysis and multidisciplinary consensus statement: exome sequencing is a first-tier clinical diagnostic test for individuals with neurodevelopmental disorders. Genet Med. 2019 Nov;21(11):2413-2421. PMID: 31182824
- Calame DG, Marafi D, Lupski JR. Neurogenetics for the Practitioner (in press)



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