Assessment of evidence for candidate pathogenic variant

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Candidate genes: ClinGen

- Definitive
- Limited
- Not yet curated

Gene-Disease Clinical Validity Curation

The ClinGen Gene-Disease Clinical Validity curation process involves evaluating the strength of evidence supporting or refuting a claim that variation in a particular gene causes a particular disease.

Gene	Disease	MOI	Expert Panel	SOP	Classification	Last Eval.
A2ML1	Noonan syndrome	AD	RASopathy	SOP5	Disputed	06/07/2018
AARS1	undetermined early-onset epileptic encephalopathy	AR	Epilepsy	SOP6	Limited	11/20/2018
AARS2	mitochondrial disease	AR	Mitochondrial Diseases	SOP8	Definitive	04/18/2022
AASS	hyperlysinemia	AR	Aminoacidopathy	SOP9	Definitive	10/14/2022
ABAT	developmental and epileptic encephalopathy	AR	Epilepsy	SOP8	Moderate	04/19/2022

Various parameters: Candidate variant

- MAF
- Protein function altering
- Mechanism of disease
- Variant type
- Disease variant database
- Population databases
- Variant quality
- Alignment quality
- Local knowledge base

Minor Allele Frequency

- 0.05 (>5%)
- AD/AR
- 0.0025% for RASopathies

ClinGen's RASopathy Expert Panel Consensus Methods for Variant Interpretation

Bruce D. Gelb, MD,[1] Hélène Cavé, PharmD, PhD,[2] Mitchell W. Dillon, MS,[3] Karen W. Gripp, MD,[4] Jennifer A. Lee, PhD,[5] Heather Mason-Suares, PhD,[6] Katherine A. Rauen, MD, PhD,[7] Bradley Williams, MS,[8] Martin Zenker, MD,[9] Lisa M. Vincent, PhD,[8] and for the ClinGen RASopathy Working Group

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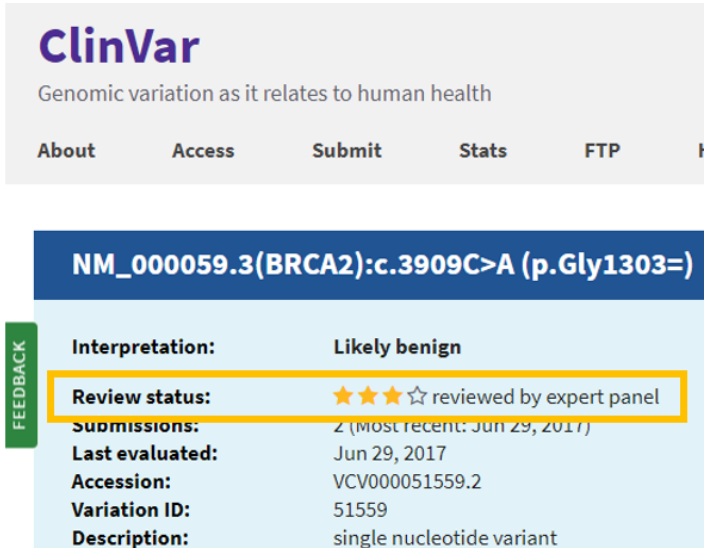
Protein Function altering

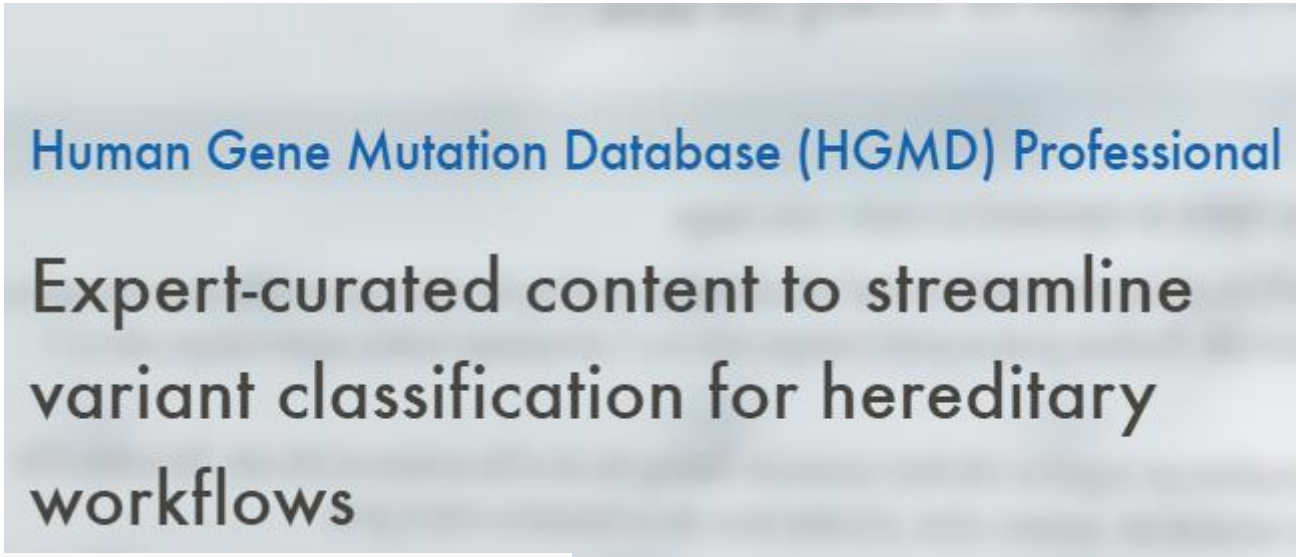
- Frameshifts, Nonsense/stop-gain, indel – truncating
- LoF/GoF variants
- Some missense variants
- *In-silico* predictors
  - SIFT = ~0
  - Polyphen = ~1
  - FATHMM = >0.5
  - CADD = >30

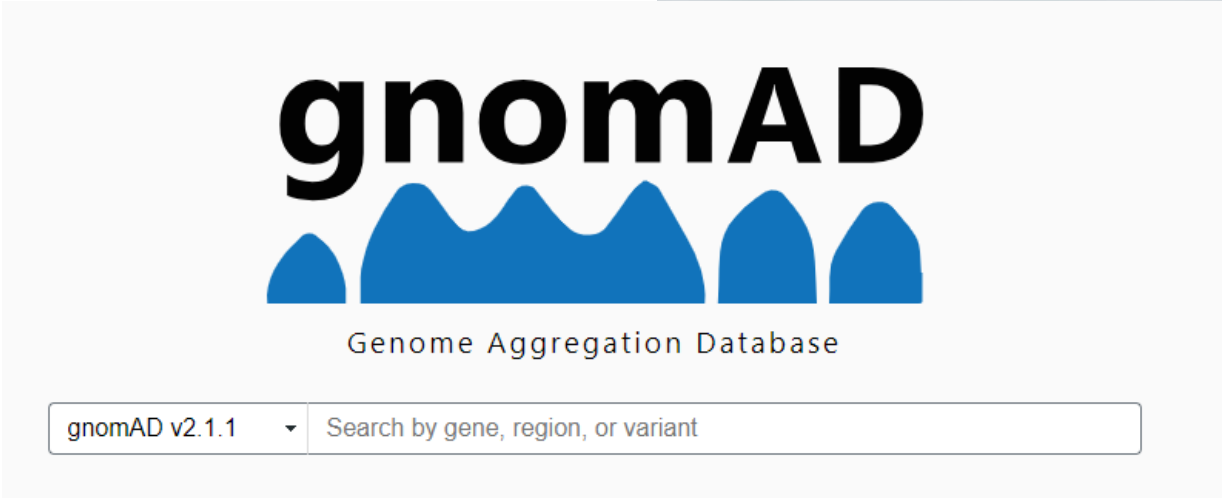
Mechanism of disease and variant

- AD,AR, XL etc…
- Synonymous / non-synonymous
- Frameshifts/stop-gained
- GoF/LoF

Disease variant and population DB

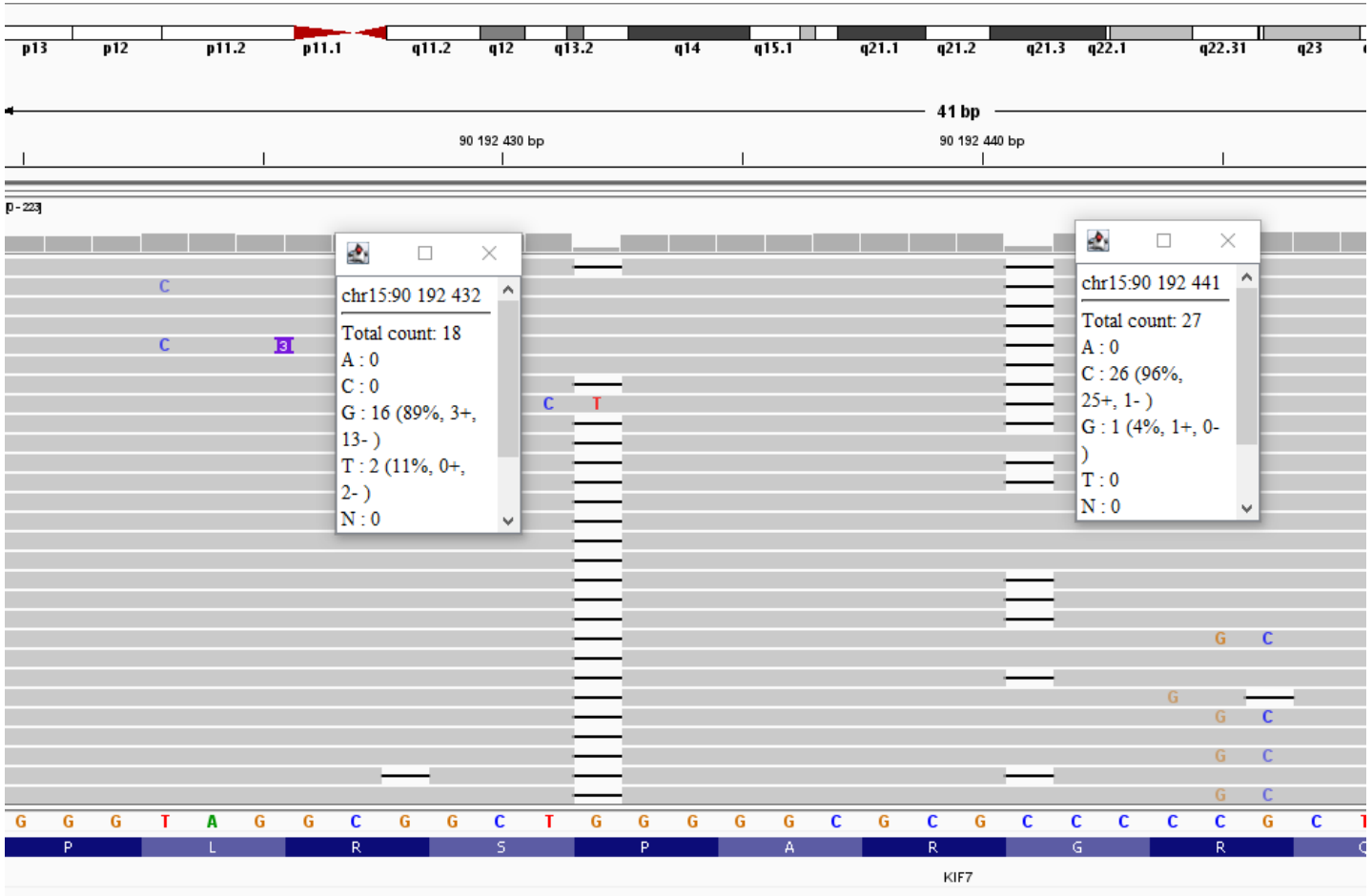




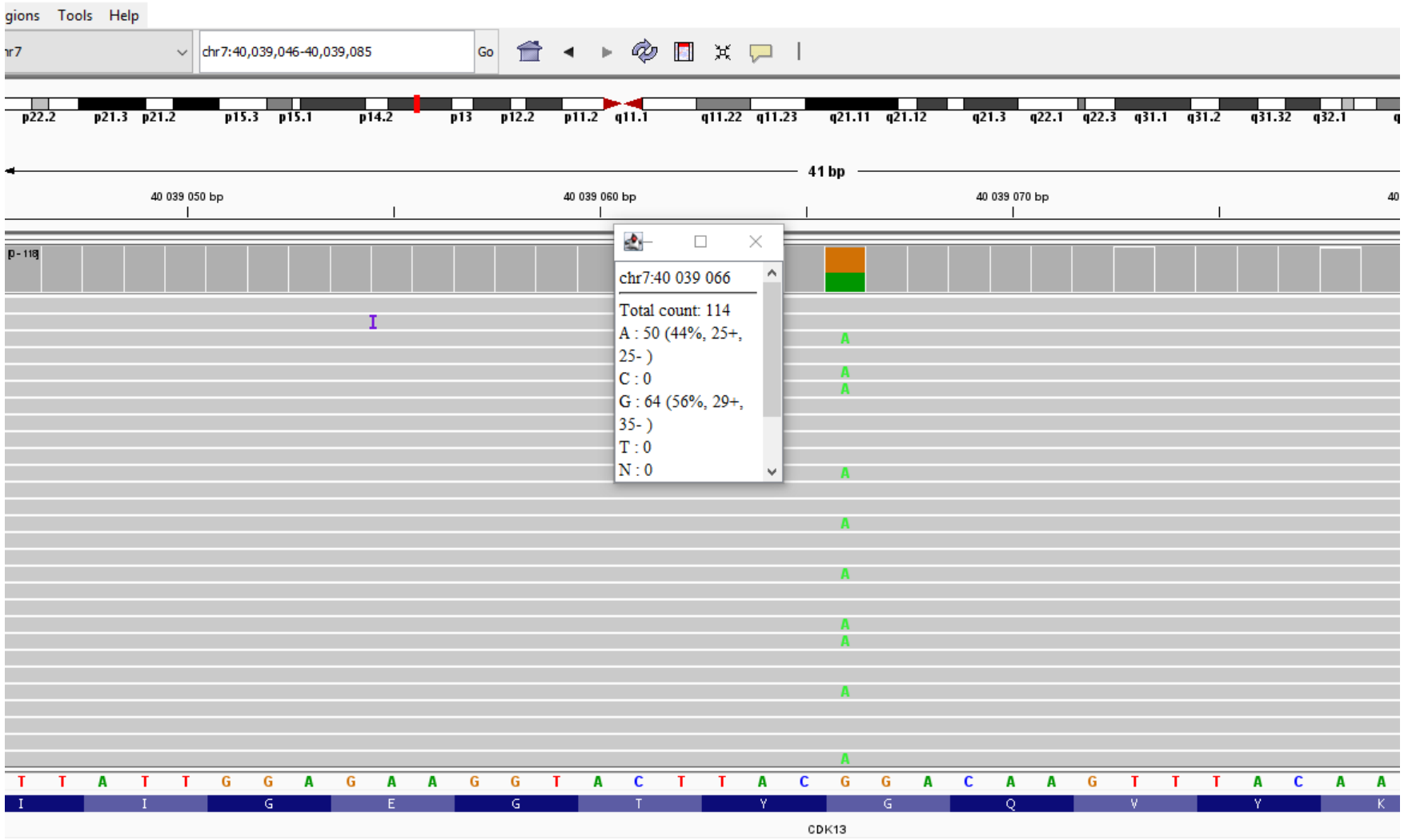


Alignment and variant quality

- Poor quality CG rich region



Good coverage: read depth



Published: 05 March 2015

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

Sue Richards PhD ✉, Nazneen Aziz PhD, Sherri Bale PhD, David Bick MD, Soma Das PhD, Julie Gastier-Foster PhD, Wayne W. Grody MD, PhD, Madhuri Hegde PhD, Elaine Lyon PhD, Elaine Spector PhD, Karl Voelkerding MD & Heidi L. Rehm PhD on behalf of ; on behalf of the ACMG Laboratory Quality Assurance Committee

ACMG-AMP

Pathogenicity	Code	Rule
Very Strong	PVS1	Null (truncating/splicing) = LoF
Strong	PS1	Same AA regardless of nucleotide
	PS2	*De novo*
	PS3	Functional evidence
	PS4	Control vs Cases variant prevalence
Moderate	PM1	Hot spot/functional domain
	PM2	Absent from controls in population databases
	PM3	AR = *trans*
	PM4	Affects Protein length
	PM5	Novel variant, Previous AA change =pathogenic
	PM6	Assumed de novo
Supporting	PP1	Segregates with affected relatives
	PP2	Missense variant cause disease: Gene has low rate of benign
	PP3	*In-silico* protein function predictors: damaging/not-tolerated
	PP4	Patients phenotype = single gene atiology
	PP5	Disease variant databases

Rules for Combining Criteria to Classify Sequence Variants

Pathogenic

1 1 Very Strong (PVS1) *AND*

- **a.** ≥1 Strong (PS1–PS4) *OR*
- **b.** ≥2 Moderate (PM1–PM6) *OR*
- **c.** 1 Moderate (PM1–PM6) and 1 Supporting (PP1–PP5) *OR*
- **d.** ≥2 Supporting (PP1–PP5)

2 ≥2 Strong (PS1–PS4) *OR*

3 1 Strong (PS1–PS4) *AND*

- **a.** ≥3 Moderate (PM1–PM6) *OR*
- **b.** 2 Moderate (PM1–PM6) *AND* ≥2 Supporting (PP1–PP5) *OR*
- **c.** 1 Moderate (PM1–PM6) *AND* ≥4 Supporting (PP1–PP5)

Likely Pathogenic

1 1 Very Strong (PVS1) *AND* 1 Moderate (PM1–PM6) *OR*

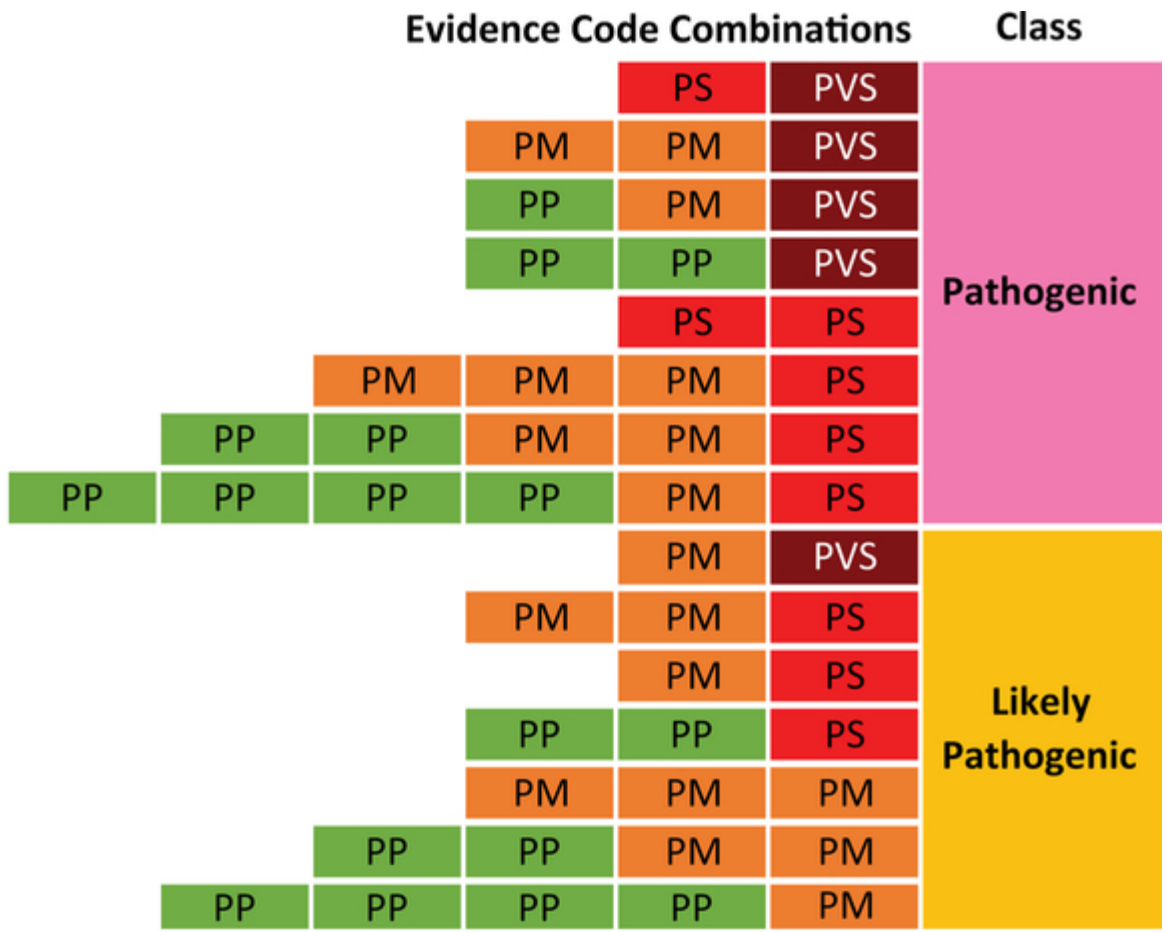
2 1 Strong (PS1–PS4) *AND* 1–2 Moderate (PM1–PM6) *OR*

3 1 Strong (PS1–PS4) *AND* ≥2 Supporting (PP1–PP5) *OR*

4 ≥3 Moderate (PM1–PM6) *OR*

5 2 Moderate (PM1–PM6) *AND* ≥2 Supporting (PP1–PP5) *OR*

6 1 Moderate (PM1–PM6) *AND* ≥4 Supporting (PP1–PP5)



Clinical utility: Case 1

- D.O.B 07/11/2014 (3 years and 8 months old at recruitment).
- First born child to non-consanguineous parents. Parents had first trimester miscarriage in 2010. Has a similarly affected brother.
- First seen at genetics in 2016.
- Main problem list : Macrocephaly (>3SD), moderate DD, flat nasal bridge, down slanted palpebral fissures, strabismus, hypotonia, café au lait spots.
- Development: Sat at 4/5 months, walked at 2 years, speech delay.

Previous investigations

- Hearing test: normal
- Renal: VCU, MAG3,
- Ultrasound: normal
- Cardiac echo normal
- CT brain: normal and no hydrocephalus
- Ophthalmology: Right eye esotropia
- FRAX: negative
- MLPA: negative
- Karyotype: 46, XY

WES

- Result: Heterozygous pathogenic variant (Class V) c.5395G>A:p.Glu1799Lysvariant in the *mTOR* gene
- Molecular diagnosis: Smith-Kingsmore syndrome
- Missense variant – recurrent variant
- ACMG Codes: PP5, PP3, PM2, PS3
- Two star ClinVar variant

Smith-kingsmore syndrome

- Rare genetic syndrome, so far reported in about 30 patients.
- It is associated with macrocephaly, hemi/megalencepahly, variable intellectual disability, autism spectrum disorder and seizures.
- Demonstrates high clinical variability.
- It is an Overgrowth-intellectual disability syndrome.
- Belongs to group of mTORopathies – neurological disorders characterized by altered cortical architecture, abnormal neuronal morphology, intractable epilepsy.

Management

- Received genetic counselling – there is now a name for her son's diagnosis.
- Received full clinical examination by a medical geneticist documenting features that are specific to the condition.
- Brother was assessed as having similar features – most likely has the same genetic condition, thus genetic testing is indicated.
- MRI Brain for the younger brother indicated.
- Prenatal testing in future pregnancies is an option.

Clinical utility: Case 2

- D.O.B 19/04/2014 (4 years and 6 months old at recruitment).
- Third born to non-consanguineous parents.
- First genetics assessment in 2017.
- Main problem list: moderate intellectual disability, colobomas and bilateral strabismus, bilateral sensory neural hearing loss, microcephaly, pigmentary abnormalities.
- Development: Sat at 1 year 6 months, crawled at 2 years, walked at 3 years, only 1 word at 4 years and 6 months.

Previous investigations

- MLPA: normal
- Karyotype: normal
- Visual assessment: visual loss due to structural abnormalities (colobomas)
- Hearing assessment: Profound sensorineural hearing loss
- CT scan: Bilateral posterior colobomas at origin of optic nerve, no intracranial pathology, anterior and posterior eye chambers normal

WES

- Result: Heterozygous pathogenic variant (Class V) variant in the *CHD7* gene (c.4480C>T, p.Arg1494Ter)
- Molecular diagnosis: *CHD7* Disorder
- Stop gain variant
- ACMG Codes: PVS1 PP3, PM2,PM6

- CHARGE - *c*oloboma, *h*eart defects, choanal *a*tresia, *r*etarded growth and development, *g*enital hypoplasia, *e*ar anomalies including deafness.

Management

- Received genetic counselling result provided parents with answers to the cause of the condition, they felt that with an answer their daughter will be better managed.
- Received full clinical examination by a medical geneticist documenting features that are specific to the condition
- Endocrine referral to screen for hypogonadotropic hypogonadism, hypothyroidism, hypoparathyroidism, hypocalcaemia
- X-ray referral to screen for renal abnormalities (horseshoe kidneys, renal agenesis, renal hypoplasia
- Monitoring of blood pressure
- Anaesthetic risk management