Variant interpretation using the ACMG-AMP guidelines

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WCEP Wessex Clinical Exome Pilot Study

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WRGL Wessex Regional Genetics Laboratory

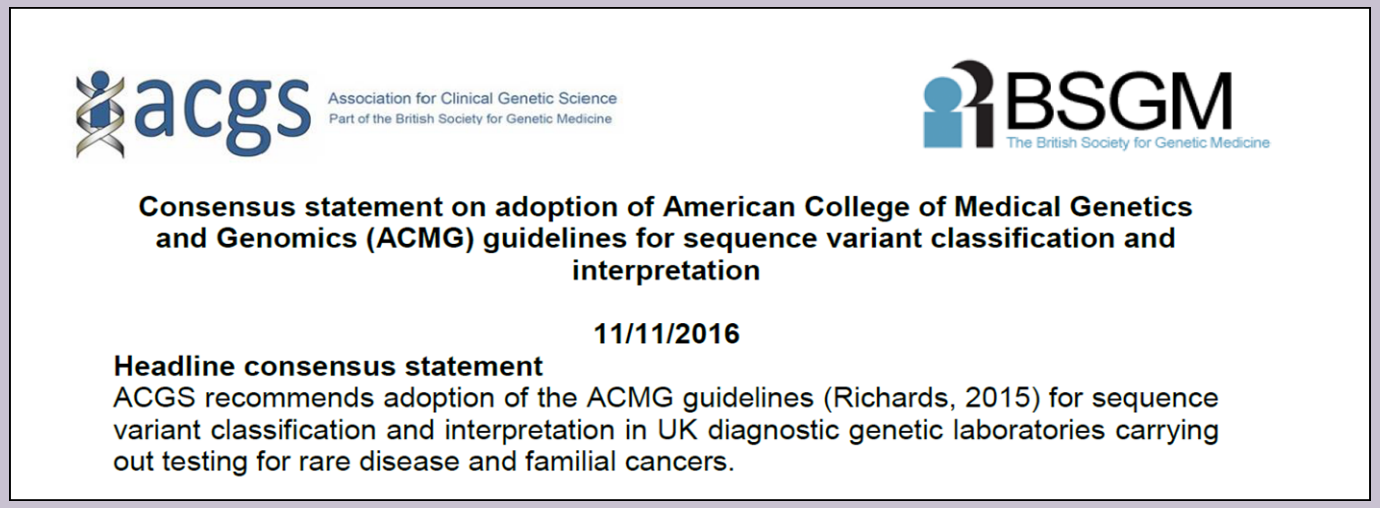
Talk outline

- Overview of the ACMG-AMP guidelines.
- Advantages and disadvantages.
- Case studies.
- Tools to help apply criteria.
- The WRGL experience.
- MSc project findings.
- Further work.

Variant interpretation prior to the release of the ACMG guidelines

At the WRGL we followed the 2013 ACGS BPGs for the evaluation of pathogenicity and reporting of sequence variants. These provided guidelines on types of evidence to collect, variant nomenclature, types of variants to report, the classification system which should be used and the associated wording on the report.
BUT…
No guidance as to what evidence fulfils which pathogenicity class, at WRGL we exercised professional judgement on a case by case basis.

In November 2016:


acgs Association for Clinical Genetic Science
Part of the British Society for Genetic Medicine

BSGM
The British Society for Genetic Medicine

Consensus statement on adoption of American College of Medical Genetics and Genomics (ACMG) guidelines for sequence variant classification and interpretation

11/11/2016

Headline consensus statement

ACGS recommends adoption of the ACMG guidelines (Richards, 2015) for sequence variant classification and interpretation in UK diagnostic genetic laboratories carrying out testing for rare disease and familial cancers.

The ACMG-AMP guidelines

ACMG STANDARDS AND GUIDELINES | Genetics inMedicine

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[1], Nazneen Aziz, PhD[2,16], Sherri Bale, PhD[3], David Bick, MD[4], Soma Das, PhD[5], Julie Gastier-Foster, PhD[6,7,8], Wayne W. Grody, MD, PhD[9,10,11], Madhuri Hegde, PhD[12], Elaine Lyon, PhD[13], Elaine Spector, PhD[14], Karl Voelkerding, MD[13] and Heidi L. Rehm, PhD[15]; on behalf of the ACMG Laboratory Quality Assurance Committee

Disclaimer: These ACMG Standards and Guidelines were developed primarily as an educational resource for clinical laboratory geneticists to help them provide quality clinical laboratory services. Adherence to these standards and guidelines is voluntary and does not necessarily assure a successful medical outcome. These Standards and Guidelines should not be considered inclusive of all proper procedures and tests or exclusive of other procedures and tests that are reasonably directed to obtaining the same results. In determining the propriety of any specific procedure or test, the clinical laboratory geneticist should apply his or her own professional judgment to the specific circumstances presented by the individual patient or specimen. Clinical laboratory geneticists are encouraged to document in the patient's record the rationale for the use of a particular procedure or test, whether or not it is in conformance with these Standards and Guidelines. They also are advised to take notice of the date any particular guideline was adopted and to consider other relevant medical and scientific information that becomes available after that date. It also would be prudent to consider whether intellectual property interests may restrict the performance of certain tests and other procedures.

The ACMG-AMP guidelines

- 28 criteria.
- Covers a variety of evidence: All evidence should be assessed and included (current case, previous cases and cases in the literature).
  - Population data
  - Functional data
  - Segregation studies
  - *De novo* observations
  - Allelic data
  - Computational predictions
- Be cautious of using the same piece of information twice.
- All criteria have default weightings, but professional judgement can be used to change the strength of evidence for most of the criteria.

Pathogenic evidence categories		**Benign evidence categories**	
Very strong	PVS1	Stand-alone	BA1
Strong	PS1-4	Strong	BS1-4
Moderate	PM1-6		
Supporting	PP1-5	Supporting	BP1-6

The ACMG-AMP guidelines

- Accumulated criteria is then compared to the classification rules table.
- If insufficient evidence to reach pathogenic/likely pathogenic of benign/likely benign then the variant defaults to VUS.

Table 5 Rules for combining criteria to classify sequence variants

Pathogenic	(i) 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)
	(ii) ≥2 Strong (PS1–PS4) *OR*
	(iii) 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	(i) 1 Very strong (PVS1) *AND* 1 moderate (PM1–PM6) *OR*
	(ii) 1 Strong (PS1–PS4) *AND* 1–2 moderate (PM1–PM6) *OR*
	(iii) 1 Strong (PS1–PS4) *AND* ≥2 supporting (PP1–PP5) *OR*
	(iv) ≥3 Moderate (PM1–PM6) *OR*
	(v) 2 Moderate (PM1–PM6) *AND* ≥2 supporting (PP1–PP5) *OR*
	(vi) 1 Moderate (PM1–PM6) *AND* ≥4 supporting (PP1–PP5)
Benign	(i) 1 Stand-alone (BA1) *OR*
	(ii) ≥2 Strong (BS1–BS4)
Likely benign	(i) 1 Strong (BS1–BS4) and 1 supporting (BP1–BP7) *OR*
	(ii) ≥2 Supporting (BP1–BP7)
Uncertain significance	(i) Other criteria shown above are not met OR
	(ii) the criteria for benign and pathogenic are contradictory

Advantages and disadvantages

- Weighted evidence framework promotes consistent variant interpretation.
- Facilitates resolution of discrepancies.
- Enables iterative building of evidence over time.
  - Easy to identify what additional information is required to upgrade or downgrade classification (useful at MDT).

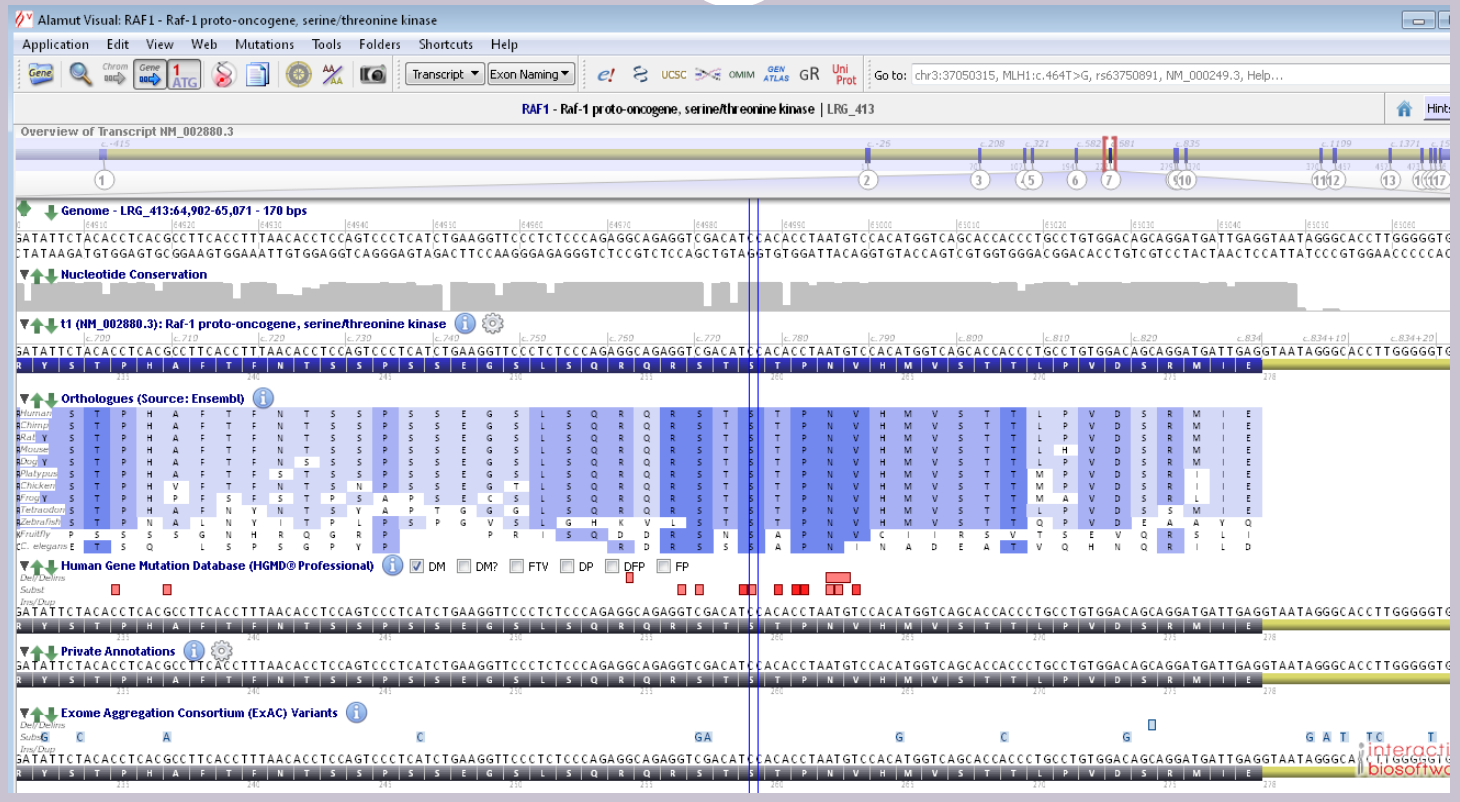
But…

- The ACMG-AMP guidelines lack specific details/parameters/cut offs.
  - Makes some criteria subjective
    - Evaluation of the guidelines by Amendola 2016 showed that inter-laboratory concordance was 34% (a similar rate to prior in-house methods).
- Evidence is now split and not considered as a whole.
- Guidelines are suitable primarily for Mendalian disorders, they are not suitable for disorders with variable penetrance.

Case 1 (case 1 from the Train the Trainers workshop)

- ?CHARGE syndrome/Rasopathy
- Features: Polyhydramnios and ventricular septal defect prenatally, severe left ventricular outflow tract obstruction, 2/3 syndactyly of left foot, nevus on chest wall, cutis aplasia of one foot, unusual ears.
- Tested on a clinical exome for CHARGE syndrome (*CHD7*) and Rasopathy gene panel (*BRAF, CBL, HRAS, KRAS, MAP2K1, MAP2K2, NF1, NRAS, PTPN11, RAF1, RIT1, SHOC2, SOS1* and *SPRED1*.
- Result: Heterozygous for a *RAF1* variant c.776C>G p.(Ser259Cys).
- Parents not yet tested.

Evidence for Case 1
RAF1 c.776C>G p.(Ser259Cys).



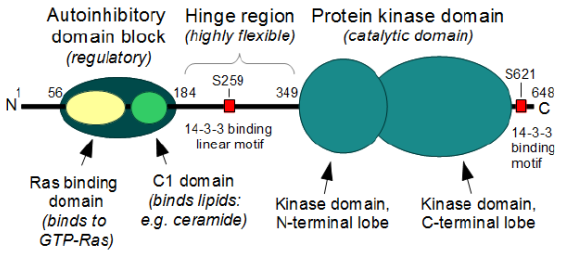
Alamut screen shot showing: conserved amino acid with conserved surrounding amino acids, very little ExAC variation, a cluster of HGMD Pro variants. ?hotspot.

Evidence for Case 1
RAF1 c.776C>G p.(Ser259Cys).

HGMD Pro

CM073300	TCC-TTC	Ser259Phe	c.776C>T	p.S259F	DM	Noonan syndrome
CM137735	TCC-CCC	Ser259Pro	c.775T>C	p.S259P	DM	Noonan syndrome
CM086899	TCC-ACC	Ser259Thr	c.775T>A	p.S259T	DM	Noonan syndrome

Schematic architecture of the human c-Raf kinase



Between the autoinhibitory domain block and the catalytic kinase domain, a long segment - characteristic to all Raf proteins - can be found. It is highly enriched in serine amino acids, but its precise sequence is poorly conserved across related Raf genes. This region appears to be intrinsically unstructured, and very flexible. Its most likely role is to act as a natural "hinge" between the rigidly folded autoinhibitory and catalytic domains, enabling complex movements and profound conformational rearrangements within the molecule.[27] This hinge region contains a small, conserved island of amino acids, that are responsible for 14-3-3 protein recognition, but only when a critical serine (Ser259 in human c-Raf) is phosphorylated. A second, similar motif is found on the extreme C-terminus (centered around the phosphorylatable Ser 621) of all Raf enzymes, but downstream of the kinase domain.

Pandit et al (2007) Nature Genetics, 39 (8): 1007-1012.

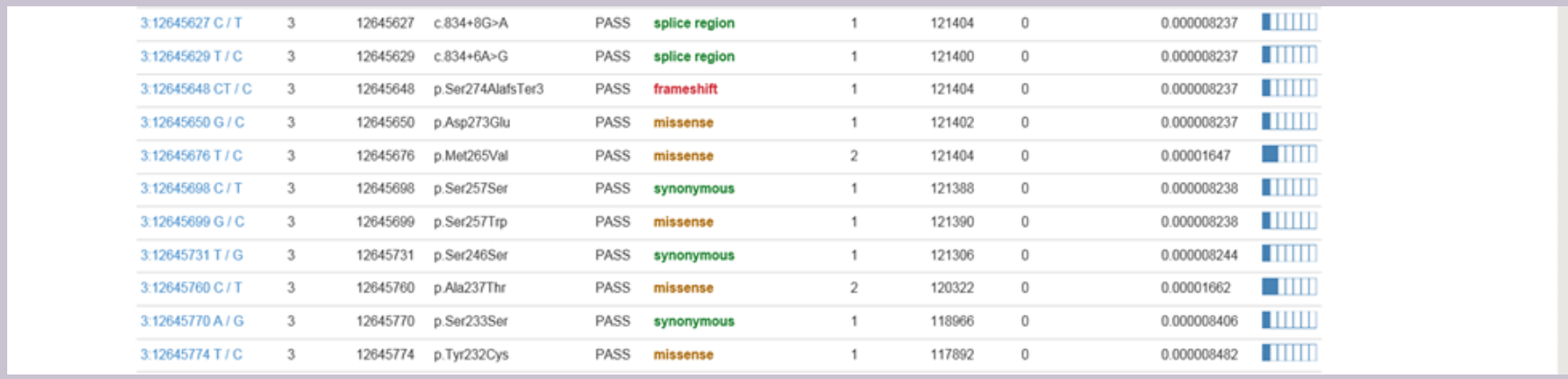
RAF1 is highly regulated, with numerous serine and threonine residues that can be phosphorylated, resulting in activation or inactivation[15]. Among these, Ser259, which resides in conserved region 2 (CR2; **Fig. 1**), is particularly important. In its inactive conformation, the N-terminal portion of RAF1 is thought to interact with and inactivate the kinase domain at the C terminus. This conformation is stabilized by 14-3-3 protein dimers that bind to phosphorylated Ser259 and Ser621 (ref. 19). Dephosphorylation of

PM1 can be applied

PM1 Located in a mutational hot spot and/or critical and well-established functional domain (e.g., active site of an enzyme) without benign variation.

Codon 259 has been proved functionally to be a critical residue, 3 disease causing mutations in HGMD Pro and no variation at codon 259 in ExAC.

Evidence for Case 1
RAF1 c.776C>G p.(Ser259Cys).



3:12645627 C / T	3	12645627	c.834+8G>A	PASS	splice region	1	121404	0	0.000008237
3:12645629 T / C	3	12645629	c.834+6A>G	PASS	splice region	1	121400	0	0.000008237
3:12645648 CT / C	3	12645648	p.Ser274AlafsTer3	PASS	frameshift	1	121404	0	0.000008237
3:12645650 G / C	3	12645650	p.Asp273Glu	PASS	missense	1	121402	0	0.000008237
3:12645676 T / C	3	12645676	p.Met265Val	PASS	missense	2	121404	0	0.00001647
3:12645698 C / T	3	12645698	p.Ser257Ser	PASS	synonymous	1	121388	0	0.000008238
3:12645699 G / C	3	12645699	p.Ser257Trp	PASS	missense	1	121390	0	0.000008238
3:12645731 T / G	3	12645731	p.Ser246Ser	PASS	synonymous	1	121306	0	0.000008244
3:12645760 C / T	3	12645760	p.Ala237Thr	PASS	missense	2	120322	0	0.00001662
3:12645770 A / G	3	12645770	p.Ser233Ser	PASS	synonymous	1	118966	0	0.000008406
3:12645774 T / C	3	12645774	p.Tyr232Cys	PASS	missense	1	117892	0	0.000008482

PM2 can be applied

PM2 Absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project,
1000 Genomes Project, or Exome Aggregation Consortium.

Evidence for Case 1
RAF1 c.776C>G p.(Ser259Cys).

HGMD Pro

CM073300	TCC-TTC	Ser259Phe	c.776C>T	p.S259F	DM	Noonan syndrome
CM137735	TCC-CCC	Ser259Pro	c.775T>C	p.S259P	DM	Noonan syndrome
CM086899	TCC-ACC	Ser259Thr	c.775T>A	p.S259T	DM	Noonan syndrome

PM5 can be applied:

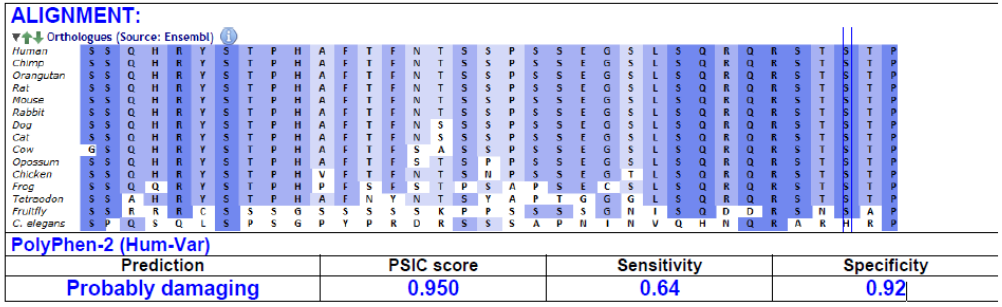
PM5 Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before.

When investigating the other variants at codon 259, one of them had functional data and had been shown to have occurred *de novo* (pathogenic in it's own right).

Evidence for Case 1
RAF1 c.776C>G p.(Ser259Cys).

Pathogenicity clues

- Highly conserved nucleotide (phyloP: 6.26 [-14.1;6.4])
- Highly conserved amino acid, up to Fruitfly (considering 15 species)
- Moderate physicochemical difference between Ser and Cys (Grantham dist.: 112 [0-215])
- Align GVGD: C15 (GV: 88.94 - GD: 109.21)
- SIFT: Deleterious (score: 0.03, median: 3.43)
- MutationTaster: disease causing (p-value: 1)



PolyPhen-2 (Hum-Var)

Prediction	PSIC score	Sensitivity	Specificity
Probably damaging	0.950	0.64	0.92

PP3 can be applied

PP3 Multiple lines of computational evidence support a deleterious effect on the gene or gene product
(conservation, evolutionary, splicing impact, etc.)

Evidence which can't be applied to Case 1 *RAF1* variant c.776C>G p.(Ser259Cys).

PS4 (moderate) can not be applied

PS4 The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls.

The exact same variant has not been reported in multiple individuals with disease.

PS3 can not be applied

PS3 Well-established in vitro or in vivo functional studies supportive of a damaging effect on the gene or gene product.

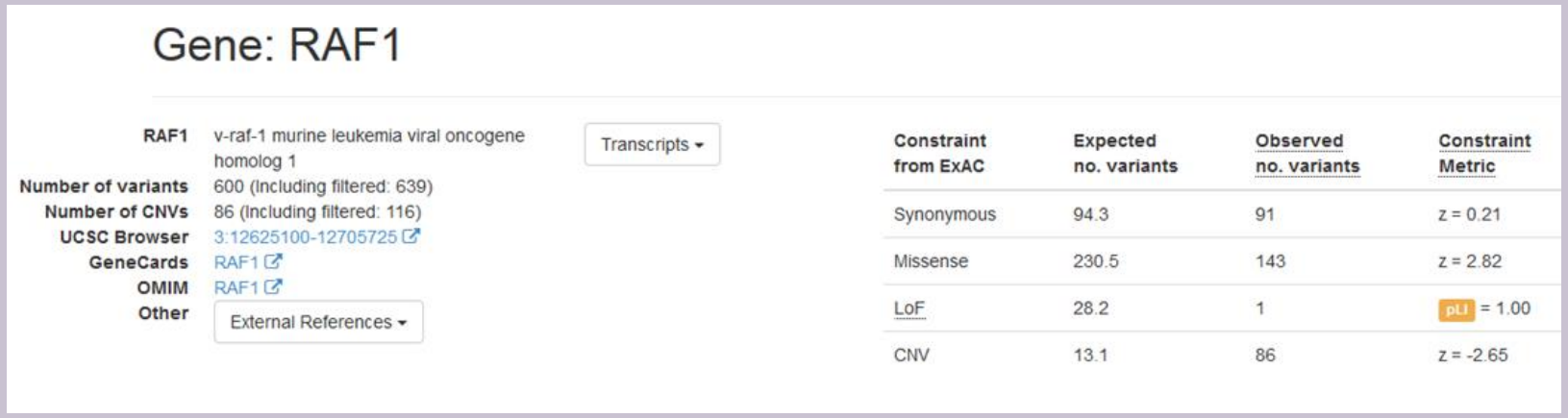
No functional evidence that this exact variant has a deleterious effect on the gene product and although a functional effect has been proved for variants at this codon this information has already been used for the mutation hotspot criteria.

Evidence which can't be applied to Case 1 *RAF1* variant c.776C>G p.(Ser259Cys).

PP2 can not be applied

PP2 Missense variant in a gene that has a low rate of benign missense variation and in which missense variants
are a common mechanism of disease

The missense constraint score for RAF1 is high but does not reach a statistically significant Z score.



Gene: RAF1

RAF1	v-raf-1 murine leukemia viral oncogene homolog 1
Number of variants	600 (including filtered: 639)
Number of CNVs	86 (including filtered: 116)
UCSC Browser	3:12625100-12705725
GeneCards	RAF1
OMIM	RAF1
Other	External References

Transcripts

Constraint from ExAC	Expected no. variants	Observed no. variants	Constraint Metric
Synonymous	94.3	91	z = 0.21
Missense	230.5	143	z = 2.82
LoF	28.2	1	pLI = 1.00
CNV	13.1	86	z = -2.65

Applying the criteria to the classification rules.

We have satisfied PM2, PM5, PM1 and PP3. (3 x moderate and 1 x supporting).

= Class 4 (likely pathogenic)

In order to reach class 5, a strong piece of evidence is required. Testing the parents and proving the variant had occurred *de novo* (and checking maternity and paternity) would provide the additional criteria required for a class 5.

Table 5 Rules for combining criteria to classify sequence variants

Pathogenic	(i) 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)
	(ii) ≥2 Strong (PS1–PS4) *OR*
	(iii) 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	(i) 1 Very strong (PVS1) *AND* 1 moderate (PM1–PM6) *OR*
	(ii) 1 Strong (PS1–PS4) *AND* 1–2 moderate (PM1–PM6) *OR*
	(iii) 1 Strong (PS1–PS4) *AND* ≥2 supporting
	(iv) ≥3 Moderate (PM1–PM6) *OR*
	(v) 2 Moderate (PM1–PM6) *AND* ≥2 supporting (PP1–PP5) *OR*
	(vi) 1 Moderate (PM1–PM6) *AND* ≥4 supporting (PP1–PP5)
Benign	(i) 1 Stand-alone (BA1) *OR*
	(ii) ≥2 Strong (BS1–BS4)
Likely benign	(i) 1 Strong (BS1–BS4) and 1 supporting (BP1–BP7) *OR*
	(ii) ≥2 Supporting (BP1–BP7)
Uncertain significance	(i) Other criteria shown above are not met OR
	(ii) the criteria for benign and pathogenic are contradictory

Case 2

- MDT case.
- 9 year old boy with severe micropthalmia, complete arhinia, microcephaly, and marked midface hypoplasia with jaw misalignment (a clear diagnosis of Bosma Arhinia Micropthalmia (BAM)).
- Gene found to be responsible recently discovered.
- French research group e-mailed referring clinician to say this patient had been found to be heterozygous for c.1043A>G p.(His348Arg) variant in *SMCHD1*.
- Research group also said it had arisen *de novo*.

Evidence for Case 2
SMCHD1 c.1043A>G p.(His348Arg)

Gene: SMCHD1

SMCHD1	structural maintenance of chromosomes flexible hinge domain containing 1
Number of variants	1237 (Including filtered: 1435)
Number of CNVs	24 (Including filtered: 91)
UCSC Browser	18:2655737-2805015
GeneCards	SMCHD1
OMIM	SMCHD1
Other	External References

Transcripts

Constraint from ExAC	Expected no. variants	Observed no. variants	Constraint Metric
Synonymous	201.7	215	z = -0.58
Missense	505.5	407	z = 2.14
LoF	69.4	3	pLI = 1.00
CNV	10.3	24	z = -1.03

Can't use PP2 as the missense constraint score has not reached significance

PP2 Missense variant in a gene that has a low rate of benign missense variation and in which missense variants are a common mechanism of disease

18:2694669 C / T	18	2694669	p.His340Tyr	PASS	missense	2	119834	0	0.00001669
18:2694670 A / C	18	2694670	p.His340Pro	PASS	missense	2	119850	0	0.00001669
18:2694682 G / A	18	2694682	p.Arg344Gln	PASS	missense	6	119670	0	0.00005014
18:2694690 G / A	18	2694690	p.Ala347Thr	PASS	missense	4	119504	0	0.00003347
18:2697036 T / G	18	2697036	p.Ile349Met	PASS	missense	1	19578	0	0.00005108
18:2697075 A / G	18	2697075	p.Ile362Met	PASS	missense	3	20410	0	0.0001470
18:2697115 A / T (rs185618962)	18	2697115	p.Ile376Phe	PASS	missense	2	19046	0	0.0001050
18:2697835 A / G	18	2697835	p.Met380Val	PASS	missense	1	120138	0	0.000008324

Variant not present on the databases of normal variation and coverage of the site is good so PM2 can be applied

PM2 Absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium.

Evidence for Case 2
SMCHD1 c.1043A>G p.(His348Arg)

Table 1 ***SMCHD1*** **mutations observed in the arhinia cohort**

Chr.	Nucleotide mutation	Exon	Inheritance (sample ID)	Number of subjects	Sample ID[a]	Amino acid alteration	Sex (sample ID)
18	g.2666926T>C	3	N/A	1	K1	p.Leu107Pro	F
18	g.2666992T>A	3	N/A	1	D1	p.Met129Lys	M
18	g.2667009A>T	3	*De novo* (AF1) N/A (M1)	2	M1, AF1	p.Ser135Cys	F (M1, AF1)
18	g.2667010G>A	3	*De novo* (I1) N/A[b] (R1)	2	I1, R1	p.Ser135Asn	F (R1), M (I1)
18	g.2667010G>T	3	*De novo*	1	AK1	p.Ser135Ile	M
18	g.2667014A>C	3	Father[b]	1	T1	p.Glu136Asp	M
18	g.2667016G>A	3	N/A	1	AG1	p.Gly137Glu	F
18	g.2667021A>C	3	*De novo* (A1) N/A (Y1)	2	A1, Y1	p.Asn139His	F (A1, Y1)
18	g.2667029G>C	3	N/A	3	C1, E1, S1	p.Leu141Phe	F (S1), M (C1, E1)
18	g.2667029G>T	3	*De novo*	1	V1	p.Leu141Phe	M
18	g.2674017T>G	5	N/A[b]	1	AB1	p.Phe171Val	M
18	g.2688478C>G	6	*De novo*	1	AA1	p.Ala242Gly	M
18	g.2694685A>G	8	Mother[b]	2	O1, O4[c]	p.Gln345Arg	F (O1, O4)
18	g.2697032A>G	9	*De novo* (X1, AC1, AE1) N/A (F1, L1, N1, Z1)	7	F1, L1, N1, Z1, X1, AC1, AE1	p.His348Arg	F (L1, X1), M(F1, N1, Z1, AC1, AE1)
18	g.2697896A>T	10	Father[b]	1	AH1	p.Gln400Leu	F
18	g.2697956A>T	10	*De novo*	1	P1	p.Asp420Val	M
18	g.2700611G>C	11	N/A	1	W1	p.Glu473Gln	M
18	g.2700837C>A	12	N/A	2	J1, U1	p.Thr523Lys	F (U1), M (J1)
18	g.2700840A>G	12	N/A	1	B1	p.Asn524Ser	M
18	g.2703697G>A	13	N/A	1	AJ1	p.Arg552Gln	M

[a]Samples L1, M1, N1, P1, AF1 and AJ1 overlap with those studied in Gordon *et al.*[6] . [b]Multiplex family. [c]Sibling. Subjects G1, H1, H2, Q1, AD1, AI and AL1 did not show a rare missense mutation in *SMCHD1*. N/A, parental samples not available; M, male; F, female.

PS4 (moderate) can be applied – Multiple cases of this variant in disease and absent in controls

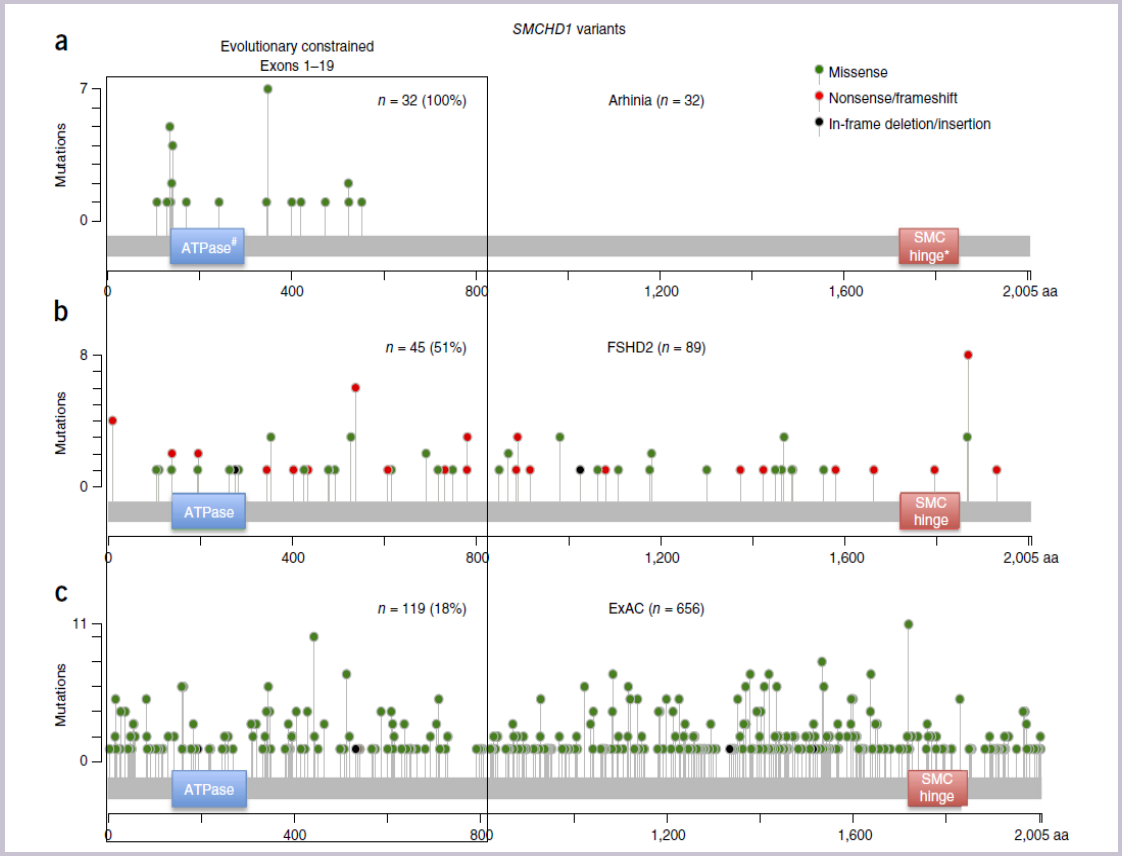
PS4 The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls.

PM6 can be applied – even though it has not been proved in our case, the Shaw paper documents 3/7 cases that have been found to be *de novo*.

PM6 Assumed de novo, but without confirmation of paternity and maternity.

Table taken from Shaw *et al Nature Genetics* 2017;49(2):239-247

Evidence for Case 2
SMCHD1 c.1043A>G p.(His348Arg)



PM1 (downgraded to supporting) was applied to this case.

PM1 Located in a mutational hot spot and/or critical and well-established functional domain (e.g., active site of an enzyme) without benign variation

The evidence in the literature shows that all the variants identified in the affected patients are clustered at the 5' end of the gene between exons 3 and 12. Even though there is substantial benign variation the evidence suggests that this area of the gene shows a higher constraint.

Figure taken from Shaw *et al Nature Genetics* 2017;49(2):239-247

Evidence for Case 2
SMCHD1 c.1043A>G p.(His348Arg)

- PP4 can be applied in this case – The phenotype is very clinically distinguishable and the vast majority of cases have been found to have a missense variant in the *SMCHD1* gene and no alternative causes identified.
- *PP4 Patient's phenotype or family history is highly specific for a disease with a single genetic etiology*

- PP3 can be applied as all the *in silico* programs support pathogenicity.
- *PP3 Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.)*

Applying the criteria to the classification rules.

We have fulfilled the following criteria: PS4 (mod), PM2, PM6, PM1(supporting), PP4 and PP3 (3 x moderate and 3 x supporting)

= Class 4 (Likely pathogenic)

Would require a strong piece of evidence to reach class 5 could be achieved by:

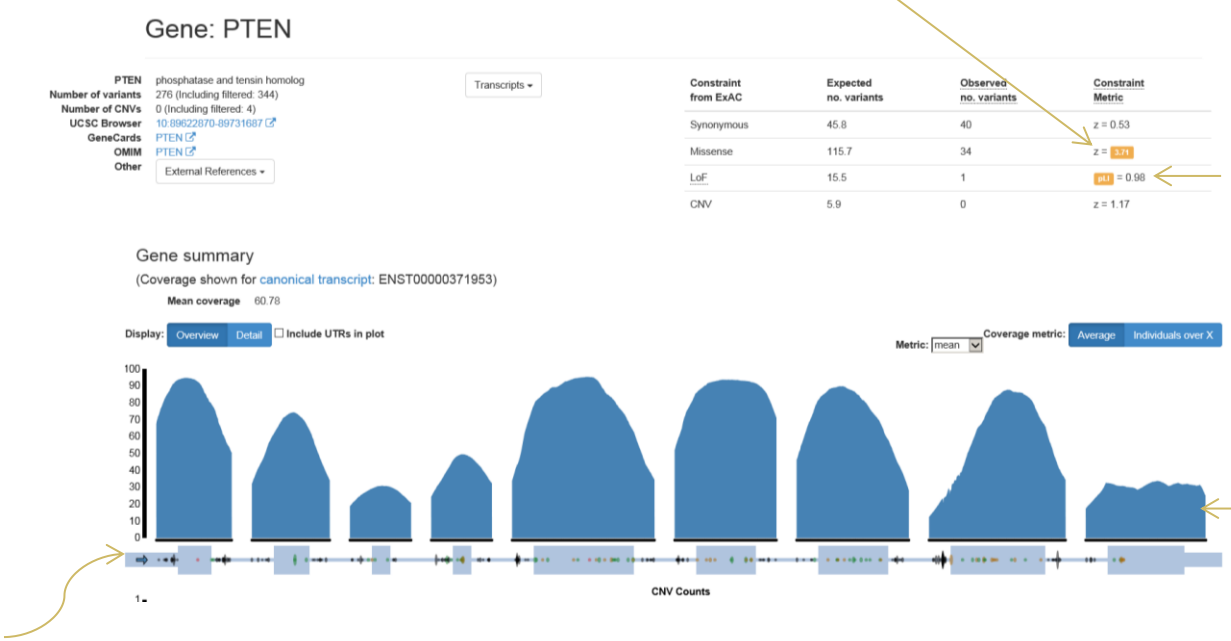
- Confirming maternity and paternity in our case (although out of the 4 known *de novo* cases it is unlikely to be false parentage in all 4 cases. So ? Safe to upgrade anyway)
- Possible co-segregation data (on OMIM it mentions 4 cases of segregation with this variant, but unable to find evidence of this).

Table 5 Rules for combining criteria to classify sequence variants

Pathogenic	(i) 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) (ii) ≥2 Strong (PS1–PS4) *OR* (iii) 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	(i) 1 Very strong (PVS1) *AND* 1 moderate (PM1–PM6) *OR* (ii) 1 Strong (PS1–PS4) *AND* 1–2 moderate (PM1–PM6) *OR* (iii) 1 Strong (PS1–PS4) *AND* ≥2 supporting (PP1–PP5) *OR* (iv) ≥3 Moderate (PM1–PM6) *OR* (v) 2 Moderate (PM1–PM6) *AND* ≥2 supporting (PP1–PP5) *OR* (vi) 1 Moderate (PM1–PM6) *AND* ≥4 supporting (PP1–PP5)
Benign	(i) 1 Stand-alone (BA1) *OR* (ii) ≥2 Strong (BS1–BS4)
Likely benign	(i) 1 Strong (BS1–BS4) and 1 supporting (BP1–BP7) *OR* (ii) ≥2 Supporting (BP1–BP7)
Uncertain significance	(i) Other criteria shown above are not met OR (ii) the criteria for benign and pathogenic are contradictory

Helpful tools - Gene/variant information in ExAC

Missense Z score, significant (highlighted) if >3.09, this means than there are statistically less observed missense variants in this gene than expected. **PP2**



The LOF score pLI is the probability of the gene being loss of function tolerant and can give an indication as to whether LOF mutations are tolerated in your gene of interest. For genes that cause dominant disorders only. Can <u>support</u> **PVS1**

Is graphical representation of all the variants listed in ExAC for your gene, this can be useful for looking at mutation hotspots (you may see clustering of LOF mutations) or for variant deserts (areas of no variation which may indicate an area of the gene which is functionally very important and variation is not tolerated) This information can be used to support literature information for **PM1** = Mutational hotspot or well-studied functional domain, without benign variation.

ExAC shows you how well your gene is covered, ExAC will warn you if your variant is covered by less than 80% of the entire data.

Looking at the list of variants in ExAC - MECP2

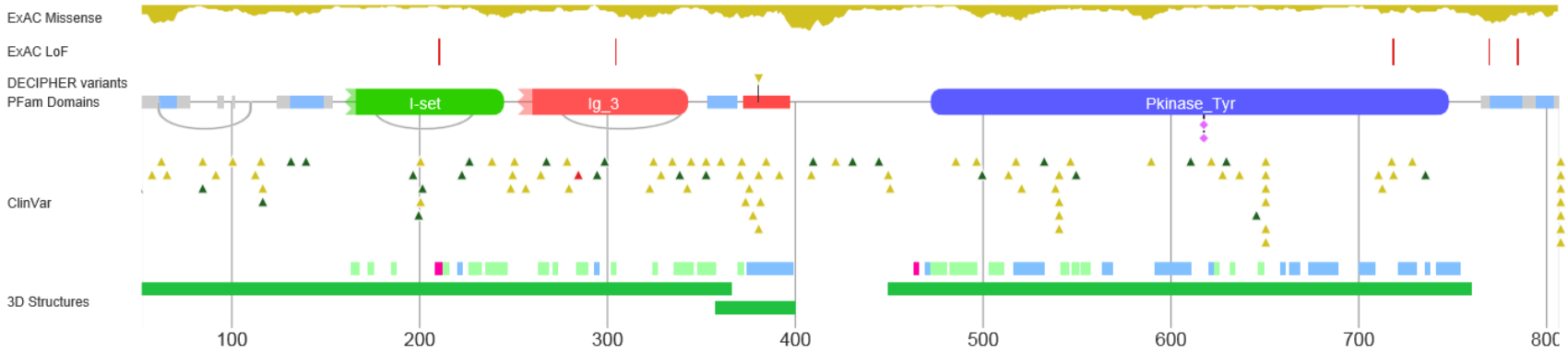
Variant	Chrom	Position	Consequence	Filter	Annotation	Flags	Allele Count	Allele Number	Number of Homozygotes	Number of Hemizygotes	Allele Frequency
X:153296161 G / T	X	153296161	p.Ser385Ter	PASS	stop gained		1	81695	0	0	0.00001224
X:153296689 G / A (rs61749714)	X	153296689	p.Arg161Ter†	PASS	stop gained		48	87676	0	20	0.0005475
X:153296070 A / AG	X	153296070	p.Glu416Ter	PASS	frameshift		1	84912	0	0	0.00001178
X:153296090 CGGAGCTCTCGGGCTCAGGT... / C	X	153296090	p.Pro400ArgfsTer12	PASS	frameshift		1	83272	0	0	0.00001201
X:153296104 TCAGG / T	X	153296104	p.Pro403SerfsTer17	PASS	frameshift		1	82363	0	1	0.00001214
X:153296112 AGGTGGGG / A	X	153296112	p.Pro399LeufsTer20	PASS	frameshift		1	81362	0	1	0.00001229
X:153296651 T / C	X	153296651	p.Ter173TrpextTer38†	PASS	stop lost		1	87723	0	0	0.00001140
X:153357723 T / A	X	153357723	p.Ter35CysextTer103†	PASS	stop lost		1	75580	0	1	0.00001323
X:153363075 G / GCCT	X	153363075	p.Gly16dup	PASS	inframe insertion		53	27350	0	15	0.001938
X:153363099 C / CGCGGCG	X	153363099	p.Ala7_Ala8dup	PASS	inframe insertion		10	31037	0	1	0.0003222
X:153363099 C / CGCG	X	153363099	p.Ala8dup	PASS	inframe insertion		2	31037	0	0	0.00006444
X:153363099 C / CGCGGCGGCG	X	153363099	p.Ala6_Ala8dup	PASS	inframe insertion		3	31037	0	0	0.00009666
X:153295944 CGTGGCGGCG / C	X	153295944	p.Thr457_Ala459del	PASS	inframe deletion		2	87733	0	1	0.00002280
X:153296090 CGGAGCTCTCGGGCTCAGGT... / C	X	153296090	p.Pro400_Ser408del	PASS	inframe deletion		1	83272	0	0	0.00001201
X:153296093 AGCTCTC / A	X	153296093	p.Glu406_Ser407del	PASS	inframe deletion		1	83326	0	1	0.00001200
X:153296099 CGGGCTCAGGTGGAGGTGG / C	X	153296099	p.Pro400_Pro405del	PASS	inframe deletion		4	82722	0	1	0.00004835
X:153296105 CAGGTGG / C (rs61753008)	X	153296105	p.Pro402_Pro403del	PASS	inframe deletion		7	82287	0	2	0.00008507

The list of variants for your gene of interest in ExAC can provide a lot of supporting evidence for mode of inheritance and mechanism of pathogenesis.

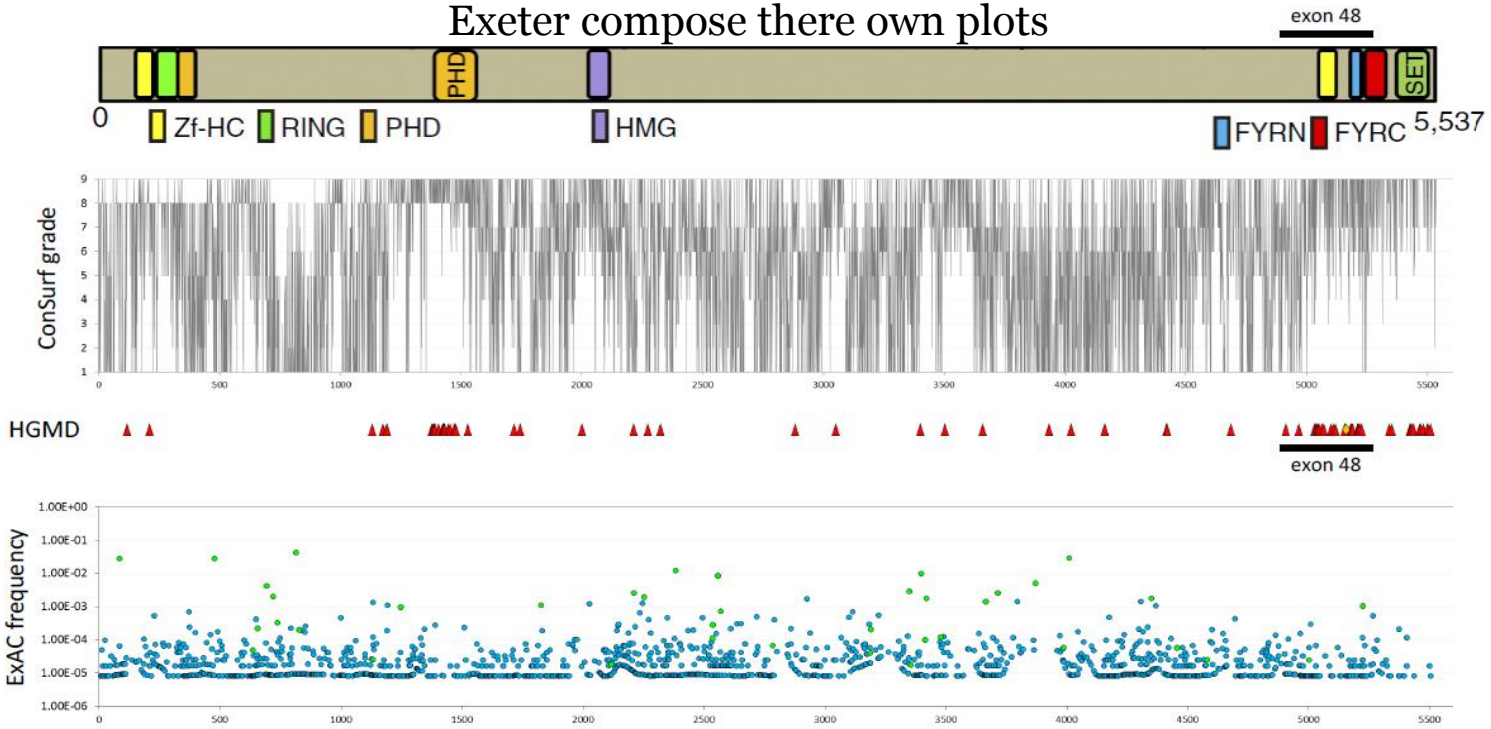
- It can indicate whether LOF mutations at the end of the gene are likely to be tolerated.
- If your variant is listed it may satisfy BS2 (observed in healthy adults) or allele frequency may be higher than the prevalence of the disorder – BS1.
- Would not expect to find a disease causing variant in males.

P22607 806aa Links ?

Taken from DECIPHER



Exeter compose there own plots

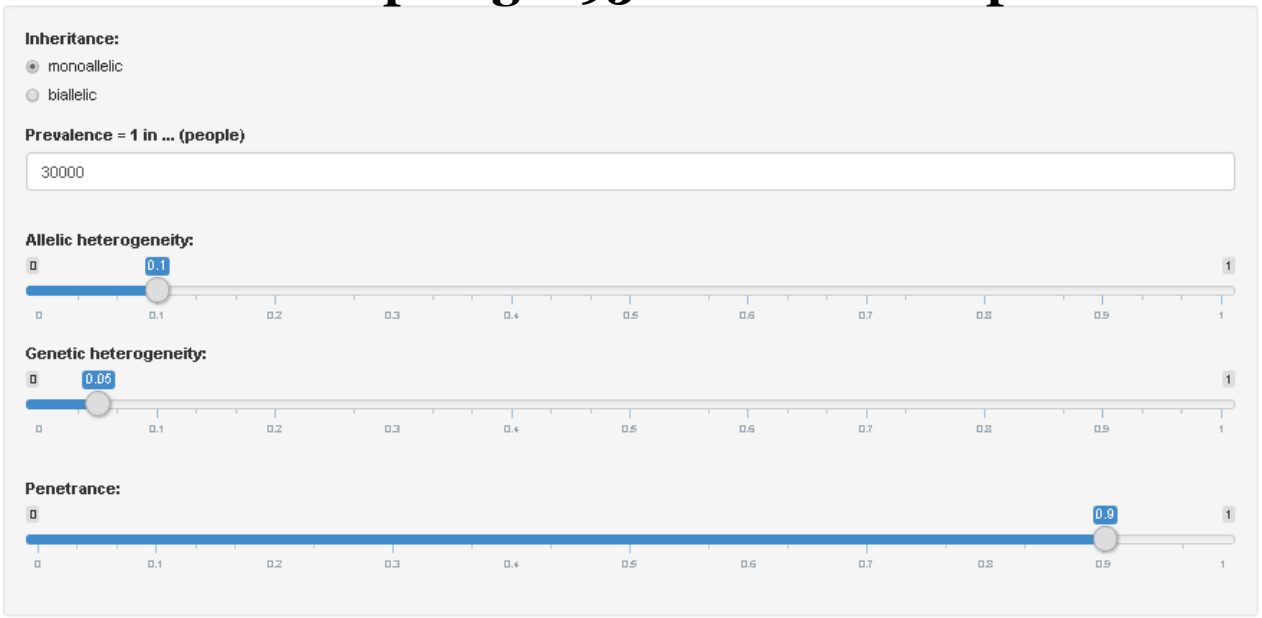


Using high-resolution variant frequencies to empower clinical genome interpretation

bioRxiv beta
THE PREPRINT SERVER FOR BIOLOGY

Nicola Whiffin[1,2,*],Eric Minikel[3,4,*],Roddy Walsh[1,2],Anne O'Donnell-Luria[3,4],Konrad Karczewski[3,4],Alexander Y Ing[5,6],Paul JR Barton[1,2],Birgit Funke[6,7],Stuart A Cook[1,2,8,9,†],Daniel MacArthur[3,4,10,†],James S Ware[1,2,4,11,†,§]

NOTCH2 p.Arg1895His variant in patient with ?Alagille syndrome



Maximum credible population AF:

9.26e-08

Population Frequencies

Population	Allele Count	Allele Number
East Asian	6	8640
European (Non-Finnish)	9	66596
South Asian	1	16508
African	0	10360
European (Finnish)	0	6606
Latino	0	11564
Other	0	906
Total	**16**	**121180**

Maximu

0

https://jamesware.shinyapps.io/alleleFrequencyApp/

Slide taken from Sian Ellard's talk at the variant interpretation workshop 28/02/17

Experience of implementing the guidelines.

- Two Clinical Scientists (molecular and arrays), 2 days each.
- We utilised previously unanalysed clinical exome data – we comprehensively analysed and classified the variants of 27 cases (identified after gene and MAF filtering).
- Used this experience to draft an SOP, which provided some extra/clearer descriptions of the criteria, set a few parameters, and documented the associated cautions of using certain criteria.
- We swapped 3 cases with Exeter to ensure we had interpreted the guidelines in the same way they had.
- We redesigned the variant interpretation table, which made it easier to see what additional information was required to make a different classification.
- We generated a checklist/evidence recording spreadsheet.
- ACMG-AMP interpretation rolled out to MDT cases and we are currently in the process of changing our diagnostic reports to reflect the evidence collected to satisfy the ACMG-AMP criteria.
- Training – we have delivered a 1 hour seminar to all the scientists, feedback is given to all scientists who interpret MDT cases and some individuals have had one to one training.

At the WRGL – SOP to support the use of the guidelines

PS4 (moderate) = variant observed in multiple patients with the same phenotype and absent in controls.

For vary rare variation (where case control studies cannot reach statistical significance due to low numbers), if your variant of interest has been observed in ≥2 affected unrelated individuals (in-house and/or in the literature) AND it is absent in controls (not listed on ExAC) the PS4 criteria can be applied at moderate level evidence.

PS1 = Same amino-acid change as an established pathogenic variant (different nucleotide change).

Search the literature (usually the platform based (Alamut/Sapientia) google searches will pick these up as they search for the amino acid change) and LSDBs (the ClinVar track on Alamut/Sapientia or the ClinVar gene page can be useful) for a similar mutation which has the same p. nomenclature as your variant of interest but has been caused by a different nucleotide change. This similar mutation then needs to have been shown to be pathogenic (by functional studies or classed as 4/5 by the ACMG guidelines by their own merit).
Caution: be cautious if the mechanism of pathogenesis of the similar mutation is splicing, as this suggests that the defect is at the nucleotide level not at the amino acid so a different nucleotide change may not have the same effect.

PS2 = *De novo* (both paternity and maternity confirmed) in a patient with the disease and no family history. (The phenotype must fit first to use this)

Use in-house information or information in the literature to prove that your variant of interest has truly arisen *de novo*. Most trio NGS based tests/studies (e.g. DDD) will perform an inheritance check on the data before analysing it, so once the variant confirmation by Sanger sequencing has been undertaken and confirmed to be *de novo* this criteria can be applied. If the NGS method has not performed an inheritance check as part of its analysis then until inheritance is checked by another method (e.g. QStar identity test) only **PM6** can be applied.

At the WRGL – checklist/evidence collection

Benign/Pathogenic	Strength	Criteria Code	Criteria	Applies to Variant	Evidence
Pathogenic	Very Strong	PVS1	Predicted null variant where LOF is known pathogenic effect (PVS1)		
Pathogenic	Moderate	PM4	Protein length change variant (in-frame dels/ins or stop-loss variants (run-on)) (PM4)		
Pathogenic	Supporting	PP2	Missense variant in a gene where pathogenic missenses are common and benign are rare (PP2)	1	Exac obs vs expected is 30/111 Z score = 3.71
Pathogenic	Strong	PS4	Prevalence in affecteds statistically increased over controls (PS4)		
Pathogenic	Moderate	PS4 (mod)	moderate if observed in multiple patients with same phenotype and absent in controls		
Pathogenic	Moderate	PM2	Absent in population databases (or extremely rare in recessive disorders (PM2)	1	Not listed on ExAC or ESP
Pathogenic	Strong	PS1	Same amino-acid change as an established pathogenic variant (different nucleotide change) (PS1)	1	The same amino acid change caused by a C>T change is a well established mutation in the literature and in LSDBs.
Pathogenic	Moderate	PM5	Novel missense change at an amino acid residue where a different missense change has been seen before (PM5)		
Pathogenic	Strong	PS3	Well-established functional studies show a deleterious effect (PS3)		
Pathogenic	Moderate	PM1	Mutational hot spot or well-studied functional domain without benign variation (PM1)		
Pathogenic	Supporting	PP1 (supp)	Co-segregation with disease in multiple affected family members (PP1) calculated based on Jarvik paper	1	segregation in affected mum, affected aunt and affected grandfather within same family ($1/2^3$=1/8)
Pathogenic	Moderate	PP1 (mod)	Co-segregation with disease in multiple affected family members (PP1) calculated based on Jarvik paper		
Pathogenic	Strong	PP1 (strong)	Co-segregation with disease in multiple affected family members (PP1) calculated based on Jarvik paper		
Pathogenic	Strong	PS2	*De novo* (paternity and maternity confirmed) (PS2)		
Pathogenic	Moderate	PM6	*De novo* (without paternity and maternity confirmed) (PM6)		
Pathogenic	Moderate	PM3	For recessive disorders, detected in *trans* with a pathogenic variant (PM3)		
Pathogenic	Strong	PM3 (strong)	For recessive disorders, multiple observations detected in *trans* with a pathogenic variant (PM3)		
Pathogenic	Supporting	PP4	Patient's phenotype or FH highly specific for gene (PP4)	1	Patients phenotype fulfil the diagnostic criteria for a mutation in this gene
Pathogenic	Supporting	PP3	In silico supports deleterious effect (PP3)	1	SIFT, Polyphen and conservation supports deleterious effect
Pathogenic	Supporting	PP5	Reputable source = pathogenic but evidence not available for independent evaluation(PP5)		

At the WRGL – variant classification table

Pathogenicity Class	1	2	3	4	5
Combinations of criteria to qualify for classification Blue: stand alone Purple: very strong evidence Red: strong evidence Orange: moderate evidence Green: supporting evidence	1xBlue ≥2xRed	1xRed&1xGreen ≥2xGreen		1xPurple&1xOrange 1xRed&1-2xOrange 1xRed&≥2xGreen ≥3xOrange 2xOrange&≥2xGreen 1xOrange&≥4xGreen	1xPurple&≥1xRed 1xPurple&≥2xOrange 1xPurple&1xOrange&1xGreen 1xPurple&≥2xGreen ≥2xRed 1xRed&≥3xOrange 1xRed&2xOrange&≥2xGreen 1xRed&1xOrange&≥4xGreen
Variant frequency and Population data	MAF is >5% in ESP, 1000 GP or ExAC (BA1) MAF is too high for the disorder(BS1) Observed in healthy adults (with full penetrance expected at early age) (BS2)	MAF is too high for the disorder(BS1) Observed in healthy adults (with full penetrance expected at early age) (BS2)		Absent in population databases (PM2) Prevalence in affecteds statistically increased over controls (PS4) moderate if observed in multiple patients with same phenotype and absent in controls	Absent in population databases (PM2) Prevalence in affecteds statistically increased over controls (PS4) moderate if observed in multiple patients with same phenotype and absent in controls
Mutation/Gene info.		Missense in gene where only truncation cause disease (BP1) Synonymous (silent) variant with non-predicted splice impact and nucleotide is not highly conserved(BP7) In-frame indels in repeat without known function (BP3)		Predicted null variant where LOF is known pathogenic effect (PVS1) Protein length change variant (in-frame ins/dels or stop-loss) (PM4) Missense variant in a gene where pathogenic missenses are common and benign are rare (PP2)	Predicted null variant where LOF is known pathogenic effect (PVS1) Protein length change variant (in-frame ins/dels or stop-loss) (PM4) Missense variant in a gene where pathogenic missenses are common and benign are rare (PP2)
Similar mutation				Same amino-acid change as an established pathogenic variant (PS1) Novel missense change at an amino acid residue where a different missense change has been seen before (PM5)	Same amino-acid change as an established pathogenic variant (PS1) Novel missense change at an amino acid residue where a different missense change has been seen before (PM5)

Did all this work lead to more consistent classification?

We gave the 3 cases that were distributed prior to the train the trainers workshop to 10 different colleagues with various levels of scientist and ACMG guideline experience and different amounts of ACMG training. – full returns from 6 analysts.

	Case 1	Case 2	Case 3	Total changes	Number of classification differences	Estimated time taken to analyse (mins)	Scientist experience	ACMG experience	Training
Analyst 1	2	2	3	7	1	285	high	low	low
Analyst 2	0	1	2	3	1	390	low	low	high
Analyst 3	4	3	3	10	2	270	low	low	low
Analyst 4*	1	1	0	2	0	120	low	med	med
Analyst 5	0	2	0	2	0	360	high	high	high
Analyst 7	2	0	2	4	0	300	high	med	low

So yes, but not as much as we had hoped!

Reasons for inconsistency

Criteria in model answer for cases 1-3	Number of times correctly applied	Percentage	Criteria applied (not in model answer)	Number of times incorrectly applied
PP2	5/6	83%	PP4	4
PM2	13/13	100%	PS2	3
PM1 (moderate)	6/7	86%	PS4(mod)	3
PM1 (supporting)	2/6	33%	BS2	3
PP3	15/19	79%	PM2	2
PM5	7/7	100%	PM5, PM1, PP2, PS1, PS3 and PP5	1 time each

- Criteria was applied wrongly when a similar variant was found rather than the exact variant (PS4 (mod – seen more times in disease and absent in normal population) and PS3 (functional studies)).
- Not reading the SOP properly (or perhaps not at all!) PS2 (*de novo*) clearly states in the SOP that the phenotype must fit in order to use this criterion and I had told all the analysts that the phenotype did not fit in this case. 3/6 analysts applied PS2 and all 3 subsequently ended up with the wrong classification.
- Not using professional judgement, many appreciated that case 3 had a mutational hotspot but felt it was not strong enough to fulfil PM1, but only 2 analysts exercised the right to downgrade the evidence to supporting.
- BS2 was applied in a reduced penetrance/late onset scenario.

Further work

- A few more SOP adjustments.
- Lots more training and practice.
- Try to speed up analysis.
  - Implement automation to fulfil many of the criteria.
  - ? Implement tiering system.
- Implement one of the pathogenicity calculators to reduce errors.
- Collaborate (within the lab, with other labs and nationally)
- Where specific cut-offs/parameters need to be made, these should be applied nationally.
- Data sharing will be critical to consistent variant interpretation.

Acknowledgments

- Emma-Jane Taylor
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- Sian Ellard (Exeter)

WCEP
Wessex Clinical Exome Pilot Study

University of Southampton

WRGL
Wessex Regional Genetics Laboratory