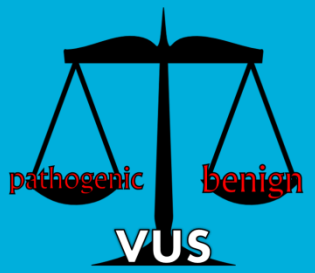
Variant classification using the ACMG recommendations

Rolph Pfundt
Human Genetics
RadboudUMC, Nijmegen



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-Why classify?

-recommendations

-ACMG guidelines

-Examples / inconsistencies

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-Why classify?

Why classify?

Variant classification affects medical management

Whether or not a variant is considered to be (likely) pathogenic influences:

-reported yes/no

-prenatal analysis yes/no
-preimplantation genetics yes/no
-preconception screening yes/no

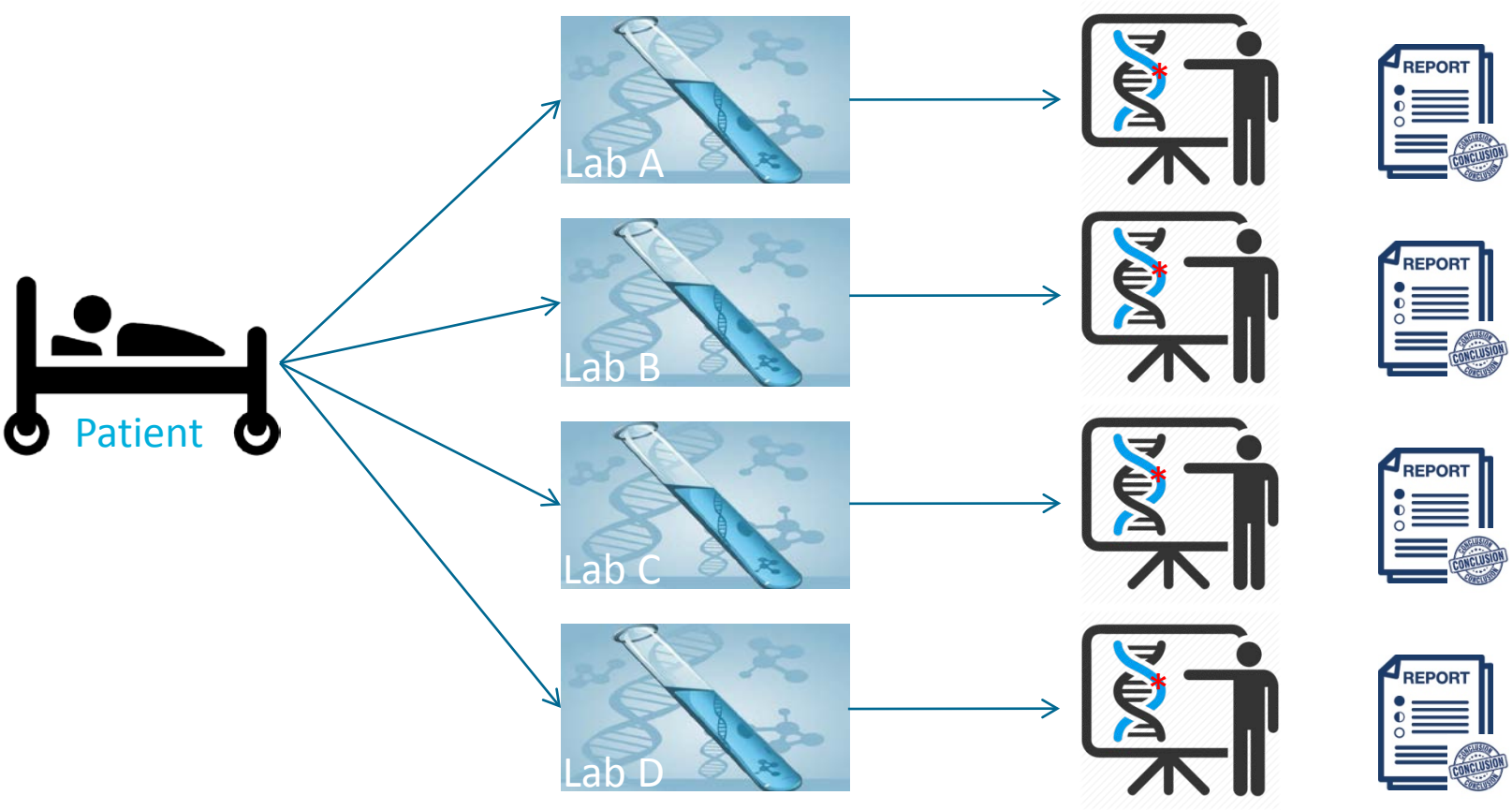
-presymptomatic testing yes/no
-presymptomatic risk management (surveillance or prophylactic surgery)

-evaluation of incidental/secondary findings
-…
-…

Why classify?

With objective classification criteria

Same patiënt different labs, same variant, same interpretation / conclusion



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Why classify?

Variant classification affects medical treatment

Williams versus athena diagnostics

This Woman Says Her 2-Year-Old Died Because Of A False DNA Test

Amy Williams believes her son didn't have to die from a rare genetic condition. Now she's suing one of the world's largest laboratories.

Her son Christian died on Jan. 5, 2008, at age 2 due to violent and mysterious seizures
Mitochondrial disease or Dravet syndrome?
Testing SCN1A (Dravet) at Athena diagnostics
Result: c.1237T>A; p.Tyr413Asn, classified in 2007 as a VUS
2014 copy of report requested by new husband
2015 Athena issued a revised report stating that the variant that was previously classified as a VUS can now be interpreted as a disease-causing mutation (2007 in paper).

-parental testing was recommended
-When Dravet was diagnosed medication would have changed (stop sodium channel block)

https://www.documentcloud.org/documents/2791611-Williams-v-Quest-Athena.html
https://www.genomeweb.com/
https://www.buzzfeed.com

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Why classify?

Proper classification and terminology provides clarity

Example KANSL2

Research Lab result WGS 2014:

De novo SNV change:

KANSL2; Chr12(GRCh37):g.49072911C>A; NM_017822.3:c.453G>T; p.(Gly151Gly)

KANSL2 is family of KANSL1 (causative for KdVS). De novo KANSL2 mutation has been reported in another WES study. Silent change that might lead to a new splice donor site (predictions), but RNA studies on fibroblasts have not (yet) confirmed this. The intellectual disability could be caused by the de novo mutation in KANSL2.

Clinical files / letters:

2014: Clinical geneticist: One variant with possible relevance, but with the current knowledge inconclusive.

2015: Pediatrician: De novo KANSL2 mutation probably causing the clinical phenotype

2016: Genetic counseler: Known with Koolen de Vries syndrome (caused by KANSL1 LOF variants)

2017: Psychomotor retardation due to KANSL2 mutation

Proper classification, terminology and counseling provides clarity

HEALTH • MEDICINE

‘I’m Permanently Damaged.’ Woman Sues After She Says Doctors Unnecessarily Removed Her Breasts and Uterus

Based on the genetic tests, the Gold Beach, Ore. resident says she had been told she had MLH1 and BRCA1 gene mutations, as well as Lynch syndrome, which together gave her a 50% chance of developing breast cancer and an up to 80% chance of developing uterine cancer. Based on those results, she went through with a double mastectomy and a hysterectomy.

After the surgeries, however, she was unhappy with the results of her mastectomy and reached out to a lawyer, who suggested that she see another doctor about breast reconstruction. After examining her case file, the new doctor informed Cooke-Moore that her genetic test results were actually negative, says Christopher Cauble, Cooke-Moore’s lawyer. She called the lab to confirm, and felt her world crash down when she learned that the doctor was right: The operations that had pushed her into early menopause, forced multiple follow-up surgeries, and left her with post-traumatic stress disorder were likely unnecessary.

-recommendations

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Struggling with how to weigh variants

CMGS CLINICAL MOLECULAR GENETICS SOCIETY

2007

VKGL

Practice guidelines for the Interpretation and Reporting of Unclassified Variants (UVs) in Clinical Molecular Genetics.

Prepared and edited by Jennie Bell[1], Danielle Bodmer[2], Erik Sistermans[3] and Simon C Ramsden[4]

1. West Midlands Regional Genetics Laboratory, Birmingham Women's Hospital NHS Trust, Metchley Park Road, Edgbaston, Birmingham, B15 2TG, United Kingdom.
2. Dept of human genetics, Radboud University Nijmegen Medical Centre, PO box 9101, 6500 HB Nijmegen, The Netherlands
3. Dept of clinical genetics, VU University Medical Center, van der Boechorststraat 7, 1081 BT Amsterdam, The Netherlands
4. National Genetics Reference Laboratory (Manchester), Dept of Medical Genetics, Saint Mary's Hospital, Hathersage Road, Manchester, M13 OJH, United Kingdom.

Guidelines ratified by the UK Clinical Molecular Genetics Society (11th January, 2008) and the Dutch Society of Clinical Genetic Laboratory Specialists (Vereniging Klinisch Genetische Laboratoriumspecialisten; VKGL) (22nd October, 2007).

Class 1 – Certainly not pathogenic
Class 2 – Unlikely to be pathogenic but cannot be formally proven
Class 3 – Likely to be pathogenic but cannot be formally proven
Class 4 - Certainly pathogenic

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Struggling with how to weigh variants

CMGS CLINICAL MOLECULAR GENETICS SOCIETY

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Class 1 – Certainly not pathogenic
Class 2 – Unlikely to be pathogenic but cannot be formally proven
Class 3 – Likely to be pathogenic but cannot be formally proven
Class 4 - Certainly pathogenic

A four class system did not provide the proper tools

Class 3* - almost class 4 ☺

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2013: 5-class system adopted by genetic societies NL / UK

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

VKGL

Practice Guidelines for the Evaluation of Pathogenicity and the Reporting of Sequence Variants in Clinical Molecular Genetics.

Yvonne Wallis[1], Stewart Payne[2], Ciaron McAnulty[3], Danielle Bodmer[4], Erik Sistermans[5], Kathryn Robertson[6], David Moore[7], Stephen Abbs[8], Zandra Deans[9] Devereau[6]

1. West Midlands Regional Genetics Laboratory, Birmingham Women's NHS Foundatio sohn Way, Edgbaston, Birmingham, B15 2TG, United Kingdom.
2. Kennedy-Galton Centre (NW Thames) Regional Genetics Centre, Level 8V, Northw Mark's Hospitals, Watford Road, Harrow, HA1 3UJ, United Kingdom.
3. Northern Genetics Service, Institute of Human Genetics, Central Parkway, Newcastle-u 3BZ, United Kingdom.
4. Dept of Human Genetics, Radboud University Nijmegen Medical Centre, PO b HB Nijmegen, The Netherlands
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6. National Genetics Reference Laboratory (Manchester), Dept of Medical Genetics, Sain tal, Hathersage Road, Manchester, M13 OJH, United Kingdom.
7. South East Scotland Genetic Service, David Brock Building, Western General Hospita Edinburgh, EH4 2XU
8. East Anglian Medical Genetics Service, Genetics Laboratories, Addenbrooke's Hospi CB2 0QQ
9. UK NEQAS for Molecular Genetics, UK NEQAS [Edinburgh], Department of Labora Royal Infirmary of Edinburgh, Edinburgh, EH16 4SA

Original guidelines ratified by the UK Clinical Molecular Genetics Societ ary, 2008) and the Dutch Society of Clinical Genetic Laboratory Specialists (Verenigning Klinisch Genetische Laboratoriumspecialisten; VKGL) (22nd October, 2007).

Guidelines updated by the Association for Clinical Genetic Science (formally Clinical Molecular Genetics Society and Association of Clinical Cytogenetics) and the Dutch Society of Clinical Genetic Laboratory Specialists (approved September 2013).

Choosing the right (combination of) words

Class	Wording to include within reports
1	**Not** pathogenic "Common" polymorphism and therefore not reported
2	**Unlikely** to be pathogenic Diagnosis not confirmed molecularly
3	**Uncertain** pathogenicity **Does not** confirm or exclude diagnosis
4	**Likely** to be pathogenic **Consistent** with the diagnosis
5	Predicted to be **pathogenic** This result **confirms** the diagnosis

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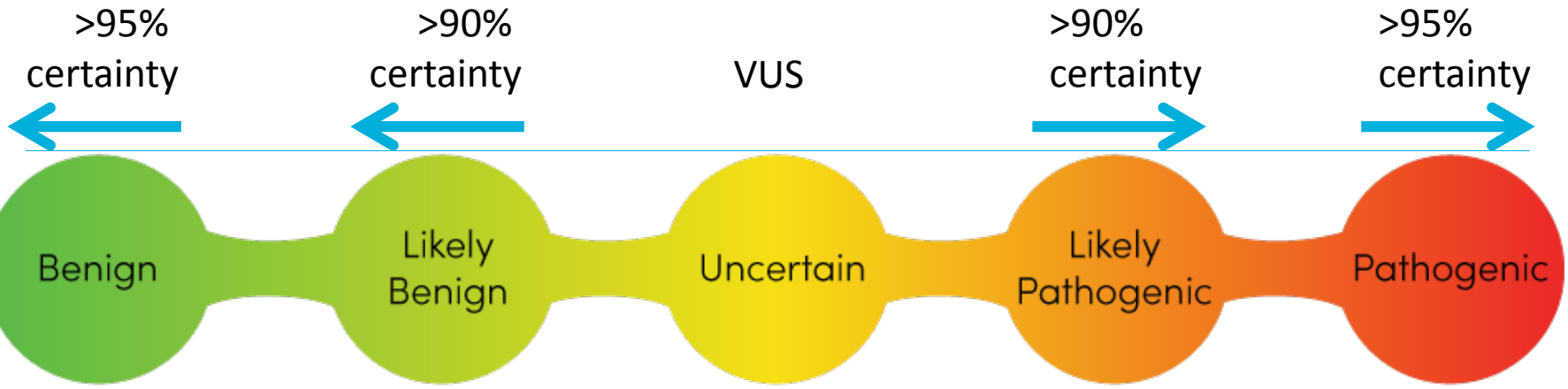
-ACMG guidelines

2015 ACMG standards and guidelines published

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



Benign variant	Class 1	‘neutral’, or clearly not pathogenic’
Likely benign variant	Class 2	‘unlikely to be pathogenic’
Variant of Uncertain significance	Class 3	‘not class 1, 2, 4, 5’ VUS / VOUS
Likely pathogenic variant	Class 4	‘likely to be pathogenc’
Pathogenic variant	Class 5	‘clearly pathogenic’

https://www.ashg.org/education/csertoolkit/intro.html

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Class 1 and 2 are not reported

Class 3 can be reported (targeted/NGS)

-segregation analysis (reimburesement on index)
-functional testing / RNA analysis
-no presymptomatic / prenatal / PGD testing

Class 4 is reported

-segregation analysis (reimburesement on index)
-presymptomatic testing in family is possible
-prenatal testing / PGD is possible

Class 5 is reported

-segregation analysis (reimburesement on counselee)
-presymptomatic testing in family is possible
-prenatal testing / PGD is possible

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Criteria for classification

ACMG provides two sets of criteria

1. For the classification of pathogenic / likely pathogenic variants
2. For the classification of benign / likely benign variants

Gathering information / arguments
to the estimate the pathogenicity of a variant

Criteria for pathogenic classification

Very strong PVS1
Strong PS1, PS2, PS3, PS4
Moderate PM1, PM2, PM3, PM4, PM5, PM6
Supporting PP1, PP2, PP3, PP4, PP5

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Criteria for pathogenic classification

Very strong PVS1
null variant (nonsense, frameshift, canonical ±1 or 2 splice sites, initiation codon, single or multiexon deletion) in a gene where LOF is a known mechanism of disease Caveats: • Beware of genes where LOF is not a known disease mechanism (e.g., GFAP, MYH7) • Use caution interpreting LOF variants at the extreme 3' end of a gene • Use caution with splice variants that are predicted to lead to exon skipping but leave the remainder of the protein intact • Use caution in the presence of multiple transcripts

Strong PS1, PS2, PS3, PS4

Moderate PM1, PM2, PM3, PM4, PM5, PM6

Supporting PP1, PP2, PP3, PP4, PP5

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Criteria for pathogenic classification

Very strong PVS1

Strong PS1, PS2, PS3, PS4

PS1

Same amino acid change as a previously established pathogenic variant regardless of nucleotide change

Example: Val→Leu caused by either G>C or G>T in the same codon

Caveat: Beware of changes that impact splicing rather than at the amino acid/protein level

PS2

De novo (both maternity and paternity confirmed) in a patient with the disease and no family history

Note: Confirmation of paternity only is insufficient. Egg donation, surrogate motherhood, errors in embryo transfer, and so on, can contribute to nonmaternity.

PS3

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

Note: Functional studies that have been validated and shown to be reproducible and robust in a clinical diagnostic laboratory setting are considered the most well established.

PS4

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

Note 1: Relative risk or OR, as obtained from case–control studies, is >5.0, and the confidence interval around the estimate of relative risk or OR does not include 1.0. See the article for detailed guidance.

Note 2: In instances of very rare variants where case–control studies may not reach statistical significance, the prior observation of the variant in multiple unrelated patients with the same phenotype, and its absence in controls, may be used as moderate level of evidence.

Moderate PM1, PM2, PM3, PM4, PM5, PM6

Supporting PP1, PP2, PP3, PP4, PP5

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Criteria for benign classification

Stand-alone BA1
Strong BS1, BS2, BS3, BS4
Supporting BP1, BP2, BP3, BP4, BP5, BP6, BP7

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Criteria for benign classification

Stand-alone BA1
Allele frequency is >5% in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium

Strong BS1, BS2, BS3, BS4

Supporting BP1, BP2, BP3, BP4, BP5, BP6, BP7

Criteria for benign classification

Stand-alone BA1

Strong BS1, BS2, BS3, BS4
BS1 Allele frequency is greater than expected for disorder (see Table 6) **BS2** Observed in a healthy adult individual for a recessive (homozygous), dominant (heterozygous), or X-linked (hemizygous) disorder, with full penetrance expected at an early age **BS3** Well-established in vitro or in vivo functional studies show no damaging effect on protein function or splicing **BS4** Lack of segregation in affected members of a family Caveat: The presence of phenocopies for common phenotypes (i.e., cancer, epilepsy) can mimic lack of segregation among affected individuals. Also, families may have more than one pathogenic variant contributing to an autosomal dominant disorder, further confounding an apparent lack of segregation.

Supporting BP1, BP2, BP3, BP4, BP5, BP6, BP7

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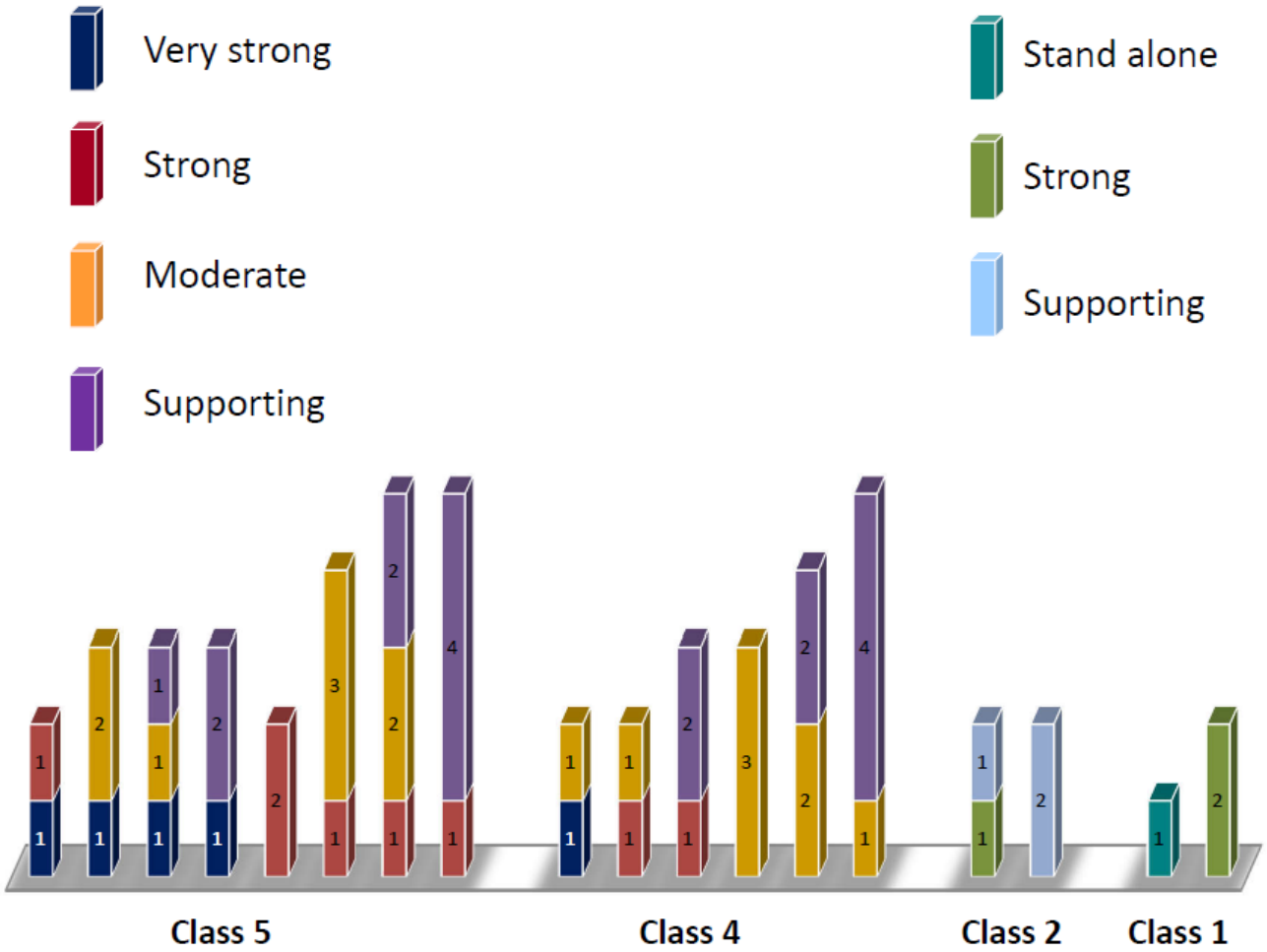
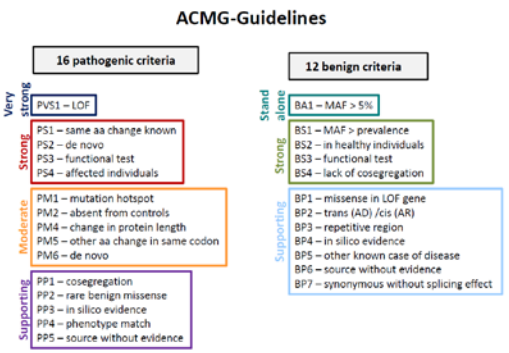
Overview of Criteria

	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 In-frame indels in repeat w/out known function BP3	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	Nonsegregation with disease BS4		Cosegregation with disease in multiple affected family members PP1	Increased segregation data →		
De novo data				De novo (without paternity & maternity confirmed) PM6	De novo (paternity and 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			

16 pathogenic criteria

12 benign criteria

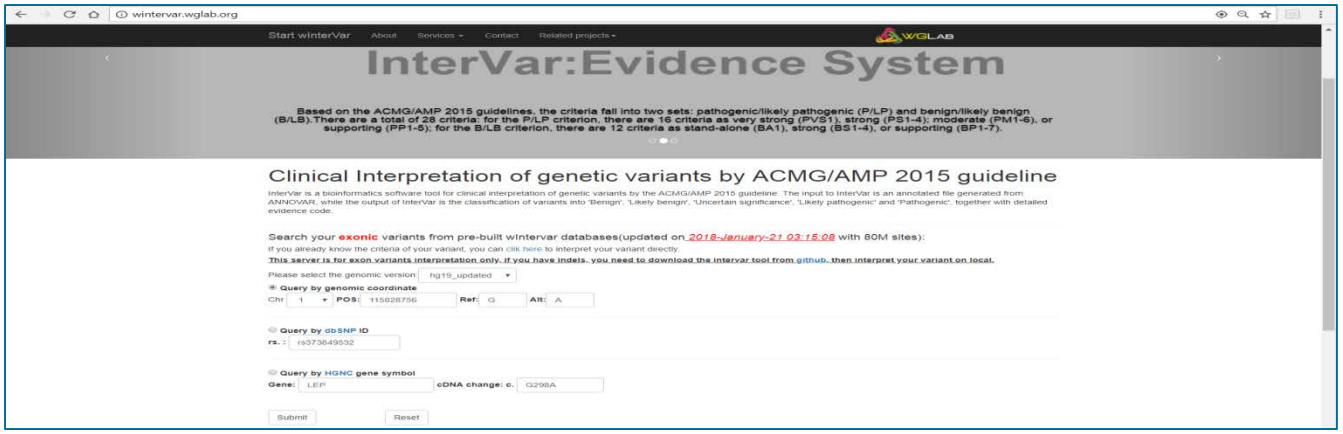




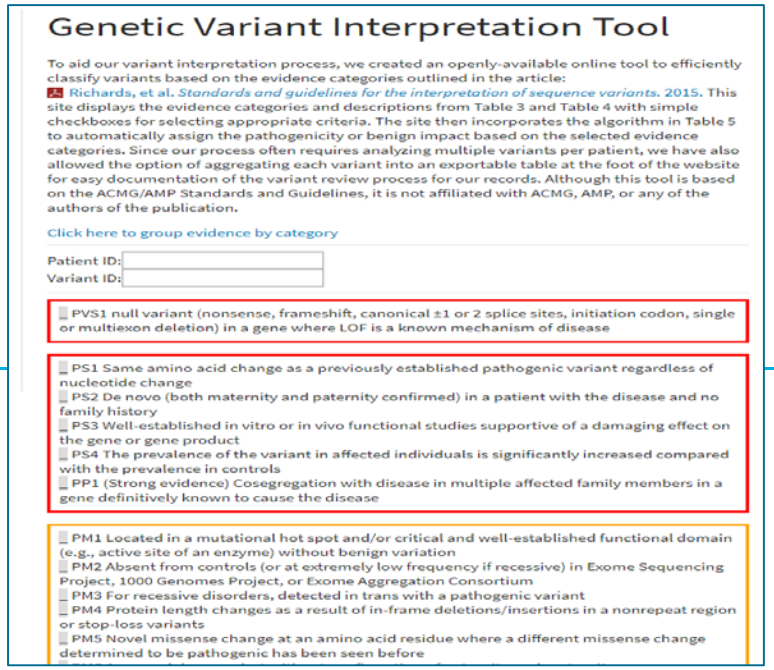
From presentation by Mari Ann Kulseth, Oslo

Helpfull websites / tools (guidance not truth)

InterVAR (http://wintervar.wglab.org/)



http://www.medschool.umaryland.edu/Genetic_Variant_Interpretation_Tool1.html/



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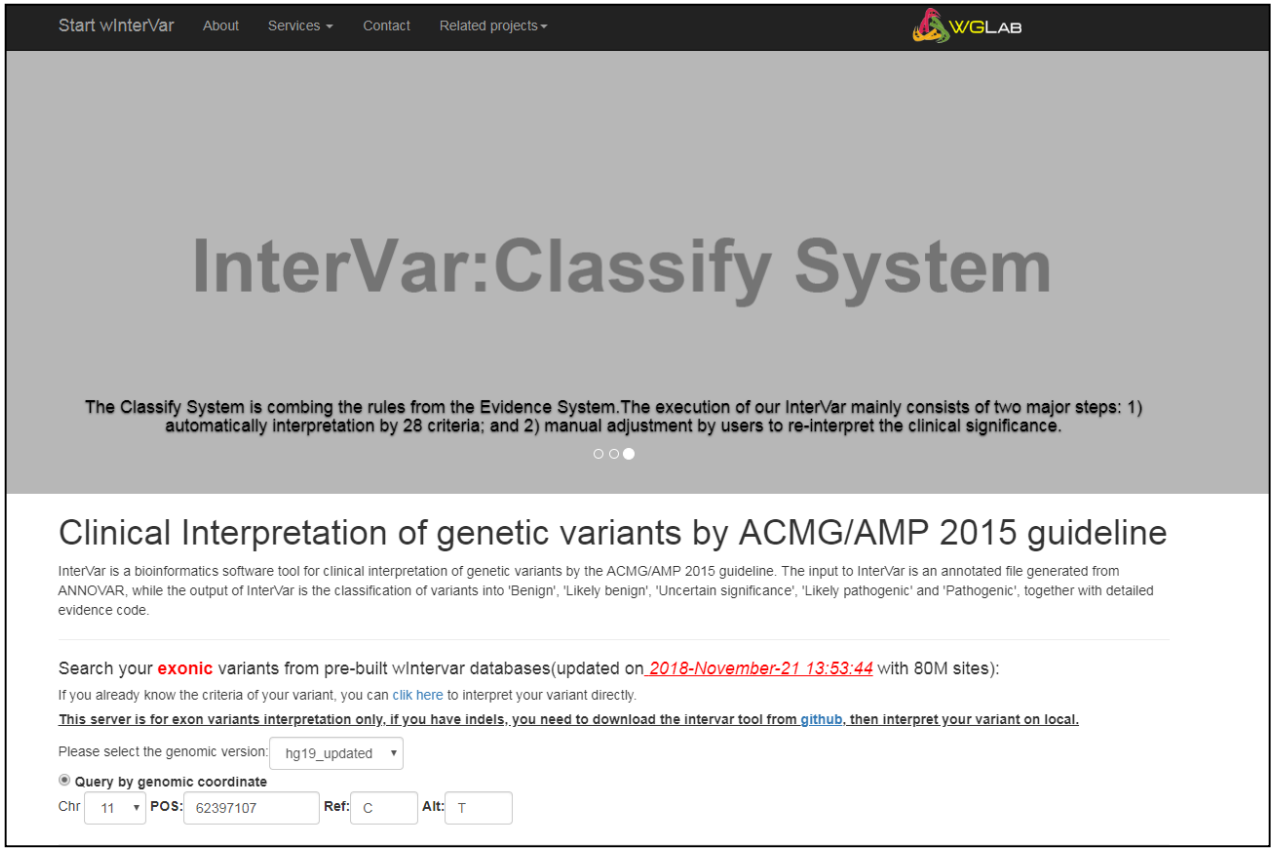
What is it and What does it do?

Web-tool for automated ACMG classification of variants



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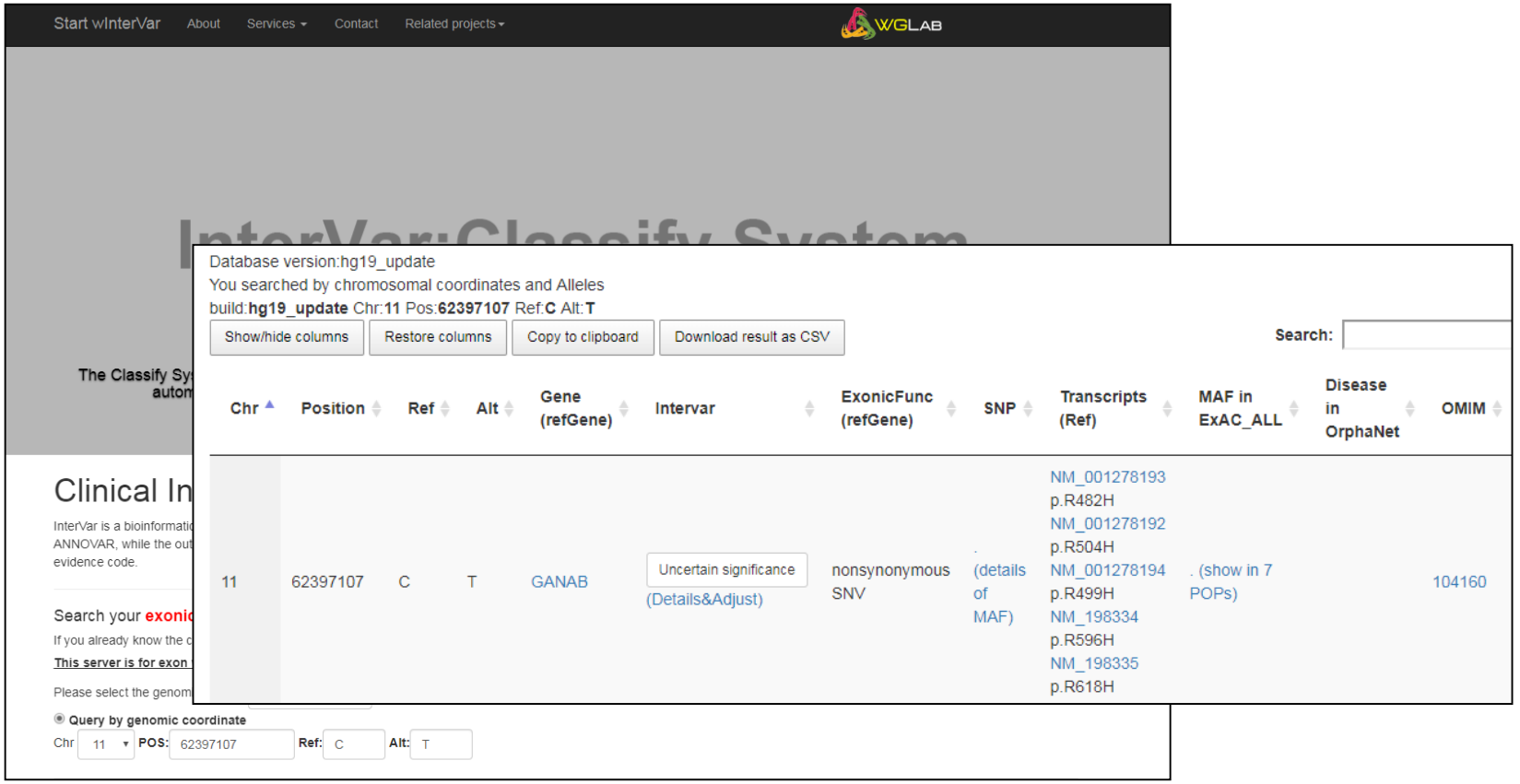

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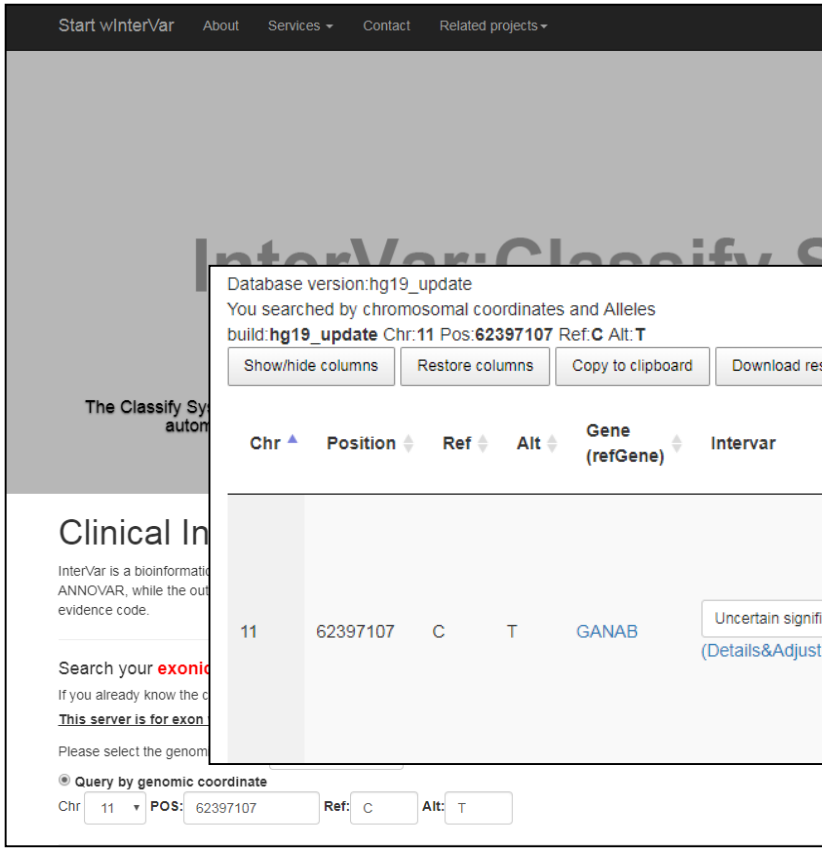
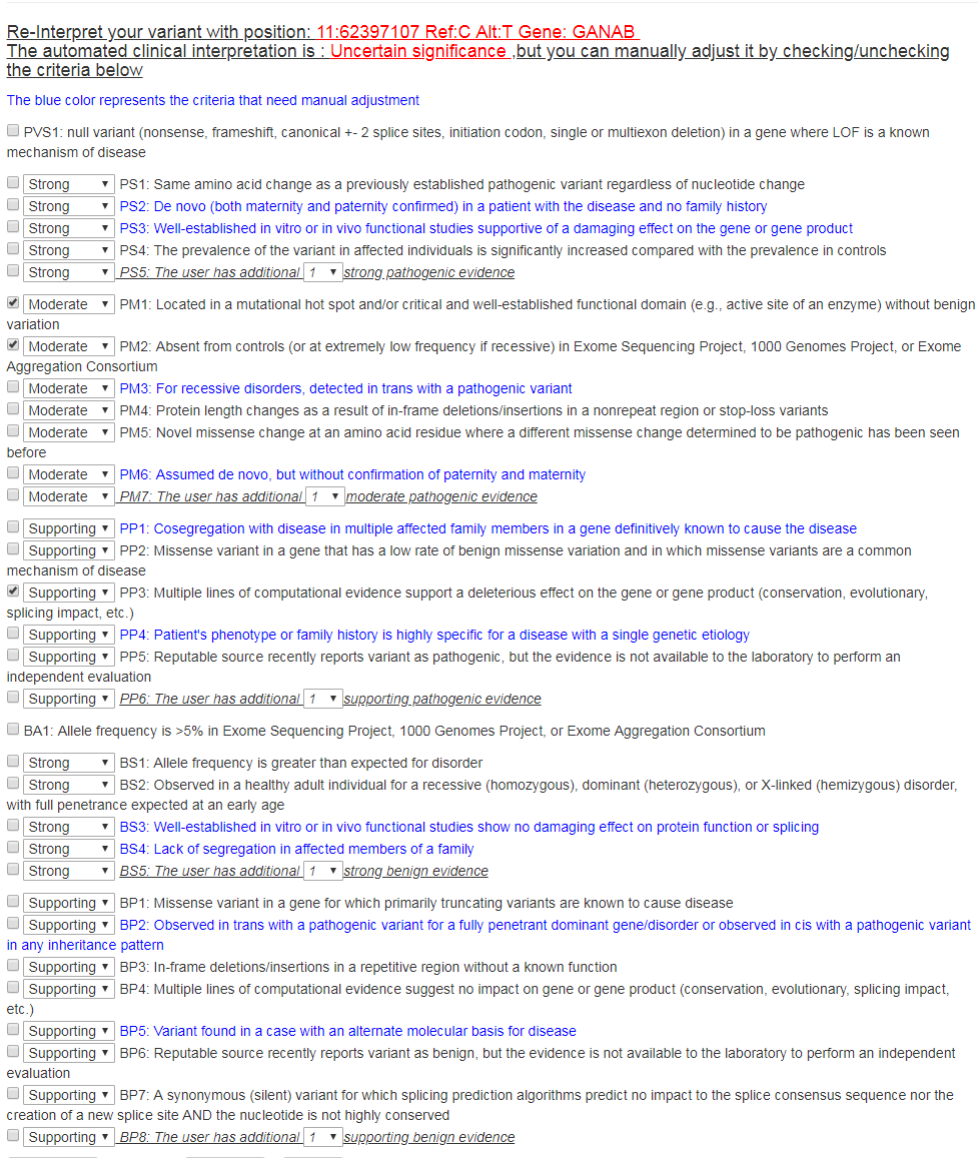
Web-tool for automated ACMG classification of variants





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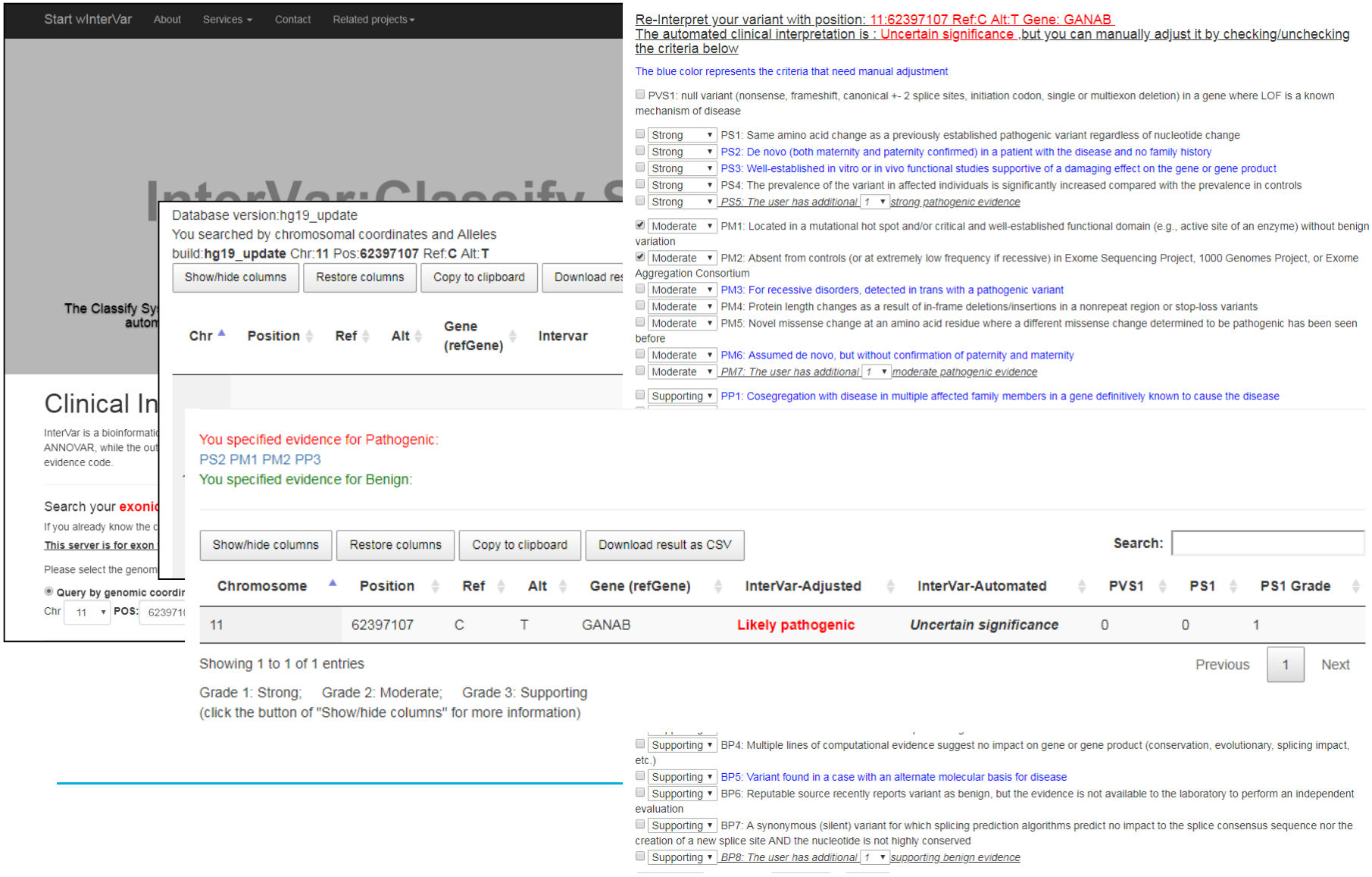

General Considerations:

-The terms “mutation” and “polymorphism”“ should not be used because of incorrect assumptions of pathogenic and benign effects

instead use the term variant (pathogenic (Class 5), likely pathogenic(Class 4), uncertain significance (Class 3), likely benign (Class 2) and benign(Class 1)

-Be carefull with “Genes of Uncertain (clinical) Significance” (GUS)

-Variants should be reported using the HGVS nomenclature (http://www.hgvs.org/mutnomen)

-Working in specific disease groups should continue to develop more focused guidance regarding the classification of variants in specific genes given that the applicability and weight assigned to certain criteria may vary by gene and disease.

-Example (if possible….)

Example: young girl with mild ID

Variant detected in the GNAS gene:
Chr20(GRCh37):g.57485432G>T
NM_000516.4:c.1014G>T
p.(Lys338Asn)

Autosomal dominant ID syndrome gene

103580

PSEUDOHYPOPARATHYROIDISM, TYPE IA; PHP1A

Alternative titles; symbols

PHP IA
ALBRIGHT HEREDITARY OSTEODYSTROPHY WITH MULTIPLE HORMONE RESISTANCE

Phenotype-Gene Relationships

Location	Phenotype	Phenotype MIM number	Inheritance	Phenotype mapping key	Gene/Locus	Gene/Locus MIM number
20q13.32	Pseudohypoparathyroidism Ia	103580	AD	3	GNAS	139320

- ☐ PVS1
- ☐ PS1
- ☐ PS2
- ☐ PS3
- ☐ PS4
- ☐ PP1
- ☐ PM1
- ☐ PM2
- ☐ PM3
- ☐ PM4
- ☐ PM5
- ☐ PM6
- ☐ PP1
- ☐ PP1
- ☐ PP2
- ☐ PP3
- ☐ PP4
- ☐ PP5
- ☐ BA1
- ☐ BS2
- ☐ BS3
- ☐ BS4
- ☐ BP1
- ☐ BP2
- ☐ BP3
- ☐ BP4
- ☐ BP5
- ☐ BP6
- ☐ BP7

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Example: young girl with mild ID

Variant detected in the GNAS gene:
Chr20(GRCh37):g.57485431A>G
NM_000516.4:c.1013A>G
p.(Lys338Arg)

Not present in EXAC/gnomAD

- ☐ PVS1
- ☐ PS1
- ☐ PS2
- ☐ PS3
- ☐ PS4
- ☐ PP1
- ☐ PM1
- ☒ PM2
- ☐ PM3
- ☐ PM4
- ☐ PM5
- ☐ PM6
- ☐ PP1
- ☐ PP1
- ☐ PP2
- ☐ PP3
- ☐ PP4
- ☐ PP5
- ☐ BA1
- ☐ BS2
- ☐ BS3
- ☐ BS4
- ☐ BP1
- ☐ BP2
- ☐ BP3
- ☐ BP4
- ☐ BP5
- ☐ BP6
- ☐ BP7

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

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Example: young girl with mild ID

Variant detected in the GNAS gene:
Chr20(GRCh37):g.57485431A>G
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- ☐ PVS1
- ☐ PS1
- ☐ PS2
- ☐ PS3
- ☐ PS4
- ☐ PP1
- ☐ PM1
- ☒ PM2
- ☐ PM3
- ☐ PM4
- ☒ PM5
- ☐ PM6
- ☐ PP1
- ☐ PP1
- ☐ PP2
- ☐ PP3
- ☐ PP4
- ☐ PP5
- ☐ BA1
- ☐ BS2
- ☐ BS3
- ☐ BS4
- ☐ BP1
- ☐ BP2
- ☐ BP3
- ☐ BP4
- ☐ BP5
- ☐ BP6
- ☐ BP7

Same AA other variant

CM102487	AAA-ATA	Lys300Ile	c.899A>T	p.K300I	DM	Pseudohypoparathyroidism 1a	Reis Oliveira (2010) An Pediatr (Barc) ,
CM013734	CCA-CTA	Pro313Leu	c.938C>T	p.P313L	DM	Albright hereditary osteodystrophy	Ahrens (2001) J Clin Endocrinol Metab **86**, 4630
CM116450	TAC-CAC	Tyr318His	c.952T>C	p.Y318H	DM	Pseudohypoparathyroidism 1a	Miao (2011) Int J Endocrinol **2011**, 509549
CM152418	GAG-AAG	Glu327Lys	c.979G>A	p.E327K	DM	Pseudohypoparathyroidism 1a	Thiele (2015) Mol Genet Genomic Med **3**, 111
CM013736	CGG-TGG	Arg336Trp	c.1006C>T	p.R336W	DM	Albright hereditary osteodystrophy	Ahrens (2001) J Clin Endocrinol Metab **86**, 4630
CM032936	AAG-AAC	Lys338Asn	c.1014G>C	p.K338N	DM	Pseudohypoparathyroidism 1a	Pohlenz (2003) Eur J Endocrinol **148**, 463
CM020281	CGA-TGA	Arg342Term	c.1024C>T	p.R342*	DM	Pseudohypoparathyroidism 1a	Linglart (2002) J Clin Endocrinol Metab **87**, 189 Goto (2010) *J Pediatr Endocrinol Metab* **23**: 303 [Additional phenotype] Lin (2015) *J Pediatr Endocrinol Metab* **28**: 911 [Additional phenotype] 2 more reference(s)...
CM152419	TTT-TTG	Phe345Leu	c.1035T>G	p.F345L	DM	Pseudohypoparathyroidism 1a	Thiele (2015) Mol Genet Genomic Med **3**, 111
CM013735	CAC-CTC	His357Leu	c.1070A>T	p.H357L	DM	Albright hereditary osteodystrophy	Ahrens (2001) J Clin Endocrinol Metab **86**, 4630
CM067779	CAT-CCT	His362Pro	c.1085A>C	p.H362P	DM	Pseudohypoparathyroidism 1a	Linglart (2006) Endocrinology **147**, 2253

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

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Example: young girl with mild ID

Variant detected in the GNAS gene:
Chr20(GRCh37):g.57485431A>G
NM_000516.4:c.1013A>G
p.(Lys338Arg)

- ☐ PVS1
- ☐ PS1
- ☐ PS2
- ☐ PS3
- ☐ PS4
- ☐ PP1
- ☐ PM1
- ☒ PM2
- ☐ PM3
- ☐ PM4
- ☒ PM5
- ☐ PM6
- ☐ PP1
- ☐ PP1
- ☐ PP2
- ☒ PP3
- ☐ PP4
- ☐ PP5
- ☐ BA1
- ☐ BS2
- ☐ BS3
- ☐ BS4
- ☐ BP1
- ☐ BP2
- ☐ BP3
- ☐ BP4
- ☐ BP5
- ☐ BP6
- ☐ BP7

Predictions hint towards pathogenicity

Transition from A to G in exon 12.
Missense substitution: Lys338 is changed to Arg.

HGVS Nomenclature (v15.11)

cDNA Level:	**NM_000516.4:c.1013A>G**
gDNA Level:	**Chr20(GRCh37):g.57485431A>G**
Protein Level:	**p.(Lys338Arg)**

Pathogenicity clues

- Highly conserved nucleotide (phyloP: 4.40 [-14.1;6.4])
- Highly conserved amino acid, up to Baker's yeast (considering 12 species)
- Small physicochemical difference between Lys and Arg (Grantham dist.: 26 [0-215])
- This variant is in protein domains:
  - Guanine nucleotide binding protein (G-protein), alpha subunit
  - G-protein alpha subunit, group S
  - P-loop containing nucleoside triphosphate hydrolase
- Align GVGD (v2007): C0 (GV: 238.71 - GD: 13.90)
- SIFT (v6.2.0): Deleterious (score: 0, median: 3.56)
- MutationTaster (v2013): disease causing (p-value: 1)

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

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NM_000516.4:c.1013A>G
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- ☐ PM3
- ☐ PM4
- ☒ PM5
- ☐ PM6
- ☐ PP1
- ☐ PP1
- ☐ PP2
- ☒ PP3
- ☐ PP4
- ☐ PP5
- ☐ BA1
- ☐ BS2
- ☐ BS3
- ☐ BS4
- ☐ BP1
- ☐ BP2
- ☐ BP3
- ☐ BP4
- ☐ BP5
- ☐ BP6
- ☐ BP7

PM2 + PM5 + PP3 = VUS - not enough evidence (class 3)

When occuring the novo

PS2 + PM2 + PM5 + PP3 = likely pathogenic (class 4)

PS2 De novo (both maternity and paternity confirmed) in a patient with the disease and no family history

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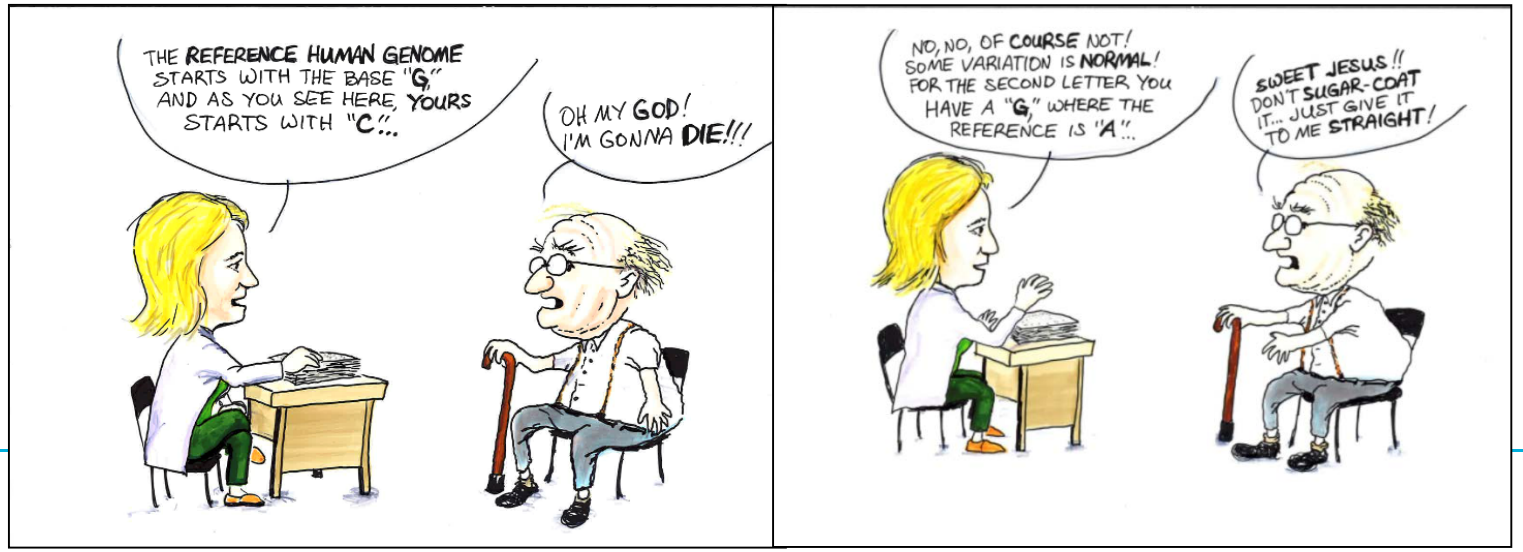
Concluding remarks (I)

ACMG system is usefull, adapt to own situation
Use it, get used to it!
Sending the right message is difficult
Be as clear and consistent as possible
Try not to overinterpret (good clinical care)
Difficult to control the final interpretation of the receiver
Impossible to control the final communication to the patient



https://goodsciencewriting.wordpress.com/

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Concluding remarks (II)

March 2018

COMMENTARY | Genetics inMedicine

Interpretation of genomic sequencing: variants should be considered uncertain until proven guilty

Karen E. Weck, MD

Multiple clinical laboratories and research studies have advertised their high sensitivity or diagnostic "hit rate" and in some cases have advertised low rates of reporting variants of uncertain significance (VUS). This is dangerous. It is more likely that the laboratories with higher rates of VUS are in fact doing a better job with variant classification

The potential harms of overclassifying variants as pathogenic include:
(i) making an incorrect diagnosis in an individual, which may prevent further testing to identify the correct diagnosis and/or result in ineffective treatment
(ii) conducting family studies that erroneously assign risk to relatives, which may lead to inappropriate screening or actions
(iii) making reproductive decisions based on incorrect information
(iv) false annotation of variants in the literature and/or variant databases, which may affect interpretation of future patient results

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Very
Unhelpful
Statement

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Allele frequency information is very helpful

The data set provided on this website spans 60,706 unrelated individuals sequenced as part of various disease-specific and population genetic studies. We have removed individuals affected by severe pediatric disease, so this data set should serve as a useful reference set of allele frequencies for severe disease studies.

The data set provided on this website spans 123,136 exomes and 15,496 genomes from unrelated individuals sequenced as part of various disease-specific and population genetic studies. We have removed individuals known to be affected by severe pediatric disease, as well as their first-degree relatives, so this data set should serve as a useful reference set of allele frequencies for severe disease studies - however, note that some individuals with severe disease may still be included in the data set, albeit likely at a frequency equivalent to or lower than that seen in the general population.

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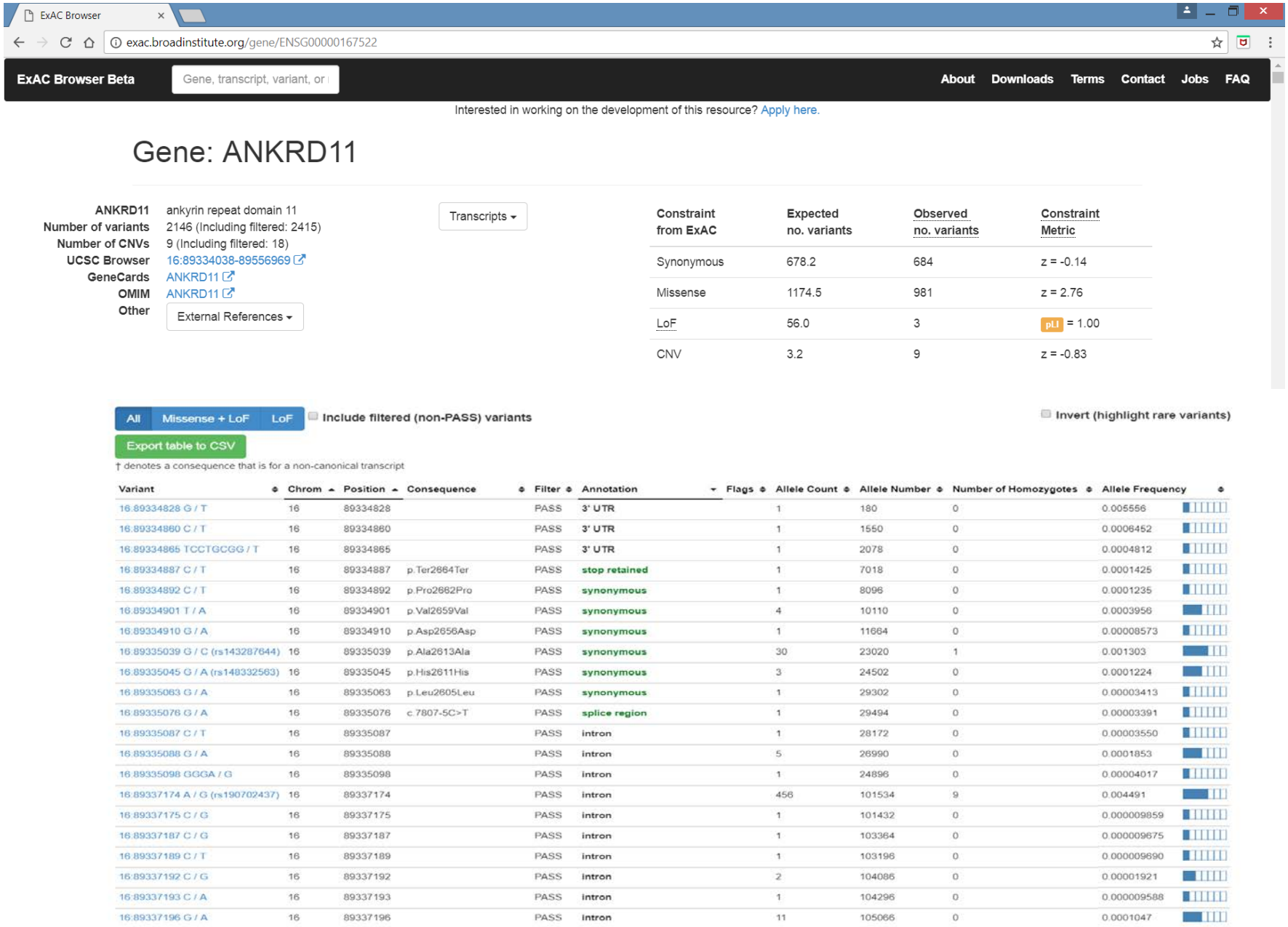
Information on allele frequencies per gene



ExAC Browser

exac.broadinstitute.org/gene/ENSG00000167522

ExAC Browser Beta | Gene, transcript, variant, or | About | Downloads | Terms | Contact | Jobs | FAQ

Interested in working on the development of this resource? Apply here.

Gene: ANKRD11

ANKRD11 ankyrin repeat domain 11
Number of variants 2146 (Including filtered: 2415)
Number of CNVs 9 (Including filtered: 18)
UCSC Browser 16:89334038-89556969
GeneCards ANKRD11
OMIM ANKRD11
Other External References

Transcripts

Constraint from ExAC	Expected no. variants	Observed no. variants	Constraint Metric
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Missense	1174.5	981	z = 2.76
LoF	56.0	3	pLI = 1.00
CNV	3.2	9	z = -0.83

All | Missense + LoF | LoF Include filtered (non-PASS) variants Invert (highlight rare variants)

Export table to CSV

† denotes a consequence that is for a non-canonical transcript

Variant	Chrom	Position	Consequence	Filter	Annotation	Flags	Allele Count	Allele Number	Number of Homozygotes	Allele Frequency
16:89334828 G / T	16	89334828		PASS	3' UTR		1	180	0	0.005556
16:89334860 C / T	16	89334860		PASS	3' UTR		1	1550	0	0.0006452
16:89334865 TCCTGCGG / T	16	89334865		PASS	3' UTR		1	2078	0	0.0004812
16:89334887 C / T	16	89334887	p.Ter2664Ter	PASS	stop retained		1	7018	0	0.0001425
16:89334892 C / T	16	89334892	p.Pro2662Pro	PASS	synonymous		1	8096	0	0.0001235
16:89334901 T / A	16	89334901	p.Val2659Val	PASS	synonymous		4	10110	0	0.0003956
16:89334910 G / A	16	89334910	p.Asp2656Asp	PASS	synonymous		1	11664	0	0.00008573
16:89335039 G / C (rs143287644)	16	89335039	p.Ala2613Ala	PASS	synonymous		30	23020	1	0.001303
16:89335045 G / A (rs148332563)	16	89335045	p.His2611His	PASS	synonymous		3	24502	0	0.0001224
16:89335063 G / A	16	89335063	p.Leu2605Leu	PASS	synonymous		1	29302	0	0.00003413
16:89335076 G / A	16	89335076	c.7807-5C>T	PASS	splice region		1	29494	0	0.00003391
16:89335087 C / T	16	89335087		PASS	intron		1	28172	0	0.00003550
16:89335088 G / A	16	89335088		PASS	intron		5	26990	0	0.0001853
16:89335098 GGGA / G	16	89335098		PASS	intron		1	24896	0	0.00004017
16:89337174 A / G (rs190702437)	16	89337174		PASS	intron		456	101534	9	0.004491
16:89337175 C / G	16	89337175		PASS	intron		1	101432	0	0.000009859
16:89337187 C / G	16	89337187		PASS	intron		1	103364	0	0.000009675
16:89337189 C / T	16	89337189		PASS	intron		1	103196	0	0.000009690
16:89337192 C / G	16	89337192		PASS	intron		2	104086	0	0.00001921
16:89337193 C / A	16	89337193		PASS	intron		1	104296	0	0.000009588
16:89337196 G / A	16	89337196		PASS	intron		11	105066	0	0.0001047

How many entries can you expect / accept in e.g. EXAC

Submitted 7 November 2016; accepted 2 February 2017; advance online publication 18 May 2017. doi:10.1038/gim.2017.26

Official journal of the American College of Medical Genetics and Genomics **ORIGINAL RESEARCH ARTICLE** Genetics inMedicine

Open

Using high-resolution variant frequencies to empower clinical genome interpretation

Nicola Whiffin, PhD[1,2], Eric Minikel, MS[3,4], Roddy Walsh, MSc[1,2], Anne H. O'Donnell-Luria, MD, PhD[3,4], Konrad Karczewski, PhD[3,4], Alexander Y. Ing, MS, CGC[5,6], Paul J.R. Barton, PhD[1,2], Birgit Funke, PhD, FACMG[5,6], Stuart A. Cook, PhD, MRCP[1,2,7,8], Daniel MacArthur, PhD[3,4,9] and James S. Ware, PhD, MRCP[1,2,4,10]

Variant: 15:48725102 C / T

Filter Status	PASS
dbSNP	rs112084407
Allele Frequency	0.0007913
Filtering AF	0.001042 (European (Non-Finnish))
Allele Count	96 / 121322
UCSC	15-48725102-C-T
ClinVar	Click to search for variant in Clinvar

Filtering allele frequency (AF): a threshold for filtering variants that are too common to plausibly cause disease. If the variant filtering AF is greater than the maximum credible population AF for the disease of interest, the variant is too common to be causative and may be filtered. Click here to see the filtering AF calculator app and citation.

Site Quality Metrics

Annotations

This variant falls on 5 transcripts in 1 genes:

missense

- FBN1 Transcripts

3' UTR

- FBN1 - ENST00000537463

Note: This list may not include additional transcripts in the same gene that the variant does not overlap.

Population Frequencies

Population	Allele Count	Allele Number	Number of Homozygotes	Allele Frequency
European (Non-Finnish)	84	66710	0	0.001259
Latino	10	11534	0	0.000867
South Asian	2	16512	0	0.0001211
African	0	10406	0	0
East Asian	0	8638	0	0
European (Finnish)	0	6614	0	0
Other	0	908	0	0
Total	**96**	**121322**	**0**	**0.0007913**

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Calculations based on allelic and genetic heterogeneity and penetrance

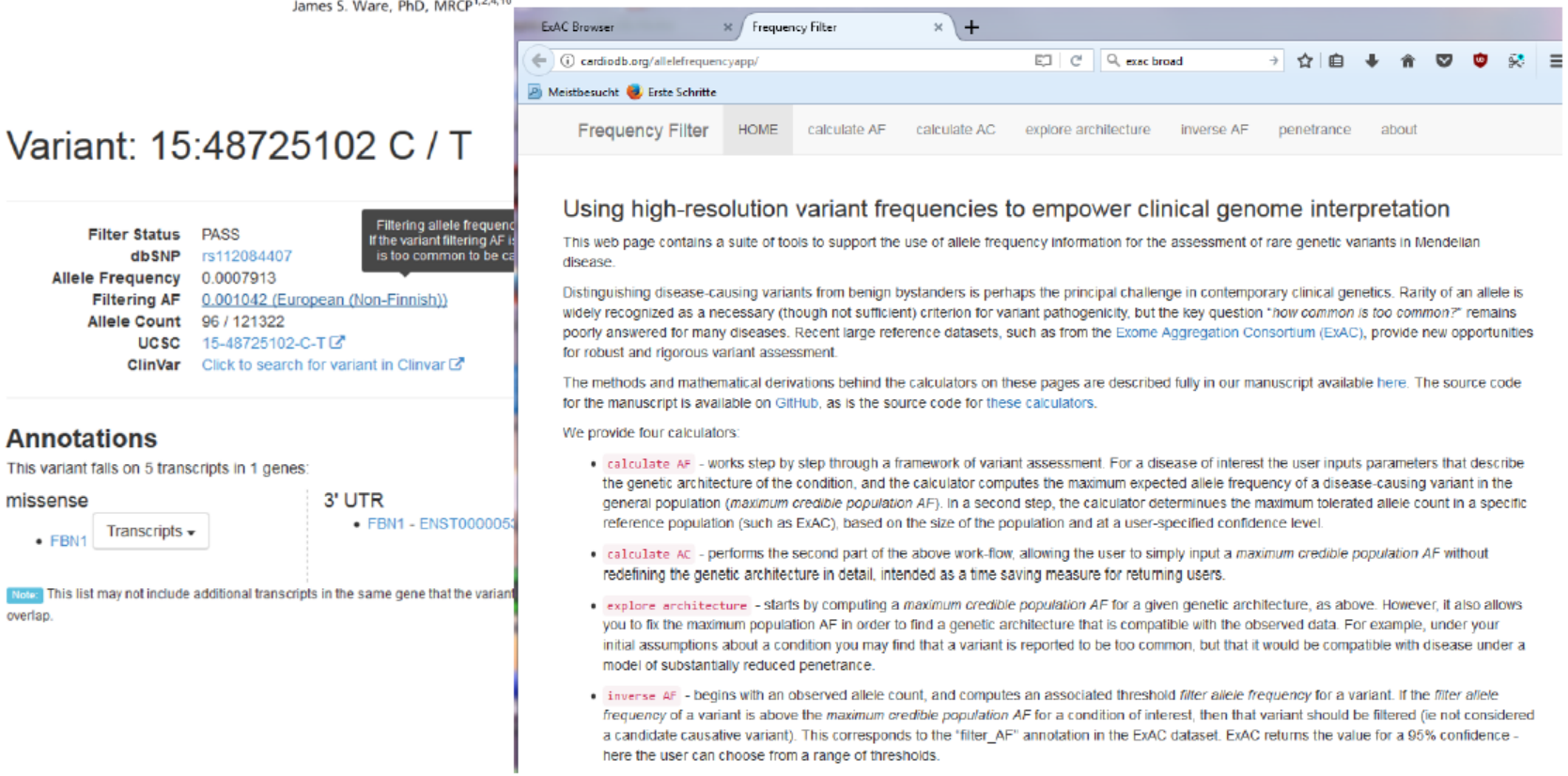

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Table 2 Maximum credible population frequencies and maximum tolerated ExAC allele counts for variants causative of exemplar inherited cardiac conditions, assuming a penetrance of 0.5 throughout

Disease	Maximum allelic contribution	Prevalence	Penetrance	Maximum population frequency	Maximum tolerated ExAC allele count
Marfan	0.015	1/3,000	0.5	5.0×10^{-6}	2
Noonan	0.10	1/1,000	0.5	1.0×10^{-4}	18
CPVT	0.10	1/10,000	0.5	1.0×10^{-5}	3
Classic Ehlers-Danlos	0.40	1/20,000	0.5	2.0×10^{-5}	5

CPVT,catecholaminergic polymorphic ventricular tachycardia; ExAC, Exome Aggregation Consortium database.
Prevalence estimates (taken as the highest value reported) were obtained from Marfan,[80] Noonan,[18] CPVT,[19] and classical Ehlers-Danlos.[20]

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European (Finnish)	0	6614	0	0
Other	0	908	0	0
Total	**96**	**121322**	**0**	**0.0007913**

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Information on allele frequencies per gene

Very informative tables in EXAC

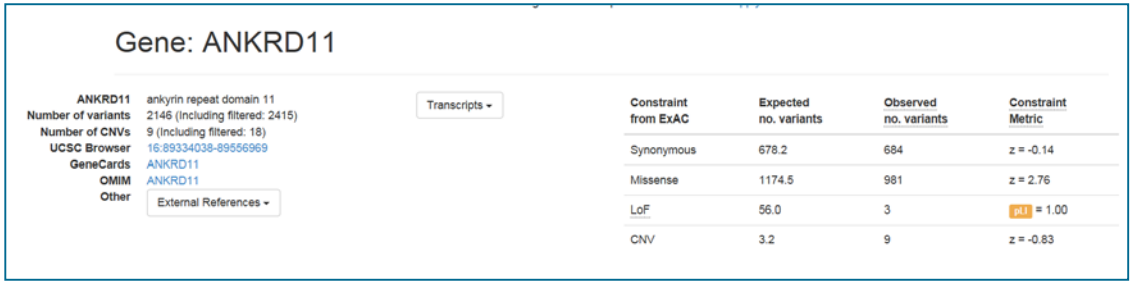
Constraint from ExAC	Expected no. variants	Observed no. variants	Constraint Metric
Synonymous	678.2	684	z = -0.14
Missense	1174.5	981	z = 2.76
LoF	56.0	3	pLI = 1.00
CNV	3.2	9	z = -0.83

For synonymous and missense, we created a signed Z score for the deviation of observed counts from the expected number. Positive Z scores indicate increased constraint (intolerance to variation) and therefore that the gene had fewer variants than expected. Negative Z scores are given to genes that had a more variants than expected. Highlighted when >3.09 (PP2 argument)

The closer pLI is to one, the more LoF intolerant the gene appears to be. We consider pLI >= 0.9 as an extremely LoF intolerant set of genes (PVS1 support).

What to expect? *(in an ideal world)*

Autosomal dominant disease
Due to loss of function mutations



Gene: ANKRD11

ANKRD11	ankyrin repeat domain 11
Number of variants	2146 (Including filtered: 2415)
Number of CNVs	9 (Including filtered: 18)
UCSC Browser	16:89334038-89556969
GeneCards	ANKRD11
OMIM	ANKRD11
Other	External References

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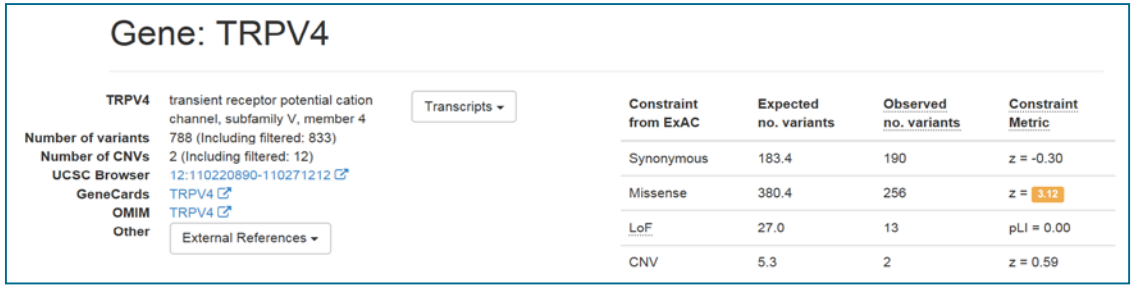
Autosomal dominant disease
Due to gain of function mutations



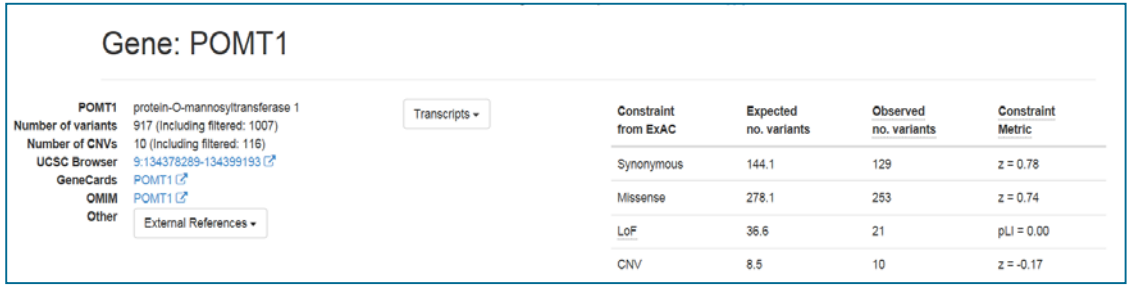
Gene: TRPV4

TRPV4	transient receptor potential cation channel, subfamily V, member 4
Number of variants	788 (Including filtered: 833)
Number of CNVs	2 (Including filtered: 12)
UCSC Browser	12:110220890-110271212
GeneCards	TRPV4
OMIM	TRPV4
Other	External References

Transcripts

Constraint from ExAC	Expected no. variants	Observed no. variants	Constraint Metric
Synonymous	183.4	190	z = -0.30
Missense	380.4	256	z = 3.12
LoF	27.0	13	pLI = 0.00
CNV	5.3	2	z = 0.59

Autosomal recessive disease



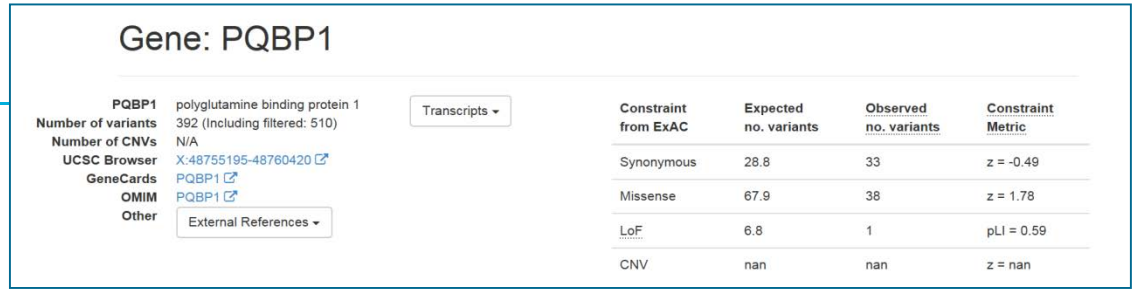
Gene: POMT1

POMT1	protein-O-mannosyltransferase 1
Number of variants	917 (Including filtered: 1007)
Number of CNVs	10 (Including filtered: 116)
UCSC Browser	9:134378289-134399193
GeneCards	POMT1
OMIM	POMT1
Other	External References

Transcripts

Constraint from ExAC	Expected no. variants	Observed no. variants	Constraint Metric
Synonymous	144.1	129	z = 0.78
Missense	278.1	253	z = 0.74
LoF	36.6	21	pLI = 0.00
CNV	8.5	10	z = -0.17

X-linked recessive disease



Gene: PQBP1

PQBP1	polyglutamine binding protein 1
Number of variants	392 (Including filtered: 510)
Number of CNVs	N/A
UCSC Browser	X:48755195-48760420
GeneCards	PQBP1
OMIM	PQBP1
Other	External References

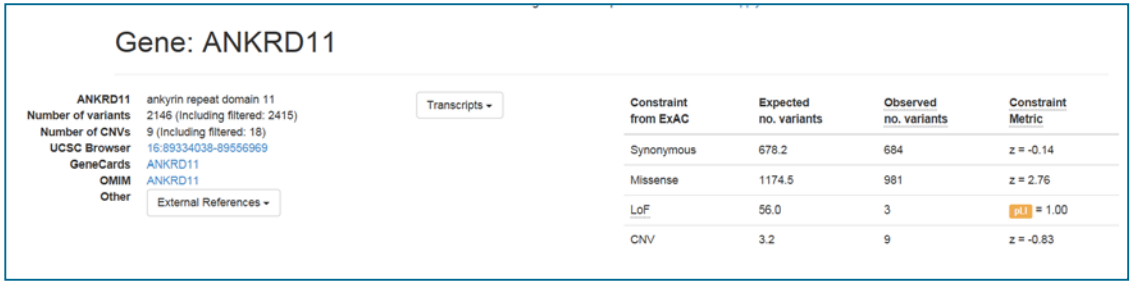
Transcripts

Constraint from ExAC	Expected no. variants	Observed no. variants	Constraint Metric
Synonymous	28.8	33	z = -0.49
Missense	67.9	38	z = 1.78
LoF	6.8	1	pLI = 0.59
CNV	nan	nan	z = nan

Radboudumc

What to expect? *(in an ideal world)*

Autosomal dominant disease
Due to loss of function mutations



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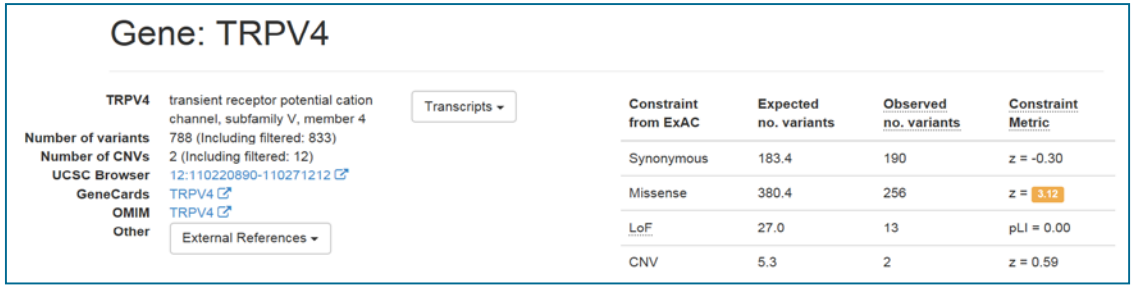

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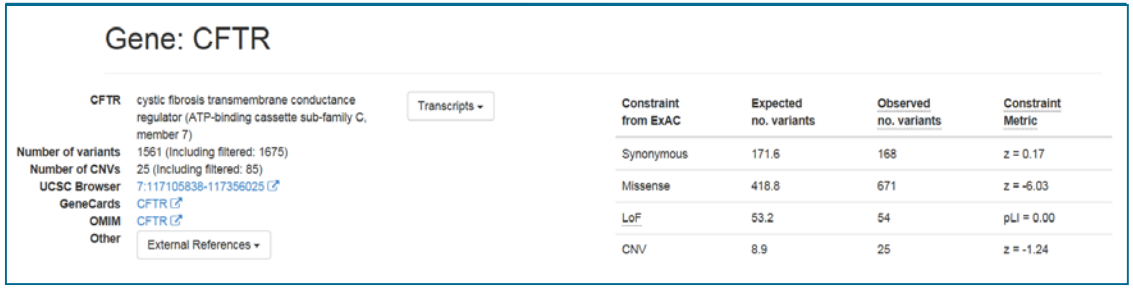
CNV	5.3	2	z = 0.59

Autosomal recessive disease



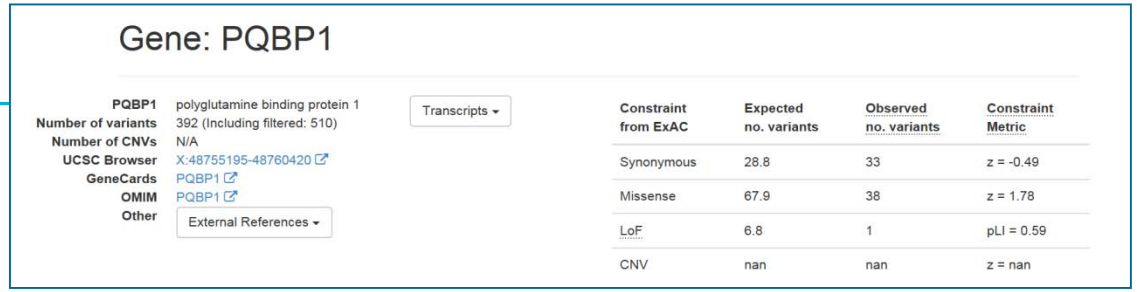
Gene: CFTR

CFTR	cystic fibrosis transmembrane conductance regulator (ATP-binding cassette sub-family C, member 7)
Number of variants	1561 (Including filtered: 1675)
Number of CNVs	25 (Including filtered: 85)
UCSC Browser	7:117105838-117356025
GeneCards	CFTR
OMIM	CFTR
Other	External References

Transcripts

Constraint from ExAC	Expected no. variants	Observed no. variants	Constraint Metric
Synonymous	171.6	168	z = 0.17
Missense	418.8	671	z = -6.03
LoF	53.2	54	pLI = 0.00
CNV	8.9	25	z = -1.24

X-linked recessive disease



Gene: PQBP1

PQBP1	polyglutamine binding protein 1
Number of variants	392 (Including filtered: 510)
Number of CNVs	N/A
UCSC Browser	X:48755195-48760420
GeneCards	PQBP1
OMIM	PQBP1
Other	External References

Transcripts

Constraint from ExAC	Expected no. variants	Observed no. variants	Constraint Metric
Synonymous	28.8	33	z = -0.49
Missense	67.9	38	z = 1.78
LoF	6.8	1	pLI = 0.59
CNV	nan	nan	z = nan

Radboudumc