# Variant Classification: ACMG recommendations

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# **OVERVIEW**

- Introduction
- ACMG-AMP Classification System
- Evaluation of inter-laboratory concordance in variant classification

#### Link for download the ACMG Standards and Guidelines

https://www.acmg.net/docs/Standards\_Guidelines\_for\_the\_Interpretation\_of\_Sequence\_Variants.pdf

search: "acmg standards and guidelines"

### Why Classification systems?



#### NIH Public Access

Author Manuscript

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#### Sequence variant classification and reporting: recommendations

for improving the interpretation of cancer susceptibility genetic

test results

Sharon E, Plon<sup>1,\*,#</sup>, Diana M. Eccles<sup>2,\*</sup>, Douglas Easton<sup>3</sup>, William D. Foulkes<sup>4</sup>, Maurizio Genuardi<sup>5</sup>, Marc S. Greenblatt<sup>6</sup>, Frans B.L. Hogervorst<sup>7</sup>, Nicoline Hoogerbrugge<sup>8</sup>, Amanda B. Spurdle<sup>9</sup>, and Sean Tavtigian<sup>10</sup> for the IARC Unclassified Genetic Variants Working Group<sup>1</sup>

Proposed Classification System for Sequence Variants Identified by Genetic Testing

Class	Description	Probability of being Pathogenic
5	Definitely Pathogenic	>0.99
4	Likely Pathogenic	0.95-0.99
3	Uncertain	0.05-0.949
2	Likely Not Pathogenic or of Little Clinical Significance	0.001-0.049
1	Not Pathogenic or of No Clinical Significance	<0.001

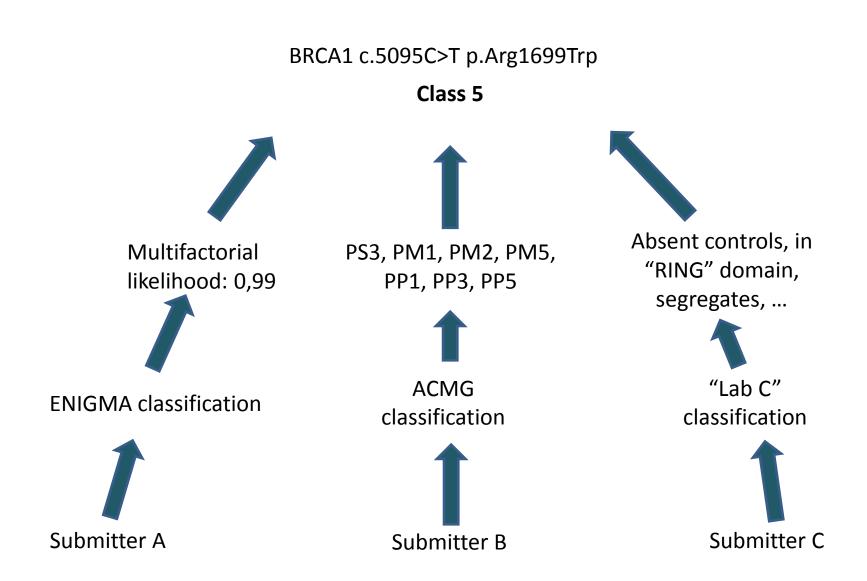
#### 5 classes linked to validated quantitative measures of causality/ pathogenicity

Class	Clinical Testing	Surveillance Recommendations if At-Risk Relative is Positive	Research Testing of Family Members
5	Test at-risk relatives for variant	Full high-risk surveillance guidelines	Not indicated
4	Test at-risk relatives for variant*	Full high-risk surveillance guidelines	May be helpful to further classify variant
3	Do not use for predictive testing in at-risk relatives *	Based on family history (and other risk factors)	May be helpful to further classify variant
2	Do not use for predictive testing in at-risk relatives *	Treat as "no mutation detected" for this disorder	May be helpful to further classify variant
1	Do not use for predictive testing in at-risk relatives *	Treat as "no mutation detected" for this disorder	Not indicated

All 5 classes are linked to clinical recommendations

Goal of IARC: To give actionable clinical recommendations to genetic data Accurate and consistent variant classification is prerequisite for Dx & Precision Medicine

### Why Classification systems?



### Example of pathogenic variant BRCA1 p.Cys61Gly (ClinVar)

Clinical significance Last evaluated)	Review status (Assertion method)	Collection method	Condition(s) (Mode of inheritance)	Origin	Citations	Submitter - Study name							
Pathogenic (Aug 10, 2015)	reviewed by expert panel • ENIGMA BRCA1/2 Classification Criteria (2015)		Breast-ovarian cancer, familial 1 [ <u>MedGen</u>   <u>OMIM</u> ]	germline	PubMed (1)     [See all records that cite     this PMID]     Other citation	Evidence-based Network fo Interpretation of Germline N Alleles (ENIGMA) Study description							
Pathogenic (Nov 3, 2014)	criteria provided, single submitter = ACMG Guidelines, 2015 = ACMG Guidelines, 2015	clinical testing	Breast-ovarian cancer, familial 1 [ <u>MedGen</u>   <u>OMIM</u> ]	germline		Michigan Medical Genetics Laboratories, University of Michigan							
Pathogenic (Feb 11, 2016)	criteria provided, single submitter • LMM Criteria	clinical testing	Hereditary breast and ovarian cancer syndrome (Autosomal dominant inheritance) [MedGen   Orphanet]	germline	PubMed (8) [See all records that cite these PMIDs]	Laboratory for Molecular Medicine,Pertners HealthCi Personalized Medicine	are						
Pathogenic (Dec 30, 2014)	criteria provided,	clinical testing		germline		Color Genomics, Inc.,							
(Dec 30, 2014)	single submitter • ACMG Guidelines, 2015 • ACMG Guidelines, 2015		predisposing syndrome [MedGen]				Pathogenic	criteria provided, single submitter • <u>Carraro et al.</u> (PLoS One. 2013)	research	Breast cancer [MedGen]	germline	PubMed (2)     [See all records that cite     these PMIDs]	Laboratory of Genomics and Molecular Biology,A. C. Came Cancer Center Study description
Pathogenic (Feb 18, 2015)	criteria provided, single submitter • Quest pathogenicity assessment criteria	clinical testing	Breast-ovarian cancer, familial 1 (Autosomal dominant inheritance) [MedGen   OMIM]	germline	PubMed (11)     [See all records that cite     these PMIDs]	Quest Diagnostics Nichols Institute San Juan Capistra	Pathogenic (Feb 22, 2016)	criteria provided, single submitter • Ambry Autosomal Dominant and X-Linked criteria (10)0045	clinical testing	Hereditary cancer- predisposing syndrome [MedGen]	germline		Ambry Genetics
Likely	criteria provided,	clinical testing		germline		GeneKor MSA		<u>(10/2015)</u>					
pathogenic (Jul 1, 2016)	single submitter   ACMG Guidelines, 2015  ACMG Guidelines, 2015		[MedGen   Orphanet   OMIM]				Pathogenic (Jan 20, 2017)	criteria provided, single submitter • <u>GeneDx Variant</u> <u>Classification</u> (06012015)	clinical testing	not provided [MedGen]	germline		<u>GeneDx</u>
Pathogenic (Oct 2, 2015)	criteria provided, single submitter • CIMBA Mutation <u>Classification</u> <u>guidelines May 2016</u>	clinical testing	Breast-ovarian cancer, familial 1 [ <u>MedGen</u>   <u>OMIM</u> ]	germline		Consortium of Investigator Modifiers of BRCA1/2 (CII/ c/o University of Cambridg	Pathogenic	criteria provided, single submitter • <u>ACMG guidelines,</u> <u>2007</u>	clinical testing	Hereditary breast and ovarian cancer syndrome [MedGen   Orphanet]	germline		Genetics Diagnostic Laboratory, Children's Hospi Eastern Ontario Study description
							Pathogenic (May 13, 2015)	criteria provided, single submitter • EGL Classification Definitions	clinical testing	Breast-ovarian cancer, familial 1 [MedGen   OMIM]	germline	• Other citation	Emory Genetics Laboratory,Emory University
<b>★</b> /	ACMG-A	MP CI	lassificatio	วท Sy	stem		Pathogenic (Jan 18, 2017)	criteria provided, single submitter • <u>Invitae Variant</u> <u>Classification</u> <u>Sherloc (09022015)</u>	clinical testing	Hereditary breast and ovarian cancer syndrome [MedGen   Orphanet]	germline	PubMed (2)     [See all records that cite     these PMIDs]	Invitae
<b>★</b> (	Different	: Class	sification S	Syste	ms		Pathogenic (Feb 23, 2017)	criteria provided, single submitter • <u>ACMG Guidelines,</u> 2015 • <u>ACMG Guidelines,</u> 2015	clinical testing	Familial cancer of breast (Autosomal dominant inheritance) [MedGen   Orphanet   OMIM]	germline		Baylor Miraca Genetics Laboratories Study description
						c	$\star$	2015 ACMG Guidelines,		[MedGen   Orphanet			Study description

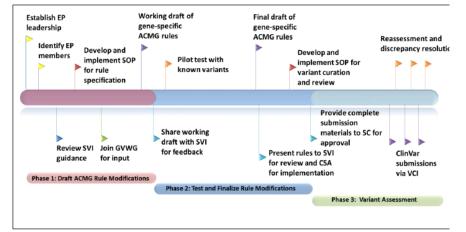
14 submissions / 10 different classification systems

# **EXPERT GROUP CLASSIFICATION**

#### ClinGen promotes formation of gene/ disease specific Expert Panels (EP)



#### Figure 2: Expert Panel milestones



#### 3.1 Overarching Goals

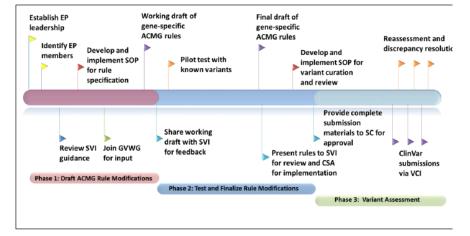
- i. Define the set of conditions and associated genes that fall within the Clinical Domain WG.
  - Evaluate the clinical validity (strength of evidence) of gene-disease associations for condition(s) within the working group domain (see 3.2).
  - Prioritize genes and conditions for attention by the WG, considering those that have not been sufficiently evaluated and annotated for clinical use as potential priorities.
  - c. Identify other groups with overlapping interests in gene-disease associations relevant to the conditions that are the responsibility of the WG in order to coordinate efforts.
- Facilitate deposition of variants from clinically relevant genes into ClinVar (see 3.3).
  - Identify existing professional guidelines and community-organized efforts that are curating variants in genes related to the specific disease domain.
  - b. Serve as a liaison to locus specific databases (LSDBs) and similar research efforts in order to facilitate reciprocal exchange of data between LSDBs and ClinVar.
  - c. Identify clinical laboratories that perform testing in the clinical domain and facilitate interactions with ClinGen staff for data submission to ClinVar.
- iii. Encourage development of Expert Panels to evaluate the clinical significance of genetic variants for submission to ClinVar.
  - Identify and encourage external groups that are already involved in curating genetic variants within the domain, and coordinate with them to avoid duplicating effort.
  - Review and evaluate the information provided from external curation groups for 3-star "Expert Panel" status in ClinVar based on the review process developed by the ClinGen Steering Committee
  - c. Establish ClinGen Expert Panels to focus on conditions deemed important by the CDWG but not currently served by an existing community effort (see 3.4).
  - d. Perform specification of the ACMG/AMP sequence variant interpretation guidelines framework for variant classification to the respective diseases/genes.

# **EXPERT GROUP CLASSIFICATION**

#### ClinGen promotes formation of gene/ disease specific Expert Panels (EP)



#### Figure 2: Expert Panel milestones



InSiGHT classification rules (4 MMR genes) ENIGMA classification rules (BRCA1/BRCA2) CFTR2 classification rules CDH1, JPS, STK11, ....

#### 3.1 Overarching Goals

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#### **Mother Of All Classification Systems**

#### ACMG recommendations

September/October 2000 · Vol. 2 · No. 5

© American College of Medical Genetics and Genomics ACMG STANDARDS AND GUIDELINES

# ACMG recommendations for standards for interpretation of sequence variations

ACMG I

These pretation course of (1) to pro ing of su educating may info I. Interpre Increas quence va tainty with cance falls which the in which i Committe quence Va variations

### ACMG recommen interpretation an Revisions 2007

ACMG Standards and

C. Sue Richards, PhD<sup>1</sup>, Sherri Bale, Ph Madhuri R. Hegde, PhD<sup>6</sup>, Elaine Lyon Laboratory Quality Assurance Commit

Key Words: clinical genetic testi

- Sequence variation is previously reported and is a recognized cause of the disorder. Review of the literature central mutation databases, e.g., Human Gene Mutatio Database (HGMD), or the locus-specific database, to as sess the current degree of certainty that the sequence variation causative of the disorder should be undertaken prior to reporting. Concordance studies between phenotype and ge notype within a family may provide acceptable criteria i the absence of more definitive functional studies.
- Sequence variation is previously unreported and is a the type which is expected to cause the disorder. Examples include variation that is predicted to shift the mRN reading frame; result in the introduction of a stop codo..

#### 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

Genetics

in Medicine

Sue Richards, PhD<sup>1</sup>, Nazneen Aziz, PhD<sup>2,16</sup>, Sherri Bale, PhD<sup>3</sup>, David Bick, MD<sup>4</sup>, Soma Das, PhD<sup>5</sup>, Julie Gastier-Foster, PhD<sup>6,7,8</sup>, Wayne W. Grody, MD, PhD<sup>9,10,11</sup>, Madhuri Hegde, PhD<sup>12</sup>, Elaine Lyon, PhD<sup>13</sup>, Elaine Spector, PhD<sup>14</sup>, Karl Voelkerding, MD<sup>13</sup> and Heidi L. Rehm, PhD<sup>15</sup>; 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 geneticists 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.

A American College of Medical Genetics and Genomics
 ACMG STANDARDS AND GUIDELINES
 in Medicine

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

Qualitative evaluation of different data types (28 defined criteria with assigned code)

Each code is assigned a weight (stand-alone, very strong, strong, moderate, or supporting) and direction (benign or pathogenic)

Variants then can be assigned in one of 5 classes (IARC 5-tier system)

If not enough lines of evidence are invoked to classify a variant as P, LP, LB, or B, or there are <u>valid</u> but contradictory lines of evidence, a variant is interpreted as a VUS

	< Ben	<sup>iign</sup> ←		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 <i>trans</i> with a dominant variant BP2 Observed in <i>cis</i> 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			

### Table 5 Rules for combining criteria to classify sequence variants

- an i an i a	
Pathogenic	(i) 1 Very strong (PVS1) AND
	(a) $\geq$ 1 Strong (PS1–PS4) OR
	(b) $\geq 2$ Moderate (PM1–PM6) OR
	(c) 1 Moderate (PM1–PM6) and 1 supporting (PP1–PP5) OR
	(d) $\geq 2$ Supporting (PP1-PP5)
	(ii) $\geq$ 2 Strong (PS1–PS4) <i>OR</i>
	(iii) 1 Strong (PS1–PS4) AND
	(a)≥3 Moderate (PM1–PM6) <i>OR</i>
	(b)2 Moderate (PM1–PM6) $AND \ge 2$ Supporting (PP1–PP5) $OR$
	(c)1 Moderate (PM1–PM6) $AND \ge 4$ supporting (PP1–PP5)
Likely pathogenic	<ul> <li>(i) 1 Very strong (PVS1) AND 1 moderate (PM1– PM6) OR</li> </ul>
	<ul> <li>(ii) 1 Strong (PS1–PS4) AND 1–2 moderate (PM1–PM6) OR</li> </ul>
	(iii) 1 Strong (PS1–PS4) AND≥2 supporting (PP1–PP5) OR
	(iv) ≥3 Moderate (PM1–PM6) OR
	<ul> <li>(v) 2 Moderate (PM1–PM6) AND ≥2 supporting (PP1–PP5) OR</li> </ul>
	(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	(i) Other criteria shown above are not met OR
significance	<ul> <li>(ii) the criteria for benign and pathogenic are contradictory</li> </ul>

### ACMG STANDARDS AND GUIDELINES in Medicin

### **General Considerations:**

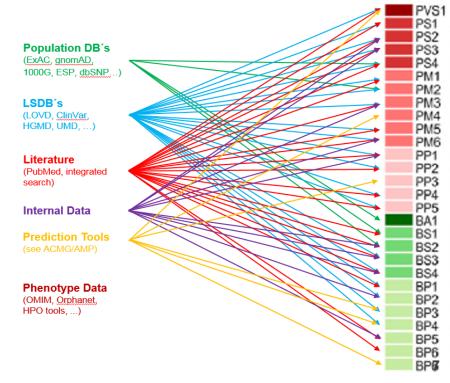
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- for variants in all Mendelian genes (single gene, gene panel, exome, genome or transcriptome)
- not for somatic, Px, multigenic/complex disorders and not for low/ moderate penetrance variants
- be carefull with candiate genes ("genes of uncertain significance"; "GUS"; Sept.2017: OMIM 3.803 genes)
- the terms "mutation" and "polymorphism" should not be used
- instead use "variant" (pathogenic (5); likely pathogenic (4); uncertain significance (3); likely benign (2); benign (1))
- variants should be reported using the HGVS nomenclature (http://www.hgvs.org/mutnomen)
- to provide flexibility: some criteria listed as one weight can be moved to another weight using professional judgment, depending on the evidence collected (multiple observations of a variant in trans with path. variant PM3 to PS)

"Pathogencity should be determined by the entire body of evidence in aggregate, including all cases studied, arriving at a single conclusion"

### What are the requirements?

- Detailed population frequency data (ExAC, 1000G, now gnomAD)
- Clinical databases / LSDB's
- Thorough literature search (find AND correctly interprete the literature)
- Access to your internal DB (hopefully these data are soon published!)
- Bioinformatic prediction integrated (protein, splice sites)



27 ACMG/AMP criteria from strong pathogenic to supporting benign (Richards et al.; Genet Med. 2015)

Selected SNP								Co	ogle OMIM
SNP (TP53:NM_	000546:c.704A>G:p	o.Asn235Ser ; I	het; AD) I	Benign	( lana, 2016-06-2	27 17:53:33 )		Gene R	
								Genera	
PatientID 103274	Coverage	Quality 222.0	/	Subpanel Cancer Mam		Associated Disease			OMIMGENE
						li-fraumeni syndrome esophageal canceres squamous cell carcin adrenocortical carcino papilloma of choroid basal cell carcinoma, b-cell chronic lympho small cell lung cance	r 9 lymphoblastic leukemia	e of li and fraumeni;;sb oma, susceptibility to, i cal carcinoma, pediatri	OMIMGENE OMIMGENE OMIMGENE OMIMGENE
ClinDB		Grading/Phe	enotype			Name		Info	
ClinVar		_	n;Uncertain sig	inificance			.704A>G (p.Asn235Ser)	Submitter: 6	
Emory		VOUS	in,oncertain sig	Jimedilee		NM_000546(TP53):c.7		-	
PopDB	rsID	Ref/Alt	AF/	AC.	AC Hom	Subpopulations			
dbSNP (134/144)	rs144340710	T/C	AFI	AC	AC HOIT				
ExAC	rs144340710					Subpopulations			
ESP		T/C	C=(	0.0002/29	0		AFR=0, EAS=0,SAS=0, AMR=0, FI	N=0.00091269, OTH=0	
	rs144340710	T/C T/C		0.0002/29 0.0002/2	₀ BS1			N=0.00091269, OTH=0	
Protein Domain	rs144340710				ů.	NFE=0.00034465, A		N=0.00091269, OTH=0 Value	
Protein Domain p53, DNA-binding			C=(	0.0002/2	ů.	NFE=0.00034465, A eurAMR=0.0001, afr predProg AGVGD	Prediction	Value	
			C=(	phyloP	ů.	NFE=0.00034465, A eurAMR=0.0001, afr predProg AGVGD SIFT	Prediction	Value C0 0.08	
p53, DNA-binding	domain	T/C	C=( phastCons 0.992	0.0002/2 phyloP 1.82	BS1	NFE=0.00034465, A eurAMR=0.0001, afr predProg AGVGD SIFT MAPP	Prediction Tolerated good	Value C0 0.08 0.0481	
p53, DNA-binding nearestSSType	domain distNearest	T/C SS n	C=( phastCons 0.992 naxEntScore	0.0002/2 phyloP 1.82 ssfSc	BS1	NFE=0.00034465, A eurAMR=0.0001, afr predProg AGVGD SIFT	Prediction	Value C0 0.08	
p53, DNA-binding	domain	T/C SS n	C=( phastCons 0.992	0.0002/2 phyloP 1.82	BS1	NFE=0.00034465, A eurAMR=0.0001, afr predProg AGVGD SIFT MAPP	Prediction Tolerated good	Value C0 0.08 0.0481	

Information								
Selected SNP							Coog	еомім
SNP (TP53:N	IM_000546:c.704A>G:p	Asn235Ser ; het; AD).	Benign	( lana, 2016-06-27	17:53:33 )		Gene Revie	
PatientID	Coverage	Quality	Subpanel		Associated Disease			Source
103274	423	222.0	Cancer_Mam	maErweitert	lung canceralveolar cell carci li-fraumeni syndrome 1; lfs1;;			
					esophageal canceresophage			
	Allele Fr	equency i	s greate	er than e	expected for	disorder		
		. ,	U		•			IGENE anet
	TP53 p.(As	sn235Ser)						anet anet
	11 33 p.(/ %	5112333617						anet
		SP MAF = 0	0002 (-)	0 02%)				
ClinDB ClinVar	-	-	•		10,000 or $0,00$		0/)	
Emory HGMD				•	10.000) or 0,00	1005 (= <b>0,005</b>	70)	706467
	• nigniy pe	enetrant and	a early on	set				
PopDB dbSNP (134/14	rsID (4) rs144340710	Ref/Alt T/C	AF/AC	AC Hom	Subpopulations			
ExAC ESP	rs144340710 rs144340710	T/C T/C	C=0.0002/29 C=0.0002/2	0	NFE=0.00034465, AFR=0, eurAMR=0.0001, afrAMR=0		0.00091269, OTH=0	
201	13144340110	110	0-0.000212	DC1		.0002		
				BS1				
Protein Domair		phastCor			predProg	Prediction	Value	
p53, DNA-bindi	ing domain	0.992	1.82		AGVGD SIFT	- Tolerated	C0 0.08	
nearestSSType	e distNearestS	S maxEntScor	e ssfSco	ore	MAPP Polyphen	good benign	0.0481 0.144	
3'	32	0%	0%					
Patient Remar	ks		Variant Rema	arks		van Hest LP et al.; F 7;6(3):311-6.: co-oct		<b>^</b>
						uncating TP53 varia tHuusko et al.; Canc		
						net. 1999 Jul 1;112( ot segregate in fami	yPMID: 2012869	
						1, 21343334, 15580 nctiona studies like		
							Add Literature	

formation									
Selected SNP							Co	ogle or	мім
SNP (TP53:NM_0	000546:c.704A>G:p	Asn235Ser ; het; AD)	Benign	( lana, 2016-06-2	7 17:53:33 )				
							Gener	LC LC	
PatientID	Coverage	Quality	Subpanel		Associated Disease			Source	
103274	423	222.0	Cancer_Mam	macrwellen	li-fraumeni syndrome esophageal canceres squamous cell carcin adrenocortical carcino papilloma of choroid y basal cell carcinoma, b-cell chronic lympho small cell lung cancer	r lymphoblastic leukemia	of li and fraumeni;;sb. ma, susceptibility to, i al carcinoma, pediatri	OMIMGENE OMIMGENE OMIMGENE OMIMGENE	
		1				01368363	I		
ClinDB		Grading/Phenotype			Name		Info		
ClinVar		Likely benign;Uncerta	in significance			.704A>G (p.Asn235Ser)	Submitter: 6		
Emory HGMD		VOUS Rhabdomvosarcoma			NM_000546(TP53):c.7	04A>G	-	omed: 7706467	
PopDB dbSNP (134/144) ExAC ESP	rsID rs144340710 rs144340710 rs144340710	Ref/Alt T/C T/C T/C	AF/AC C=0.0002/29 C=0.0002/2	AC Hom	Subpopulations NFE=0.00034465, A eurAMR=0.0001, afr	AFR=0, EAS=0, SAS=0, AMR=0, FIN AMR=0.0002	=0.00091269, OTH=0		
Protein Domain p53, DNA-binding of	domain	phastCo 0.992	ns phyloP 1.82	BS1	predProg AGVGD	Prediction	Value C0		
					SIFT	Tolerated	0.08	BP4	
					APP Polyphen	good benign	0.0481 0.144		
nearestSSType 3'	distNearestS 32	SS   maxEntSco 0%	re ssfSc 0%	ore		Dongi	0.111		
Patient Remarks			Variant Rem	arks		nctiona studies like	curence with tr ant in LFS-patien cer Genet Cytoge (1):9-14. does n ilyPMID: 2012869 0553, 21232794: fu		

SNP (TP53:NM_000546:c.704A>G;p.Asn235Ser ; het, AD)       Benign       (lana, 2016-06-27 17:53:33 )         PatientID       Coverage       Quality       Subpanel         103274       423       222.0       Cancer_MammaErweitert         I-fraumeni syndrome 1; lfs1;;sarcoma family syndrome of li and fraumeni;;sb       OMIMGENE esophageal canceresophageal squamous cell carcinoma, susceptibility to, i       OMIMGENE oMIMGENE         adrenocortical carcinoma, head and neck; hnscc       OMIMGENE adrenocortical carcinoma, hereditary; adccadrenocortical carcinoma, pediatri       OMIMGENE oMIMGENE	formation									
SMP (TPS3 ML_000546: 704A-G p.Ant2358er; ML AD)       Beingin (lan2, 2016-06-27 17.55.33)       Gene Reviews         Patientity       Coverage       Quality       Subpanel         103274       433       2230       Cancer Maintain Evention         Harmeri Standing, Induéd, adenciación ma, succeptibility 0.       OMMCENE         Patientity       Scancer Maintain Evention       Harmeri Standing, Induéd, adenciación ma, succeptibility 0.       OMMCENE         Patientity       Fasoral Antonio Symphocyte Later and the standing and concers compage ad guannas cell carcomma, succeptibility 0.       OMMCENE         Popole       PD52??       Cancer Mainmain Evention       Standing and cell une st	Selected SNP							1	ole 0	мім
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PpDD       Tell       PPDS?       Numcesses         ClinicB       Grading/Phenotype       Name       ClinicB	<mark>103274</mark>	<mark>423</mark>	222.0	Cancer_Mam	maErweitert	-			OMIMGENE	
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ClinDB       GradingPhenolype       Name       Info         ClinVar       Likely benign(Juncertain significance       NM_000548.5(TP53):c.704A>G       Submitter: 6         Emoy       VOUS       NM_000548.5(TP53):c.704A>G       Submitter: 6         PopDB       IsID       Ret/Alt       AF/AC       AC Hom       Subpopulations         disSNP (134/144)       rst44340710       TC       C.0002229       0       NFE=0.00034465, AFR=0, EAS=0, SAS=0, AMR=0, FIN=0.00091269, OTH=0         EAX       rst44340710       TC       C=0.000229       0       NFE=0.00034465, AFR=0, EAS=0, SAS=0, AMR=0, FIN=0.00091269, OTH=0         ESP       rst44340710       TC       C=0.000229       0       NFE=0.00034465, AFR=0, EAS=0, SAS=0, AMR=0, FIN=0.00091269, OTH=0         ESP       rst44340710       TC       C=0.000229       0       NFE=0.00034465, AFR=0, EAS=0, SAS=0, AMR=0, FIN=0.00091269, OTH=0         ESP       rst44340710       TC       C=0.000229       0       NFE=0.00034465, AFR=0, EAS=0, SAS=0, AMR=0, FIN=0.00091269, OTH=0         Licin Invest 1995 Apr.95(4):1606-11.       BS1        0001       standard       0001         J.Clin Invest 1995 Apr.95(4):1606-11.       Susponter Licin Arrows and the spondic rhabdomyosarcoma (RMS) carry constitutional mutations of the p53       00       00       00       00				22		aiant coll alioblactoma			Ornhonot	
ClinVar       Likely benign;Uncertain significance       NM_000546 5(TP53):c704A>G (p.4sn2358er)       Submitter: 6         Emony       VOUS       NM_000540(TP53):c704A>G       CM951230, Pubmed: 7706467         PopDB       rs1D       RefMitt       AF/AC       AC Hom       Subpopulations         dbSNP (134/144)       rs144340710       TC       CM951230, Pubmed: 7706467         EARC       rs144340710       TC       C=0.0002/29       NFE=0.00034465, AFR=0, EAS=0, SAS=0, AMR=0, FIN=0.00091269, OTH=0         ESP       rs144340710       TC       C=0.0002/29       NFE=0.0001, affAMR=0.0002         BS1       Uninterest, 1995 Apr:95(4):1606-11.       BS1         Germline p53 mutations are frequently detected in young children with rhabdomyosarcoma.       Diller L <sup>1</sup> , Sexsmith E, Gottlieb A, Li FP, Malkin D.         Author information       Abstract       We investigated the possibility that a proportion of children with sporadic rhabdomyosarcoma (RMS) carry constitutional mutations of the p53 gene. Amplified genomic DNA was extracted from peripheral blood leukocytes and PCR was used to amplify exons 2-11 of the p53 gene. Amplified genomic DNA was extracted from peripheral blood leukocytes and PCR was used to amplify exons 2-11 of the p53 gene. Amplified genomic DNA was extracted from peripheral blood leukocytes and PCR was used to amplify exors 2-11 of the p53 gene. Amplified genomic DNA was extracted from peripheral blood sub using single-strand conformation polymorphism (SSCP) analysis. The DNA sequence of an approximatic ampridox at the more 2			PP:			Search Associated Diseases				
Emory       VOUS       NNE_000546(TP53):c704A+G         HGMD       Rhabdomyosarcoma       CM951230, Pubmed: 7706467         PopDB       rsiD       RetiAtt       AF/AC       AC Hom       Subpopulations         dbSNP (134/144)       rs144340710       TC       Exac       Subpopulations         dbSNP (134/144)       rs144340710       TC       C=0.0002/20       NEE-0.00034465, AFR=0, EAS=0, SAS=0, AMR=0, FIN=0.00091269, OTH=0         EP       rs144340710       TC       C=0.0002/20       NEE-0.00034465, AFR=0, EAS=0, SAS=0, AMR=0, FIN=0.00091269, OTH=0         EVE       rs144340710       TC       C=0.0002/2       BS1         J_Clin Invest, 1995 Apr.95(4); 1606-11.       BS1       BS1         J_Clin Invest, 1995 Apr.95(4); 1606-11.       Generation       Jule 1, Sexamith E, Gottlieb A, Li EP, Malkin D.         Immoving Autor information       Abstract       Diller 1, Sexamith E, Gottlieb A, Li EP, Malkin D.       BP4         We investigated the possibility that a proportion of children with sporadic rhabdomyosarcoma (RMS) carry constitutional mutations of the p53 tumor suppressor gene. 33 patients with sporadic RMS at two large outpatient pediatric oncology clinics submitted blood samples. Genomic DNA was screened for the presence of germline p53 mutations using single-strand conformation polymorphism (SSCP) analysis. The DNA sequence of the p53 mutations using single-strand conformation polymorphism (SSCP) analysis. The DNA sequence of tho p56 ancor	ClinDB		Grading/Phenotype			Name	Info	)		
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PopDB       rsiD       Ref/Att       AF/AC       AC Hom       Subpopulations         dbSNP (134/144)       rs144340710       T/C       C=0.0002/29       0       NFE=0.00034465, AFR=0, EAS=0, SAS=0, AMR=0, FIN=0.00091269, OTH=0         ESP       rs144340710       T/C       C=0.0002/29       0       NFE=0.00034465, AFR=0, EAS=0, SAS=0, AMR=0, FIN=0.00091269, OTH=0         ESP       rs144340710       T/C       C=0.0002/29       0       NFE=0.00034465, AFR=0, EAS=0, SAS=0, AMR=0, FIN=0.00091269, OTH=0         LCIn Invest.       1995 Apr;95(4):1606-11.       BS1       Image: Comparison of Compari						NM_000546(TP53):c.704A>G	- CM	051220 Pub	mod: 7706467	
JClin Invest. 1995 Apr;95(4):1606-11.         Germline p53 mutations are frequently detected in young children with rhabdomyosarcoma.         Diller L <sup>1</sup> , Sexsmith E, Gottlieb A, Li FP, Malkin D.            • Author information          Abstract         We investigated the possibility that a proportion of children with sporadic rhabdomyosarcoma (RMS) carry constitutional mutations of the p53 tumor suppressor gene. 33 patients with sporadic RMS at two large outpatient pediatric oncology clinics submitted blood samples. Genomic DNA was extracted from peripheral blood leukocytes and PCR was used to amplify exons 2-11 of the p53 gene. Amplified genomic DNA was screened for the presence of germline p53 mutations using single-strand conformation polymorphism (SSCP) analysis. The DNA sequence of those samples that showed aberrant migration of bands on SSCP analysis was determined to identify the precise nature of the gene mutations. Patient records were reviewed to assess clinical correlates of the mutant p53 carrier state. Heterozygous constitutional mutations using single-strand conformation polymorphism (SSCP) analysis. The DNA sequence of mean mutations. Patient records were reviewed to assess clinical correlates of the mutant p53 carrier state. Heterozygous constitutional mutations were diverged on again groups 0 of the p53 enditional mutations	dbSNP (134/144) ExAC	rs144340710 rs144340710	T/C T/C	C=0.0002/29	AC Hom	NFE=0.00034465, AFR=0, EAS=0		269, OTH=0		
J Clin Invest. 1995 Apr,95(4):1606-11.         Germline p53 mutations are frequently detected in young children with rhabdomyosarcoma.         Diller L <sup>1</sup> , Sexsmith E, Gottlieb A, Li FP, Malkin D. <ul> <li>Author information</li> </ul> Abstract           We investigated the possibility that a proportion of children with sporadic rhabdomyosarcoma (RMS) carry constitutional mutations of the p53 tumor suppressor gene. 33 patients with sporadic RMS at two large outpatient pediatric oncology clinics submitted blood samples. Genomic DNA was extracted from peripheral blood leukocytes and PCR was used to amplify exons 2-11 of the p53 gene. Amplified genomic DNA was screened for the presence of germline p53 mutations using single-strand conformation polymorphism (SSCP) analysis. The DNA sequence of neutron suppressor line p53 mutations on SSCP analysis was determined to identify the precise nature of the gene mutations. Patient records were reviewed to assess clinical correlates of the mutant p53 carrier state. Heterozygous constitutional mutations was one theorem increme are leaded in aven 0 and one in overo 0 4 of the p53 gene.	ESP	rs144340710	T/C	C=0.0002/2	DC1	eurAMR=0.0001, afrAMR=0.0002				
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PatientiD       Coverage       Ouality       Subpanel         103274       423       222.0       Cancer_Mammatrivelteti         Ivig cancerstepholic cell carcinoma, included; adenocarcinoma, susceptibility to, supmones cell carcinoma, susceptibility to, supmones cell carcinoma, susceptibility to, supmones cell carcinoma, susceptibility to, tecc?         PDE5P??       Cancer_Mammatrivelteti         PDE5P??       Cancer_Mammatrivelteti         PDE5P??       Cancer_Mammatrivelteti         PDE5P??       Cancer_Mammatrivelteti         ClinDB       Gradingt-Henotype         ClinDB       G											formation
BMP (TPS3 ML_000546 c.704A+Cz p.Aar.2356r; het, AD)     Benign     (Iana, 2015-06-27 17.53.33)       PatientID     Coverage     Quality     Subpanel       103274     423     222.0     Cancer MammaErweitott       PBD5 272     423     222.0     Cancer MammaErweitott       PPD5 272     Cancer MammaErweitott     Associated Disease       PDF5 272     Cancer MammaErweitott     Higt cancer alweita and netch finds: a subceptibility to, subceptibility to, subceptibility to, subceptibility to, subceptibility to, rocc?       PBD5 272     PD5 272       ClinOB     GradingPEhenotyse       ClinOB     GradingPEhenotyse       ClinOB     GradingPEhenotyse       HGMD     RefMt       HGMD     RefMt       HGMD     RefMt       AFAC     AC Hom       Subpopulations     CM951230, P       PopDB     rsid4340710       T/C     C=0.000222       BS11       Protein Domain     phastCons       pf33, OM+ binding domain     0.992       122     Poptiben       Poptiben     Desides       Protein Domain     phastCons       pf33, OM+ binding domain     0.992       122     P       Poptiben     Desides       Popode     0.001       Starter Hemark	ogle OMIM										Selected SNP
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PDDB     rsiD     RefiAlt     AFAC     AC Hom     Subpopulations       PopDB     rsiD     RefiAlt     AFAC     AC Hom       PopDB     rsiD     RefiAlt     AFAC       PopDB     rsiD     RefiAlt     AFAC       PopDB     rsiD     RefiAlt     AC Hom       PopDB     rsiD     RefiAlt     AFAC       RefiAlt     AFAC     AC Hom     Subpopulations       PopDB     rsiD     RefiAlt     AFAC     AC Hom       Subpopulations     Subpopulations     Subpopulations     Subpopulations       Populational     0.992     1.82     Populations       Protein Domain     phastCore     ssfStcore<						maErweitert	Cancer_Mam	) (	222.0	423	103274
PPD5??       Search Associated Diseases         ClinDB       Grading/Phenotype       Name         ClinDB       Grading/Phenotype       Name         ClinVar       Likely benign/Juncertain significance       NM_000546 (UPs3);c; 704A+G (p.Asr;2558er)         Subopulations       -       ClinVar         HGMD       Ret/Att       AF/AC       AC Hom         VOUS       NM_000546 (UPs3);c; 704A+G (p.Asr;2558er)       Submitter: 6         MM_000546 (UP53);c; 704A+G       -       Clin9512230, P         PopDB       rsiD       Ret/Att       AF/AC       AC Hom       Suboppulations         HGMD       Rhabdomyosarcoma       -       Clin9512;c; 704A+G       -         PopDB       rsiD       Ret/Att       AF/AC       AC Hom       Suboppulations         HGMD       Rhabdomyosarcoma       -       -       Clin9512;c; 704A+G       -         PopDB       rsiD       Ret/Att       AF/AC       AC Hom       Suboppulations       -         HGMD       Rabdomyosarcoma       0       -       -       -       -         PopDB       rsiD       Ret/Att       AF/AC       AC Hom       Suboppulations       -         PopDB       rsiD       Ret/At											
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PP5??       basa cell carcinoma, susceptibility 0, 7, bc7         Beside cell carcinoma, susceptibility 0, 7, bc7       basa cell carcinoma, susceptibility 0, 7, bc7         Beside cell carcinoma, susceptibility 0, 7, bc7       basa cell carcinoma, susceptibility 0, 7, bc7         Beside cell chronic tympholic leukemia       small cell lung cancer         Search Associated Diseases       info         ClinOB       Grading/Phenolype       Name         ClinVar       Likely benign;Uncertain significance       NM_000545 (TP53); c704A+G       Submitter: 6         MU_000545 (TP53); c704A+G       CM951230, P       Submitter: 6       -         HGND       Rhabdomyosarcoma       NM_000546 (TP53); c704A+G       CM951230, P         PopDB       rs1D       Ret/Att       AF/AC       AC Hom       Subpopulations         HGND       Rhabdomyosarcoma       NFE-0.0003446; AFR=0, EAS=0, SAS=0, AMR=0, FIN=0.00091269, OTH=         ExAC       rs144340710       T/C       C       C       0002/29       0         Potein Domain       phastCons       phyoP       podd       0.0048         p33, DNA-binding domain       0.992       1.82       Polythen       AGYCO       -         Polythen       0.992       1.82       Ofer       Ofer       Ofer         Po											
PD5??       Search Associated Diseases         ClinDB       Grading/Phenotype       Name       Info         ClinVar       Likely benign/lucertain significance       NML_000546.5(TP53): C704A>G (p.Asn235Ser)       Submitter 6         HGMD       Rhabdomyosarcoma       -       CM951230.P       Submitter 6         HGMD       Rhabdomyosarcoma       -       CM951230.P       Submitter 6         HGMD       Rhabdomyosarcoma       -       CM951230.P       Subpopulations         HGMD       RetHAt       AF/AC       AC Hom       Subpopulations       CM951230.P         PopDB       rsiLD       RetHAt       AF/AC       AC Hom       Subpopulations       CM951230.P         PopDB       rsiLd4340710       TrC       C=0.0002/29       0       NFE=0.00034465.AFR=0, EAS=0, SAS=0, AMR=0, FIN=0.00091269, OTH=         ESP       rs144340710       TrC       C=0.0002/29       0       NFE=0.00034465.AFR=0, EAS=0, SAS=0, AMR=0, FIN=0.00091269, OTH=         BS1       Protein Domain       phastCons       phyloP       SFT       Tolerated       0.08         MAPP       good       0.0481       Polyphen       0.144       SFT       Tolerated       0.08         MAPP       good       0.046       SFT       Concocc	OMIMGENE										
PD5??       Search Associated Diseases         ClinDB       Grading/Phenotype       Name       Info         ClinDB       Grading/Phenotype       Name       Info         ClinVar       Likely benignt/incertain significance       NIL<000546 5(TP53): 7.04A+G (p.Asn.2558r)	Orphanet		emia								
PD5??       issarch Associated Diseases         ClinDB       Grading/Phenotype       Name       Info         ClinVar       Likely benign/Incertain significance       NML_000546 (TP53): 704A-G (p.4sn235Ser)       Submitter: 6         MGDD       Rhabdomyosarcoma       NML_000546 (TP53): 704A-G (p.4sn235Ser)       Submitter: 6         HGMD       Rhabdomyosarcoma       -       CM951230, P         PopDB       rs1D       Ref/Att       AF/AC       AC Hom       Subpopulations         dbSNP (134/144)       rs144340710       TC       C=0.0002/29       0       NFE=0.00034465, AFR=0, EAS=0, SAS=0, AMR=0, FIN=0.00091269, OTH=ESP       rs144340710       TC       C=0.0002/29       0       NEE=0.00034465, AFR=0, EAS=0, SAS=0, AMR=0, FIN=0.00091269, OTH=ESP       Ref/Atr       GVGD       -       CO       SUBDOW         Protein Domain       phastCons       phyloP       BS1       NEE=0.00034465, AFR=0, EAS=0, SAS=0, AMR=0, FIN=0.00091269, OTH=ESP       CO       SUBS1         Protein Domain       phastCons       phyloP       BS1       NET       OC       SUBDOW         Potein Domain       phastCons       phyloP       SUGO       -       CO       SUB         Ref.at       32       0%       0%       0%       -       CO       7(6/3)11-6: c	Orphanet Orphanet		lastic leukemia								
PP5??       Search Associated Diseases         ClinDB       Grading/Phenotype       Name       Info         ClinVar       Likely benignt/incertain significance       NML 000546 5(TP53):c.704A+G (p.4sn2358er)       Submitter: 6         Emory       VOUS       NL       ML       Submitter: 6       NML 000546 5(TP53):c.704A+G (p.4sn2358er)       Submitter: 6         PopDB       rsiD       Ret/Alt       AF/AC       AC Hom       Subpopulations       CM951230, P         PopDB       rsiD       Ret/Alt       AF/AC       AC Hom       Subpopulations       CM951230, P         PopDB       rsiD       Ret/Alt       AF/AC       AC Hom       Subpopulations          dbSNP (134/144)       rsi44340710       TC       C=0.0002/29       0           ESP       rsi44340710       TC       C=0.0002/29       0            BS1       Protein Domain       phastCons       phyloP       pedProg       Prediction       Value         p3_3       DA-binding domain       0.992       1.82        AGVGD       -       CD         startsSType       distNearesISS       maxEntScore       ssfScore        0% <td>Ornhanet</td> <td></td> <td>astic reukernia</td> <td>ute lymphobia</td> <td></td> <td></td> <td></td> <td></td> <td>_</td> <td></td> <td></td>	Ornhanet		astic reukernia	ute lymphobia					_		
ClinDB       Grading/Phenolype       Name       Info         ClinVar       Likely benign/Incertain significance       NM_000546.5(TP53):c.704A~G (p.Asn235Ser)       Submitter. 6         Emory       VOUS       NM_000546.5(TP53):c.704A~G       -       -         HGMD       Rhabdomyosarcoma       -       CM951230, P       -         PopDB       rsID       Ret/Alt       AF/AC       AC Hom       Subpopulations         dbSNP (134/144)       rs144340710       T/C       EAC       rs144340710       T/C       C=0.0002/29       0       NFE=0.00034465, AFR=0, EAS=0, SAS=0, AMR=0, FIN=0.00091269, OTH=       eurAMR=0.0001, afrAMR=0.0002         ESP       rs144340710       T/C       C=0.0002/29       0       NFE=0.00034465, AFR=0, EAS=0, SAS=0, AMR=0, FIN=0.00091269, OTH=       eurAMR=0.0001, afrAMR=0.0002         BS1       Protein Domain       phastCons       phyloP       P       Good       0.0481         nearestSSType       distNearestS       maxEntScore       ssfScore       SiFT       Tolerated       0.0481         3       32       0%       0%       P       phyloP       polyphen       0.144         Patient Remarks       Variant Remarks       Variant Remarks       Variant Remarks       Variant Remarks       Variant Remarks	Orphanot							00533			
ClinVar       Likely benign;Uncertain significance       NM_000546.5(TP53):c.704A>G (p.4sn235Ser)       Submitter: 6         Emory       VOUS       Rhabdomyosarcoma       -       CM951230, P         PopDB       rsID       Ret/Alt       AF/AC       AC Hom       Subpopulations         dbSNP (134/144)       rs144340710       T/C       E=0.0002/29       0       NFE=0.00034465, AFR=0, EAS=0, SAS=0, AMR=0, FIN=0.00091269, OTH=         ESP       rs144340710       T/C       C=0.0002/29       0       NFE=0.00034465, AFR=0, EAS=0, SAS=0, AMR=0, FIN=0.00091269, OTH=         eurAMR=0.0001; afrAMR=0.0002       BS11       Protein Domain       phastCons       phi/oP         p53, DNA-binding domain       0.992       1.82       StT       Tolerated       0.08         MAPP       good       0.0144       Polyphen       benign       0.144         Patient Remarks       32       0%       0%       0%       Variant Remarks       Variant Remarks       Variant Remarks       Variant Remarks       Variant Remarks       Variant in LFS-patien         Patient Remarks       Variant Remarks       Variant Remarks       Variant in LFS-patien       Variant in LFS-patien       Variant in LFS-patien			s	ed Diseases	Search Associate		_	PP5??			
ClinVar       Likely benign;Uncertain significance       NM_000546.5(TP53):c.704A>G (p.4sn235Ser)       Submitter: 6         Emory       VOUS       Rhabdomyosarcoma       -       CM951230, P         PopDB       rsID       Ret/Alt       AF/AC       AC Hom       Subpopulations         dbSNP (134/144)       rs144340710       T/C       E=0.0002/29       0       NFE=0.00034465, AFR=0, EAS=0, SAS=0, AMR=0, FIN=0.00091269, OTH=         ESP       rs144340710       T/C       C=0.0002/29       0       NFE=0.00034465, AFR=0, EAS=0, SAS=0, AMR=0, FIN=0.00091269, OTH=         eurAMR=0.0001; afrAMR=0.0002       BS11       Protein Domain       phastCons       phi/oP         p53, DNA-binding domain       0.992       1.82       StT       Tolerated       0.08         MAPP       good       0.0144       Polyphen       benign       0.144         Patient Remarks       32       0%       0%       0%       Variant Remarks       Variant Remarks       Variant Remarks       Variant Remarks       Variant Remarks       Variant in LFS-patien         Patient Remarks       Variant Remarks       Variant Remarks       Variant in LFS-patien       Variant in LFS-patien       Variant in LFS-patien		Info			Name			ienotype	Grading/Phe		ClinDB
HGMD     Rhabdomyosarcoma     CM951230, P       PopDB     rsID     Ret/Alt     AF/AC     AC Hom     Subpopulations       dbSNP (134/144)     rs144340710     T/C     ExAC     rs144340710     T/C       ESP     rs144340710     T/C     C=0.0002/29     0     NFE=0.00034465, AFR=0, EAS=0, SAS=0, AMR=0, FIN=0.00091269, OTH=eurAMR=0.0001, afrAMR=0.0002       Protein Domain     phastCons     phyloP     predProg     Prediction     Value       ACVGD     -     C0       SFT     Tolerated     0.08       MAPP     good     0.0481       Polyphen     Design     0.144       Polyphen     Design     0.144		Submitter: 6	(p.Asn235Ser)	):c.704A>G (p	NM_000546.5(TP53)		ificance	gn;Uncertain sign	Likely benig		ClinVar
PopDB       rsiD       Reti/Alt       AF/AC       AC Hom       Subpopulations         dbSNP (134/144)       rs144340710       T/C       ESP       NFE=0.0003/29       0       NFE=0.00034465, AFR=0, EAS=0, SAS=0, AMR=0, FIN=0.00091269, OTH=eur/AMR=0.0001, afrAMR=0.0002         ESP       rs144340710       T/C       C=0.0002/29       0       NFE=0.00034465, AFR=0, EAS=0, SAS=0, AMR=0, FIN=0.00091269, OTH=eur/AMR=0.0001, afrAMR=0.0002         Protein Domain       phastCons       phyloP       predProg       Prediction       Value         p53, DNA-binding domain       0.992       1.82       NFE=0.0002       SIFT       Tolerated       0.08         mearestSSType       distNearestSS       maxEntScore       ssfScore       SIFT       Tolerated       0.04         3'       32       0%       0%       0%       0%       0.144       0.144         Patient Remarks       Variant Rema		-		c.704A>G	NM_000546(TP53):c						
dbSNP (134/144)       rs144340710       T/C         EXAC       rs144340710       T/C         ESP       rs144340710       T/C         Protein Domain       phastCons       phyloP         p53, DNA-binding domain       0.992       1.82         Image: state	pmed. 7706467	CM951230, Publ			-			osarcoma	Rhabdomyo		HGMD
Protein Domain       phastCons       phyloP       predProg       Prediction       Value         p53, DNA-binding domain       0.992       1.82       AGVGD       -       CO         nearestSSType       distNearestSS       maxEntScore       ssfScore       SiFT       Tolerated       0.08         3'       32       0%       0%       O%       O%       0.144         Variant Remarks	)	0.00091269, OTH=0			NFE=0.00034465	0	0002/29	C=0.0	T/C T/C	rs144340710 rs144340710	dbSNP (134/144) ExAC
Protein Domain       phastCons       phyloP       predProg       Prediction       Value         p53, DNA-binding domain       0.992       1.82       AGVGD       -       C0         searestSSType       distNearestSS       maxEntScore       ssfScore       SiFT       Tolerated       0.08         3'       32       0%       0%       0%       Value       Value       Value         Patient Remarks       Variant Remarks       Variant Remarks       Variant Remarks       Variant n LFS-patien       Value       Value       Value         Variant match       0.992       0.992       0.992       0.992       0.0481			002	atrame=0.000	eurAMR=0.0001, a	BS1	000272	C=0.0	1/C	rs144340710	ESP
p53, DNA-binding domain       0.992       1.82       AGVGD       -       C0         nearestSSType       distNearestSS       maxEntScore       ssfScore       SiFT       Tolerated       0.08         3'       32       0%       0%       0%       0%       0%       0%         Variant Remarks         Variant Remarks         Variant Remarks         Variant Remarks         Variant Remarks         Variant in LFS-patien tHuusko et al.; Cancer Genet Cytoge net 1999 Jul; 112(1):914. does n ot segregate in familyPMID: 2012869						DUI					
Sift       Tolerated       0.08         MAPP       good       0.0481         Polyphen       benign       0.144         3'       32       0%       0%         Variant Remarks         Variant Remarks       Variant Remarks         Variant Remarks       van Hest LP et al.; Fam Cancer. 200         7;6(3):311-6: co-occurrence with tr uncating TP53 variant in LFS-patien tHuusko et al.; Cancer Genet Cytoge net 1999 Jul;112(1):9-14. does n ot segregate in familyPMID: 2012869			Prediction					-			Protein Domain
MAPP     good     0.0481       nearestSSType     distNearestSS     maxEntScore     ssfScore       3'     32     0%     0%         Patient Remarks     Variant Remarks         Variant Remarks     van Hest LP et al.; Fam Cancer. 200       7;6(3):311-6.: co-occurence with tr uncating TP53 variant in LFS-patien tHuusko et al.; Cancer Genet Cytoge net. 1999 Jul 1;112(1):9-14. does n of segregate in familyPM/DU: 2012869			- Tolesated				1.82	0.992		omain	p53, DNA-binding of
nearestSSType       distNearestSS       maxEntScore       ssfScore         3'       32       0%       0%         Patient Remarks       Variant Remarks       van Hest LP et al.; Fam Cancer. 200 7;6(3):311-6.: co-occurence with tr uncating TP53 variant in LFS-patien tHuusko et al.; Cancer Genet Cytoge net. 1999 Jul 1;112(1):9-14. does n ot segregate in famil/PMID: 2012869	BP4										
3' 32 0% 0% Patient Remarks Variant Remarks Variant Remarks Variant Remarks Variant Remarks van Hest LP et al.; Fam Cancer. 200 7;6(3):311-6.: co-occurence with tr uncating TP33 variant in LFS-patien tHuusko et al.; Cancer Genet Cytoge net. 1999 Jul 1;112(1):9-14. does n ot segregate in familyPMID: 2012869			-			0.00	eefSc	mayEntScore	2 1	distNearestS	neoractSSTvne
7;6(3):311-6.: co-occurence with tr uncating TP53 variant in LFS-patien tHuusko et al.; Cancer Genet Cytoge net. 1999 Jul 1;112(1):9-14. does n ot segregate in familyPMID: 2012869						16					
7;6(3):311-6.: co-occurence with tr uncating TP53 variant in LFS-patien tHuusko et al.; Cancer Genet Cytoge net. 1999 Jul 1;112(1):9-14. does n ot segregate in familyPMID: 2012869											
uncating TP53 variant in LFS-patien tHuusko et al.; Cancer Genet Cytoge net. 1999 Jul 1;112(1):9-14. does n ot segregate in familyPMID: 2012869		am Cancer. 200	van Hest LP et al.; Fam			arks	Variant Rem				Patient Remarks
tHuusko et al.; Cancer Genet Cytoge net. 1999 Jul 1;112(1):9-14. does n ot segregate in familyPMID; 2012869											
net. 1999 Jul 1;112(1):9-14. does n ot segregate in familyPMID: 2012869											
ot searegate in familyPMID: 2012869											
		yPMID: 2012869	ot segregate in familyP								
BS1+BS3+BS4+BP2+BP4 = class 1						ss 1	= cla	P2+BP4	4+BF	-BS3+BS	BS1-
						55 <b>1</b>	Cra			200 . 20	
BP2+BS4#BS3		54+B53	Rh5+R2								

#### Criteria for classifying pathogenic variants (Tabelle I)

Evidence pathoge		Category
rong	1	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. <i>Caveats:</i>
Very strong	PVS1	<ul> <li>Beware of genes where LOF is not a known disease mechanism (e.g., GFAP, MYH7)</li> <li>Use caution interpreting LOF variants at the extreme 3' end of a gene</li> <li>Use caution with splice variants that are predicted to lead to exon skipping but leave the remainder of the protein intact</li> </ul>
		Use caution in the presence of multiple transcripts
	PS1	<ul> <li>Same amino acid change as a previously established pathogenic variant regardless of nucleotide change</li> <li>Example: Val→Leu caused by either G&gt;C or G&gt;T in the same codon</li> <li>Caveat: Beware of changes that impact splicing rather than at the amino acid/protein level</li> </ul>
	PS3	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 non maternity.
Strong	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.
<u>s</u> –	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.

#### In-house variant interpretation tool and database: 10.500 individual panels analyzed

- 6792 "strong truncating" variants SNV's (Stop-gain, fs\*, +/- 1,2 splice)
- 946 manually graded as VUS
- 651 manually graded as Class 1 and Class 2

Val	Mat	Туре	Gene	MOI	Char	nges		Position	Ref	Alt								
	G	FS	AMPD1	AR	NM_	000036:c.104de	el:p.Pro35Leufs	*87 chr1:115238088-11523	8088 G	-								
	G	FS	651 SNV	's are shown	B +					Caselination 1								
	G	FS FS	f	Val Mat	Type	▲ Gene	MOI	Changes	Position	Ref	Alt	GT	AF	rsID	1000G	ESP	ExAC	q
	G	FS		G G	FS	MYH8	AD	NM 002472:c.3320del:p.Leu1107Hisfs*60	chr17:10304211-10304211	A	-	het	-	rs751871946	1000G	0.0001	0.0002	0
	G		1	G	FS	SLC46A1	AR	NM_080669:c.1226del:p.lle409Thrfs*10	chr17:26727722-26727722	A		hom		rs561780114	1	0.9997	1	1
	-	FS	4	G	FS	P2RX5	-	NM_002561:c.333del:p.Asn112Thrfs*36	chr17:3594277-3594277	G		hom		rs3215407	0.5735	0.5439	0.6676	
	G	FS	F	G	FS	ABCA10	-	NM_080282:c.4515_4516del:p.Gln1506Glyfs		GA	-	het	-	rs3842375	0.0881	0.0602	0.0745	
	G	FS	4	G	FS	ABCA10		NM_080282:c.1331_1334del:p.Ser444Phefs*		ACAG	1.0	het		rs113082690	0.0885	0.0731	0.0743	
	G	FS	>	G	FS	CYP2F1		NM_000774:c.15dup:p.Thr6Hisfs*22	chr19:41622107-41622108	-	С	het	-	rs3833221	0.272	0.2556	0.2148	4
	G	FS	F	G	FS	CYP2F1		NM_000774:c.15dup:p.Thr6Hisfs*22	chr19:41622107-41622108	-	C	hom	-	rs3833221	0.272	0.2556	0.2148	9
	Ğ	FS		G	FS	CARD8 SIGLEC12	-	NM_014959:c.290_291dup:p.Val98Lysfs*26 NM_053003:c.196dup:p.Ala66Glvfs*50	chr19:48735017-48735018 chr19:52004791-52004792		TT C	het het	-	rs140826611 rs66949844	0.0421	0.0427	0.0525	0
	-		C	G	FS	SIGLEC12 SIGLEC12		NM_053003:c.196dup:p.Ala66Glyfs*50 NM_053003:c.196dup:p.Ala66Glyfs*50	chr19:52004791-52004792 chr19:52004791-52004792	-	c	hom		rs66949844	0.5929	0.6503	0.6407	(
	G	FS	n i	G	FS	ZNF480	-	NM 144684:c.9 10del:p.Cvs3*	chr19:52803670-52803671	- TG	C .	het		rs3217319	0.5929	0.6503	0.6883	0
	G	FS	F	G	FS	ZNF480 ZNF480		NM_144684:c.9_10del:p.Cys3*	chr19:52803670-52803671	TG		hom		rs3217319	0.599	0.6134	0.6883	0
	G	FS	C	Ğ	FS	SBK3		NM 001199824:c.954 955insG:p.Gly318 Ph		-	С	hom		rs397738405	1	0.0104	1	-
	G	FS	1	G	FS	FMO2	-	NM 001460:c.337del:p.Val113*	chr1:171165803-171165803	G	-	het	-	rs28369860	0.1028	0.1161	0.0606	
	-			Ğ	FS	FCN3	AR	NM_003665:c.349del:p.Leu117Serfs*65	chr1:27699671-27699671	G		het		rs532781899	0.0188	0.0211	0.0166	
	G	FS		G	FS	GJB4	AD, AR	NM_153212:c.155_158del:p.Val52Alafs*55	chr1:35227008-35227011	TGTC		het		rs146812843		0.0343	0.0336	
	G	FS	4	G	FS	CYP4B1	-	NM_001099772:c.884_885del:p.Asp295Glyfs	chr1:47280747-47280748	AT	-	het	-	rs3215983	0.1354	0.1143	0.1475	
	G	FS	4	G	FS	DEFB126	-	NM_030931:c.163_166del:p.GIn55Glyfs*28	chr20:126156-126159	CAAA	-	het	-	rs11467497		0.1918	0.1523	
	G	FS	4	G	FS	DEFB126		NM_030931:c.317_318del:p.Pro106Argfs*12	7 chr20:126311-126312	CC		het	1.0	rs11467417		0.5662	0.5534	
	G	FS		G	FS	DEFB126	-	NM_030931:c.317_318del:p.Pro106Argfs*12		CC	-	hom	-	rs11467417		0.5662	0.5534	
	-		1	G	FS	ADAM33	-	NM_025220:c.2412_2419del:p.GIn804Hisfs*		TCTGG.		het	-	rs146576636	0.0705	0.0388		
	G	FS	<b>(</b>	G	FS	SCARF2	AR	NM_153334:c.2304dup:p.Glu769Argfs*9	chr22:20779973-20779974	-	G	hom		rs5844418	1	0.982	1	
	G	FS	(	G	FS FS	SCARF2	AR	NM_153334:c.2253dup:p.Pro752Alafs*26	chr22:20780024-20780025	-	C	hom		rs5844420	1	0.9031	0.0000	
	G	FS	C	G	FS	TTC21B PRKRA	AD, AR AR	NM_024753:c.21+26_21+33dup NM_003690:c.22_23del:p.Ala8Argfs*22	chr2:166810161-166810162 chr2:179315735-179315736		CCCG	het het	-	rs569432248 rs141354030	0.5084	0.3121	0.6829	
	G	FS	2	G	FS	PRKD3	-	NM_005813:c.2673dup	chr2:37480319-37480320	) GC	т	het		rs140587747	0.2362	0.0704	0.0481	
	-			G	FS	PRKD3		NM 005813:c.2673dup	chr2:37480319-37480320		T	hom		rs140587747	0.0543	0.0704	0.0481	
	G	FS	(	G	FS	PNPT1	AR	NM 033109:c.*11dup	chr2:55863360-55863361	-	A	het	-	rs35916020	0.0040	0.0704	0.4661	
	G	FS	E	Ğ	FS	PNPT1	AR	NM 033109:c.*11dup	chr2:55863360-55863361		A	hom		rs35916020			0.4661	
	G	FS	4	G	FS	FANCL	AR	NM_001114636:c.1111_1114dup:p.Thr372As	chr2:58386928-58386929	-	TAAT	het		rs759217526		0.0025	0.0029	
	G	FS	5	G	FS	CD207	-	NM_015717:c.71+2dup	chr2:71062833-71062834	-	С	hom	-	rs11450450	1	0.9998	1	
IF	G	FS	-	G	FS	ALMS1	AR	NM_015120:c.35_36insGGAGGAGGAGGAGGAGG	chr2:73613031-73613032	-	GGAG	hom	-					
	-			G	FS	RYK		NM_001005861:c.59_60insC:p.Ala20_Glu21	chr3:133969437-133969438		G	hom		rs587770426	0.9982	0.9865		
IF	G	FS	1	G	FS	RYK	-	NM_001005861:c.9_10insG:p.Gly3_Arg4fs	chr3:133969487-133969488		С	hom	-	rs587744425	0.999			
	G	FS	(	G	FS	KCNMB3	-	NM_171830:c.753del:p.Val252Tyrfs*4	chr3:178960767-178960767		-	het		rs143962239	0.0891	0.1158	0.0676	
	G	FS	7	G	FS	HTR3E	-	NM_182589:c.64del:p.Glu22Serfs*39	chr3:183818222-183818222		-	het	-	rs397897677	0.0355	0.0741	0.0661	
	Ğ	FS		G	FS	HTR3E	-	NM_182589:c.64del:p.Glu22Serfs*39	chr3:183818222-183818222			hom		rs397897677	0.0355	0.0741	0.0661	
	G	FS		G	FS FS	CLDN16 CLDN16	AR AR	NM_006580:c.166del:p.Ala56Leufs*16	chr3:190106072-190106072		-	het	-	rs368234054	0.117		0.1945	
	-		· ·	G	FS	CCR5	-	NM_006580:c.166del:p.Ala56Leufs*16 NM_000579:c.554_585del:p.Ser185llefs*32	chr3:190106072-190106072 chr3:46414944-46414975	ACAGT		hom het		rs368234054 rs333	0.0292	0.0604	0.1945	
	G	FS	L L	G	FS	CCR5		NM_000579:c.554_585del:p.Ser185ilefs*32	chr3:46414944-46414975	ACAGT.		hom		rs333	0.0292	0.0604		
	G	FS	F	G	FS	FGFRL1	-	NM 001004356:c.1454 1455del:p.His485Le	chr4:1019055-1019056	CA	1	het	1	rs145808953	0.0232	0.0004	0.1987	
	G	FS	4	Ğ	FS	FIP1L1	IC, SMu	NM_030917:c.1459_1460del:p.Arg487Glyfs*3		AG		het		rs143671659			0.1141	
	G	FS		G	FS	SLC22A1	-	NM_003057:c.1276+9_1276+16del	chr6:160560898-160560905			het		rs113569197	0.6895		0.6122	
	-			G	FS	SLC22A1		NM 003057:c.1276+9 1276+16del	chr6:160560898-160560905			hom		rs113569197	0.6895		0.6122	
	G	FS	F I	G	FS	HLA-A	-	NM_002116:c.751del:p.Asp251Thrfs*46	chr6:29912029-29912029	G	-	het	-	rs45576436	0.3706	0.3678		
	G	FS	E	G	FS	HLA-B	Mu	NM_005514:c.206_207insC:p.Glu69Aspfs*30	chr6:31324601-31324602	-	G	het	1.1	rs9281379			0.122	
	G	FS	F	G	FS	HLA-B	Mu	NM_005514:c.204del:p.Glu69Argfs*8	chr6:31324604-31324604	т	-	het	-	rs200186034		0.4424	0.1758	
	G	FS	c i	G	FS	MICA	-	NM_001177519:c.953_956del:p.Gly318Alafs*		GCTG	-	het	-	rs138201170	0.0958	0.2512	0.1953	
	-			G	FS	MICA	-	NM_001177519:c.953_956del:p.Gly318Alafs*		GCTG		hom		rs138201170	0.0958	0.2512	0.1953	
	G	FS	r	G	FS	MICA		NM_001177519:c.953del:p.Gly318Alafs*68	chr6:31380161-31380161	G		het		rs67841474	0.2049		0.3188	
				G	FS	MICA	-	NM_001177519:c.953del:p.Gly318Alafs*68	chr6:31380161-31380161	G	-	hom	-	rs67841474	0.2049		0.3188	
				G	FS	MICA	-	NM_001177519:c.952_953insCT:p.Gly318Ala		-	CT	het	-	rs41293539	0.2338		0.319	
				G	FS	MICA	-	NM_001177519:c.952_953insCT:p.Gly318Ala		-	CT	hom		rs41293539	0.2338		0.319	
				G	FS FS	MICA	-	NM_001177519:c.952_953InsCTGCTGCTGC NM_001177519:c.952_953InsCTGCTGCTGC		-	CTGCT		-	rs41293539 rs41293539	0.2117		0.2387	

#### Criteria for classifying pathogenic variants (Tabelle I)

Evidence of pathogenicity		Category	
Very strong	PVS1	<ul> <li>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.</li> <li>Caveats:         <ul> <li>Beware of genes where LOF is not a known disease mechanism (e.g., GFAP, MYH7)</li> </ul> </li> </ul>	
Very s	PV	<ul> <li>Use caution interpreting LOF variants at the extreme 3' end of a gene</li> <li>Use caution with splice variants that are predicted to lead to exon skipping but leave the remainder of the protein intact</li> </ul>	
		Use caution in the presence of multiple transcripts	]
	PS1	<ul> <li>Same amino acid change as a previously established pathogenic variant regardless of nucleotide change</li> <li>Example: Val→Leu caused by either G&gt;C or G&gt;T in the same codon</li> <li>Caveat: Beware of changes that impact splicing rather than at the amino acid/protein level</li> </ul>	
	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 non maternity.	
Strong	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.	+
S	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.	

### Only for a few genes "well established functional studies" have been defined

#### InSiGHT (MMR genes)

Assays assessing	MMR protein repa	ir capacity as a complete p	process		
Functional assays using cell-free systems	In vitro MMR complementation assays	An <i>in vitro</i> test of the repair of mismatched DNA by protein extracts. Baculovirus infected insect cell extracts are used to complement MMR-deficient cell extract; MMR genes transfected into MMR-deficient cell line; or IVTT of PCR fragments complement MMR-deficient cell extracts. DNA repair substrates: mismatch within restriction site or LacZ domain.	False negative results possible for variants that are pathogenic due to poor expression or protein stability. Variants defective in nuclear import may yield false-positive results. Subtle defects will not be detected if amount of protein is saturating.	Wild type; known defective (untransfected MMR deficient cell line or pathogenic control). Transfection efficiency for assays involving transient expression in cell lines.	49,79,112,117-134 <sup>135-137</sup> Transfection efficiency not measured <sup>66</sup> Level of MMR activity not quantified
Functional assays using mammalian cell-based systems	Cellular-based MMR functional assay using a human/mouse expression system	Monitor the repair capacity as a whole through expression of mutant human MMR gene constructs in human/mouse cell lines. MMR status measured using: cellular response to methylating agents (MMR- deficient cells have acquired tolerance to these agents), spontaneous mutation rate at the endogenous <i>HPRT</i> gene, repair of an exogenously added mismatch-containing GFP plasmid, or measuring microsatellite instability.	Best to use cell lines that lack endogenous expression of the MMR protein. Level of protein expression is critical: poor expression can produce false- negative results; variant MMR gene expression is unregulated and may be toxic to cells. "Knock- in" of the variant allele through oligonucleotide gene targeting avoids unregulated expression.	Wild type; known defective.	85,133,138-141

	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.
te	PM2	Absent from controls (or at extremely low frequency if recessive) (Table 6) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium
		<ul> <li>Caveat: Population data for insertions/deletions may be poorly called by next-generation sequencing.</li> </ul>
	PM3	For recessive disorders, detected in trans with a pathogenic variant
ate	Ы	Note: This requires testing of parents (or offspring) to determine phase.
Moderate	PM4	Protein length changes as a result of in-frame deletions/insertions in a nonrepeat region or stop-loss variants
	PM5	Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before
		<ul> <li>Example: Arg156His is pathogenic; now you observe Arg156Cys</li> </ul>
		<ul> <li>Caveat: Beware of changes that impact splicing rather than at the amino acid/protein level.</li> </ul>
	PM6	Assumed de novo, but without confirmation of paternity and maternity
	PP1	Co segregation with disease in multiple affected family members in a gene definitively known to cause the disease Note: May be used as stronger evidence with increasing segregation data
50	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
Supporting	PP3	<ul> <li>Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.)</li> <li>Caveat: Because many in-silico algorithms use the same or very similar input for their predictions, each algorithm should not be counted as an independent criterion. PP3 can be used only once in any evaluation of a variant.</li> </ul>
	PP4	Patient's phenotype or family history is highly specific for a disease with a single genetic etiology
	PP5	Reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation

#### Criteria for classifying benign variants (Tabelle 2)

Evidence of benign impact	Category
Stand alone BA1	Allele frequency is >5% in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium

	BS1	Allele frequency is greater than expected for disorder (see Table 6)						
50	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						
Strong	Well-established in vitro or in vivo functional studies show no damaging effect on protein function or s							
S	BS4	<ul> <li>Lack of segregation in affected members of a family</li> <li>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.</li> </ul>						

	BP1	Missense variant in a gene for which primarily truncating variants are known to cause disease
	BP2	Observed in trans with a pathogenic variant for a fully penetrant dominant gene/disorder or observed in cis with a pathogenic variant in any inheritance pattern
	BP3	In-frame deletions/insertions in a repetitive region without a known function
Supporting	BP4	<ul> <li>Multiple lines of computational evidence suggest no impact on gene or gene product (conservation, evolutionary, splicing impact, etc.)</li> <li>Caveat: Because many in silico algorithms use the same or very similar input for their predictions, each algorithm cannot be counted as an independent criterion. BP4 can be used only once in any evaluation of a variant.</li> </ul>
S	BP5	Variant found in a case with an alternate molecular basis for disease
	BP6	Reputable source recently reports variant as benign, but the evidence is not available to the laboratory to perform an independent evaluation
	BP7	A synonymous (silent) variant for which splicing prediction algorithms predict no impact to the splice consensus sequence nor the creation of a new splice site AND the nucleotide is not highly conserved

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

Open

### Using high-resolution variant frequencies to empower clinical genome interpretation

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

#### Variant: 15:48725102 C / T

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:



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

#### **Population Frequencies**

Population	Allele Count	<ul> <li>Allele</li> <li>Number</li> </ul>	<ul> <li>Number of Homozygotes</li> </ul>	\$ 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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#### Variant: 15:48725102 C / T

Filter Status dbSNP	PASS rs112084407	Filtering allele fre If the variant filterin is too common to
Allele Frequency	0.0007913	
Filtering AF	0.001042 (European (N	<u>lon-Finnish))</u>
Allele Count	96 / 121322	
UCSC	15-48725102-C-T 🗗	
ClinVar	Click to search for varia	ant in Clinvar 🗗

#### Annotations

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

ExAC Browser	× Frequer	ncy Filter	× +								
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🧕 Meistbesucht 🧕 Erste Schritte											
Frequency Filter	HOME	calculate AF	calculate AC	explore architecture	inverse AF	penetrance	about				

#### Using high-resolution variant frequencies to empower clinical genome interpretation

This web page contains a suite of tools to support the use of allele frequency information for the assessment of rare genetic variants in Mendelian disease.

Distinguishing disease-causing variants from benign bystanders is perhaps the principal challenge in contemporary clinical genetics. Rarity of an allele is widely recognized as a necessary (though not sufficient) criterion for variant pathogenicity, but the key question "*how common is too common?*" remains poorly answered for many diseases. Recent large reference datasets, such as from the Exome Aggregation Consortium (ExAC), provide new opportunities for robust and rigorous variant assessment.

The methods and mathematical derivations behind the calculators on these pages are described fully in our manuscript available here. The source code for the manuscript is available on GitHub, as is the source code for these calculators.

We provide four calculators:

- calculate AF works step by step through a framework of variant assessment. For a disease of interest the user inputs parameters that describe
  the genetic architecture of the condition, and the calculator computes the maximum expected allele frequency of a disease-causing variant in the
  general population (maximum credible population AF). In a second step, the calculator determinues the maximum tolerated allele count in a specific
  reference population (such as ExAC), based on the size of the population and at a user-specified confidence level.
- calculate AC performs the second part of the above work-flow, allowing the user to simply input a *maximum credible population AF* without redefining the genetic architecture in detail, intended as a time saving measure for returning users.
- explore architecture starts by computing a maximum credible population AF for a given genetic architecture, as above. However, it also allows
  you to fix the maximum population AF in order to find a genetic architecture that is compatible with the observed data. For example, under your
  initial assumptions about a condition you may find that a variant is reported to be too common, but that it would be compatible with disease under a
  model of substantially reduced penetrance.
- inverse AF begins with an observed allele count, and computes an associated threshold *filter allele frequency* for a variant. If the *filter allele frequency* of a variant is above the *maximum credible population AF* for a condition of interest, then that variant should be filtered (ie not considered a candidate causative variant). This corresponds to the "filter\_AF" annotation in the ExAC dataset. ExAC returns the value for a 95% confidence here the user can choose from a range of thresholds.

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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 <sup>-6</sup>	2
Noonan	0.10	1/1,000	0.5	1.0 x 10	10
CPVT	0.10	1/10,000	0.5	$1.0 \times 10^{-5}$	3
Classic Ehlers-Danlos	0.40	1/20,000	0.5	2.0×10 <sup>-5</sup>	5

#### Variant: 15:48725102 C / T

CPVT,catecholaminergic polymorphic ventricular tachycardia; ExAC, Exome Aggregation Consortium database. Prevalence estimates (taken as the highest value reported) were obtained from Marfan,<sup>40</sup> Noonan,<sup>18</sup> CPVT,<sup>19</sup> and classical Ehlers-Dankos.<sup>20</sup>

Filter Status dbSNP	PASS rs112084407	If the variant filtering AF is greater than the maximum or	variants that are too common to plausibly cause disease. edible population AF for the disease of interest, the variant lick here to see the filtering AF calculator app and citation.		
Allele Frequency	0.0007913				
Filtering AF	0.001042 (European (Non-Finnish))		Site Quality Metrics		
Allele Count	96 / 121322				
UCSC	15-48725102-C-T 🗹				
ClinVar	Click to search for vari	iant in Clinvar 🗹			

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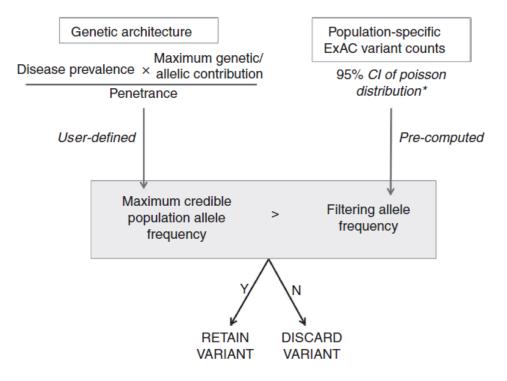
Official journal of the American College of Medical Genetics and Genomics ORIGINAL RESEARCH ARTICLE

Genetics inMedicine

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### Using high-resolution variant frequencies to empower clinical genome interpretation

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### **ACMG-AMP CRITERIA KNOWN TO BE PROBLEMATIC**

Box 1. Recommendations and Additional Resources for Increasing Consistency in the Usage of ACMG-AMP Rules

- Develop disease-specific allele-frequency thresholds to enable lowering of the stand-alone benign criteria from a MAF of ≥5% to values specific to each disorder.
- Establish a resource of all genes to define whether LOF is a known mechanism of disease.
- Make recommendations for which computational algorithms are best in practice.
- Better define "well-established" functional data and/or distribute a resource that lists functional assays that meet the well-established threshold. Also define when to use reduced strength of the rule.
- Develop quantitative thresholds of evidence for and against segregation of different strengths.
- Promote the development of software tools that automate computable aspects of the ACMG-AMP guidelines to improve accurate use.

Amendola et al.; The American Journal of Human Genetics 98, 1067–1076, June 2, 2016

#### Table 1

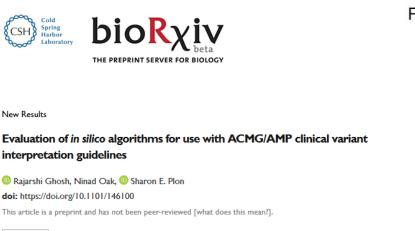
Flexibility allotted for in 'lines of evidence'

Lines of evidence <sup>a</sup>	Type of data	Flexibility		
BS1/PM2	Allele frequency in general population	Determining what frequency is consistent with 'greater than expected for disorder' or 'at extremely low prevalence for recessive disorders'		
BS2/PP4	Observed in unaffected/affected	Determining the extent of medical work-up necessary to conclude disease status		
BS3/PS3	Functional studies	Determining what constitutes 'well-established' functional studies		
BS4/PP1	Segregation studies	Determining the extent of medical work-up necessary to conclude disease status Determining number of non-segregations-or segregations needed to meet criteria		
BP2/BP5	Other pathogenic variant identified	May need to account for phenotype or disease severity		
BP6/PP5	Reputable source classification	Determining what constitutes a 'reputable source'		
PM1	Gene-specific information	Determining what constitutes 'a critical or well-established' functional domain		

Hoskins et al.; Current Opinion in Genetics & Development, volume 42. 33-39. 2017

### **ACMG-AMP CRITERIA KNOWN TO BE PROBLEMATIC**

Preview PDF



Supplementary material

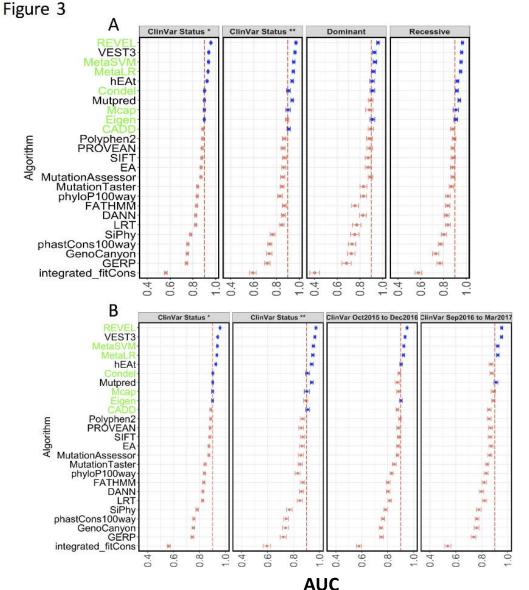
Abstract

Abstract

Info/History

Metrics

The ACMG/AMP variant classification guidelines for clinical reporting recommend complete concordance of predictions among all in silico algorithms used without specifying the number or types of algorithms. The subjective nature of this recommendation contributes to discordance of variant classification among clinical laboratories. Using 14,819 benign or pathogenic missense variants from the ClinVar database, we compared performance of 25 algorithms across datasets differing in distinct biological and technical variables. There was wide variability in concordance for benign variants. We identified recently developed algorithms with high predictive power and robust to variables like disease mechanism, gene constraint and mode of inheritance, although poorer performing algorithms are more frequently used based on review of the clinical genetics literature (2011-2017). We describe high performing algorithm combinations with increased concordance in variant assertion, which should lead to more informed *in silico* algorithm usage by diagnostic laboratories.



### **ACMG-AMP VARIANT CLASSIFICATION TOOLS**

Public gov US National Library of Medicine National Institutes of Health	PubMed	Advanced		
Format: Abstract -				
Am J Hum Genet. 2017 Feb 2;100(			Step 1: Automatic prediction	Step 2: Manual adjustment
InterVar: Clinical Inte				

Li Q<sup>1</sup>, Wang K<sup>2</sup>.

#### Author information

#### Abstract

In 2015, the American Colleg updated standards and guide criteria. However, variability b these guidelines and the lack are not available. To address called InterVar to help human and generate automated inte friendly variant interpretation addressing severe congenital sequencing studies, we demo variants.

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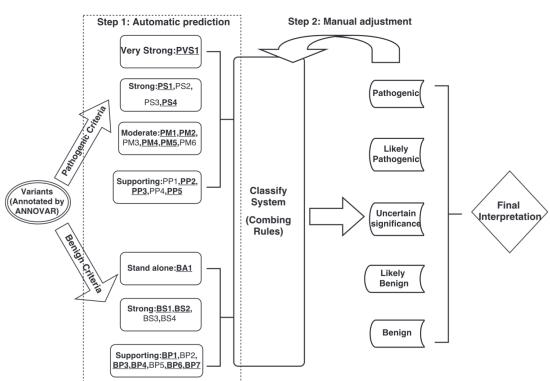
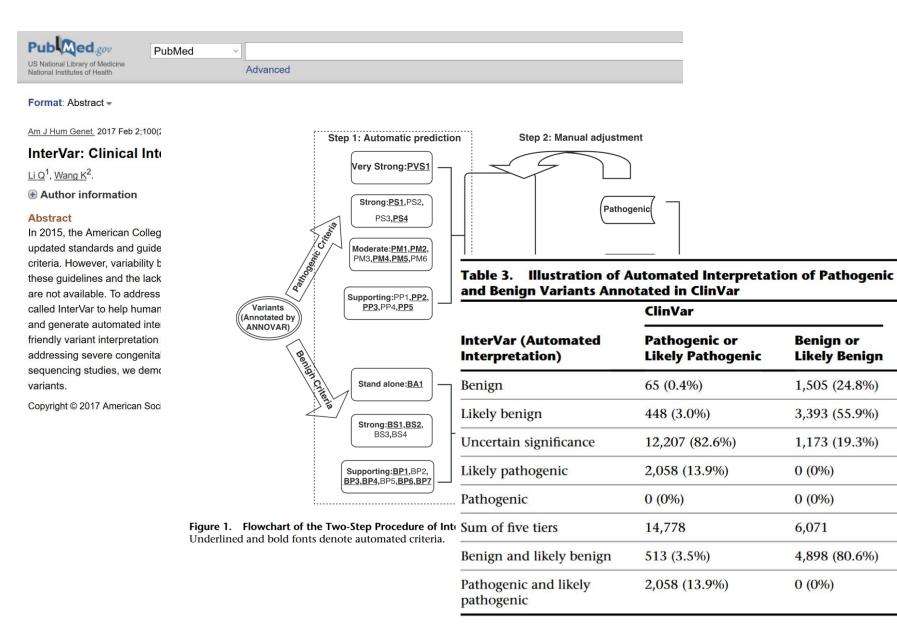


Figure 1. Flowchart of the Two-Step Procedure of InterVar Underlined and bold fonts denote automated criteria.

### **ACMG-AMP VARIANT CLASSIFICATION TOOLS**



### **EVALUATION OF INTER-LABORATORY CONCORDANCE**

Study	Description	Observed Concordance	Reasons for Inconsistency	Remarks
Amendola et al. Am J Hum Genet 2016	Comparison of concordance of 9 CSER- labs classifying 99 variants	<b>34% before</b> and <b>71% after</b> consensus discussion / only 5% of differences are clinically relevant	Correct use of several ACMG rules was not clear / challenging variants	training is necessary for consistent classification / underscores importance of not only having a standardized approach to variant assessment but also sharing variant interpretations for identifying and resolving discordance
Harrison et al. Genet Med 2017	ClinVar Laboratory comparison and consistency assessment	83% initially concordant 87% of discordant variants could be resolved	ACMG rules not applied to ClinVar variants (53%) Internal data not published (33%) Differences in use/ weighting of data (14%)	Participating laboratories increased their overall concordance from 88.3 to 91.7%, sharing variant interpretations in ClinVar is critical to moving toward more consistent variant interpretations
Pepin et al. Genet Med 2016	Comparison and evaluation of consistent variant classifications (outside labs vs in house) in a distinct disease field (COLx)	29% complete, 29% "moderate" 58% not actionable	Lack of reference of the biology (48%) Lack of access to unpublished data (33%)	In diseases with a "special biology" expert knowledge is important for accurate classification / unpublished data are a major source of inconsistent classification
Balmana et al. J Clin Oncol 2016	ClinVar study comparing variant classifications of 603 variants in non-BRCA cancer genes	74% concordance 11% clinically relevant	many observed differences were because of variants in low- penetrance genes (RR<2)	Conflicting interpretation of genetic findings is frequent and may have implications for medical management decision
Yang et al. Genet Med 2017	ClinVar search of discordant actionable classifications, evaluation of reasons for inconsistencies	96% major consensus 94% complete consensus	Non-clinical lab subm. Clinical areas differ Old data points Literature citations	Recent variant classifications from clinical testing laboratories have high overall concordance.

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

Open

#### Sources of discordance among germ-line variant classifications in ClinVar

Shan Yang, PhD<sup>1</sup>, Stephen E. Lincoln, BS<sup>1</sup>, Yuya Kobayashi, PhD<sup>1</sup>, Keith Nykamp, PhD<sup>1</sup>, Robert L. Nussbaum, MD, FACP, FACMG<sup>1,2</sup> and Scott Topper, PhD, FACMG<sup>1</sup>

**Purpose:** ClinVar is increasingly used as a resource for both genetic variant interpretation and clinical practice. However, controversies exist regarding the consistency of classifications in ClinVar, and questions remain about how best to use these data. Our study systematically examined ClinVar to identify common sources of discordance and thus inform ongoing practices.

Methods: We analyzed variants that had multiple classifications in ClinVar, excluding benign polymorphisms. Classifications were categorized by potential actionability and pathogenicity. Consensus interpretations were calculated for each variant, and the properties of the discordant outlier classifications were summarized.

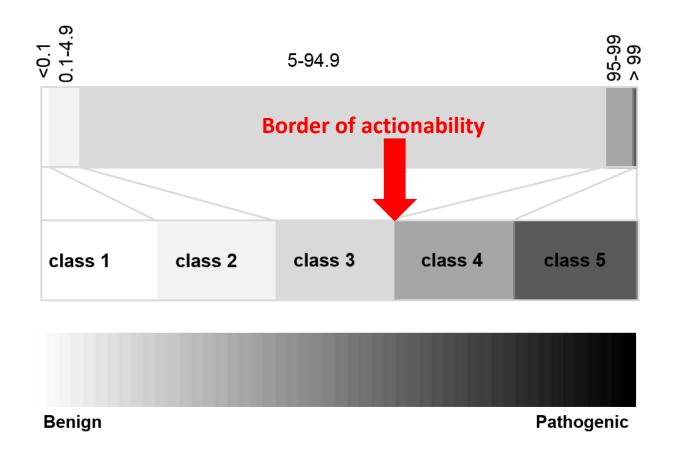
**Results:** Our study included 74,065 classifications of 27,224 unique variants in 1,713 genes. We found that (i) concordance rates differed among clinical areas and variant types; (ii) clinical testing

methods had much higher concordance than basic literature curation and research efforts; (iii) older classifications had greater discordance than newer ones; and (iv) low-penetrance variants had particularly high discordance.

**Conclusion:** Recent variant classifications from clinical testing laboratories have high overall concordance in many (but not all) clinical areas. ClinVar can be a reliable resource supporting variant interpretation, quality assessment, and clinical practice when factors uncovered in this study are taken into account. Ongoing improvements to ClinVar may make it easier to use, particularly for nonexpert users.

Genet Med advance online publication 1 June 2017

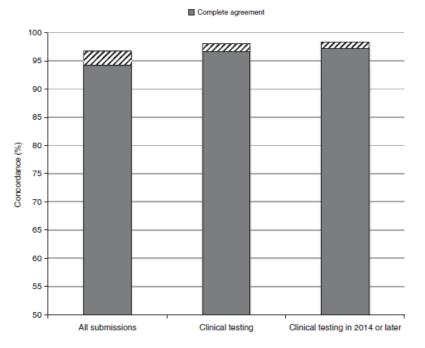
Key Words: clinical genetic testing; ClinVar; concordance; data sharing; variant interpretation



InSiGHT: posterior probability of pathogenicity derived by multifactorial likelihood analysis https://www.insight-group.org/

#### **ORIGINAL RESEARCH ARTICLE**

YANG et al | Sources of discordance in ClinVar

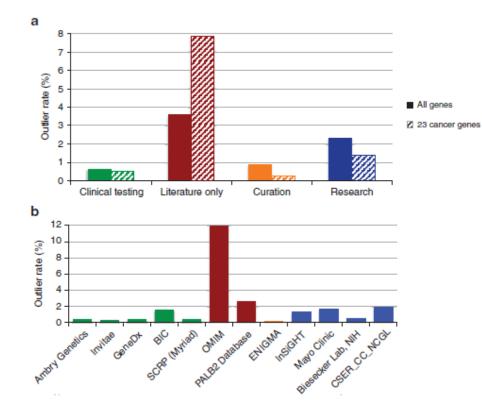


Majority consensus

Figure 5 Concordance for ClinVar and subsets. Variant classification concordance measured as a fraction of variants for all of ClinVar and for subsets of ClinVar filtered by submission type and classification date. Concordance is calculated on an actionability basis (see text).

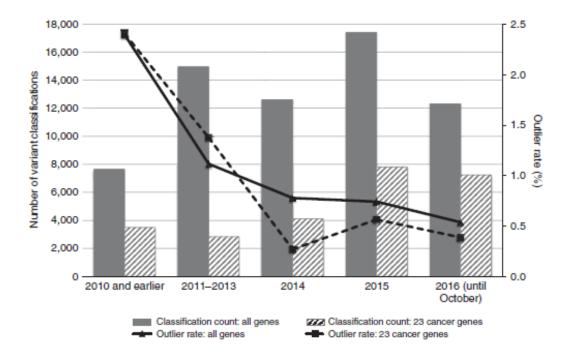
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# TAKE HOME MESSAGE ....

Like every new method/ technology the ACMG-AMP classification rules need training and time

Eventually we will get used to it .....

