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
Hum Mutat. Author manuscript; available in PMC 2011 April 13.

Published in final edited form as:
Hum Mutat. 2008 November ; 29(11): 1282–1291. doi:10.1002/humu.20880.

Sequence variant classification and reporting: recommendations for improving the interpretation of cancer susceptibility genetic test results

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

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

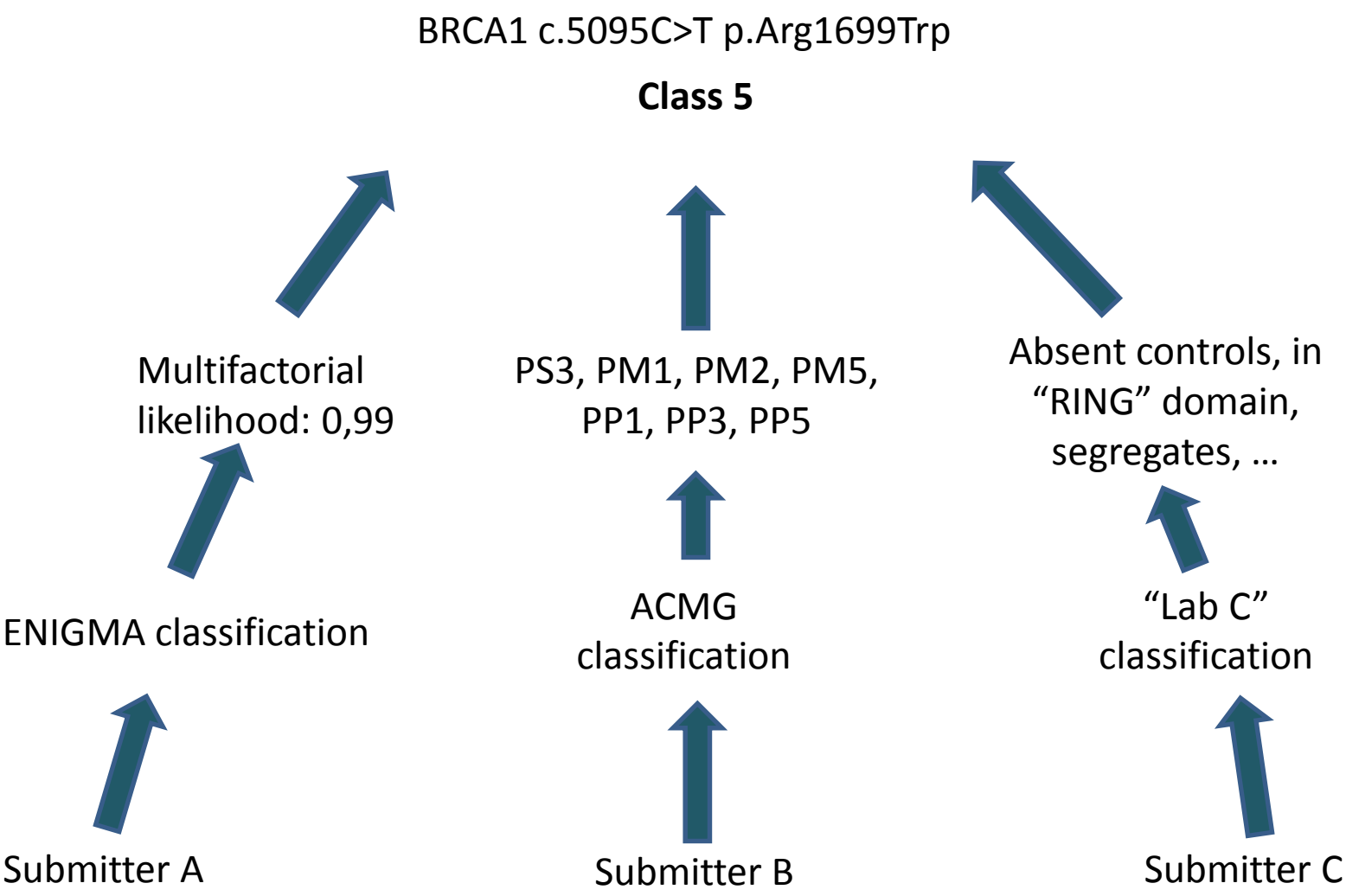
Class	Clinical Testing	Surveillance Recommendations if At-Risk Relative is Positive	Research Testing of Family Members
5	Test at-risk relatives for variant	Full high-risk surveillance guidelines	Not indicated
4	Test at-risk relatives for variant*	Full high-risk surveillance guidelines	May be helpful 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)	curation	Breast-ovarian cancer, familial 1 [MedGen | OMIM]	germline	• PubMed (1) [See all records that cite this PMID] • Other citation	Evidence-based Network for the Interpretation of Germline Mutant 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 [MedGen | OMIM]	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,Partners HealthCare Personalized Medicine
Pathogenic (Dec 30, 2014)	criteria provided, single submitter • ACMG Guidelines, 2015 • ACMG Guidelines, 2015	clinical testing	Hereditary cancer-predisposing syndrome [MedGen]	germline		Color Genomics, Inc.,
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
Likely pathogenic (Jul 1, 2016)	criteria provided, single submitter • ACMG Guidelines, 2015 • ACMG Guidelines, 2015	clinical testing	Familial cancer of breast [MedGen | Orphanet | OMIM]	germline		GeneKor MSA
Pathogenic (Oct 2, 2015)	criteria provided, single submitter • CIMBA Mutation Classification guidelines May 2016	clinical testing	Breast-ovarian cancer, familial 1 [MedGen | OMIM]	germline		Consortium of Investigators Modifiers of BRCA1/2 (CIM c/o University of Cambridg
Pathogenic	criteria provided, single submitter • Carraro et al. (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. Camargo Cancer Center Study description
Pathogenic (Feb 22, 2016)	criteria provided, single submitter • Ambry Autosomal Dominant and X-Linked criteria (10/2015)	clinical testing	Hereditary cancer-predisposing syndrome [MedGen]	germline		Ambry Genetics
Pathogenic (Jan 20, 2017)	criteria provided, single submitter • GeneDx Variant Classification (06012015)	clinical testing	not provided [MedGen]	germline		GeneDx
Pathogenic (Apr 4, 2013)	criteria provided, single submitter • ACMG guidelines, 2007	clinical testing	Hereditary breast and ovarian cancer syndrome [MedGen | Orphanet]	germline		Genetics Diagnostic Laboratory,Children's Hospital of 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
Pathogenic (Jan 18, 2017)	criteria provided, single submitter • Invitae Variant Classification Sherloc (09022015)	clinical testing	Hereditary breast and ovarian cancer syndrome [MedGen | Orphanet]	germline	• PubMed (2) [See all records that cite these PMIDs]	Invitae
Pathogenic (Feb 23, 2017)	criteria provided, single submitter • ACMG Guidelines, 2015 • ACMG Guidelines, 2015	clinical testing	Familial cancer of breast (Autosomal dominant inheritance) [MedGen | Orphanet | OMIM]	germline		Baylor Miraca Genetics Laboratories Study description

ACMG-AMP Classification System

Different Classification Systems

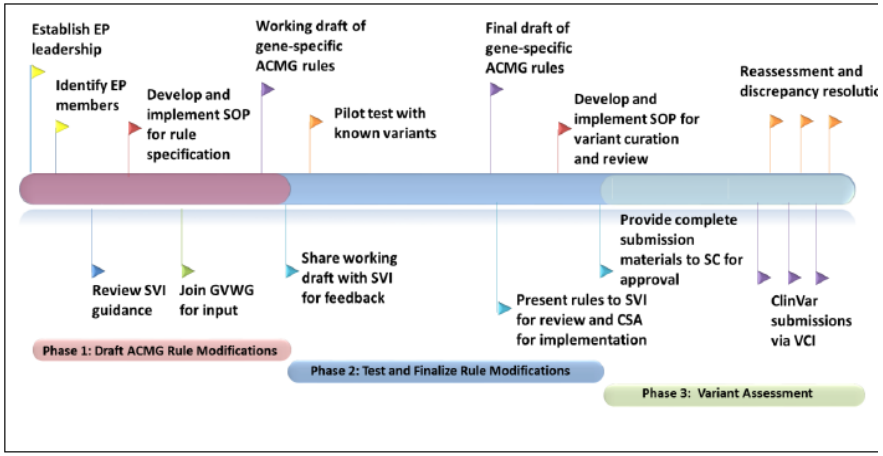
14 submissions / 10 different classification systems

EXPERT GROUP CLASSIFICATION

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

ClinGen
Clinical Genome Resource

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.
   a. Evaluate the clinical validity (strength of evidence) of gene-disease associations for condition(s) within the working group domain (see 3.2).
   b. 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.

ii. Facilitate deposition of variants from clinically relevant genes into ClinVar (see 3.3).
   a. 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.
   a. Identify and encourage external groups that are already involved in curating genetic variants within the domain, and coordinate with them to avoid duplicating effort.
   b. 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.

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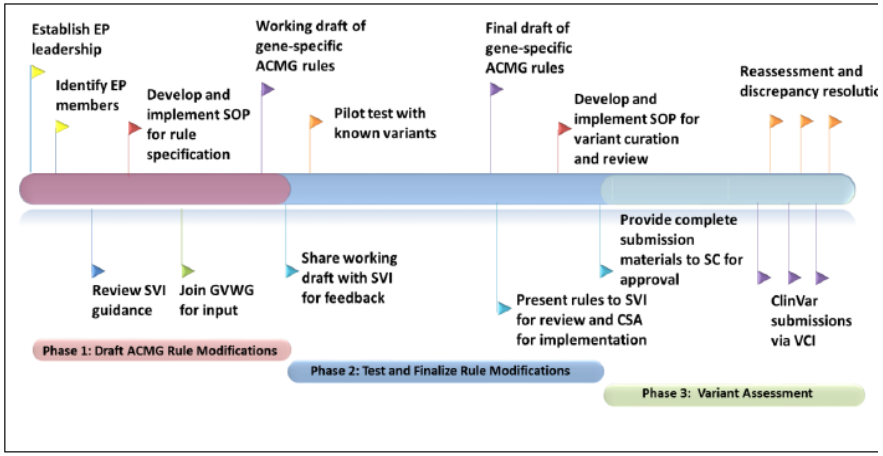

InSiGHT classification rules (4 MMR genes)
ENIGMA classification rules (BRCA1/BRCA2)
CFTR2 classification rules
CDH1, JPS, STK11, ….

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MOACS

Mother Of All Classification Systems

ACMG recommendations

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

ACMG recommendations for standards for interpretation of sequence variations

ACMG L

ACMG Standards and

These
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I. Interpre

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Committe
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variations

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

ACMG recommen interpretation an Revisions 2007

C. Sue Richards, PhD[1], Sherri Bale, Ph
Madhuri R. Hegde, PhD[6], Elaine Lyon
Laboratory Quality Assurance Commi

Key Words: *clinical genetic testi*

ACMG STANDARDS AND GUIDELINES | Genetics inMedicine

Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology

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

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

ACMG-AMP GUIDELINES

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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 valid but contradictory lines of evidence, a variant is interpreted as a VUS

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	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			

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Table 5 Rules for combining criteria to classify sequence variants

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

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General Considerations:

- 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“

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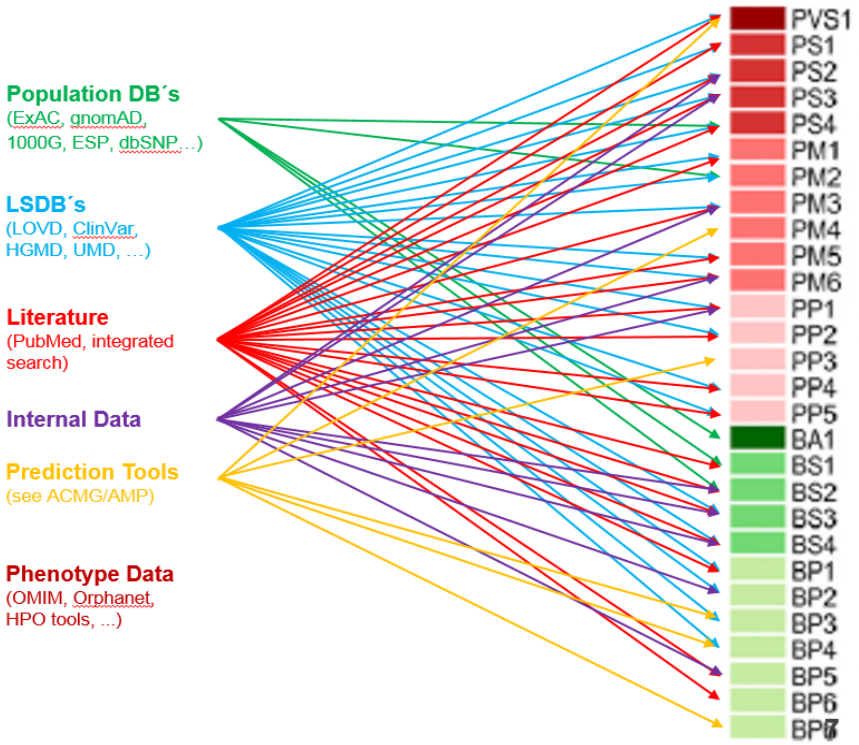
What are the requirements?

- 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)

Information

Selected SNP

SNP (TP53:NM_000546:c.704A>G:p.Asn235Ser ; het; AD) Benign (lana, 2016-06-27 17:53:33)

Google | OMIM | Gene Reviews | LOVD

PatientID	Coverage	Quality	Subpanel
103274	423	222.0	Cancer_MammaErweitert

Associated Disease	Source
lung canceralveolar cell carcinoma, included;;adenocarcinoma of lung, inclu...	OMIMGENE
li-fraumeni syndrome 1; lfs1;;sarcoma family syndrome of li and fraumeni;;sb...	OMIMGENE
esophageal canceresophageal squamous cell carcinoma, susceptibility to, i...	OMIMGENE
squamous cell carcinoma, head and neck; hnscc	OMIMGENE
adrenocortical carcinoma, hereditary; adccadrenocortical carcinoma, pediatri...	OMIMGENE
papilloma of choroid plexus; cpp;;choroid plexus papillomachoroid plexus ca...	OMIMGENE
basal cell carcinoma, susceptibility to, 7; bcc7	OMIMGENE
b-cell chronic lymphocytic leukemia	Orphanet
small cell lung cancer	Orphanet
precursor b-cell acute lymphoblastic leukemia	Orphanet
gliosarcoma	Orphanet
giant cell glioblastoma	Orphanet

☐ Search Associated Diseases

ClinDB	Grading/Phenotype	Name	Info
ClinVar	Likely benign;Uncertain significance	NM_000546.5(TP53):c.704A>G (p.Asn235Ser)	Submitter: 6
Emory	VOUS	NM_000546(TP53):c.704A>G	-
HGMD	Rhabdomyosarcoma	-	CM951230, Pubmed: 7706467

PopDB	rsID	Ref/Alt	AF/AC	AC Hom	Subpopulations
dbSNP (134/144)	**rs144340710**	T/C			
ExAC	**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=0.0002/2		eurAMR=0.0001, afrAMR=0.0002

BS1

Protein Domain	phastCons	phyloP
p53, DNA-binding domain	0.992	1.82

predProg	Prediction	Value
AGVGD	-	C0
SIFT	Tolerated	0.08
MAPP	good	0.0481
Polyphen	benign	0.144

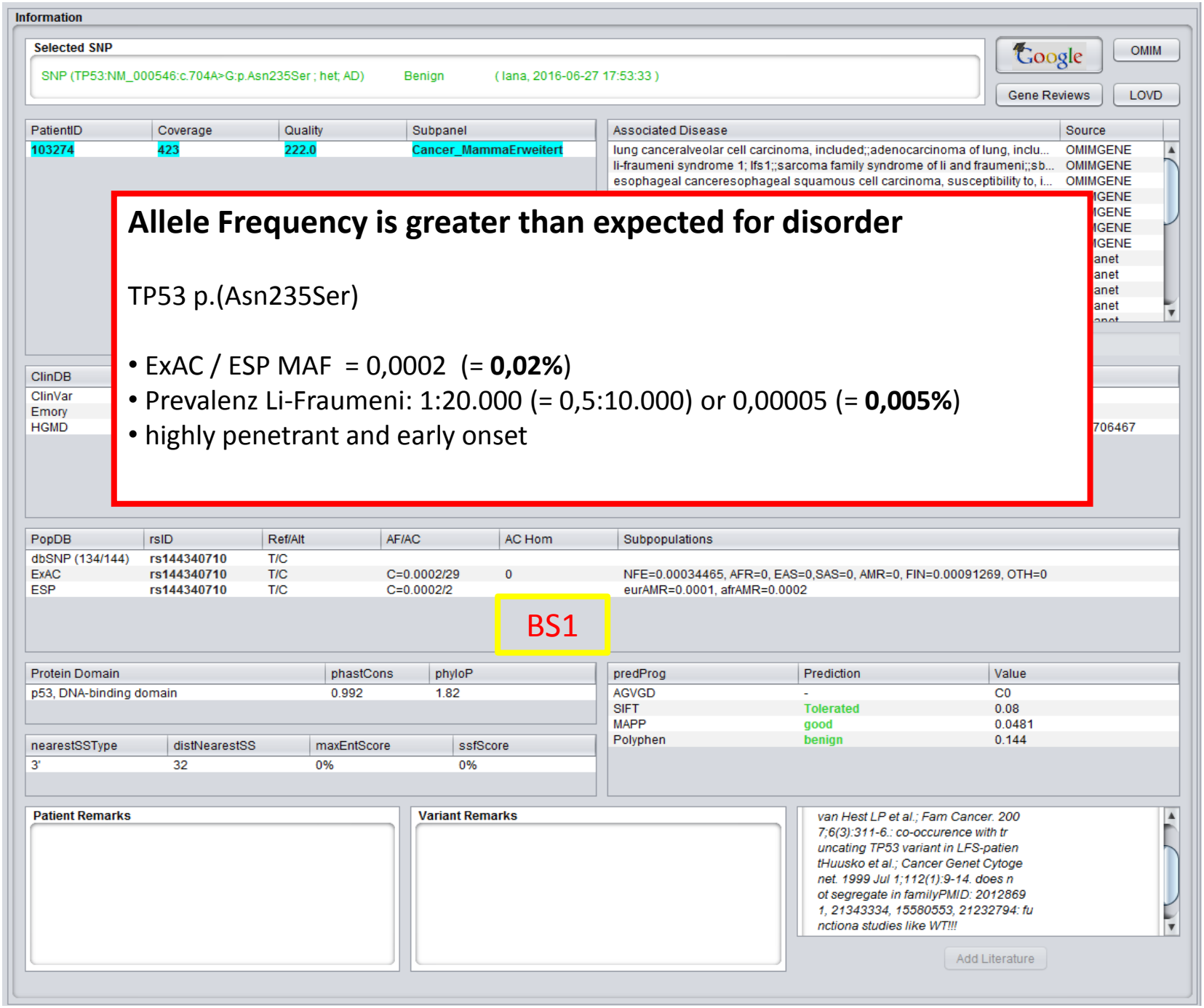
nearestSSType	distNearestSS	maxEntScore	ssfScore
3'	32	0%	0%

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 familyPMID: 2012869 1, 21343334, 15580553, 21232794: fu nctiona studies like WT!!!

Add Literature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Google
OMIM
Gene Reviews
LOVD
PatientID Coverage Quality Subpanel
103274 423 222.0 Cancer_MammaErweitert
Associated Disease Source
lung canceralveolar cell carcinoma, included;;adenocarcinoma of lung, inclu... OMIMGENE
li-fraumeni syndrome 1; lfs1;;sarcoma family syndrome of li and fraumeni;;sb... OMIMGENE
esophageal canceresophageal squamous cell carcinoma, susceptibility to, i... OMIMGENE
Allele Frequency is greater than expected for disorder
TP53 p.(Asn235Ser)
• ExAC / ESP MAF = 0,0002 (= 0,02%)
• Prevalenz Li-Fraumeni: 1:20.000 (= 0,5:10.000) or 0,00005 (= 0,005%)
• highly penetrant and early onset
ClinDB
ClinVar
Emory
HGMD
706467
PopDB rsID Ref/Alt AF/AC AC Hom Subpopulations
dbSNP (134/144) rs144340710 T/C
ExAC 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=0.0002/2 eurAMR=0.0001, afrAMR=0.0002
BS1
Protein Domain phastCons phyloP
p53, DNA-binding domain 0.992 1.82
predProg Prediction Value
AGVGD - C0
SIFT Tolerated 0.08
MAPP good 0.0481
Polyphen benign 0.144
nearestSSType distNearestSS maxEntScore ssfScore
3' 32 0% 0%
Patient Remarks
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1, 21343334, 15580553, 21232794: fu
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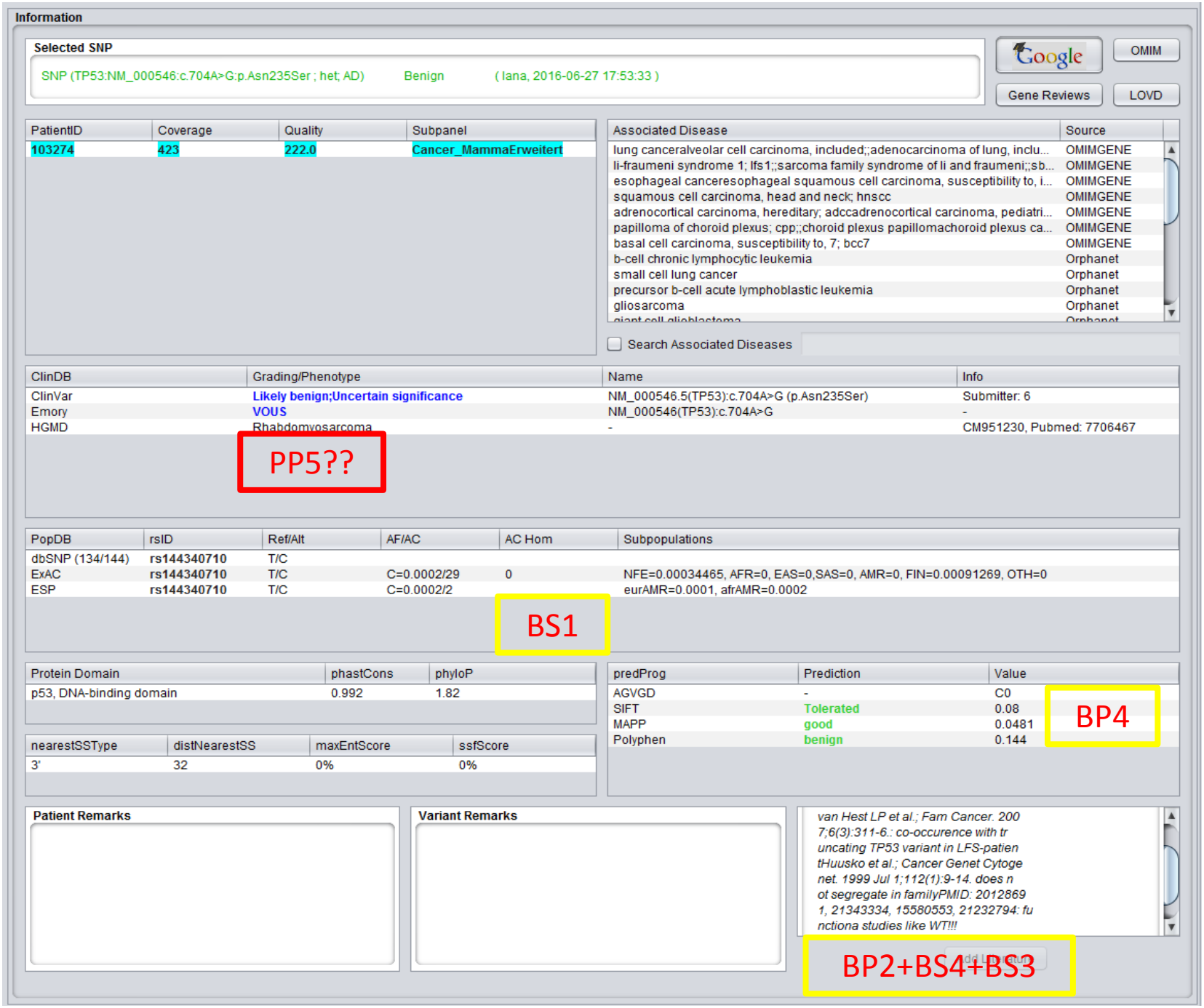
Add Literature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LOVD
PatientID Coverage Quality Subpanel
103274 423 222.0 Cancer_MammaErweitert
Associated Disease Source
lung canceralveolar cell carcinoma, included;;adenocarcinoma of lung, inclu... OMIMGENE
li-fraumeni syndrome 1; lfs1;;sarcoma family syndrome of li and fraumeni;;sb... OMIMGENE
esophageal canceresophageal squamous cell carcinoma, susceptibility to, i... OMIMGENE
squamous cell carcinoma, head and neck; hnscc OMIMGENE
adrenocortical carcinoma, hereditary; adccadrenocortical carcinoma, pediatri... OMIMGENE
papilloma of choroid plexus; cpp;;choroid plexus papillomachoroid plexus ca... OMIMGENE
basal cell carcinoma, susceptibility to, 7; bcc7 OMIMGENE
b-cell chronic lymphocytic leukemia Orphanet
small cell lung cancer Orphanet
precursor b-cell acute lymphoblastic leukemia Orphanet
gliosarcoma Orphanet
Search Associated Diseases
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Emory VOUS NM_000546(TP53):c.704A>G -
HGMD Rhabdomyosarcoma - CM951230, Pubmed: 7706467
PP5??
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ExAC 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=0.0002/2 eurAMR=0.0001, afrAMR=0.0002
BS1
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nearestSSType distNearestSS maxEntScore ssfScore
3' 32 0% 0%
predProg Prediction Value
AGVGD - C0
SIFT Tolerated 0.08
MAPP good 0.0481
Polyphen benign 0.144
BP4
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 familyPMID: 2012869 1, 21343334, 15580553, 21232794: fu nctiona studies like WT!!!
BP2+BS4+BS3

Information

Selected SNP

SNP (TP53:NM_000546:c.704A>G:p.Asn235Ser ; het; AD) Benign (Iana, 2016-06-27 17:53:33)

Google | OMIM | Gene Reviews | LOVD

PatientID	Coverage	Quality	Subpanel
103274	423	222.0	Cancer_MammaErweitert

Associated Disease	Source
lung canceralveolar cell carcinoma, included;;adenocarcinoma of lung, inclu...	OMIMGENE
li-fraumeni syndrome 1; lfs1;;sarcoma family syndrome of li and fraumeni;;sb...	OMIMGENE
esophageal canceresophageal squamous cell carcinoma, susceptibility to, i...	OMIMGENE
squamous cell carcinoma, head and neck; hnscc	OMIMGENE
adrenocortical carcinoma, hereditary; adccadrenocortical carcinoma, pediatri...	OMIMGENE
papilloma of choroid plexus; cpp;;choroid plexus papillomachoroid plexus ca...	OMIMGENE
basal cell carcinoma, susceptibility to, 7; bcc7	OMIMGENE
b-cell chronic lymphocytic leukemia	Orphanet
small cell lung cancer	Orphanet
precursor b-cell acute lymphoblastic leukemia	Orphanet
gliosarcoma	Orphanet
giant cell glioblastoma	Orphanet

PP5??

Search Associated Diseases

ClinDB	Grading/Phenotype	Name	Info
ClinVar	Likely benign;Uncertain significance	NM_000546.5(TP53):c.704A>G (p.Asn235Ser)	Submitter: 6
Emory	VOUS	NM_000546(TP53):c.704A>G	-
HGMD	Rhabdomyosarcoma	-	CM951230, Pubmed: 7706467

PopDB	rsID	Ref/Alt	AF/AC	AC Hom	Subpopulations
dbSNP (134/144)	rs144340710	T/C			
ExAC	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=0.0002/2		eurAMR=0.0001, afrAMR=0.0002

BS1

J Clin Invest. 1995 Apr;95(4):1606-11.

Germline p53 mutations are frequently detected in young children with rhabdomyosarcoma.

Diller L[1], Sexsmith E, Gottlieb A, Li FP, Malkin D.

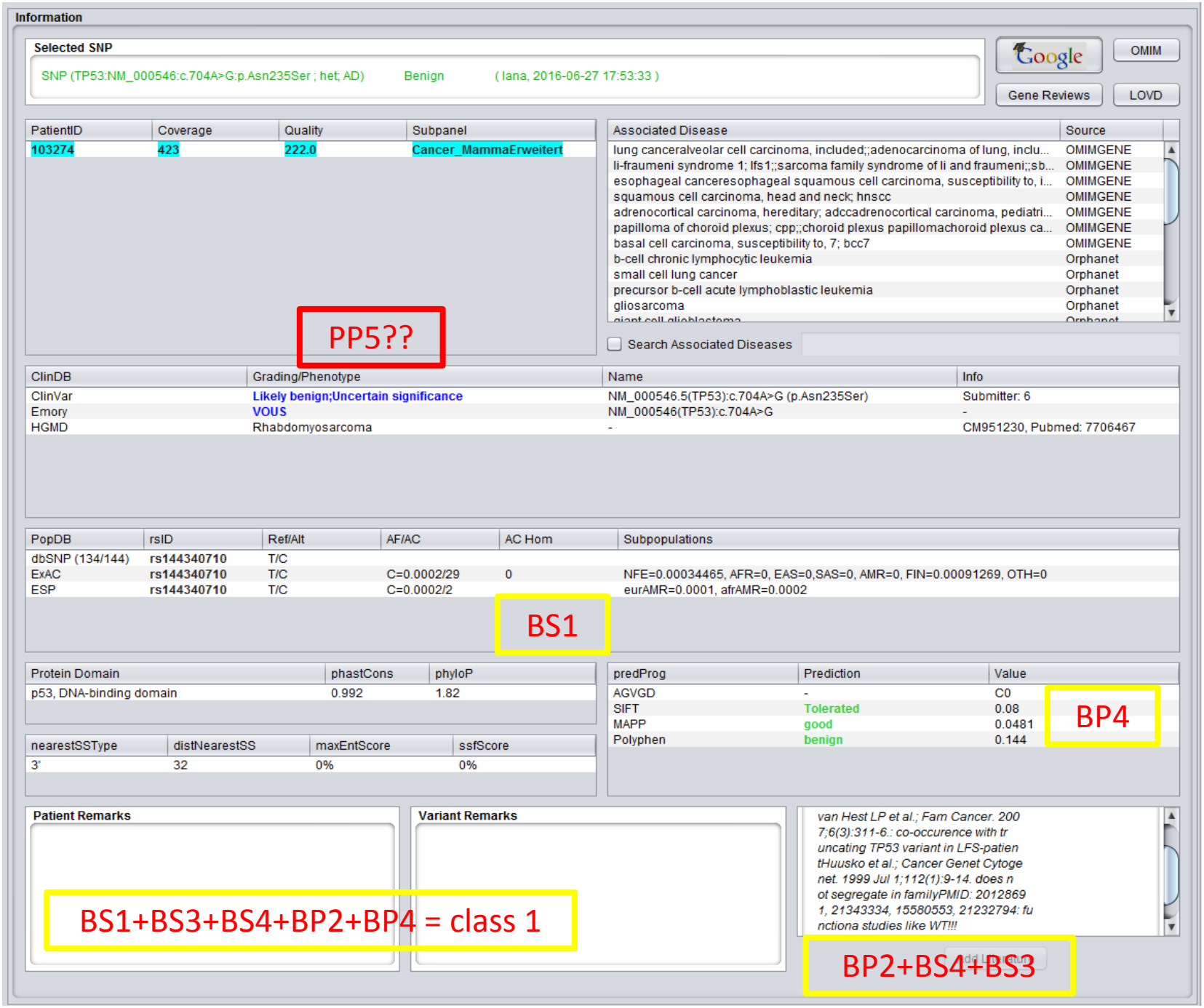
Author information

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 were detected in 3/33 patient samples screened. Two of these missense mutations are located in exon 7 and one in exon 8 of the p53 gene.

BP4

BP2+BS4+BS3


Information
Selected SNP
SNP (TP53:NM_000546:c.704A>G:p.Asn235Ser ; het; AD) Benign (lana, 2016-06-27 17:53:33)
Google
OMIM
Gene Reviews
LOVD
PatientID Coverage Quality Subpanel
103274 423 222.0 Cancer_MammaErweitert
Associated Disease Source
lung canceralveolar cell carcinoma, included;;adenocarcinoma of lung, inclu... OMIMGENE
li-fraumeni syndrome 1; lfs1;;sarcoma family syndrome of li and fraumeni;;sb... OMIMGENE
esophageal canceresophageal squamous cell carcinoma, susceptibility to, i... OMIMGENE
squamous cell carcinoma, head and neck; hnscc OMIMGENE
adrenocortical carcinoma, hereditary; adccadrenocortical carcinoma, pediatri... OMIMGENE
papilloma of choroid plexus; cpp;;choroid plexus papillomachoroid plexus ca... OMIMGENE
basal cell carcinoma, susceptibility to, 7; bcc7 OMIMGENE
b-cell chronic lymphocytic leukemia Orphanet
small cell lung cancer Orphanet
precursor b-cell acute lymphoblastic leukemia Orphanet
gliosarcoma Orphanet
PP5??
Search Associated Diseases
ClinDB Grading/Phenotype Name Info
ClinVar Likely benign;Uncertain significance NM_000546.5(TP53):c.704A>G (p.Asn235Ser) Submitter: 6
Emory VOUS NM_000546(TP53):c.704A>G -
HGMD Rhabdomyosarcoma - CM951230, Pubmed: 7706467
PopDB rsID Ref/Alt AF/AC AC Hom Subpopulations
dbSNP (134/144) rs144340710 T/C
ExAC 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=0.0002/2 eurAMR=0.0001, afrAMR=0.0002
BS1
Protein Domain phastCons phyloP
p53, DNA-binding domain 0.992 1.82
predProg Prediction Value
AGVGD - C0
SIFT Tolerated 0.08
MAPP good 0.0481
Polyphen benign 0.144
BP4
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
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net. 1999 Jul 1;112(1):9-14. does n
ot segregate in familyPMID: 2012869
1, 21343334, 15580553, 21232794: fu
nctiona studies like WT!!!
BS1+BS3+BS4+BP2+BP4 = class 1
BP2+BS4+BS3

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Criteria for classifying pathogenic variants (Tabelle 1)

Evidence of pathogenicity		Category
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	**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 non maternity.*
	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.*

ACMG GUIDELINES

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

946 SNVs are shown

Val	Mat	Type	Gene	MOI	Changes	Position	Ref	Alt
	G	FS	AMPD1	AR	NM_000036:c.104del:p.Pro35Leufs*87	chr1:115238088-115238088	G	-

651 SNVs are shown

Val	Mat	Type	Gene	MOI	Changes	Position	Ref	Alt	GT	AF	rsID	1000G	ESP	ExAC	gnomAD
	G	FS	MYH8	AD	NM_002472:c.3320del:p.Leu1107Hisfs*60	chr17:10304211-10304211	A	-	het	-	rs751871946		0.0001	0.0002	0.0002
	G	FS	SLC46A1	AR	NM_080669:c.1226del:p.Ile409Thrfs*10	chr17:26727722-26727722	A	-	hom	-	rs561780114	1	0.9997	1	1
	G	FS	P2RX5	-	NM_002561:c.333del:p.Asn112Thrfs*36	chr17:3594277-3594277	G	-	hom	-	rs3215407	0.5735	0.5439	0.6676	0.6761
	G	FS	ABCA10	-	NM_080282:c.4515_4516del:p.Gln1506Glyfs*	chr17:67145191-67145192	GA	-	het	-	rs3842375	0.0881	0.0602	0.0745	0.0808
	G	FS	ABCA10	-	NM_080282:c.1331_1334del:p.Ser444Phefs*	chr17:67190537-67190540	ACAG	-	het	-	rs113082690	0.0885	0.0731	0.0743	0.0804
	G	FS	CYP2F1	-	NM_000774:c.15dup:p.Thr6Hisfs*22	chr19:41622107-41622108	-	C	het	-	rs3833221	0.272	0.2556	0.2148	0.2077
	G	FS	CYP2F1	-	NM_000774:c.15dup:p.Thr6Hisfs*22	chr19:41622107-41622108	-	C	hom	-	rs3833221	0.272	0.2556	0.2148	0.2077
	G	FS	CARD8	-	NM_014959:c.290_291dup:p.Val98Lysfs*26	chr19:48735017-48735018	-	TT	het	-	rs140826611	0.0421	0.0427	0.0525	0.0507
	G	FS	SIGLEC12	-	NM_053003:c.196dup:p.Ala66Glyfs*50	chr19:52004791-52004792	-	C	het	-	rs66949844	0.5929	0.6503	0.6407	0.6458
	G	FS	SIGLEC12	-	NM_053003:c.196dup:p.Ala66Glyfs*50	chr19:52004791-52004792	-	C	hom	-	rs66949844	0.5929	0.6503	0.6407	0.6458
	G	FS	ZNF480	-	NM_144684:c.9_10del:p.Cys3*	chr19:52803670-52803671	TG	-	het	-	rs3217319	0.599	0.6134	0.6883	0.6923
	G	FS	ZNF480	-	NM_144684:c.9_10del:p.Cys3*	chr19:52803670-52803671	TG	-	hom	-	rs3217319	0.599	0.6134	0.6883	0.6923
	G	FS	SBK3	-	NM_001199824:c.954_955insG:p.Gly318_Phe	chr19:56052337-56052338	-	C	hom	-	rs397738405	1		1	1
	G	FS	FMO2	-	NM_001460:c.337del:p.Val113*	chr1:171165803-171165803	G	-	het	-	rs28369860	0.1028	0.1161	0.0606	0.0561
	G	FS	FCN3	AR	NM_003665:c.349del:p.Leu117Serfs*65	chr1:27699671-27699671	G	-	het	-	rs532781899	0.0188	0.0211	0.0166	0.0162
	G	FS	GJB4	AD, AR	NM_153212:c.155_158del:p.Val52Alafs*55	chr1:35227008-35227011	TGTC	-	het	-	rs146812843		0.0343	0.0336	0.0328
	G	FS	CYP4B1	-	NM_001099772:c.884_885del:p.Asp295Glyfs*	chr1:47280747-47280748	AT	-	het	-	rs3215983	0.1354	0.1143	0.1475	0.1489
	G	FS	DEFB126	-	NM_030931:c.163_166del:p.Gln55Glyfs*28	chr20:126156-126159	CAAA	-	het	-	rs11467497		0.1918	0.1523	0.1462
	G	FS	DEFB126	-	NM_030931:c.317_318del:p.Pro106Argfs*127	chr20:126311-126312	CC	-	het	-	rs11467417		0.5662	0.5534	0.5518
	G	FS	DEFB126	-	NM_030931:c.317_318del:p.Pro106Argfs*127	chr20:126311-126312	CC	-	hom	-	rs11467417		0.5662	0.5534	0.5518
	G	FS	ADAM33	-	NM_025220:c.2412_2419del:p.Gln804Hisfs*1	chr20:3649633-3649640	TCTGG...	-	het	-	rs146576636	0.0705	0.0388		0.0418
	G	FS	SCARF2	AR	NM_153334:c.2304dup:p.Glu769Argfs*9	chr22:20779973-20779974	-	G	hom	-	rs5844418	1	0.982	1	1
	G	FS	SCARF2	AR	NM_153334:c.2253dup:p.Pro752Alafs*26	chr22:20780024-20780025	-	C	hom	-	rs5844420	1	0.9031		1
	G	FS	TTC21B	AD, AR	NM_024753:c.21+26_21+33dup	chr2:166810161-166810162	-	CCCG...	het	-	rs569432248	0.5084		0.6829	
	G	FS	PRKRA	AR	NM_003690:c.22_23del:p.Ala8Argfs*22	chr2:179315735-179315736	GC	-	het	-	rs141354030	0.2382	0.3121		0.3397
	G	FS	PRKD3	-	NM_005813:c.2673dup	chr2:37480319-37480320	-	T	het	-	rs140587747	0.0543	0.0704	0.0481	0.0455
	G	FS	PRKD3	-	NM_005813:c.2673dup	chr2:37480319-37480320	-	T	hom	-	rs140587747	0.0543	0.0704	0.0481	0.0455
	G	FS	PNPT1	AR	NM_033109:c.*11dup	chr2:55863360-55863361	-	A	het	-	rs35916020			0.4661	
	G	FS	PNPT1	AR	NM_033109:c.*11dup	chr2:55863360-55863361	-	A	hom	-	rs35916020			0.4661	
	G	FS	FANCL	AR	NM_001114636:c.1111_1114dup:p.Thr372As	chr2:58386928-58386929	-	TAAT	het	-	rs759217526		0.0025	0.0029	0.003
	G	FS	CD207	-	NM_015717:c.71+2dup	chr2:71062833-71062834	-	C	hom	-	rs11450450	1	0.9998	1	1
	G	FS	ALMS1	AR	NM_015120:c.35_36insGGAGGAGGAGGAGG	chr2:73613031-73613032	-	GGAG...	hom	-					
	G	FS	RYK	-	NM_001005861:c.59_60insC:p.Ala20_Glu21fs	chr3:133969437-133969438	-	G	hom	-	rs587770426	0.9982	0.9865		1
	G	FS	RYK	-	NM_001005861:c.9_10insG:p.Gly3_Arg4fs	chr3:133969487-133969488	-	C	hom	-	rs587744425	0.999			
	G	FS	KCNMB3	-	NM_171830:c.753del:p.Val252Tyrfs*4	chr3:178960767-178960767	T	-	het	-	rs143962239	0.0891	0.1158	0.0676	0.0619
	G	FS	HTR3E	-	NM_182589:c.64del:p.Glu22Serfs*39	chr3:183818222-183818222	G	-	het	-	rs397897677	0.0355	0.0741	0.0661	0.0668
	G	FS	HTR3E	-	NM_182589:c.64del:p.Glu22Serfs*39	chr3:183818222-183818222	G	-	hom	-	rs397897677	0.0355	0.0741	0.0661	0.0668
	G	FS	CLDN16	AR	NM_006580:c.166del:p.Ala56Leufs*16	chr3:190106072-190106072	G	-	het	-	rs368234054	0.117		0.1945	0.194
	G	FS	CLDN16	AR	NM_006580:c.166del:p.Ala56Leufs*16	chr3:190106072-190106072	G	-	hom	-	rs368234054	0.117		0.1945	0.194
	G	FS	CCR5	-	NM_000579:c.554_585del:p.Ser185Ilefs*32	chr3:46414944-46414975	ACAGT...	-	het	-	rs333	0.0292	0.0604		0.0748
	G	FS	CCR5	-	NM_000579:c.554_585del:p.Ser185Ilefs*32	chr3:46414944-46414975	ACAGT...	-	hom	-	rs333	0.0292	0.0604		0.0748
	G	FS	FGFRL1	-	NM_001004356:c.1454_1455del:p.His485Le	chr4:1019055-1019056	CA	-	het	-	rs145808953			0.1987	
	G	FS	FIP1L1	IC, SMu	NM_030917:c.1459_1460del:p.Arg487Glyfs*3	chr4:54319248-54319249	AG	-	het	-	rs143671659			0.1141	
	G	FS	SLC22A1	-	NM_003057:c.1276+9_1276+16del	chr6:160560898-160560905	TGGTA...	-	het	-	rs111569197	0.6895		0.6122	0.5938
	G	FS	SLC22A1	-	NM_003057:c.1276+9_1276+16del	chr6:160560898-160560905	TGGTA...	-	hom	-	rs111569197	0.6895		0.6122	0.5938
	G	FS	HLA-A	-	NM_002116:c.751del:p.Asp251Thrfs*46	chr6:29912029-29912029	G	-	het	-	rs45576436	0.3706	0.3678		
	G	FS	HLA-B	Mu	NM_005514:c.206_207insC:p.Glu69Aspfs*30	chr6:31324601-31324602	-	G	het	-	rs9281379			0.122	0.0875
	G	FS	HLA-B	Mu	NM_005514:c.204del:p.Glu69Argfs*8	chr6:31324604-31324604	T	-	het	-	rs200186034		0.4424	0.1758	0.1492
	G	FS	MICA	-	NM_001177519:c.953_956del:p.Gly318Alafs*i	chr6:31380158-31380161	GCTG	-	het	-	rs138201170	0.0958	0.2512	0.1953	0.1591
	G	FS	MICA	-	NM_001177519:c.953_956del:p.Gly318Alafs*i	chr6:31380158-31380161	GCTG	-	hom	-	rs138201170	0.0958	0.2512	0.1953	0.1591
	G	FS	MICA	-	NM_001177519:c.953del:p.Gly318Alafs*68	chr6:31380161-31380161	G	-	het	-	rs67841474	0.2049		0.3188	0.2124
	G	FS	MICA	-	NM_001177519:c.953del:p.Gly318Alafs*68	chr6:31380161-31380161	G	-	hom	-	rs67841474	0.2049		0.3188	0.2124
	G	FS	MICA	-	NM_001177519:c.952_953insCT:p.Gly318Ala	chr6:31380161-31380162	-	CT	het	-	rs41293539	0.2338		0.319	
	G	FS	MICA	-	NM_001177519:c.952_953insCT:p.Gly318Ala	chr6:31380161-31380162	-	CT	hom	-	rs41293539	0.2338		0.319	
	G	FS	MICA	-	NM_001177519:c.952_953insCTGCTGCTGC	chr6:31380161-31380162	-	CTGCT...	het	-	rs41293539	0.2117		0.2387	
	G	FS	MICA	-	NM_001177519:c.952_953insCTGCTGCTGC	chr6:31380161-31380162	-	CTGCT...	hom	-	rs41293539	0.2117		0.2387	
	G	FS	CYP21A2	AR	NM_000500:c.1443delG:p.Pro481fs	chr6:31976139-31976139	G	-	hom	-	rs374273480	0.0795	0.0711	0.1264	0.0826

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Criteria for classifying pathogenic variants (Tabelle 1)

Evidence of pathogenicity		Category
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	**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 non maternity.*
	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.*

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Only for a few genes „well established functional studies“ have been defined

InSiGHT (MMR genes)

Assays assessing MMR protein repair capacity as a complete process					
Functional assays using cell-free systems	In vitro MMR complementation assays	An *in vitro* 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.	49,79,112,117-134 135-137 Transfection efficiency not measured 66 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 *HPRT* 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

Thompson et al.; Nat Genet: dio:10.1038/ng.2854

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Moderate	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.
	PM2	Absent from controls (or at extremely low frequency if recessive) (Table 6) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium • *Caveat: Population data for insertions/deletions may be poorly called by next-generation sequencing.*
	PM3	For recessive disorders, detected in trans with a pathogenic variant *Note: This requires testing of parents (or offspring) to determine phase.*
	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 • *Example: Arg156His is pathogenic; now you observe Arg156Cys* • *Caveat: Beware of changes that impact splicing rather than at the amino acid/protein level.*
	PM6	Assumed de novo, but without confirmation of paternity and maternity
Supporting	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*
	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
	PP3	Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.) • *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.*
	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

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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
Strong	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	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
	BP4	Multiple lines of computational evidence suggest no impact on gene or gene product (conservation, evolutionary, splicing impact, etc.) • 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.
	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

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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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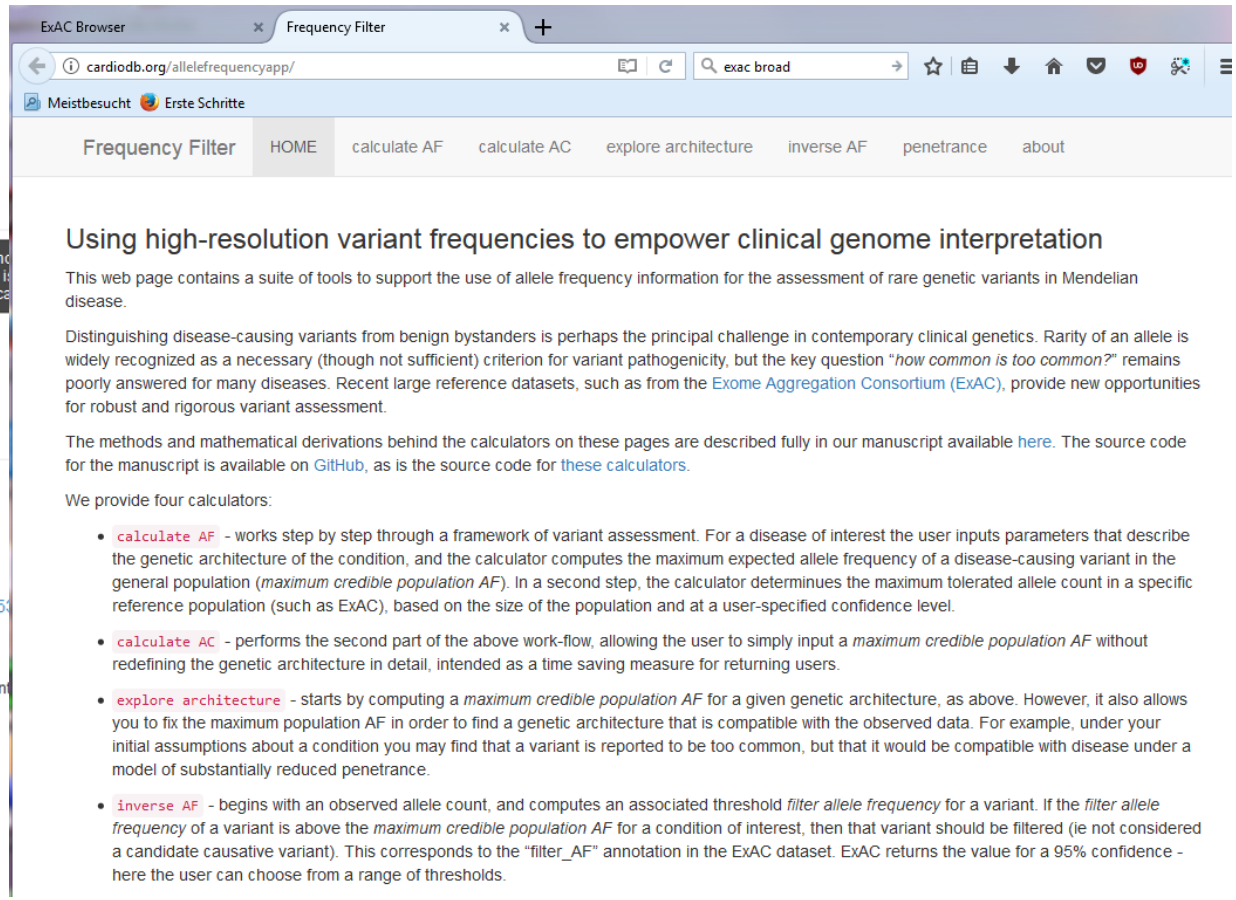

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Table 2 Maximum credible population frequencies and maximum tolerated ExAC allele counts for variants causative of exemplar inherited cardiac conditions, assuming a penetrance of 0.5 throughout

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,[40] Noonan,[18] CPVT,[19] and classical Ehlers-Danlos.[20]

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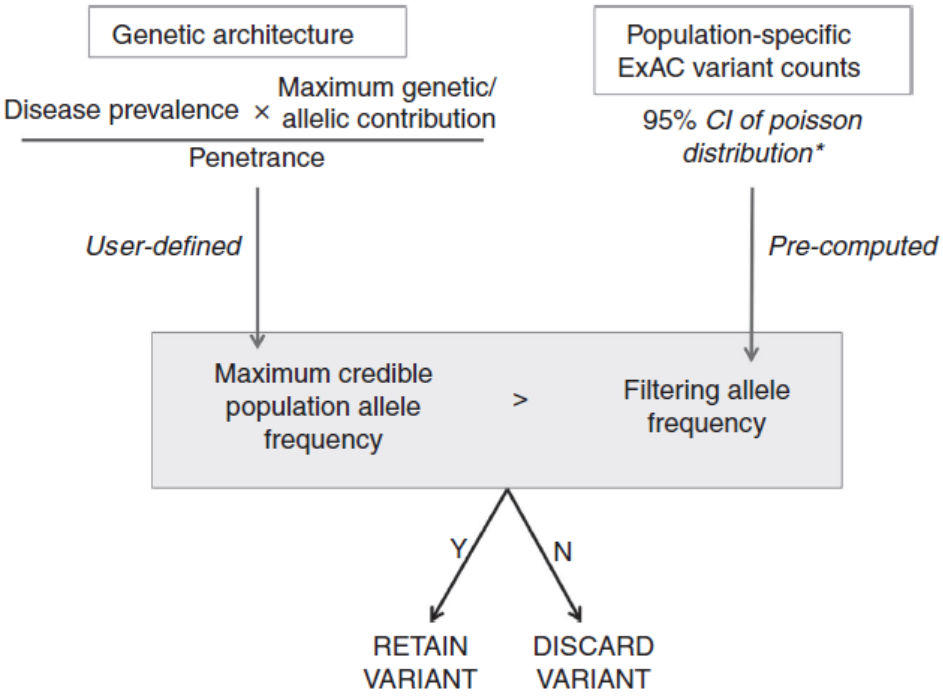

ACMG-AMP CRITERIA KNOWN TO BE PROBLEMATIC

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 $\geq 5\%$ 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[a]	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

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New Results

Evaluation of *in silico* algorithms for use with ACMG/AMP clinical variant interpretation guidelines

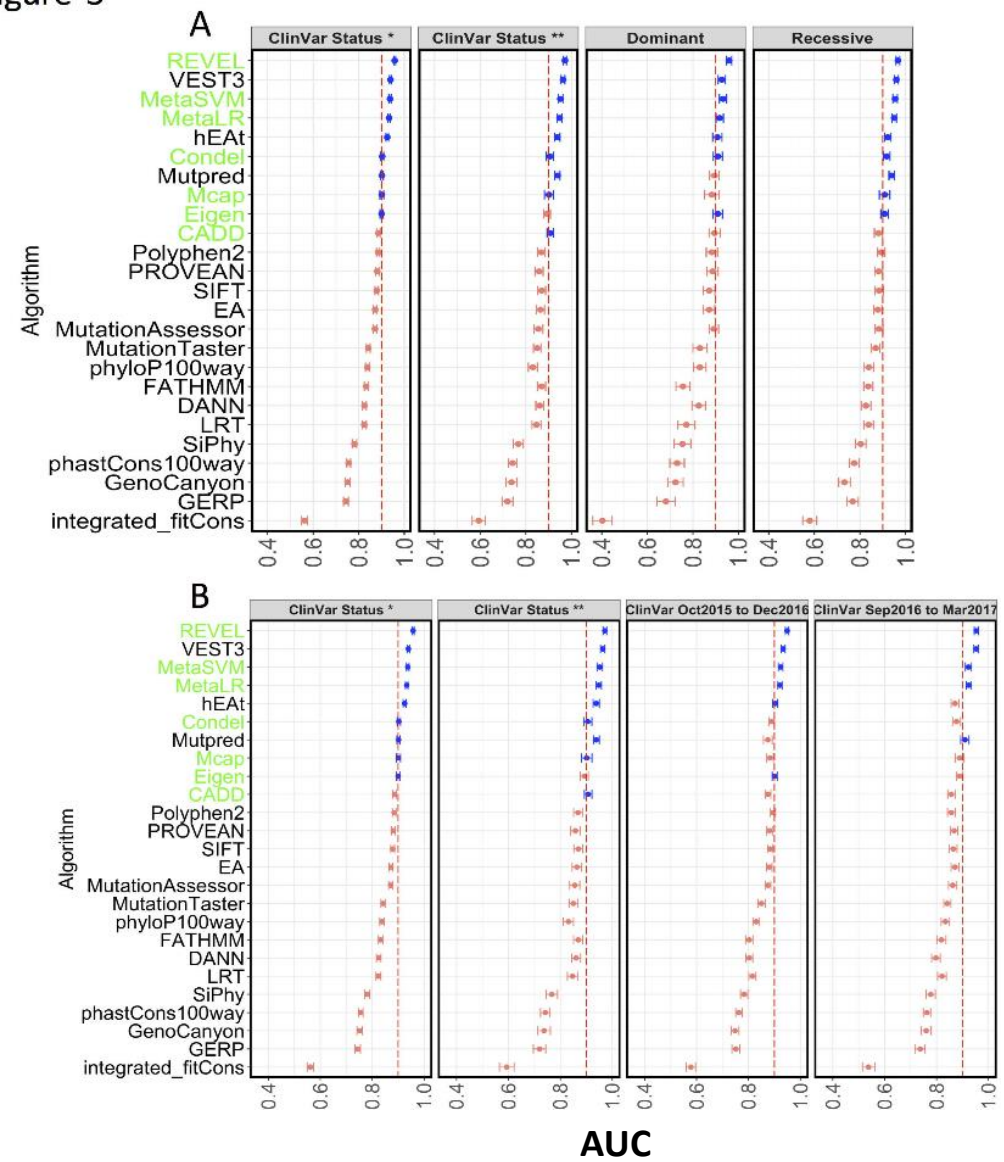
Rajarshi Ghosh, Ninad Oak, Sharon E. Plon

doi: https://doi.org/10.1101/146100

Abstract

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 among different combinations of algorithms with particularly low 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.

Figure 3



ACMG-AMP VARIANT CLASSIFICATION TOOLS

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Format: Abstract

Am J Hum Genet. 2017 Feb 2;100(2

InterVar: Clinical Int

Li Q[1], Wang K[2].

Author information

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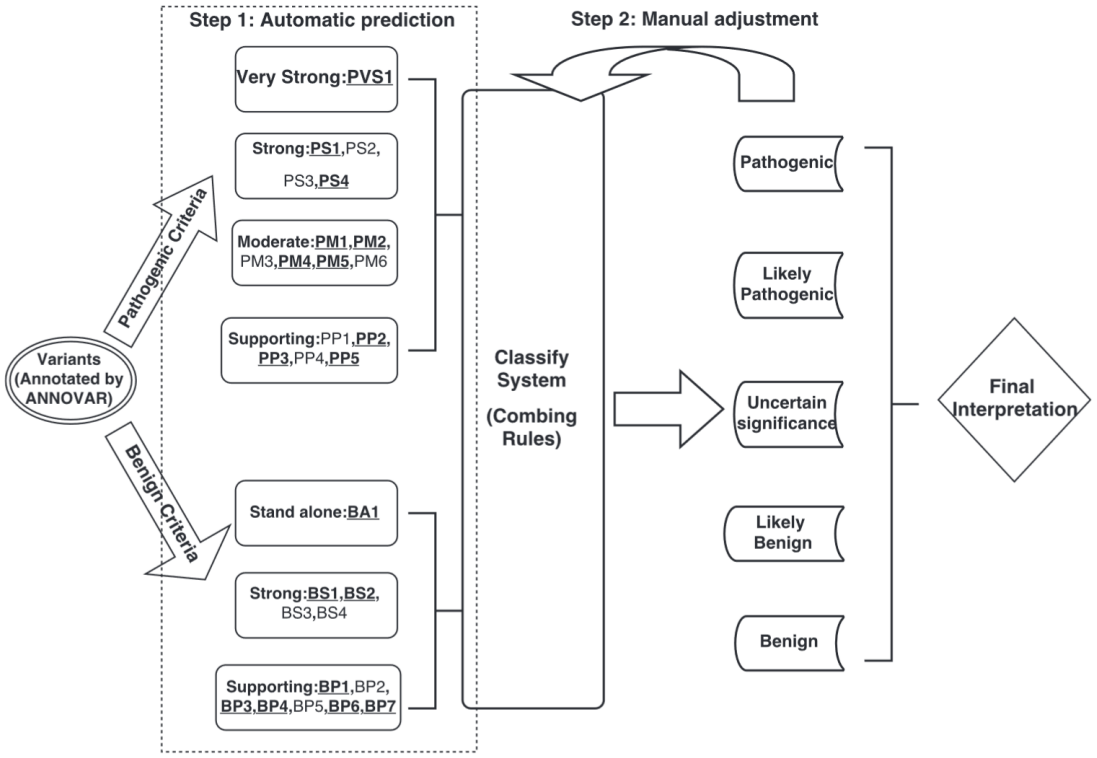

Figure 1. Flowchart of the Two-Step Procedure of InterVar
Underlined and bold fonts denote automated criteria.

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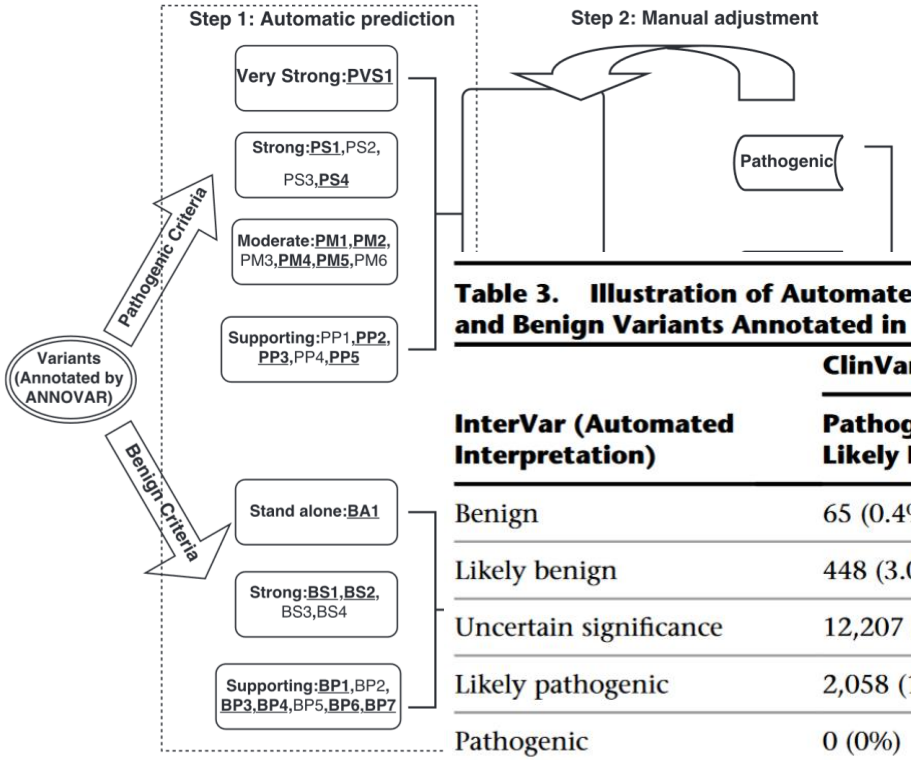

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Table 3. Illustration of Automated Interpretation of Pathogenic and Benign Variants Annotated in ClinVar

	ClinVar	
InterVar (Automated Interpretation)	**Pathogenic or Likely Pathogenic**	**Benign or Likely Benign**
Benign	65 (0.4%)	1,505 (24.8%)
Likely benign	448 (3.0%)	3,393 (55.9%)
Uncertain significance	12,207 (82.6%)	1,173 (19.3%)
Likely pathogenic	2,058 (13.9%)	0 (0%)
Pathogenic	0 (0%)	0 (0%)
Sum of five tiers	14,778	6,071
Benign and likely benign	513 (3.5%)	4,898 (80.6%)
Pathogenic and likely pathogenic	2,058 (13.9%)	0 (0%)

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	**34% before** and **71% after** 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.

Are there really large inconsistencies in ClinVar ?

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

Open

Sources of discordance among germ-line variant classifications in ClinVar

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

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.

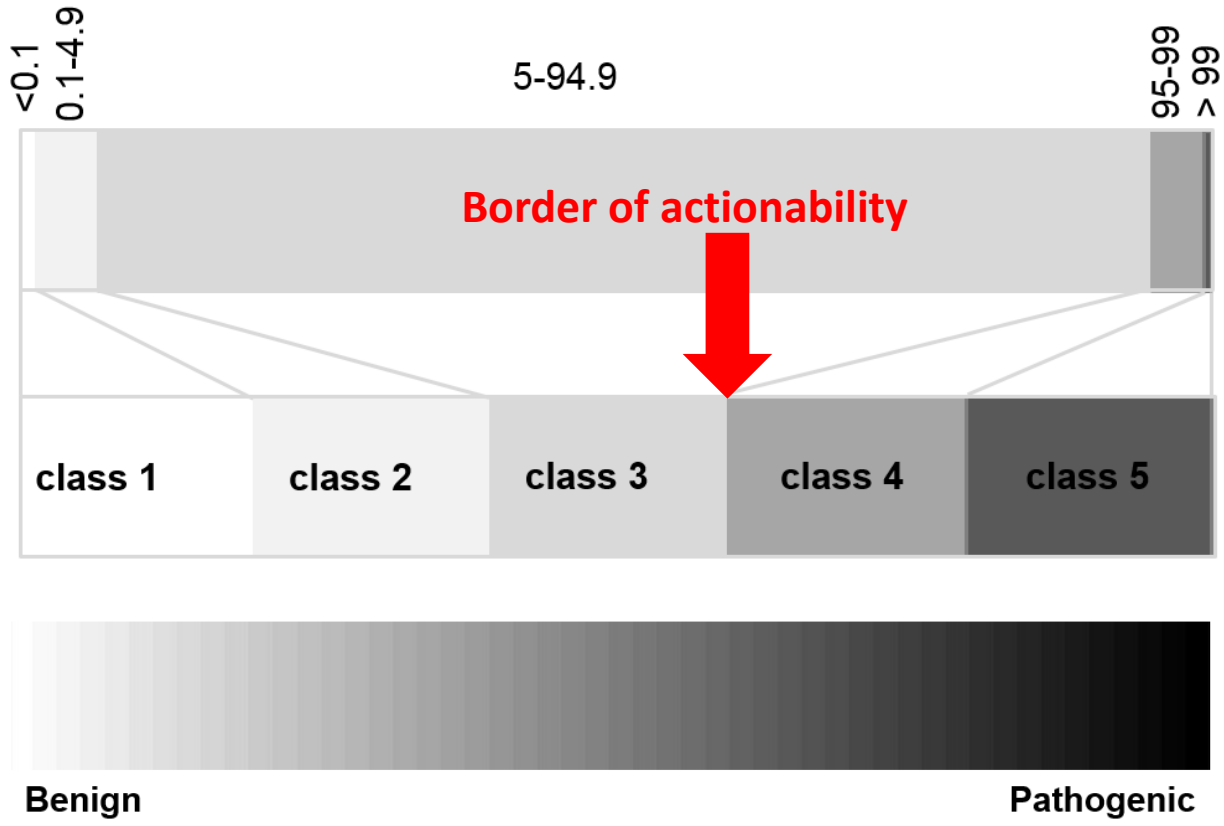
Results: Our study included 74,065 classifications of 27,224 unique variants in 1,713 genes. We found that (i) concordance rates differed among clinical areas and variant types; (ii) clinical testing methods had much higher concordance than basic literature curation and research efforts; (iii) older 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

Are there really large inconsistencies in ClinVar ?



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

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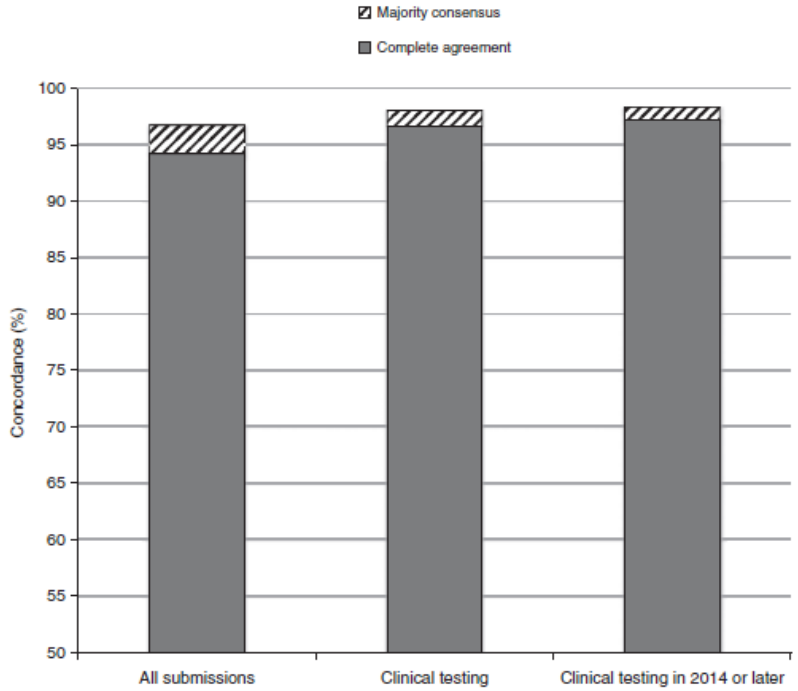
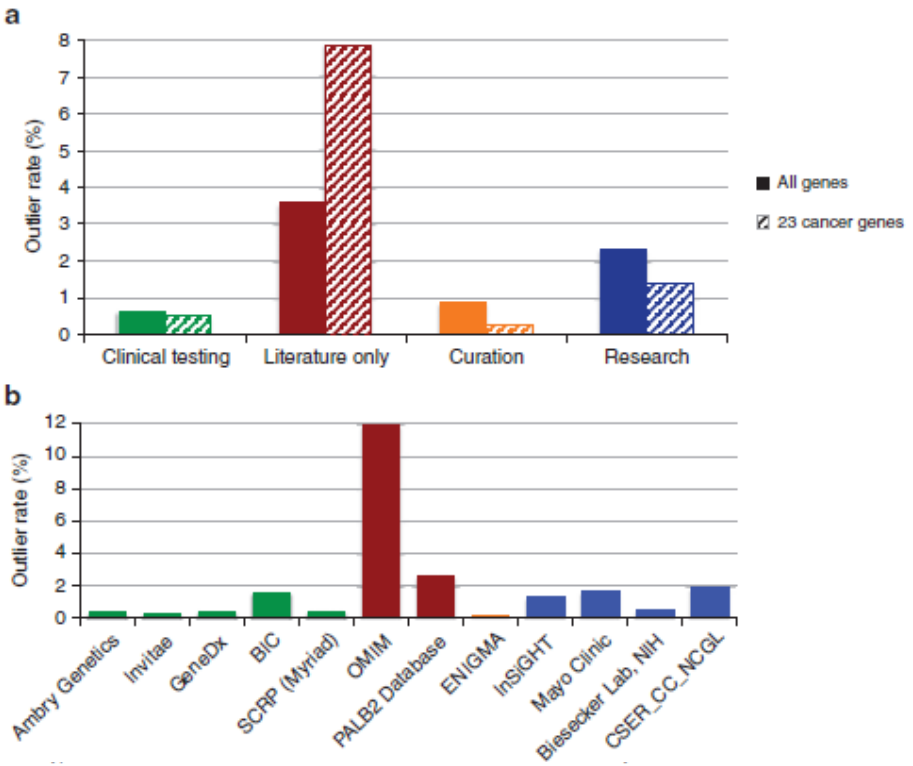


Figure 5 Concordance for ClinVar and subsets. Variant classification concordance measured as a fraction of variants for all of ClinVar and for subsets of ClinVar filtered by submission type and classification date. Concordance is calculated on an actionability basis (see text).

Are there really large inconsistencies in ClinVar ?



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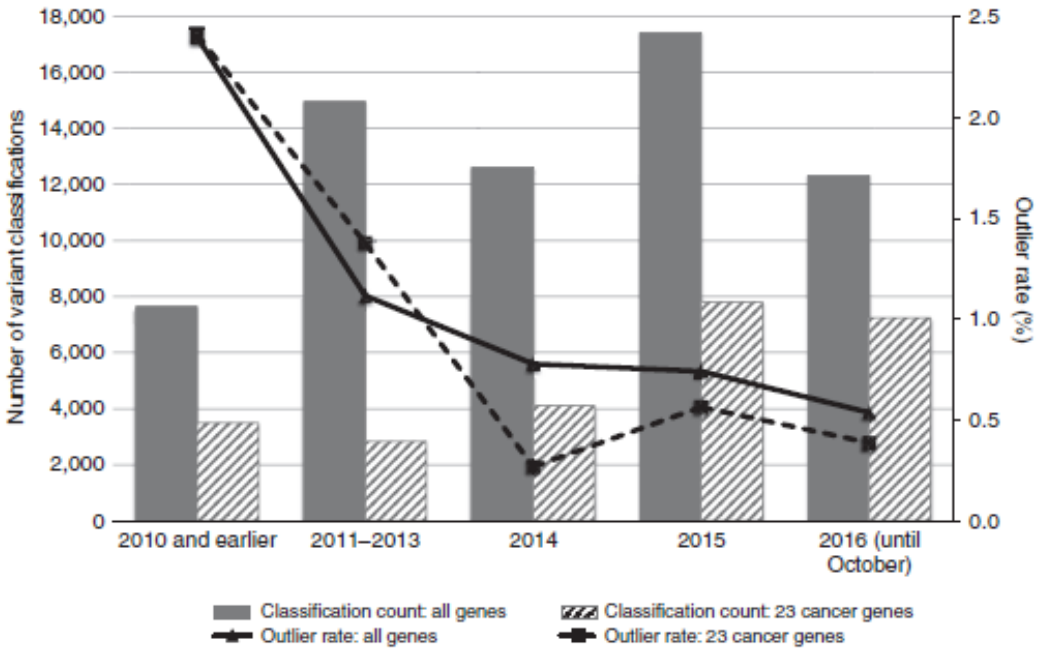

TAKE HOME MESSAGE ….

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

Eventually we will get used to it …..