SECONDARY VARIANTS

Jennifer J. Johnston, PhD
September 28, 2011

Primary Variant

Proband

Whole exome

X number of variants

Variants of interest

Gene Discovery

Secondary Variant

Proband

Whole exome

X number of variants

Secondary Variants

Variant of interest

Gene Discovery

What Do We Do With These Variants?

- Ignore them
- Analyze them and return useful results

Why return these variants?

- Secondary line of research
- Ethical obligation to research participant?

NHLBI Guidelines (should return)

- Important health implication of finding for participant, risk established and substantial
- Finding is actionable- therapy or prevention that could change course of disease
- Test analytically valid and disclosure complies with laws
- Participant has opted to receive results

Fabsitz et al. Circ Cardiovasc Genet (2010)

NHLBI Guidelines (may return)

- Benefit outweighs risk from participant's perspective
- IRB approved disclosure plan
- Test analytically valid and disclosure complies with laws
- Participant has opted to receive results

Fabsitz et al. Circ Cardiovasc Genet (2010)

CLIA

- Clinical Laboratory Improvement Amendments 1988

- "Applies to research laboratories as well if they report patient-specific results for the diagnosis, prevention, or treatment of any disease or impairment of, or the assessment of the health of, individual patients."

http://www.cms.gov/CLIA/

ASHG Childhood Testing Guidelines

- "If the medical or psychosocial benefits of a genetic test will not accrue until adulthood, as in the case of carrier status or adult-onset diseases, genetic testing generally should be deferred." - 1995

http://www.ashg.org/pages/statement_ajhg57.shtml

So you have decided to return secondary variants….

What do you return?

Diseases

- Nature of disorder
  - Severity/threat
  - Actionability/treatability
  - Alternative modes of diagnosis
  - Proband vs. descendant risk

Diseases to Consider

- Cancer predisposition- Breast/Ovarian, Colorectal, other- BRCA1/2, APC, MLH1, MSH2, MSH6, PMS2
- Hypertrophic Cardiomyopathy- MYH7, MYBPC3, TNNT2, TNNI3, TPM1, MYL2, MYL3, ACTC1, CSRP3, TNN, ACTN2, MYH6, TCAP, TNNC1
- Long QT Syndrome- KCNQ1, KCNE1, KCNH2, KCNE2, SCN5A
- Malignant Hyperthermia- RYR1, CACNA1S

Diseases to Consider

- Thrombophilia- F5 (Factor 5 Leiden, p.R506Q, 24/566), F2 (prothrombin, G20210A)
- Hemochromatosis- HFE
- Pharmacogenetics
- Adult onset neurological disorders
- Carrier variants that may affect future generations

Gene/Variant

- Gene
  - HGMD, OMIM, GeneTests

- Variant
  - Return variants ***known*** to be causative
  - Can return ***novel*** variants highly likely to be causative
  - consider effect of telling versus not telling

http://www.ncbi.nlm.nih.gov/omim

http://www.ncbi.nlm.nih.gov/sites/GeneTests/

Gene/Variant

- Gene
  - HGMD, OMIM, GeneTests

- Variant
  - Return variants ***known*** to be causative
  - Can return ***novel*** variants highly likely to be causative
  - consider effect of telling versus not telling
    - CF - BRCA1 – CDH1

http://www.ncbi.nlm.nih.gov/omim

http://www.ncbi.nlm.nih.gov/sites/GeneTests/

At this point need to start filtering variants!

How to Work with Variant Data

Annotation Source - VarSifter

Find variant in HGMD or LSDB

Analyze support for causation

Decide whether to return variant

CS Secondary Variant

572 Probands

Whole exome

X number of variants

Variant of interest

Gene Discovery

Secondary Variants

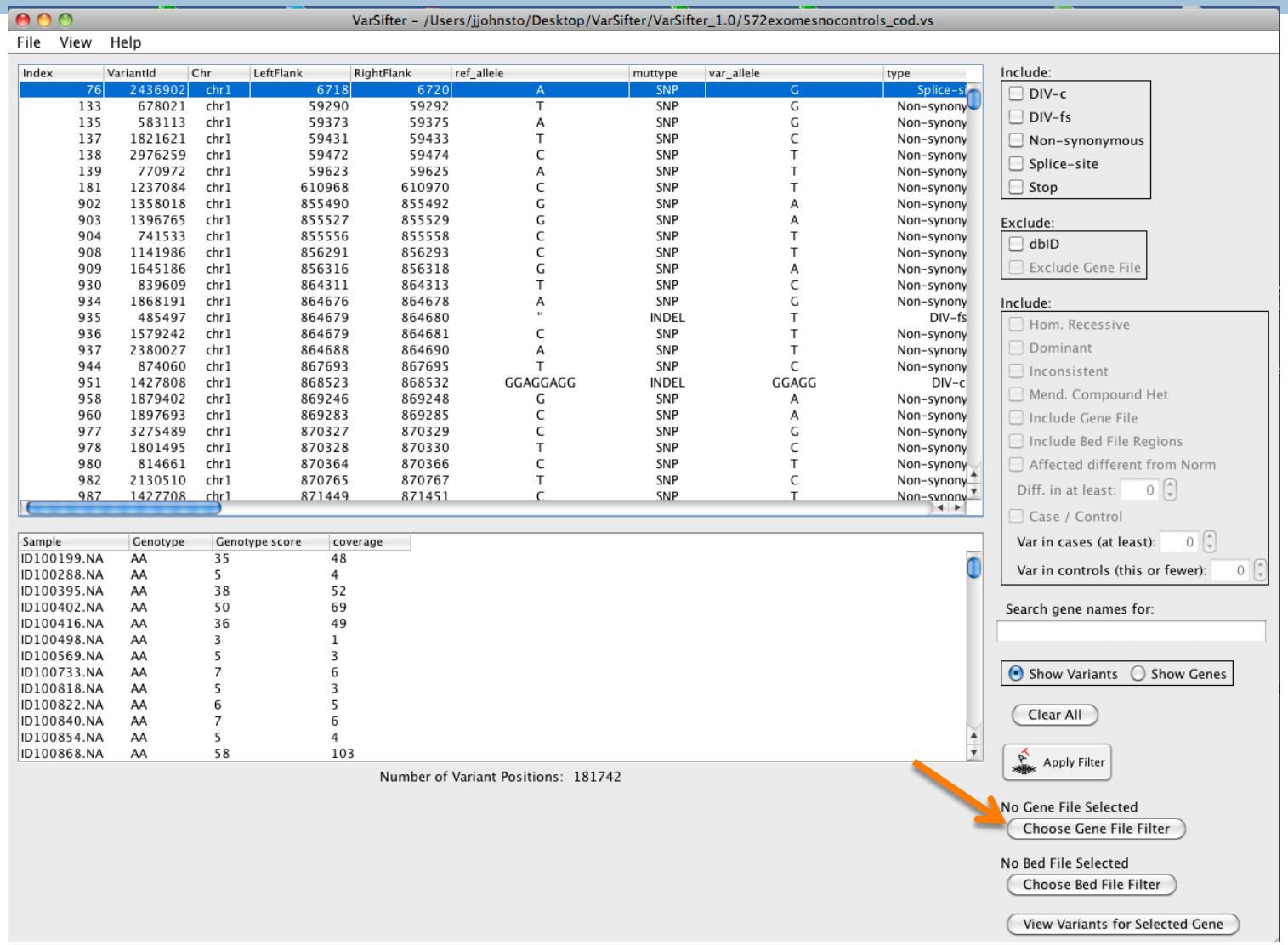
181,742

High Susceptibility Cancer Genes

APC	Familial adenomatous polyposis	*FLCN*	Birt-Hogg-Dubé syndrome	*NF1*	Neurofibromatosis type 1	*RET*	Multiple endocrine neoplasia Familial medullary thyroid cancer	*TP53*	Li-Fraumeni syndrome
BMPR1A	Familial juvenile polyposis	*KIT*	Gastrointestinal stromal tumor	*NF2*	Neurofibromatosis type 2	*SDHAF2*	Hereditary paraganglioma	*TSC1*	Tuberous sclerosis complex 1
BRCA1	Hereditary breast-ovarian cancer	*MEN1*	Multiple endocrine neoplasia type 1	*PDGFRA*	Gastrointestinal stromal tumor (GIST)	*SDHB*	Hereditary paraganglioma	*TSC2*	Tuberous sclerosis complex 2
BRCA2	Hereditary breast-ovarian cancer	*MET*	Hereditary papillary renal cell carcinoma	*PMS2*	Hereditary nonpolyposis colon cancer	*SDHC*	Hereditary paraganglioma	*VHL*	von Hippel-Lindau syndrome
CDC73 (HPRT2)	Hereditary hyperparathyroidism -jaw tumor syndrome	*MLH1*	Hereditary nonpolyposis colon cancer	*PRKAR1A*	Carney complex type 1	*SDHD*	Hereditary paraganglioma	*WT1*	Familial Wilms tumor 1
CDH1	Hereditary diffuse gastric cancer	*MSH2*	Hereditary nonpolyposis colon cancer	*PTCH1*	Nevoid basal cell carcinoma syndrome	*SMAD4*	Familial juvenile polyposis		
CDKN2A	Hereditary multiple melanoma	*MSH6*	Hereditary nonpolyposis colon cancer	*PTEN*	Cowden disease	*SMARCB1*	Schwannomatosis		
FH	Hereditary renal cell carcinoma	*MUTYH*	MYH-associated polyposis	*RB1*	Hereditary retinoblastoma	*STK11*	Peutz-Jeghers syndrome		

Lindor et al. *J Natl Cancer Inst Monogr.* (2008)

VarSifter – Gene Filter



VarSifter – Gene Filter

VarSifter - /Users/jjohnsto/Desktop/VarSifter/VarSifter_1.0/572exomesnocontrols_cod.vs

File View Help

Chr	LeftFlank	RightFlank	ref_allele	muttype	var_allele	type	Gene_nam
chr9	97269293	97269295	C	SNP	T	Non-synonymous	
chr9	97269299	97269301	T	SNP	C	Non-synonymous	
chr9	97270920	97270922	G	SNP	A	Non-synonymous	
chr9	97270930	97270932	G	SNP	A	Non-synonymous	
chr9	97271013	97271015	A	SNP	C	Non-synonymous	
chr9	97308597	97308599	C	SNP	G	Non-synonymous	
chr9	97318760	97318762	C	SNP	G	Non-synonymous	
chr9	97318792	97318794	T	SNP	C	Non-synonymous	
chr9	134761509	134761511	G	SNP	A	Non-synonymous	
chr9	134761808	134761829	GCTGCTGCTGCTGCTGCTGC	INDEL	GCTGCTGCTGCTGCTGCTG...	DIV-c	
chr9	134761834	134761836	C	SNP	T	Non-synonymous	
chr9	134762747	134762749	G	SNP	C	Non-synonymous	
chr9	134768872	134768874	G	SNP	A	Non-synonymous	
chr9	134768991	134768993	C	SNP	T	Non-synonymous	
chr9	134769000	134769002	C	SNP	T	Non-synonymous	
chr9	134770825	134770827	G	SNP	C	Non-synonymous	
chr9	134771025	134771027	T	SNP	C	Non-synonymous	
chr9	134771137	134771139	G	SNP	C	Non-synonymous	
chr9	134771325	134771327	G	SNP	C	Non-synonymous	
chr9	134775690	134775692	T	SNP	C	Non-synonymous	
chr9	134776271	134776273	G	SNP	T	Non-synonymous	
chr9	134776688	134776690	G	SNP	A	Non-synonymous	
chr9	134776724	134776726	A	SNP	G	Non-synonymous	
chr9	134777551	134777553	C	SNP	T	Non-synonymous	
chr9	134792497	134792499	G	SNP	T	Non-synonymous	

Sample	Genotype	Genotype score	coverage
ID100199.NA	AA	35	47
ID100288.NA	AA	15	18
ID100395.NA	AA	35	48
ID100402.NA	AA	38	52
ID100416.NA	AA	46	63
ID100498.NA	AA	11	13
ID100569.NA	AA	7	7
ID100733.NA	AA	12	14
ID100818.NA	AA	8	8
ID100822.NA	AA	16	20
ID100840.NA	AA	11	13
ID100854.NA	AA	4	2

Number of Variant Positions: 455

Include:
- DIV-c
- DIV-fs
- Non-synonymous
- Splice-site
- Stop

Exclude:
- dbID
- Exclude Gene File

Include:
- Hom. Recessive
- Dominant
- Inconsistent
- Mend. Compound Het
- Include Gene File
- Include Bed File Regions
- Affected different from Norm

Diff. in at least: 0

Case / Control

Var in cases (at least): 0

Var in controls (this or fewer): 0

Search gene names for:

Show Variants Show Genes

Clear All

Apply Filter

/Users/jjohnsto/Desktop/VarSifter/ClinSeqOncogenes01042011.t

Choose Gene File Filter

No Bed File Selected

Choose Bed File Filter

CS Secondary Variant

572 Probands

Whole exome

X number of variants

Variant of interest

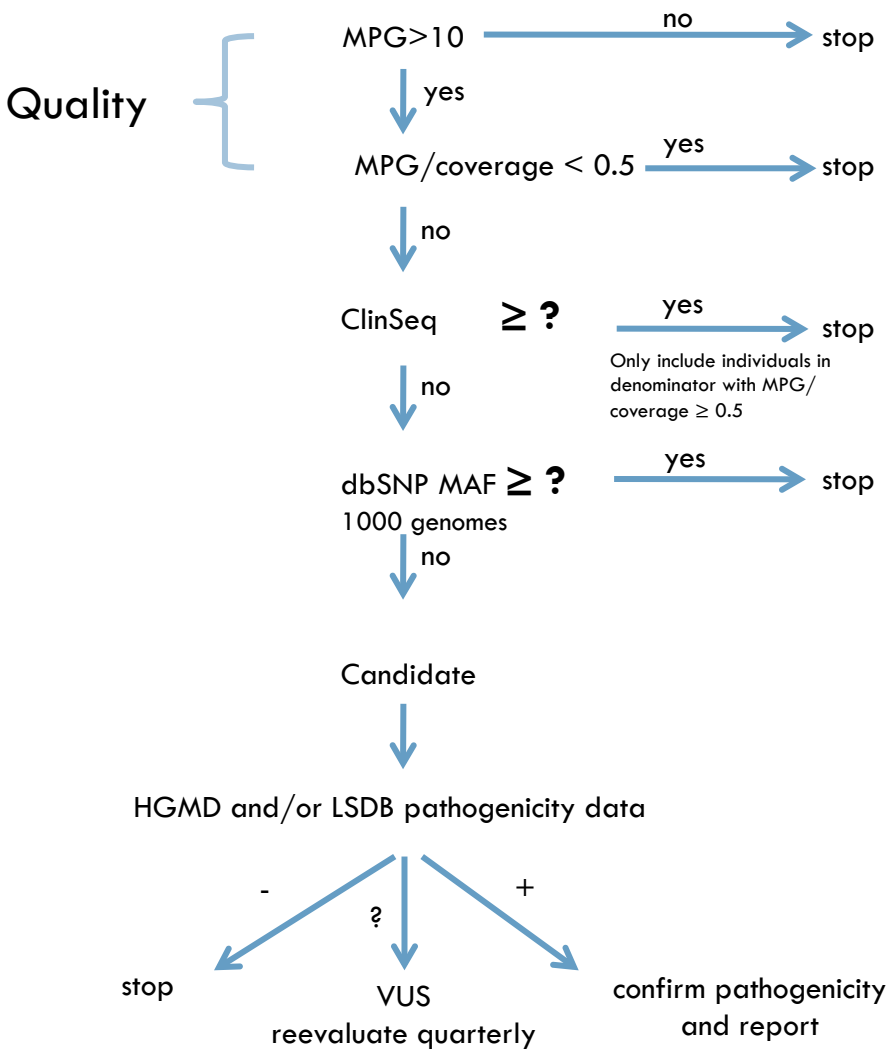
Gene Discovery

Secondary Variants
(nonsense, nonsynonymous,
frameshift, splice)
181,742

Adult onset cancer susceptibility genes

455 variants

Framework for Variant Interpretation



VarSifter – Most Probable Genotype/ Coverage

VarSifter - /Users/jjohnsto/Desktop/VarSifter/VarSifter_1.0/572exomesnocontrols_cod.vs

File View Help

Index	VariantId	Chr	LeftFlank	RightFlank	ref_allele	muttype	var_allele	typ
428194	989090	chr13	31804765	31804767	C	SNP	T	N
428199	803617	chr13	31805128	31805130	T	SNP	C	N
428200	841738	chr13	31805178	31805180	G	SNP	C	N
428201	1898438	chr13	31805406	31805408	A	SNP	G	N
428202	3182391	chr13	31805503	31805505	C	SNP	T	N
428207	1852661	chr13	31808455	31808457	C	SNP	G	N
428209	1746948	chr13	31808799	31808801	A	SNP	C	N
428210	3029987	chr13	31808841	31808843	A	SNP	G	N
428214	562236	chr13	31809462	31809464	A	SNP	G	N
428218	2500760	chr13	31810006	31810008	C	SNP	T	N
428220	1579654	chr13	31810053	31810055	A	SNP	G	N
428221	3002200	chr13	31810072	31810074	G	SNP	A	N
428224	1027379	chr13	31810749	31810751	G	SNP	T	N
428226	1746949	chr13	31811084	31811086	A	SNP	T	N
428229	1603851	chr13	31811270	31811272	A	SNP	C	N
428230	1534135	chr13	31811689	31811691	C	SNP	T	N
428233	1858193	chr13	31811803	31811805	G	SNP	A	N
428235	2322944	chr13	31811970	31811979	ATTAAATT	INDEL	ATT	
428236	1513053	chr13	31812043	31812045	T	SNP	G	N
428237	1551174	chr13	31812045	31812047	G	SNP	T	N
428239	1578933	chr13	31812223	31812225	A	SNP	T	N
428240	862096	chr13	31812235	31812237	C	SNP	T	N
428241	1623569	chr13	31812437	31812439	T	INDEL	"	
428242	718833	chr13	31812591	31812593	C	SNP	T	N
428243	1556721	chr13	31812813	31812815	C	SNP	T	N

Sample	Genotype	Genotype score	coverage
ID180290.NA	AC	17	36
ID100199.NA	AA	112	160
ID100288.NA	AA	85	120
ID100395.NA	AA	55	76
ID100402.NA	AA	92	130
ID100416.NA	AA	83	117
ID100498.NA	AA	59	82
ID100569.NA	AA	32	43
ID100733.NA	AA	66	93
ID100818.NA	AA	33	45
ID100822.NA	AA	81	114
ID100840.NA	AA	46	63

Number of Variant Positions: 455

Include:
- DIV-c
- DIV-fs
- Non-synonymous
- Splice-site
- Stop

Exclude:
- dbID
- Exclude Gene File

Include:
- Hom. Recessive
- Dominant
- Inconsistent
- Mend. Compound Het
- Include Gene File
- Include Bed File Regions
- Affected different from Norm

Diff. in at least: 0

Case / Control

Var in cases (at least): 0

Var in controls (this or fewer): 0

Search gene names for:

Show Variants Show Genes

Clear All

Apply Filter

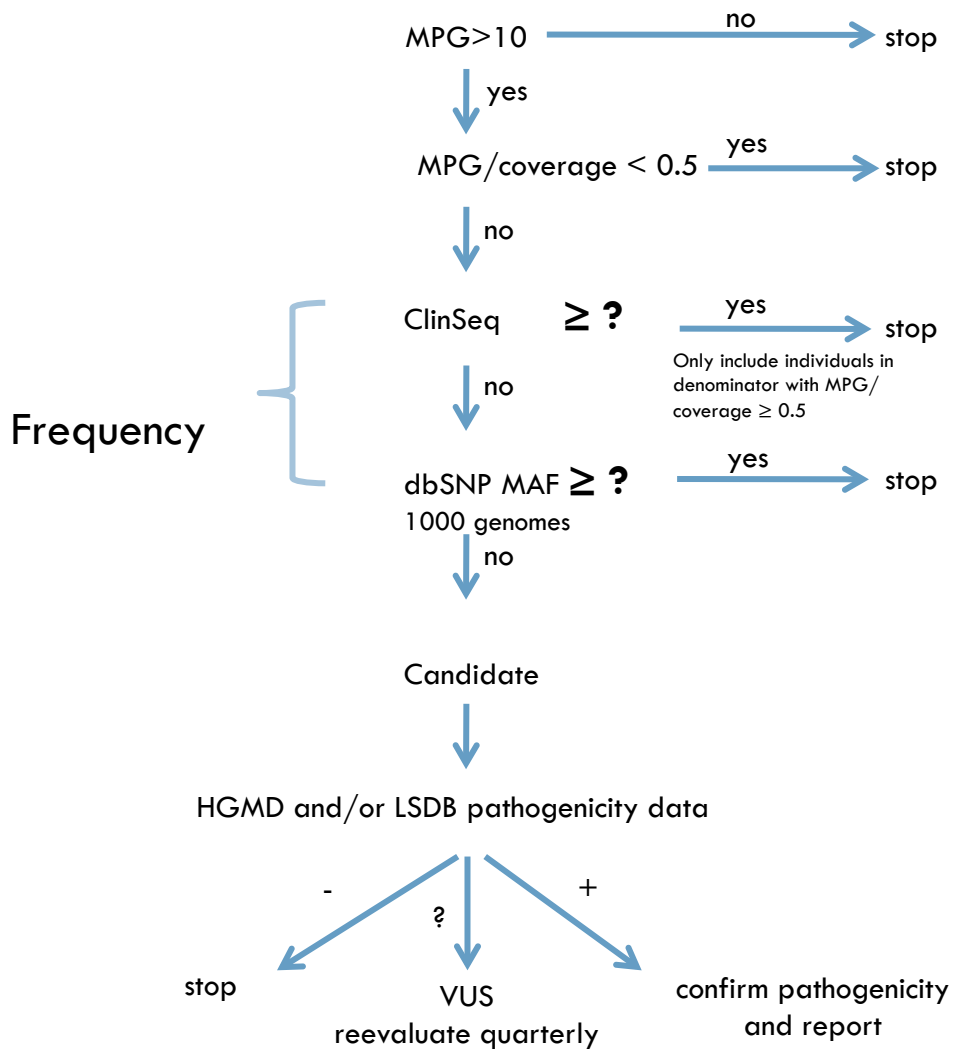
/Users/jjohnsto/Desktop/VarSifter/ClinSeqOncogenes01042011.t

Choose Gene File Filter

No Bed File Selected

Choose Bed File Filter

Framework for Variant Interpretation



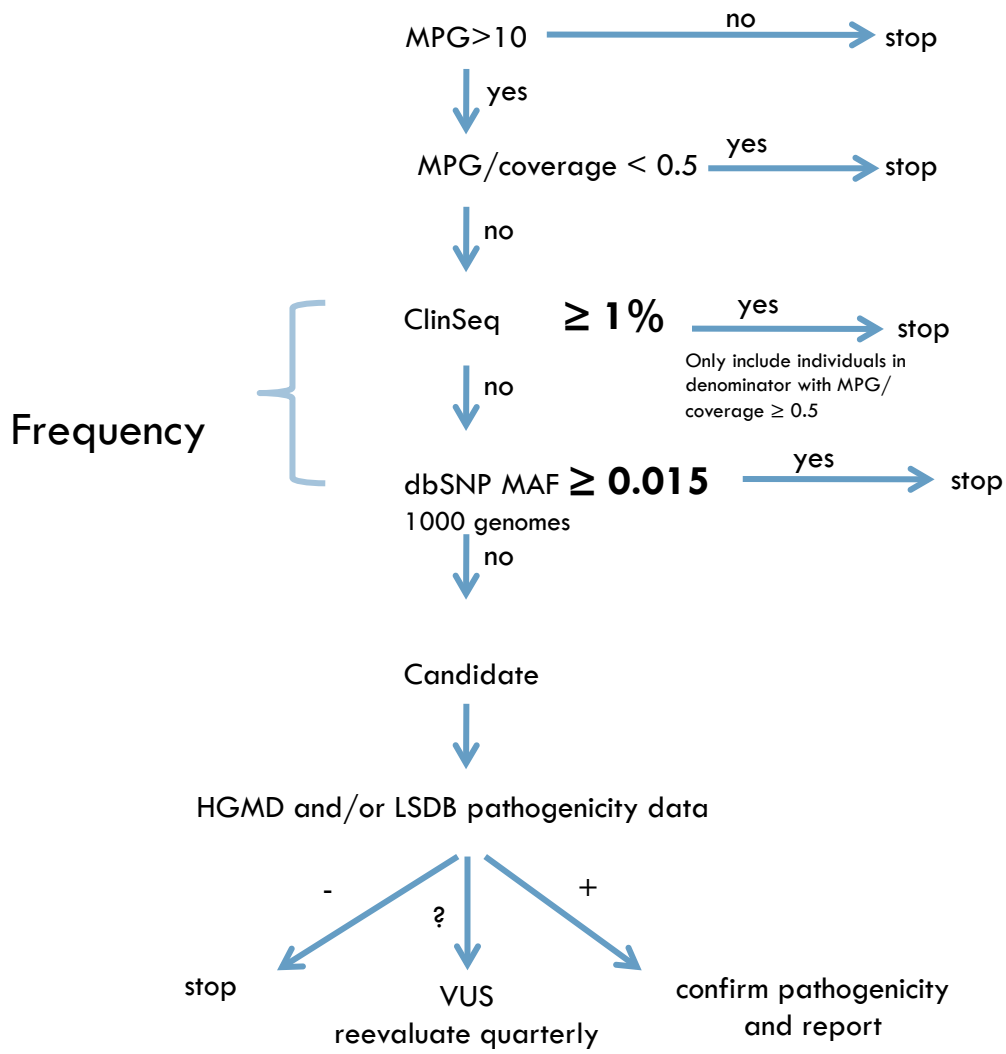
VarSifter –MPG/Coverage

VarSifter - /Users/jjohnsto/Desktop/VarSifter/VarSifter_1.0/572exomesnocontrol

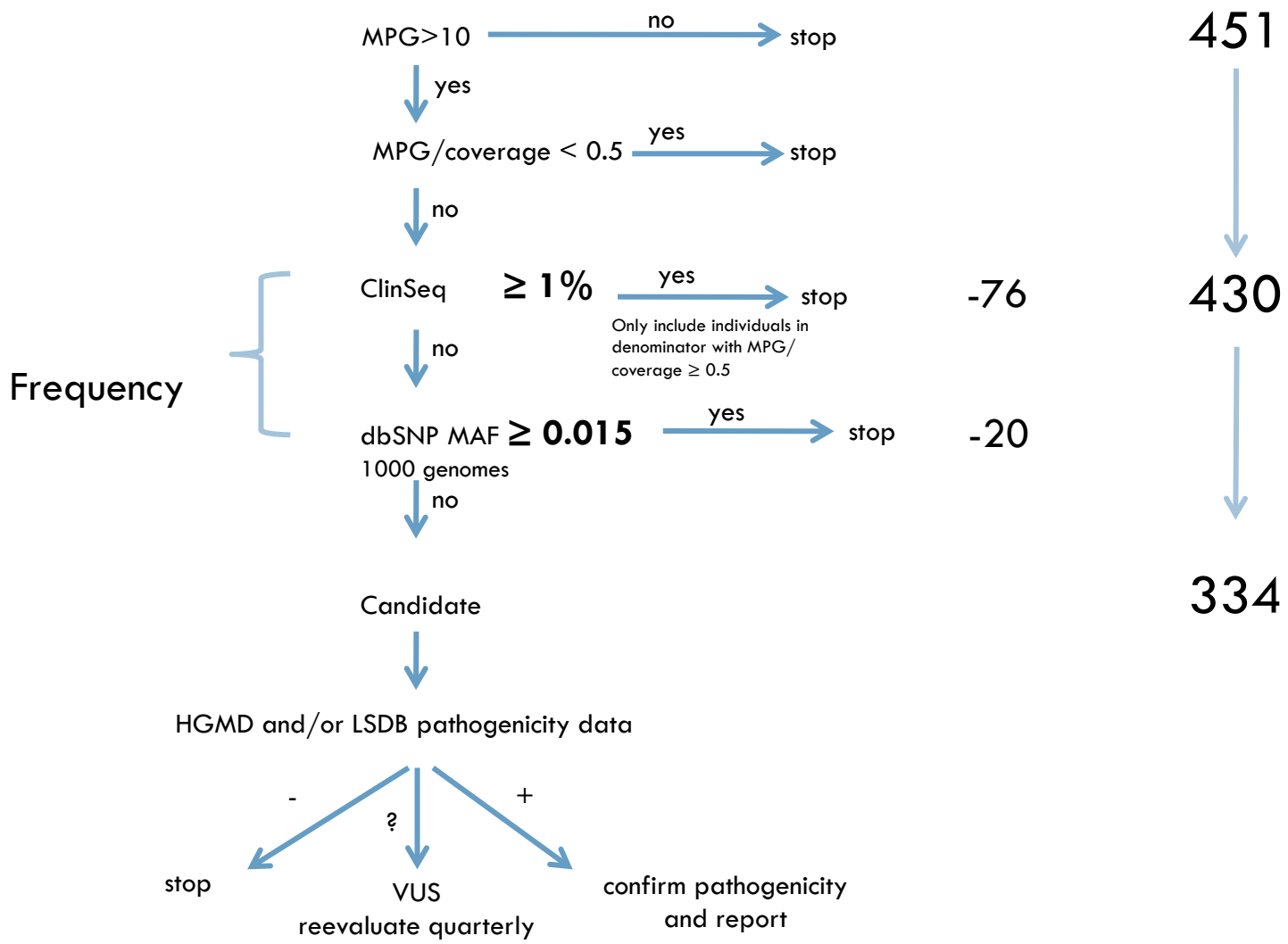
File View Help

Gene_name	ref_aa	aa_pos	var_aa	CSc_genotypes	CSc_homref	CSc_het	CSc_refallele	CSc_nonrefallele	CSmaf
BRCA2	S	1172	L	572	571	1	1143	1	0.000900
BRCA2	I	1188	V	572	571	1	1143	1	0.000900
BRCA2	G	1194	D	572	571	1	1143	1	0.000900
BRCA2	D	1420	Y	500	494	6	994	6	0.006000
BRCA2	K	1531	N	569	568	1	1137	1	0.000900
BRCA2	E	1593	D	572	571	1	1143	1	0.000900
BRCA2	S	1733	F	570	568	2	1138	2	0.001800
BRCA2	G	1771	D	562	561	1	1123	1	0.000900
BRCA2	NA	0	NA	551	550	1	1101	1	0.000900
BRCA2	I	1851	S	554	553	1	1107	1	0.000900
BRCA2	V	1852	F	555	554	1	1109	1	0.000900
BRCA2	D	1911	V	572	571	1	1143	1	0.000900
BRCA2	T	1915	M	571	551	20	1122	20	0.017500
BRCA2	NA	0	NA	572	569	3	1141	3	0.002600
BRCA2	R	2034	C	572	568	4	1140	4	0.003500
BRCA2	R	2108	C	570	569	1	1139	1	0.000900
BRCA2	V	2109	I	570	569	1	1139	1	0.000900
BRCA2	N	2113	S	568	567	1	1135	1	0.000900
BRCA2	H	2116	R	565	564	1	1129	1	0.000900
BRCA2	I	2285	V	496	495	1	991	1	0.001000
BRCA2	H	2440	R	572	571	1	1143	1	0.000900
BRCA2	V	2466	A	566	0	0	0	1132	-1.000...
BRCA2	R	2502	C	572	571	1	1143	1	0.000900
BRCA2	T	2515	I	572	571	1	1143	1	0.000900
BRCA2	A	2717	S	572	571	1	1143	1	0.000900

Cancer Variant Filtering



CS Cancer Filtering



Evaluation of Candidates

- Human Gene Mutation Database (HGMD)
- Locus Specific Database (LSDB)
  - Controls
  - Multiple reports
  - Functional data
  - Presence with other causative mutations
  - Segregation with disease (LD & linkage caveat)
    - *De novo* (assuming parentage)
    - Penetrance
    - Phenocopies

VarSifter - HGMD

File View Help

Chr	LeftFlank	RightFlank	Gene_name	HGMDids	HGMDdisease	HGMDtags	HGMDinGene	transcript
chr13	31810006	31810008	BRCA2	CM050182	Breast cancer ?	DM	y	uc001uub.1
chr13	31810053	31810055	BRCA2	-	-	-	y	uc001uub.1
chr13	31810072	31810074	BRCA2	-	-	-	y	uc001uub.1
chr13	31810749	31810751	BRCA2	CM003133	Breast and/or ovarian cancer ?	DM	y	uc001uub.1
chr13	31811084	31811086	BRCA2	-	-	-	y	uc001uub.1
chr13	31811270	31811272	BRCA2	-	-	-	y	uc001uub.1
chr13	31811689	31811691	BRCA2	-	-	-	y	uc001uub.1
chr13	31811803	31811805	BRCA2	CM041731	Breast and/or ovarian cancer ?	DM	y	uc001uub.1
chr13	31811970	31811979	BRCA2	-	-	-	y	uc001uub.1
chr13	31812043	31812045	BRCA2	-	-	-	y	uc001uub.1
chr13	31812045	31812047	BRCA2	-	-	-	y	uc001uub.1
chr13	31812223	31812225	BRCA2	-	-	-	y	uc001uub.1
chr13	31812235	31812237	BRCA2	CM010170	Breast cancer ?	DM	y	uc001uub.1
chr13	31812437	31812439	BRCA2	-	-	-	y	uc001uub.1
chr13	31812591	31812593	BRCA2	CM994286	Breast and/or ovarian cancer ?	DM	y	uc001uub.1
chr13	31812813	31812815	BRCA2	-	-	-	y	uc001uub.1
chr13	31812816	31812818	BRCA2	CM043917	Breast cancer ?	DM	y	uc001uub.1
chr13	31812829	31812831	BRCA2	-	-	-	y	uc001uub.1
chr13	31812838	31812840	BRCA2	CM022331	Breast and/or ovarian cancer	DM	y	uc001uub.1
chr13	31816705	31816707	BRCA2	-	-	-	y	uc001uub.1
chr13	31827308	31827310	BRCA2	-	-	-	y	uc001uub.1
chr13	31827386	31827388	BRCA2	CM960194	Breast cancer	DM	y	uc001uub.1
chr13	31828632	31828634	BRCA2	CM012590	Breast and/or ovarian cancer	DM	y	uc001uub.1
chr13	31828672	31828674	BRCA2	CM994287	Breast and/or ovarian cancer ?	DM	y	uc001uub.1
chr13	31835487	31835489	BRCA2	CM043984	Breast and/or ovarian cancer	DM	y	uc001uub.1
chr13	31835520	31835522	BRCA2	CM004715	Breast cancer	DM	y	uc001uub.1

Human Gene Mutation Database

http://nihlibrary.nih.gov/ResearchTools/Pages/Bioinformatics.aspx

HGMD start page

https://portal.biobase-international.com/hgmd/pro/start.php

POTS Main Page parkinson UCSC HGMD OMIM Home ...nce in Man nih Journals GeneClinics... Home Page

BIOBASE

HGMD Start

Gene

Mutation

Reference

Advanced

Information

Contact us

HGMD® Professional 2011.2

(Release date 24th June 2011)

HGMD®

To start a search choose the search option in the menu to the left.

This release comprises the following tables:

Data type:	Description:	Entries:
Missense/nonsense	Single base-pair substitutions in coding regions are presented in terms of a triplet change with an additional flanking base included if the mutated base lies in either the first or third position in the triplet.	63313
Splicing	Mutations with consequences for mRNA splicing are presented in brief with information specifying the relative position of the lesion with respect to a numbered intron donor or acceptor splice site. Positions given as positive integers refer to a 3' (downstream) location, negative integers refer to a 5' (upstream) location.	10653
Regulatory	Substitutions causing regulatory abnormalities are logged in with thirty nucleotides flanking the site of the mutation on both sides. The location of the mutation relative to the transcriptional initiation site, initiaton codon, polyadenylation site or termination codon is given.	2049
Small deletions	Micro-deletions (20 bp or less) are presented in terms of the deleted bases in lower case plus, in upper case, 10 bp DNA sequence flanking both sides of the lesion. The numbered codon is preceded in the given sequence by the caret character (^).	17807
Small insertions	Micro-insertions (20 bp or less) are presented in terms of the inserted bases in lower case plus, in upper case, 10 bp DNA sequence flanking both sides of the lesion. The numbered codon is preceded in the given sequence by the caret character (^).	7346
Small indels	Micro-indels (20 bp or less) are presented in terms of the deleted/inserted bases in lower case plus, in upper case, 10 bp DNA sequence flanking both sides of the lesion. The numbered codon is preceded in the given sequence by the caret character (^).	1671
Gross deletions	Information regarding the nature and location of each lesion is logged in narrative form because of the extremely variable quality of the original data reported.	7383
Gross insertions/duplications	Information regarding the nature and location of each lesion is logged in narrative form because of the extremely variable quality of the original data reported.	1583
Complex rearrangements	Information regarding the nature and location of each lesion is logged in narrative form because of the extremely variable quality of the original data reported.	1089
Repeat variations	Information regarding the nature and location of each lesion is logged in narrative form because of the extremely variable quality of the original data reported.	353
	Mutation total	**113247**
	With chromosomal coordinates (NCBI37.2/hg19)	90487
	With chromosomal coordinates (NCBI36.3/hg18 - REMOVAL 2012.1)	90480
	With HGVS descriptions	91159

HGMD - Search

BIOBASE

HGMD® Professional 2011.2
(Release date 24th June 2011)

HGMD®

HGMD Start

Gene
Mutation
Reference
Advanced

Information
Contact us

GENE SEARCH

Search terms [Help]

Boolean operators are [+ - * ""]

Use Boolean
Non-boolean

Concise output
Detailed output

Search fields [Help]

All fields | Submit query | Clear

APC
Exact gene symbol only

Gene summary | Submit query | Clear

Browse Genes

Alphabetical	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z
Chromosome	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X		Y	
Pre-queried	Random gene entry					Newly added genes					Newly updated genes						Mutation totals					Gene ontology				

HGMD – Mutation Page

BIOBASE

HGMD® Professional 2011.2
(Release date 24th June 2011)

HGMD®

HGMD Start
Gene
Mutation
Reference
Advanced
Information
Contact us

HGMD Accession Number CM004715

Disease/phenotype	Gene symbol	Codon change	Amino acid change	Codon number	Reference
Breast cancer	BRCA2	tGTT-ATT	Val-Ile	2728	Sinilnikova (1999) International journal of cancer. Journal international du cancer (*Int J Cancer*) **83:** p325. PubMed: 10399947 Kuznetsov (2008) *Nat Med* **14:** p.875 [Functional characterisation] PubMed: 18607349 Kuusisto (2011) *Breast Cancer Res* **13:** p.R20 [Additional report] PubMed: 21356067

The V2728I substitution does not exhibit a shift in polarity and displays an increase in Kyte-Doolittle hydrophobicity from 4.2 to 4.5. Approximately 0.67% of missense mutations in HGMD are Val-Ile.

Extra information		
Genomic sequence (build 37.2 - NEW)	ATTATTGAACTTACAGATGGGTGGTATGCT(G-A)TTAAGGCCCAGTTAGATCCTCCCCTCTTAG	
Amino acid sequence	IIELTDGWYAVKAQLDPPLLA	Amino acid alignment
Genomic coordinate (build 37.2 - NEW)	Chr 13: 32937521; Chr 13: 32937521-32937523	
Genomic coordinate (build 36.3 - REMOVAL 2012.1)	Chr 13: 31835521	
HGVS nomenclature	NM_000059.3: c.8182G>A; NP_000050.2: p.V2728I	
dbSNP number	rs28897749	
Variant class	Disease causing mutation	
Comments	No comments	
CpG	No	

Amino acid comparison table		
Trait	Val (V)	Ile (I)
Amino acid name	valine	isoleucine
Polarity/charge	non-polar	non-polar
pH	neutral	neutral
Residue weight	99	113
Hydrophobicity score	4.2	4.5
Hydrophilicity score	-1.5	-1.8
Secondary structure propensity	α former / strong β former	α former / strong β former
Grantham difference	29	
Sift prediction	TOLERATED	

HGMD – Primary Literature

Display Settings: Abstract

Send to:

Read free full text at BioMed Central | FREE full text article in PubMed Central

Breast Cancer Res. 2011 Feb 28;13(1):R20. [Epub ahead of print]

Screening for BRCA1, BRCA2, CHEK2, PALB2, BRIP1, RAD50, and CDH1 mutations in high-risk Finnish BRCA1/2-founder mutation-negative breast and/or ovarian cancer individuals.

Kuusisto KM, Bebel A, Vihinen M, Schleutker J, Sallinen SL.

Department of Pediatrics, Genetics Outpatient Clinic, Tampere University Hospital, Biokatu 8, Tampere, 33520, Finland. Satu-Leena.Sallinen@pshp.fi.

Abstract

ABSTRACT:

INTRODUCTION: Two major high-penetrance breast cancer genes, BRCA1 and BRCA2, are responsible for approximately 20% of hereditary breast cancer (HBC) cases in Finland. Additionally, rare mutations in several other genes that interact with BRCA1 and BRCA2 increase the risk of HBC. Still, a majority of HBC cases remain unexplained which is challenging for genetic counseling. We aimed to analyze additional mutations in HBC-associated genes and to define the sensitivity of our current BRCA1/2 mutation analysis protocol used in genetic counseling.

METHODS: Eighty-two well-characterized, high-risk hereditary breast and/or ovarian cancer (HBOC) BRCA1/2-founder mutation-negative Finnish individuals, were screened for germline alterations in seven breast cancer susceptibility genes, BRCA1, BRCA2, CHEK2, PALB2, BRIP1, RAD50, and CDH1. BRCA1/2 were analyzed by multiplex ligation-dependent probe amplification (MLPA) and direct sequencing. CHEK2 was analyzed by the high resolution melt (HRM) method and PALB2, RAD50, BRIP1 and CDH1 were analyzed by direct sequencing. Carrier frequencies between 82 (HBOC) BRCA1/2-founder mutation-negative Finnish individuals and 384 healthy Finnish population controls were compared by using Fisher's exact test. In silico prediction for novel missense variants effects was carried out by using Pathogenic-Or-Not -Pipeline (PON-P).

RESULTS: Three previously reported breast cancer-associated variants, BRCA1 c.5095C > T, CHEK2 c.470T > C, and CHEK2 c.1100delC, were observed in eleven (13.4%) individuals. Ten of these individuals (12.2%) had CHEK2 variants, c.470T > C and/or c.1100delC. Fourteen novel sequence alterations and nine individuals with more than one non-synonymous variant were identified. One of the novel variants, BRCA2 c.72A > T (Leu24Phe) was predicted to be likely pathogenic in silico. No large genomic rearrangements were detected in BRCA1/2 by multiplex ligation-dependent probe amplification (MLPA).

CONCLUSIONS: In this study, mutations in previously known breast cancer susceptibility genes can explain 13.4% of the analyzed high-risk BRCA1/2-negative HBOC individuals. CHEK2 mutations, c.470T > C and c.1100delC, make a considerable contribution (12.2%) to these high-risk individuals but further segregation analysis is needed to evaluate the clinical significance of these mutations before applying them in clinical use. Additionally, we identified novel variants that warrant additional studies. Our current genetic testing protocol for 28 Finnish BRCA1/2-founder mutations and protein truncation test (PTT) of the largest exons is sensitive enough for clinical use as a primary screening tool.

PMID: 21356067 [PubMed - as supplied by publisher] PMCID: PMC3109589 **Free PMC Article**

Related citations

Spectrum of mutations in BRCA1, BRCA2, CHEK2, and TP53 in families at high risk of br [JAMA. 2006]

Selected Aspects of Molecular Diagnostics of Constitutional Alte [Hered Cancer Clin Pract. 2006]

BRCA1/BRCA2 rearrangements and CHEK2 common mutation: [Breast Cancer Res Treat. 2008]

Review Pitfalls and caveats in BRCA sequencing. [Ultrastruct Pathol. 2006]

Review Breast cancer genetics in African Americans. [Cancer. 2003]

See reviews...

See all...

Related information

Related Citations

OMIM (calculated)

References for this PMC Article

Free in PMC

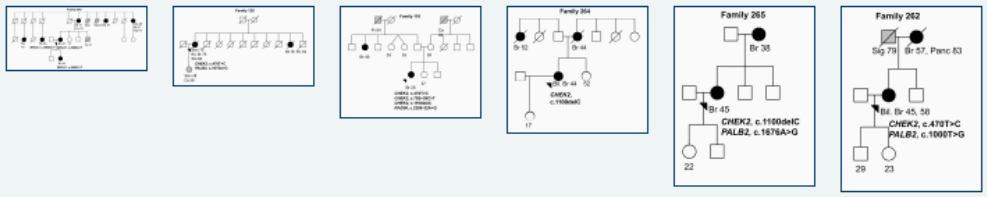
Recent activity

Turn Off Clear

Screening for BRCA1, BRCA2, CHEK2, PALB2, BRIP1, RAD50, and CDH1 muti PubMed

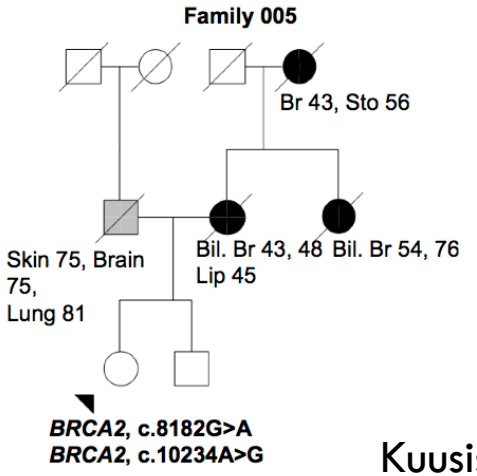
See more...

Images from this publication. See all images (8) Free text



Primary Literature

The role of the three *BRCA2* missense variants, c.8182G > A, c.9976A > T, and c.10234A > G, in HBOC risk, is uncertain [31-33]. All three heterozygous variants were observed in two healthy women with a history of BrCa, one carrying the c.9976A > T variant and the other both the c.8182G > A and c.10234A > G variants (Tables 2 and 3, Figure 8, Family 005). At this stage, because we only have samples from the index individuals, no segregation analyses of the variants have been performed, but these families clearly warrant additional studies.



Kuusisto et al.

Locus-Specific DataBases

Locus Specific Database list

Based on various online resources and direct submissions of LSDBs

LSDB list | Submit new LSDB | Log in

Locus Specific Mutation Databases

IMPORTANT NOTE: Genes are in order of HUGO APPROVED GENE DESIGNATION, not alias. e.g. "p53" will be found under "TP53" while "CD40L" or "TNFSF5" will be found under "CD40LG" and so on.

If you wish to add a gene you can do so here.

Please select the first letter of the Gene:

A B C D E F G H I J K L M N O P Q R S T U V W X Y Z

Or, specify the HGNC Gene Symbol:

Go to this gene »

397 public entries

Gene Symbol	Database	Curators	Software
A2M alpha-2-macroglobulin	Mendelian genes http://grenada.lumc.nl/LOVD2/mendelian_genes/home.php?select_db=A2M	Curator vacancy ?	LOVD 2.X
A4GALT alpha 1,4-galactosyltransferase	Mendelian genes http://grenada.lumc.nl/LOVD2/mendelian_genes/home.php?select_db=A4GALT	Curator vacancy ?	LOVD 2.X
AAAS achalasia, adrenocortical insufficiency, alacrimia (Allgrove, triple-A)	Mendelian genes http://grenada.lumc.nl/LOVD2/mendelian_genes/home.php?select_db=AAAS	Curator vacancy ?	LOVD 2.X
AANAT arylalkylamine N-acetyltransferase	Mendelian genes http://grenada.lumc.nl/LOVD2/mendelian_genes/home.php?select_db=AANAT	Curator vacancy ?	LOVD 2.X
AARS alanyl-tRNA synthetase	LOVD - Leiden Open Variation Database https://grenada.lumc.nl/LOVD2/shared1/home.php?select_db=AARS	Curator Vacancy *Leiden University Medical Center*	LOVD 2.X

http://www.hgvs.org/dblist/glsdb.html

http://grenada.lumc.nl/LSDB_list/lsdb.phpaction=view_all&symbol_start=M

LSDB

Locus Specific Database list

http://grenada.lumc.nl/LSDB_list/lsdb.php?action=view_all&symbol_start=A

POTS Main Page parkinson UCSC HGMD OMIM Home ...nce in Man nih Journals GeneClinics... Home Page

	http://fishmap2.igib.res.in/home.php?select_db=APBB2		
APC Adenomatous Polyposis Coli	Colon cancer gene variant databases http://chromium.liacs.nl/LOVD2/colon_cancer/home.php?select_db=APC	Stefan Aretz & Waltraut Friedl & Kirsten Wöllner *Institute of Human Genetics, Bonn* *Institute of Human Genetics* *Institute of Human Genetics, Bonn*	LOVD 2.X
APC	Zhejiang University Center for Genetic and Genomic Medicine http://www.genomed.org/LOVD/home.php?select_db=APC		LOVD 2.X
APC	Zhejiang University Center for Genetic and Genomic Medicine (ZJU-CGGM) http://genomed.org/LOVD/HNPCC/home.php?select_db=APC		LOVD 2.X
APC	LOVD - Leiden Open Variation Database https://australianhumanvariomedatabase.arcs.org.au/home.php?select_db=APC		LOVD 2.X
APC adenomatous polyposis coli	The UMD APC mutations database http://www.umd.be/APC/	Christophe Beroud, Laboratoire de génétique Moléculaire et Chromosomique, Montpellier, France Thierry Soussi INSERM, Hopital Necker Enfants Malades, Paris	UMD
APC adenomatous polyposis coli	Zhejiang University-Adinovo Center APC Database http://china-hvp.org/LOVD/?select_db=APC	Ming Qi, PhD, FACMG, Peikuan Cong and Yudong Gao	LOVD 2.X
APC adenomatous polyposis coli	The APC Mutation Database http://fap.taenzer.me	Dr. Stefan Aretz and Dr. Waltraut Friedl	Unknown

LOVD Gene homepage

General information	
Gene name	Adenomatous Polyposis Coli
Gene symbol	**APC**
Chromosome Location	5q22.2
Database location	chromium.liacs.nl
Curator	Kirsten Wöllner, Stefan Aretz and Waltraut Friedl
PubMed references	View all (unique) PubMed references in the APC database
Date of creation	September 09, 2009
Last update	September 21, 2011
Version	**APC110921**
Add sequence variant	Submit a sequence variant
First time submitters	Register here
Reference sequence file	coding DNA reference sequence for describing sequence variants
Genomic refseq ID	NG_008481.1
Transcript refseq ID	NM_000038.4
Exon/intron information	Exon/intron information table
Total number of unique DNA variants reported	**1191**
Total number of individuals with variant(s)	**3782**
Total number of variants reported	**3792**
Subscribe to updates of this gene	
NOTE	Aliases for APC are; BTPS2, DP2, DP2.5, DP3, GS

Graphical displays and utilities	
Summary tables	Summary of all sequence variants in the APC database, sorted by type of variant (with graphical displays and statistics)
Reading-frame checker	The Reading-frame checker generates a prediction of the effect of whole-exon changes
UCSC Genome Browser	Show variants in the UCSC Genome Browser (compact view)
Ensembl Genome Browser	Show variants in the Ensembl Genome Browser
NCBI Sequence Viewer	Show distribution histogram of variants in the NCBI Sequence Viewer

Sequence variant tables	
Unique sequence variants	Listing of all unique sequence variants in the APC database, without patient data
Complete sequence variant listing	Listing of all sequence variants in the APC database
Variants with no known pathogenicity	Listing of all APC variants reported to have no noticeable phenotypic effect (note: excluding variants of unknown effect)

Search the database	
By type of variant	View all sequence variants of a certain type
Simple search	Query the database by selecting the most important variables (exon number, type of variant, disease phenotype)
Advanced search	Query the database by selecting a combination of variables
Based on patient origin	View all variants based on your patient origin search terms
Search through hidden entries	Find the number of variant entries in the database (including hidden entries) matching your search terms.

LSDB

View unique variants | Search unique variants | View all contents | Full database search | Variant listing based on patient origin | Database statistics | Switch gene

LOVD - Variant listings

Unhide all columns | Hide Specific Columns | Hide all columns

About this overview [Show]

3783 public entries

100 entries per page

Path.	Exon	Codon_nr	DNA change	DNA_reported	RNA change	Protein	Type	Cons_predicted	DB-ID	Variant remarks	Origin	Variant reference
-/?	00	-	c.-?C>G	-47306C>G (5' of ATG)	-	-	-	-	APC_00415	numbering 5' of ATG	-	-
-/?	00	-	c.?C>T	-47287C>T	-	-	-	-	APC_00416	numbering 5' of ATG	-	-
-/?	00	-	c.?insG	-47307insG	-	-	-	-	APC_00417	numbering 5' of ATG	-	-
-/?	00	-	c.?T>G	-47408T>G	-	-	-	-	APC_00418	numbering 5' of ATG	-	-
+/?	01_15+promoter	del	cytogeneticdeletion	-	-	-	deletion, large	deletion, large	APC_00200	cytogenetic deletion	unknown	Raedle et al. 2001
+/?	01_15+promoter	del	cytogeneticdeletion	-	-	-	deletion, large	deletion, large	APC_00200	cytogenetic deletion	de novo	Aretz et al. 2005
+/?	01_15+promoter	del	cytogeneticdeletion	-	-	-	deletion, large	deletion, large	APC_00200	cytogenetic deletion	de novo	Aretz et al. 2005
+/?	01_15+promoter	del	cytogeneticdeletion	-	-	-	deletion, large	deletion, large	APC_00200	cytogenetic deletion	unknown	Aretz and Friedl (unpublished)
+/?	01	24	c.70C>T	-	-	p.Arg24X	substitution, base pair	nonsense	APC_00551	-	-	Kanter-Smoler et al. 2008
+/?	01_15	del	g.26940-?_133343+?del	-	-	-	deletion, large	deletion, large	APC_00587	-	familial	Kanter-Smoler et al. 2008
+/?	01+promoter	del	g.35041-?_52505+?del	-	-	-	deletion, large	deletion, large	APC_00526	-	familial	Aretz et al. 2005
+/?	01+promoter	del	g.35041-?_52505+?del	-	-	-	deletion, large	deletion, large	APC_00526	-	familial	Aretz et al. 2005
+/?	01+promoter	del	g.35041-?_52505+?del	-	-	-	deletion, large	deletion, large	APC_00526	-	familial	Aretz et al. 2005
+/?	01_05+promoter	del	g.35041-?_78383+?del	-	-	-	deletion, large	deletion, large	APC_00527	-	familial	Aretz et al. 2005

LSDB

View unique variants | Search unique variants | View all contents | Full database search | Variant listing based on patient origin | Database statistics | Switch gene

LOVD - Variant listings

Unhide all columns | Hide Specific Columns | Hide all columns

About this overview [Show]

3783 public entries

100 entries per page

Path.	Exon	Codon_nr	DNA change	DNA_reported	RNA change	Protein	Type	Cons_predicted	DB-ID	Variant remarks	Origin	Variant reference
-/?	00	-	c.-?C>G	-47306C>G (5' of ATG)	-	-	-	-	APC_00415	numbering 5' of ATG	-	-
-/?	00	-	c.?C>T	-47287C>T	-	-	-	-	APC_00416	numbering 5' of ATG	-	-
-/?	00	-	c.?insG	-47307insG	-	-	-	-	APC_00417	numbering 5' of ATG	-	-
-/?	00	-	c.?T>G	-47408T>G	-	-	-	-	APC_00418	numbering 5' of ATG	-	-
+/?	01_15+promoter	del	cytogeneticdeletion	-	-	-	deletion, large	deletion, large	APC_00200	cytogenetic deletion	unknown	Raedle et al. 2001
+/?	01_15+promoter	del	cytogeneticdeletion	-	-	-	deletion, large	deletion, large	APC_00200	cytogenetic deletion	de novo	Aretz et al. 2005
+/?	01_15+promoter	del	cytogeneticdeletion	-	-	-	deletion, large	deletion, large	APC_00200	cytogenetic deletion	de novo	Aretz et al. 2005
+/?	01_15+promoter	del	cytogeneticdeletion	-	-	-	deletion, large	deletion, large	APC_00200	cytogenetic deletion	unknown	Aretz and Friedl (unpublished)
+/?	01	24	c.70C>T	-	-	p.Arg24X	substitution, base pair	nonsense	APC_00551	-	-	Kanter-Smoler et al. 2008
+/?	01_15	del	g.26940-?_133343+?del	-	-	-	deletion, large	deletion, large	APC_00587	-	familial	Kanter-Smoler et al. 2008
+/?	01+promoter	del	g.35041-?_52505+?del	-	-	-	deletion, large	deletion, large	APC_00526	-	familial	Aretz et al. 2005
+/?	01+promoter	del	g.35041-?_52505+?del	-	-	-	deletion, large	deletion, large	APC_00526	-	familial	Aretz et al. 2005
+/?	01+promoter	del	g.35041-?_52505+?del	-	-	-	deletion, large	deletion, large	APC_00526	-	familial	Aretz et al. 2005
+/?	01_05+promoter	del	g.35041-?_78383+?del	-	-	-	deletion, large	deletion, large	APC_00527	-	familial	Aretz et al. 2005

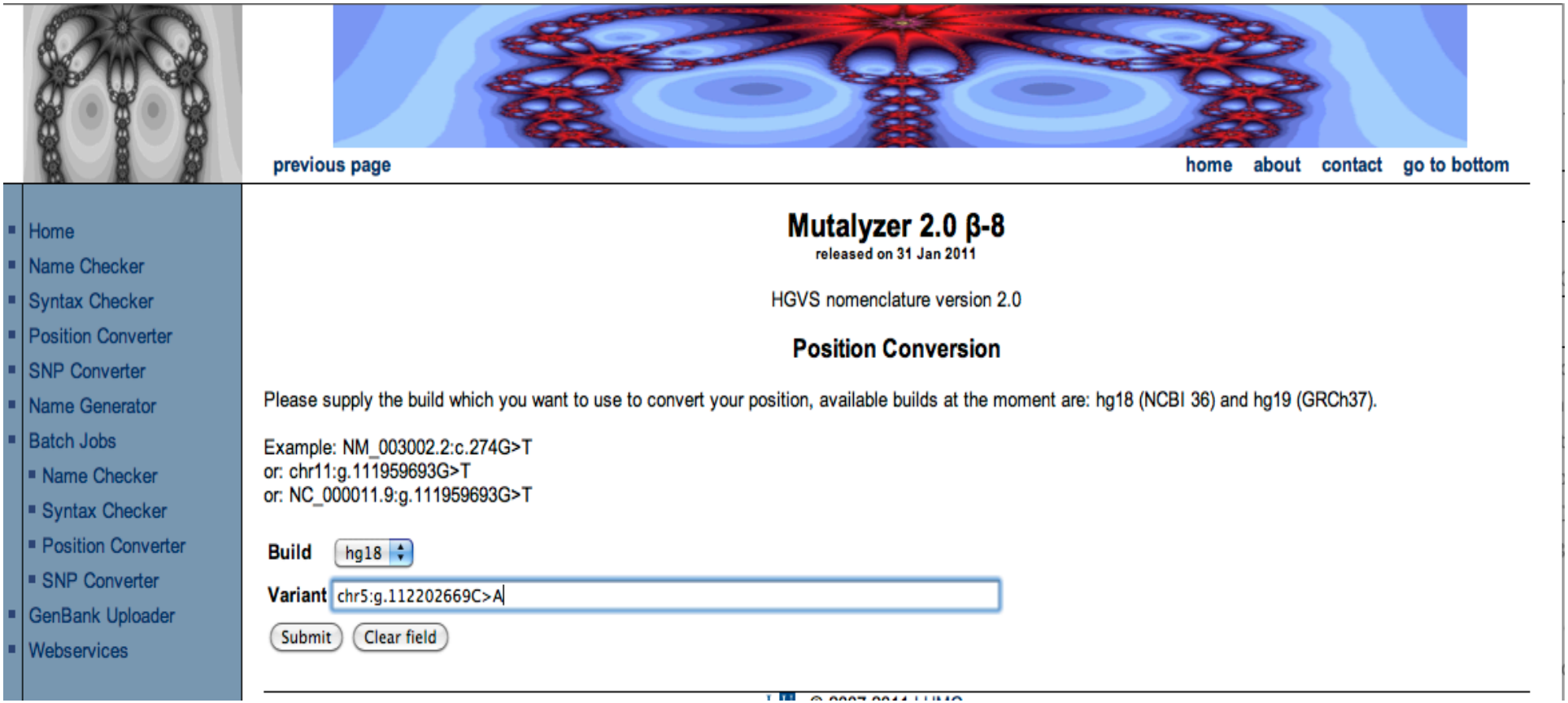
Annotation Source - VarSifter

VarSifter – /Users/jjohnsto/Desktop/VarSifter/VarSifter_1.0/572exomesnocontrols_cod.vs

File View Help

Chr	LeftFlank	RightFlank	type	Gene_name	ref_aa	aa_pos	var_aa	dbID	HGMDids	HGMDdisease	s
chr5	112204359	112204361	Non-synonymous	APC	L	1724	V	-	-	-	
chr5	112203573	112203575	Non-synonymous	APC	K	1179	E	-	-	-	
chr5	112203562	112203568	DIV-c	APC	NA	0	NA	-	-	-	
chr5	112203561	112203563	Non-synonymous	APC	[illegible]	1458	S	-	-	-	
chr5	112203138	112203140	Non-synonymous	APC	E	1317	Q	rs1801166(C,G)	CM980089	"Colorectal cancer, predisp...	
chr5	112203109	112203111	Non-synonymous	APC	I	1024	K	rs1801155(A,T)	CM970090	"Colorectal cancer, predisp...	
chr5	112202773	112202775	Non-synonymous	APC	E	912	S	-	-	-	
chr5	112202668	112202670	Non-synonymous	APC	T	877	K	-	CM080043	Colorectal adenoma	
chr5	112202649	112202661	DIV-c	APC	NA	0	NA	-	-	-	
chr5	112202575	112202577	Non-synonymous	APC	L	1129	S	-	CM045407	Adenomatous polyposis coli ?	
chr5	112202541	112202543	Non-synonymous	APC	N	1118	D	-	CM045405	Adenomatous polyposis coli	
chr5	112202438	112202440	Non-synonymous	APC	D	1083	E	-	-	-	
chr5	112202362	112202364	Non-synonymous	APC	D	1058	G	-	-	-	
chr5	112201866	112201868	Non-synonymous	APC	E	893	K	-	CM013242	Adenomatous polyposis coli	
chr5	112201797	112201799	Non-synonymous	APC	P	870	S	rs33974176(C,T)	CM080070	Colorectal adenoma	
chr5	112201627	112201629	Non-synonymous	APC	N	813	S	-	-	-	
chr5	112201486	112201488	Non-synonymous	APC	A	766	V	-	-	-	
chr5	112201393	112201395	Non-synonymous	APC	A	735	V	-	-	-	
chr5	112192455	112192457	Non-synonymous	APC	I	544	T	-	-	-	
chr5	112191579	112191581	Non-synonymous	APC	S	535	F	-	-	-	
chr5	112190789	112190791	Non-synonymous	APC	R	499	G	-	CM930023	Adenomatous polyposis coli	
chr5	112182867	112182869	Non-synonymous	APC	R	414	C	-	CM910030	Adenomatous polyposis coli	
chr5	112156122	112156124	Stop	APC	E	243	*	-	-	-	
chr5	112156090	112156092	Non-synonymous	APC	R	232	Q	-	-	-	
chr5	112144460	112144462	Non-synonymous	APC	Q	203	E	-	CM086466	Adenomatous polyposis coli	
chr5	112130983	112130985	Non-synonymous	APC	E	140	D	-	-	-	
chr5	112130951	112130953	Non-synonymous	APC	S	130	G	-	CM087822	"Colorectal cancer, severe...	
chr5	112130942	112130944	Non-synonymous	APC	S	127	G	-	CM024498	Adenomatous polyposis coli ?	
chr5	112130880	112130882	Non-synonymous	APC	R	106	H	-	CM080058	Adenomatous polyposis coli	
chr5	112118538	112118540	Non-synonymous	APC	M	18	K	-	-	-	

Check Mutation Nomenclature



Mutalyzer http://www.mutalyzer.nl/2.0/

Check Mutation Nomenclature

- Home
- Name Checker
- Syntax Checker
- Position Converter
- SNP Converter
- Name Generator
- Batch Jobs
  - Name Checker
  - Syntax Checker
  - Position Converter
  - SNP Converter
- GenBank Uploader
- Webservices

- Help
- FAQ
- Exercise
- Disclaimer
- Feedback

- External Links
  - Human Gene Nomenclature
  - HGVS Variation Nomenclature
  - HGVS Nomenclature Extension Proposal
  - LOVD
  - Mutalyzer 1.0.4

Mutalyzer 2.0 β-8
released on 31 Jan 2011

HGVS nomenclature version 2.0

Position Conversion

Please supply the build which you want to use to convert your position, available builds at the moment are: hg18 (NCBI 36) and hg19 (GRCh37).

Example: NM_003002.2:c.274G>T
or: chr11:g.111959693G>T
or: NC_000011.9:g.111959693G>T

Build hg18

Variant chr5:g.112202669C>A

Submit Clear field

Output:

Chromosomal Variant:

NC_000005.8:g.112202669C>A

Found transcripts in mutation region:

APC
NM_001127510.2:c.3479C>A
NM_001127511.2:c.3425C>A
NM_000038.5:c.3479C>A
NM_000038.4:c.3479C>A
NM_001127511.1:c.3479C>A
NM_001127510.1:c.3479C>A

Check Mutation Nomenclature

- Home
- Name Checker
- Syntax Checker
- Position Converter
- SNP Converter
- Name Generator
- Batch Jobs
  - Name Checker
  - Syntax Checker
  - Position Converter
  - SNP Converter
- GenBank Uploader
- Webservices

- Help
- FAQ
- Exercise
- Disclaimer
- Feedback

- External Links
  - Human Gene Nomenclature
  - HGVS Variation Nomenclature

Mutalyzer 2.0 β-8
released on 31 Jan 2011

HGVS nomenclature version 2.0

Name Generator

Reference

Reference: NM_000038.4:c.3479(— Reference incorrect: should be of the format "NM_002001.2"

Sequence Type: Coding DNA

Gene Symbol:

Variant 1

Mutation Type: Substitution

Start Position: Start Position required. Start Position incorrect: position notation help

Deleted Sequence: Deleted Sequence incorrect: substitution must consist of a single nucleotide / amino acid. Deleted Sequence incorrect: must consist of nucleotides [ACTG]

Inserted Sequence: Inserted Sequence incorrect: substitution must consist of a single nucleotide / amino acid. Inserted Sequence incorrect: must consist of nucleotides [ACTG]

* This field is optional

Add Variant | Clear Form

Constructed HGVS Name - Please click the link to check with the Name Checker

NM_000038.4:c.3479C>A:c.>

Check Mutation Nomenclature

- Home
- Name Checker
- Syntax Checker
- Position Converter
- SNP Converter
- Name Generator
- Batch Jobs
  - Name Checker
  - Syntax Checker
  - Position Converter
  - SNP Converter
- GenBank Uploader
- Webservices

- Help
- FAQ
- Exercise
- Disclaimer
- Feedback

- External Links
  - Human Gene Nomenclature
  - HGVS Variation Nomenclature
  - HGVS Nomenclature Extension Proposal
  - LOVD
  - Mutalyzer 1.0.4

Mutalyzer 2.0 β-8
released on 31 Jan 2011

HGVS nomenclature version 2.0

Name checker

Please insert the mutation name using the HGVS format:
<Accession Number>.<version number>(<Gene symbol>):<sequence type>.<mutation>

Example: AB026906.1:c.274G>T

NM_000038.4:c.3479C>A

Submit Clear field

Mutalyzer output:

0 Errors, 0 Warnings.

Overview of the raw variants:

Raw variant 1: substitution at 3564
CAGCATGAAGAAGAAGAGAGACCAA C AAATTATAGCATAAAATATAATGAA
CAGCATGAAGAAGAAGAGAGACCAA A AAATTATAGCATAAAATATAATGAA

Description relative to transcription start:
(Not for use in LSDBs in case of protein-coding transcripts).

NM_000038.4:n.3564C>A

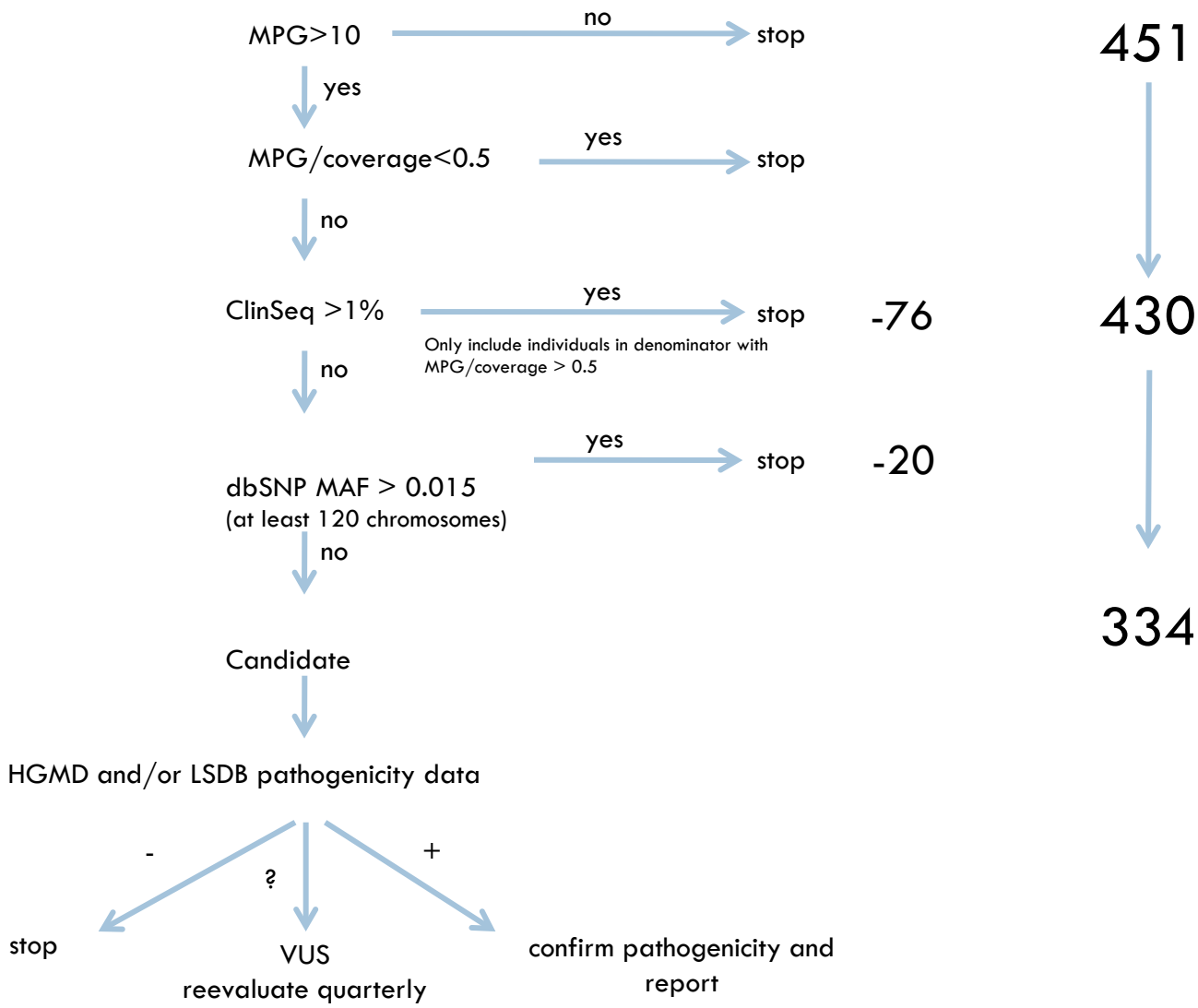
Affected transcripts:

NM_000038.4(APC_v001):c.3479C>A

Affected proteins:

NM_000038.4(APC_i001):p.(Thr1160Lys)

CS Cancer Filtering



International Association for Research on Cancer (IARC) Pathogenicity Scale

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 signi¢cance	0.001-0.049
1	Not pathogenic or of no clinical significance	<0.001
0	Insufficient information i.e. did not pass quality filter	

Plon et al. Human Mut. (2008)

Variant Decision Examples

APC chr5	112,202,668-112,202,670	NM_000038.4 c.3479C>A p.Thr1160Lys	3	1 in 258	-	CM080043 DM	Not in LSDB	two patients with CRA; rare variant hypothesis[4]
APC chr5	112,201,627-112,201,629	NM_000038.4 c.2438A>G p.Asn813Ser	3	1 in 258	-	-	Not in LSDB	-
BRCA2 chr13	31,812,437-31,812,439	NM_000059.3 c.5946del p.Ser1982ArgfsX22	5	1 in 258	-	-	In LSDB[1] (7X): (?) BIC[2] (>1000X): clinically important	↑frameshift; ↑cosegregation[11]
FLCN chr17	17,059,322-17,059,324	NM_144997.5 c.1333G>A p.Ala445Thr	1L	1 in 255	rs41419545(C,T) no frequency data	-	In LSDB (1X): (-) https://grenada.lumc.nl/LSDB2/shared1/home.php?select_db=FLCN	-
MSH6 chr2	47,879,811-47,879,813	NM_000179.2 c.1186C>G p.Leu396Val	2	1 in 258	rs2020908(C,G) MAF 0.010 in 192 chr	CM101608 probable FP	In LSDB (19X): (?)	↓3/200 control individuals; ↓no significant mismatch repair defect[24]
MUTYH chr1	45,570,704-45,570,706	NM_001048171.1 c.691C>T p.Arg231Cys	4	1 in 225	-	CM055444 DM	in LSDB (5X): (?)	↑biallelic; ↑MSH6 binding domain; 0/80 control individuals[30]
RET chr10	42,933,913-42,933,915	NM_020630.4 c.2372A>T p.Tyr791Phe	2	2 in 258	-	CM971306 DM	In LSDB (7X)	↓found in unaffected relatives; ↓found with causative mutation; 8/1000 control individuals[34]; reported in Hirschsprung[35]

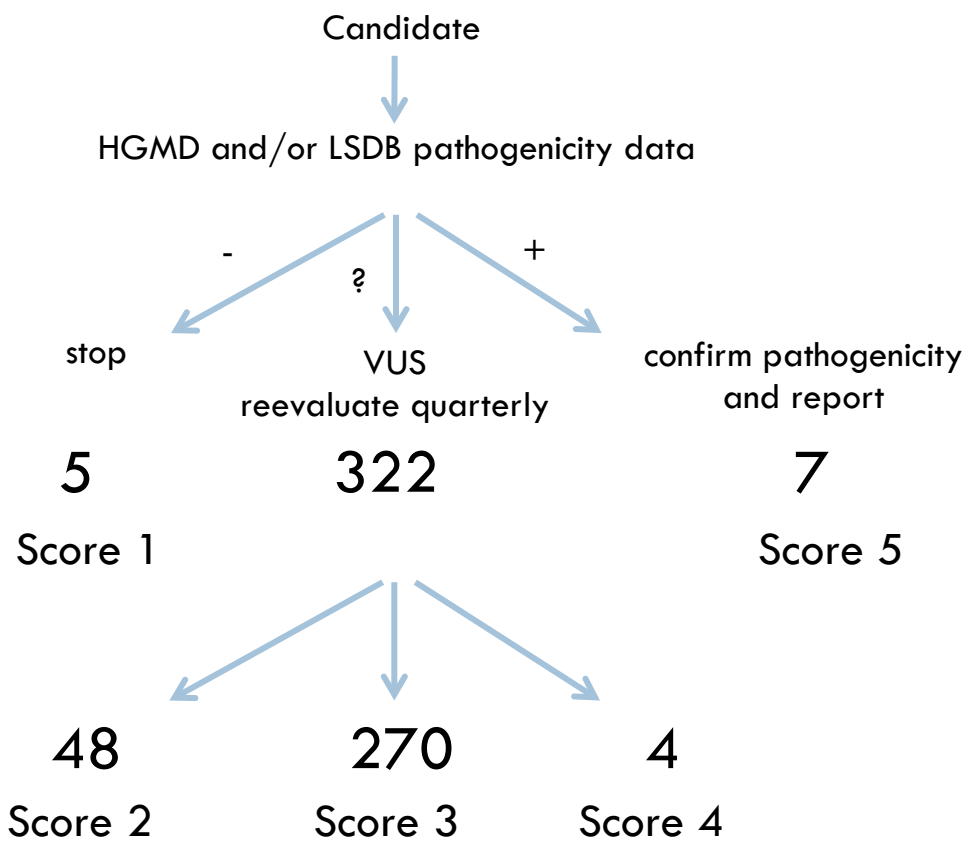
Variant Decision Examples

APC chr5	112,202,668-112,202,670	NM_000038.4 c.3479C>A p.Thr1160Lys	3	1 in 258	-	CM080043 DM	Not in LSDB	two patients with CRA; rare variant hypothesis[4]
APC chr5	112,201,627-112,201,629	NM_000038.4 c.2438A>G p.Asn813Ser	3	1 in 258	-	-	Not in LSDB	-
BRCA2 chr13	31,812,437-31,812,439	NM_000059.3 c.5946del p.Ser1982ArgfsX22	5	1 in 258	-	-	In LSDB[1] (7X): (?) BIC[2] (>1000X): clinically important	↑frameshift; ↑cosegregation[11]
FLCN chr17	17,059,322-17,059,324	NM_144997.5 c.1333G>A p.Ala445Thr	1L	1 in 255	rs41419545(C,T) no frequency data	-	In LSDB (1X): (-) https://grenada.lumc.nl/LSDB2/shared1/home.php?select_db=FLCN	-
MSH6 chr2	47,879,811-47,879,813	NM_000179.2 c.1186C>G p.Leu396Val	2	1 in 258	rs2020908(C,G) MAF 0.010 in 192 chr	CM101608 probable FP	In LSDB (19X): (?)	↓3/200 control individuals; ↓no significant mismatch repair defect[24]
MUTYH chr1	45,570,704-45,570,706	NM_001048171.1 c.691C>T p.Arg231Cys	4	1 in 225	-	CM055444 DM	in LSDB (5X): (?)	↑biallelic; ↑MSH6 binding domain; 0/80 control individuals[30]
RET chr10	42,933,913-42,933,915	NM_020630.4 c.2372A>T p.Tyr791Phe	2	2 in 258	-	CM971306 DM	In LSDB (7X)	↓found in unaffected relatives; ↓found with causative mutation; 8/1000 control individuals[34]; reported in Hirschsprung[35]

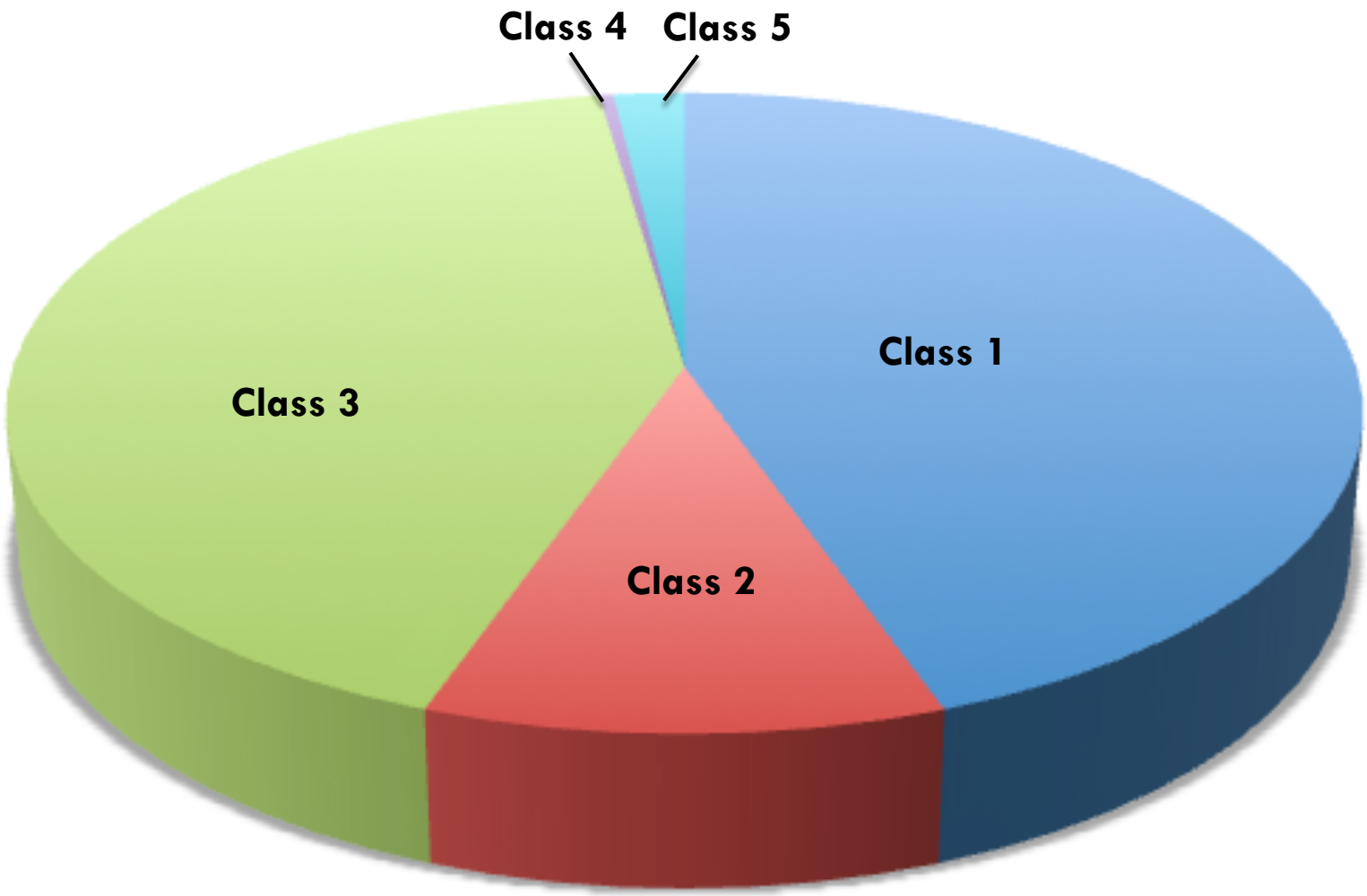
- HGMD and LSDB often have conflicting information
- References cited do not always support causation

MUST READ PRIMARY LITERATURE!!!!

CS Cancer Filtering



Summary of Variant Scores



CS Cancer Variants of Interest

- Three *BRCA2* variants, both score *5*
- Two *BRCA1* variants, both score *5*
- One *SDHC* variant, score 4- p.Arg15X, LOVD ?/+, Paraganglioma
- One *FLCN* variant, score 4- p.Lys508Arg, LOVD +/+?, Birt-Hogg-Dube syndrome
- Four variants in *MUTYH* (two 4, two 5s; AR; none biallelic)

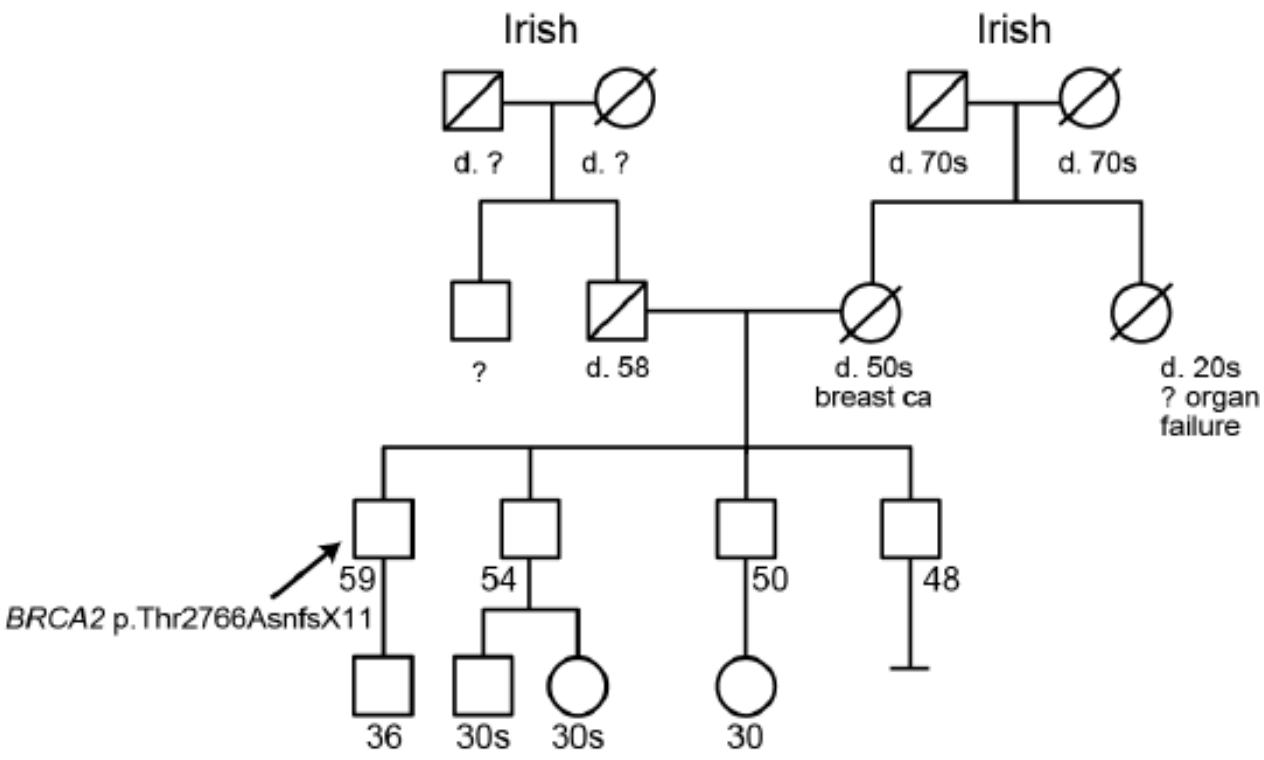
Seven is a Big Number

- Seven probands with BRCA1/2 variants in 572 ClinSeq cohort
  - All previously described
  - All associated with familial high-penetrance cancers
  - Only four had pedigrees that would lead to testing
  - Potentially life-saving results

Family History May not be Informative

BRCA2 c.8297delC
Thr2766AsnfsX11
Classification: 5

Evidence of Pathogenicity:
Reported 41 times, no debate about pathogenicity



Pathogenicity Score Criteria

Database Designation	Novel	Novel	Pathogenic	Pathogenic	VUS	Benign
Mutation Type	Missense	Nonsense Frameshift Splice	Missense	Nonsense Frameshift Splice	Any	Any
Score 5		Similar mutation type Consistent family history	Multiple reports, no evidence against	No evidence against		
Score 4		Similar mutation type Equivocal family history	Multiple reports, evidence against **OR** Single report, evidence for	Multiple reports, single evidence against	Multiple primary reports as pathogenic	
Score 3	All novel missense	Dissimilar mutation type Inconsistent family history	Single report, no supporting evidence	Multiple reports, multiple evidence against **OR** Single report, single evidence against	Primary reports as VUS	Single report OR primary reports as pathogenic
Score 2			Single report, multiple evidence against	Single report, multiple evidence against	Multiple evidence against	Multiple reports, no supporting evidence **OR** Single report, evidence against

A Cautionary Tale

Gene symbol	Disease / phenotype	Location	HGMD accession
CDH1	Gastric cancer	16q22.1	CM041745

Disease/phenotype	Gene symbol	Codon change	Amino acid change	Codon number	Reference
Gastric cancer	CDH1	tGCC-ACC	Ala-Thr	298	Brooks-Wilson (2004) Journal of medical genetics (*J Med Genet*) **41:** p508. PubMed: 15235021 Mateus (2009) *Exp Cell Res* **315:** p.1393 [Functional characterisation] PubMed: 19268661
The A298T substitution exhibits a shift in polarity from non-polar to polar and displays a decrease in Kyte-Doolittle hydrophobicity from 1.8 to -0.7. Approximately 1.77% of missense mutations in HGMD are Ala-Thr.					

Extra information		
Genomic sequence (build 37.2 - NEW)	GACGCGGACGATGATGTGAACACCTACAAT(G-A)CCGCCATCGCTTACACCATCCTCAGCCAAG	
Amino acid sequence	DADDDVNTYNAAIAYTILSQD	Amino acid alignment
Genomic coordinate (build 37.2 - NEW)	Chr 16: 68845646; Chr 16: 68845646-68845648	
Genomic coordinate (build 36.3 - REMOVAL 2012.1)	Chr 16: 67403147	
HGVS nomenclature	NM_004360.3: c.892G>A; NP_004351.1: p.A298T	
dbSNP number	No dbSNP ID	
Variant class	Disease causing mutation	
Comments	No comments	
CpG	No	

Amino acid comparison table		
Trait	Ala (A)	Thr (T)
Amino acid name	alanine	threonine
Polarity/charge	non-polar	polar
pH	neutral	neutral
Residue weight	71	101
Hydrophobicity score	1.8	-0.7
Hydrophilicity score	-0.5	-0.4
Secondary structure propensity	strong α former / β indifferent	α indifferent / β former
Grantham difference	58	
Sift prediction	TOLERATED	

Cautionary Tale

J Med Genet. 2004 Jul;41(7):508-17.

Germline E-cadherin mutations in hereditary diffuse gastric cancer: assessment of 42 new families and review of genetic screening criteria.

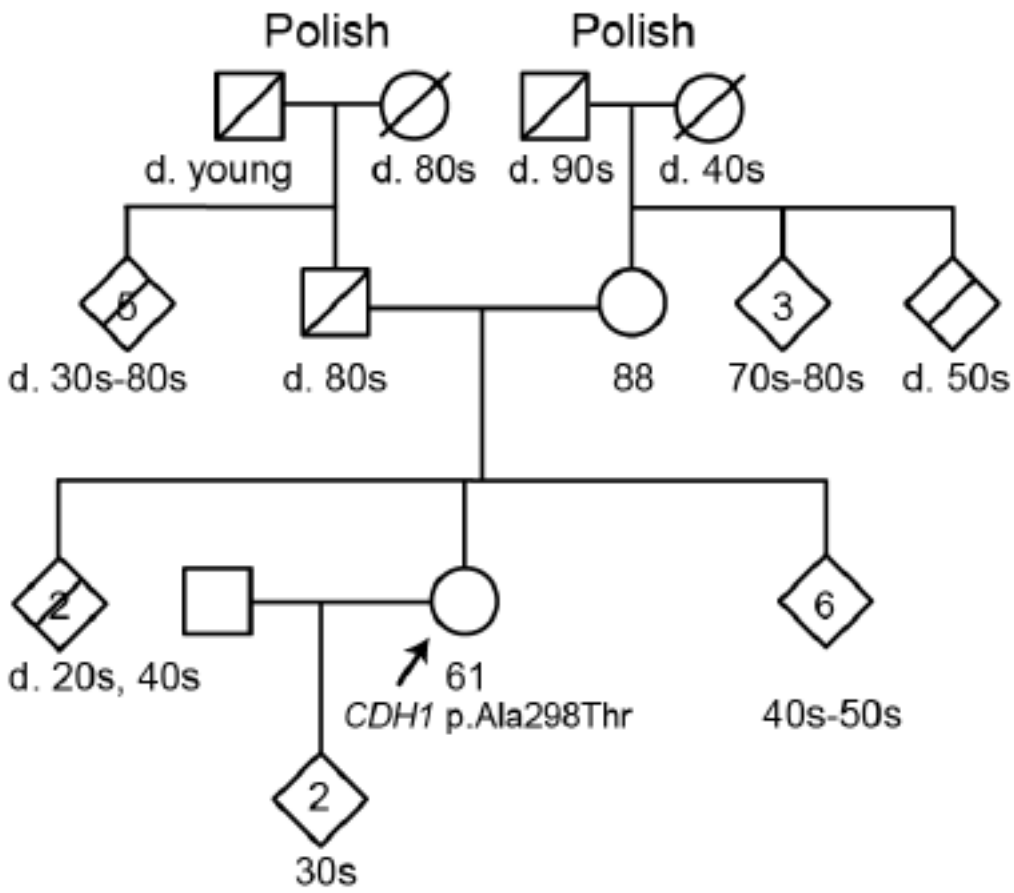
Brooks-Wilson AR, Kaurah P, Suriano G, Leach S, Senz J, Grehan N, Butterfield YS, Jeyes J, Schinas J, Bacani J, Kelsey M, Ferreira P, MacGillivray B, MacLeod P, Micek M, Ford J, Foulkes W, Australie K, Greenberg C, LaPointe M, Gilpin C, Nikkel S, Gilchrist D, Hughes R, Jackson CE, Monaghan KG, Oliveira MJ, Seruca R, Gallinger S, Caldas C, Huntsman D.

Table 2 Details of the gastric cancer families in the study and mutations detected

Family no	Cancer type, age	Study criteria met	Other family members with gastric cancers, n (ages)	Family members with breast cancer, n (confirmed lobular breast cancer)	CDH1 mutation: exon, nucleotide (amino acid)	Type of mutation
F26	DGC, 36	1	2 (32†, 33)	0	Exon 7, G892A (A298T)	Missense

The mutations found include small insertions and deletions, splice site mutations, and three non-conservative amino acid substitutions (A298T, W409R, and R732Q). All three missense mutations conferred loss of E-cadherin function in in vitro assays.

Cautionary Tale

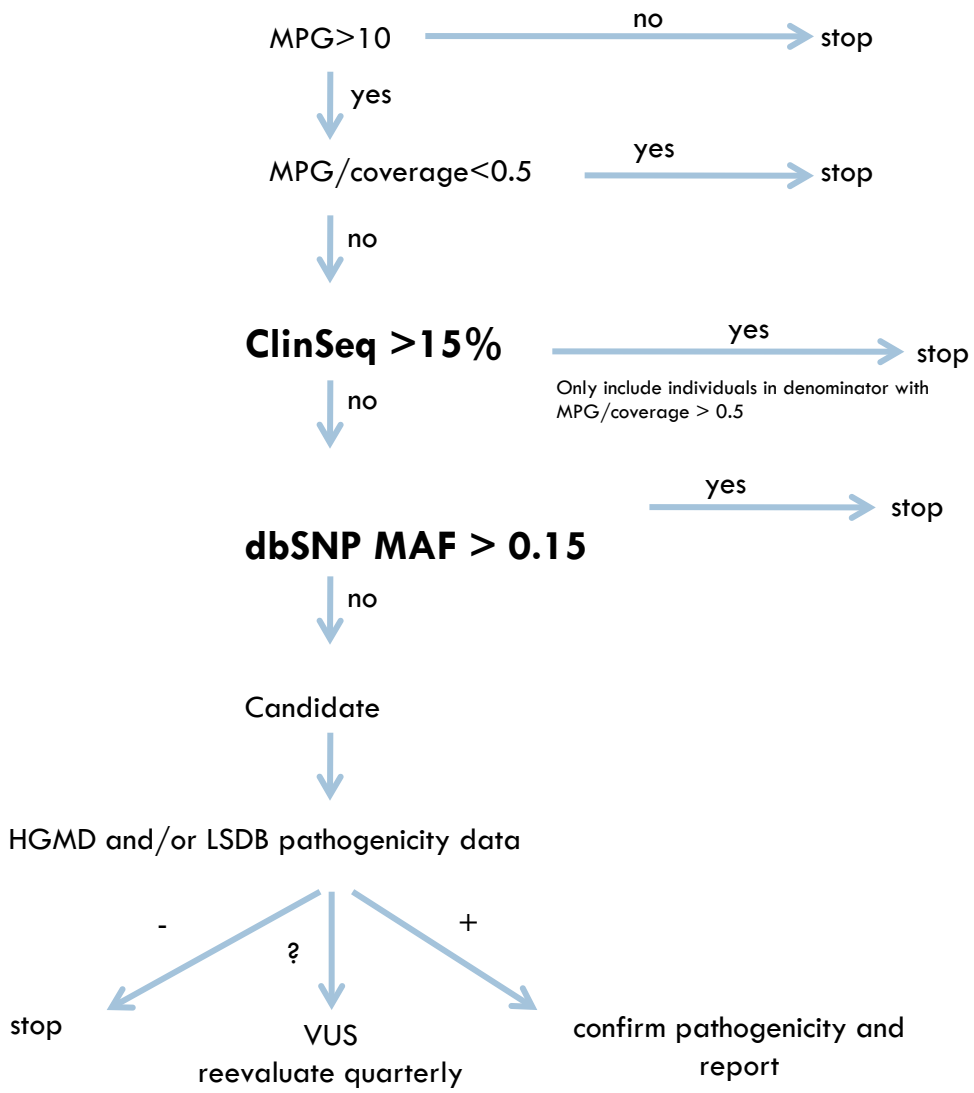


What should we consider when returning carrier variants?

Disease-Gene-Variant

- Severity of disease
- Genes proven to cause disease
- Variants with known pathogenicity
- Threshold for disease incidence?

Framework for Carrier Variants



Ambrygen Gene List

- 78 genes offered in prenatal panel
- 75 AR, 3 X-linked
- 1:2,500 for CF to 1:1,000,000 for Beta ketothiolase deficiency

http://www.ambrygen.com/

Common Recessive disease

ETHNICITY	DISEASE	CARRIER FREQUENCY
Ashkenazi Jewish:	Tay-Sachs	1/30
	Canavan	1/40
	Cystic fibrosis	1/29
	Familial Dysautonomia	1/30
Mediterranean:	Thalassemia	1/20-1/50
	Sickle cell anemia	1/30-1/50
European Caucasian:	Cystic fibrosis	1/29
African American:	Sickle cell anemia	1/10
	Thalassemia	1/30-1/75
	Cystic fibrosis	1/65
Asian:	Thalassemia	1/20-1/50
	Cystic fibrosis	1/90
Hispanic:	Cystic fibrosis	1/46
French Canadian:	Tay-Sachs	1/15
	Cystic fibrosis	1/29

Population Risk:

$1/30 * 1/30 * 1/4 = 1/3600$

Known Carrier Risk:

$1/30 * 1/4 = 1/120$

30 X population risk

http://www.hopkinsmedicine.org/fertility/resources/genetic_screening.html

Extremely Rare Recessive disease

Beta ketothiolase deficiency

Population Risk (1 in a million):

1/500 * 1/500 * 1/4 = 1/1,000,000

Known Carrier Risk:

1/500 * 1/4 = 1/2,000

500 X population risk

What did we find?

- 10 stops in HGMD
- 216 nonsynonymous in HGMD

- 11 novel stops
- 25 frame shifts
- 5 in frame deletions
- 14 splice not in HGMD

- 623 were nonsynonymous changes not present in HGMD

CS Carrier Variants - 78 Genes

CFTR – Cystic Fibrosis - p.ΔF508, 7/571

BBS10 – Bardet Biedl - c.271dup, common mutation, 2/401

ASPA – Canavan disease - p.Glu285Ala, founder AJ, 1/564

IDUA – Hurler- p.Ala327Pro, common mutation, 1/522

GALT – Galactosaemia, p.Gln188Arg, common mutation, 3/574

G6PC – Glycogen Storage 1a, p.Arg83Cys, founder AJ, 4/572

MUT – p.Asn219Tyr, common methylmalonic aciduria mutation 1/572

How might we think of things differently for a trio?

Family W04

Proband

Whole exome

89,536 number of variants

24 variants of interest

Gene Discovery

W04 Secondary Variant

Proband

Whole exome

X number of variants

Variant of interest

89,536 Secondary Variants (all)

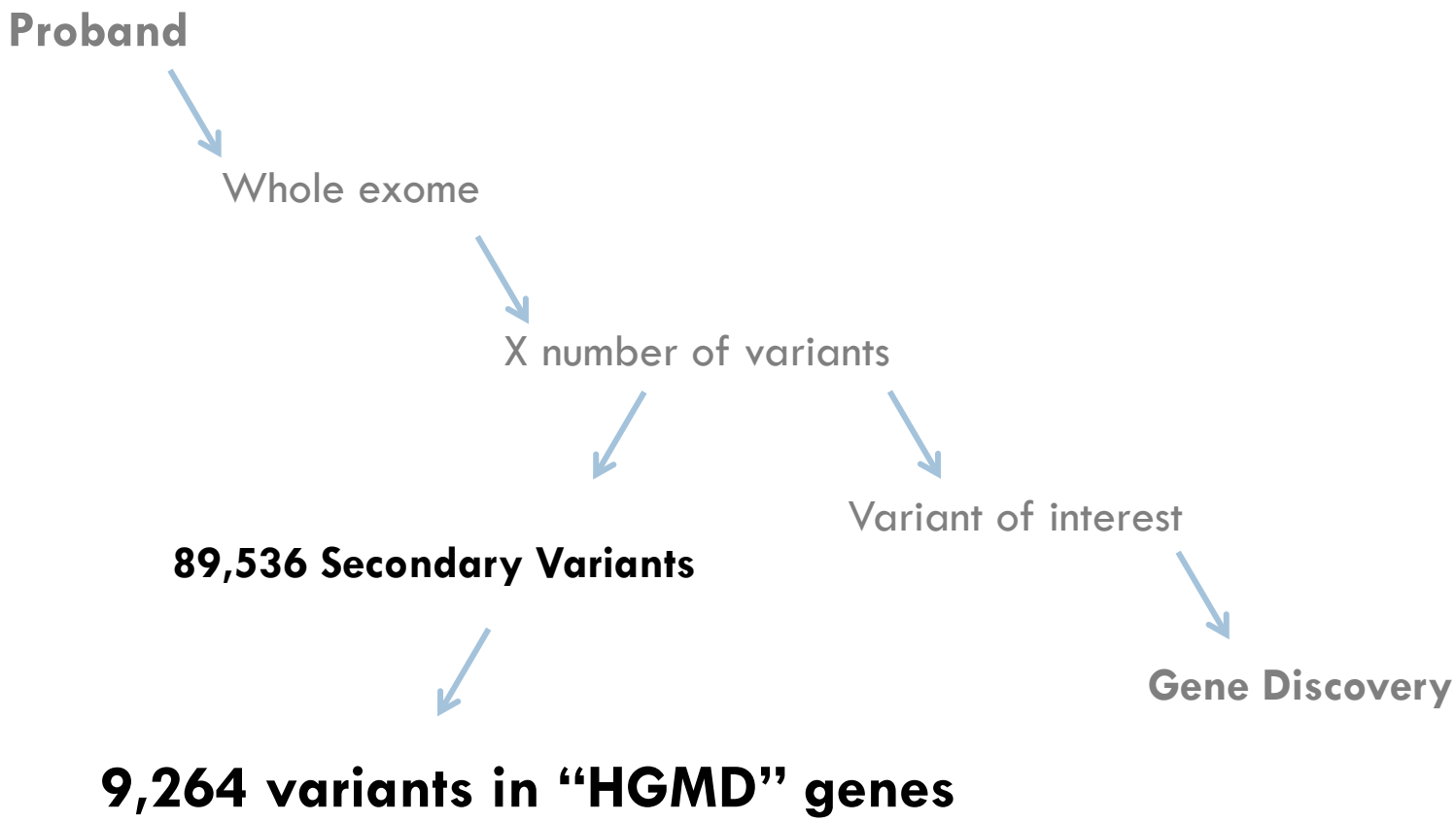
Gene Discovery

VarSifter - HGMD

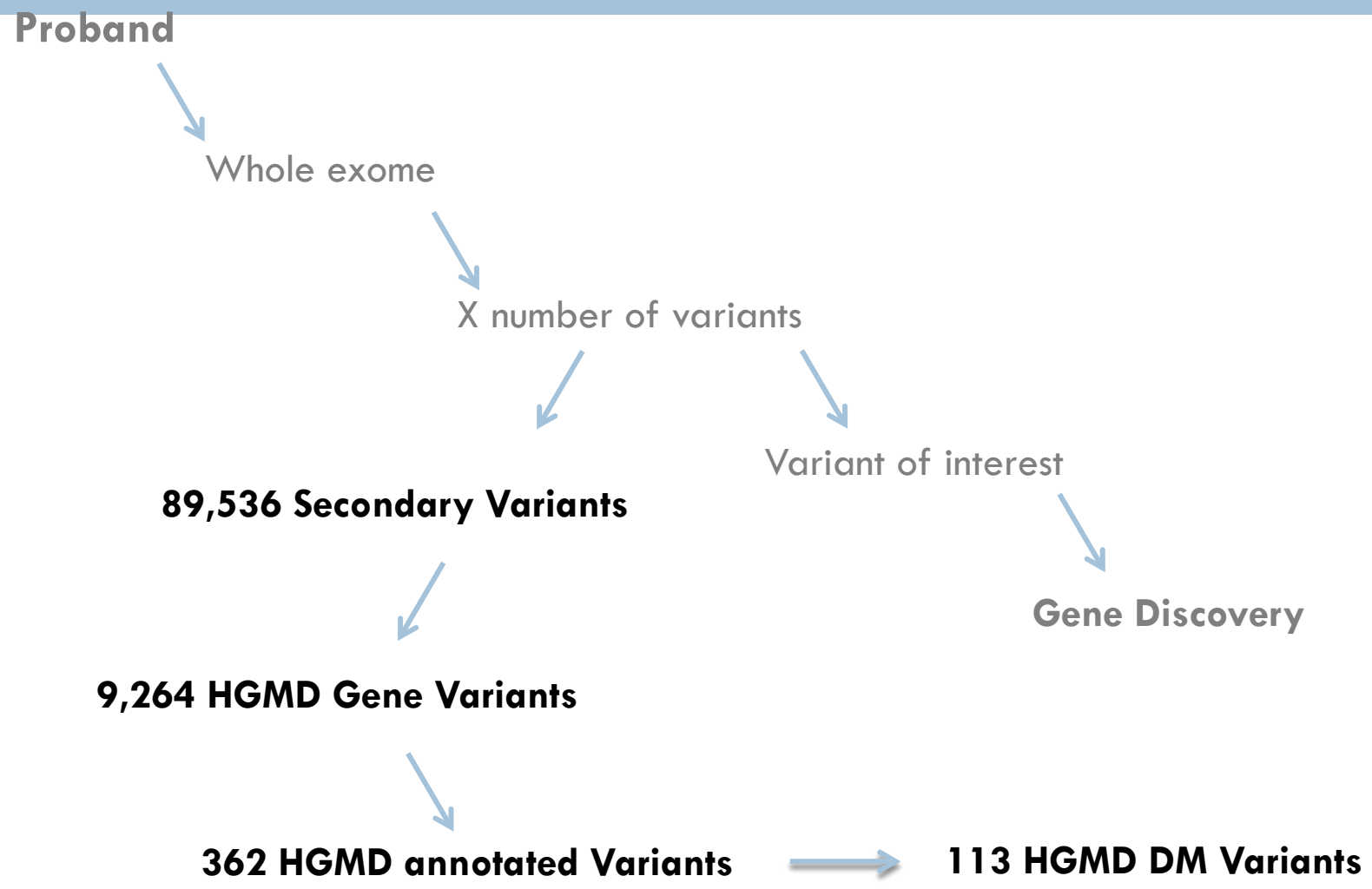
File View Help

Chr	LeftFlank	RightFlank	Gene_name	HGMDids	HGMDdisease	HGMDtags	HGMDinGene	transcript
chr13	31810006	31810008	BRCA2	CM050182	Breast cancer ?	DM	y	uc001uub.1
chr13	31810053	31810055	BRCA2	-	-	-	y	uc001uub.1
chr13	31810072	31810074	BRCA2	-	-	-	y	uc001uub.1
chr13	31810749	31810751	BRCA2	CM003133	Breast and/or ovarian cancer ?	DM	y	uc001uub.1
chr13	31811084	31811086	BRCA2	-	-	-	y	uc001uub.1
chr13	31811270	31811272	BRCA2	-	-	-	y	uc001uub.1
chr13	31811689	31811691	BRCA2	-	-	-	y	uc001uub.1
chr13	31811803	31811805	BRCA2	CM041731	Breast and/or ovarian cancer ?	DM	y	uc001uub.1
chr13	31811970	31811979	BRCA2	-	-	-	y	uc001uub.1
chr13	31812043	31812045	BRCA2	-	-	-	y	uc001uub.1
chr13	31812045	31812047	BRCA2	-	-	-	y	uc001uub.1
chr13	31812223	31812225	BRCA2	-	-	-	y	uc001uub.1
chr13	31812235	31812237	BRCA2	CM010170	Breast cancer ?	DM	y	uc001uub.1
chr13	31812437	31812439	BRCA2	-	-	-	y	uc001uub.1
chr13	31812591	31812593	BRCA2	CM994286	Breast and/or ovarian cancer ?	DM	y	uc001uub.1
chr13	31812813	31812815	BRCA2	-	-	-	y	uc001uub.1
chr13	31812816	31812818	BRCA2	CM043917	Breast cancer ?	DM	y	uc001uub.1
chr13	31812829	31812831	BRCA2	-	-	-	y	uc001uub.1
chr13	31812838	31812840	BRCA2	CM022331	Breast and/or ovarian cancer	DM	y	uc001uub.1
chr13	31816705	31816707	BRCA2	-	-	-	y	uc001uub.1
chr13	31827308	31827310	BRCA2	-	-	-	y	uc001uub.1
chr13	31827386	31827388	BRCA2	CM960194	Breast cancer	DM	y	uc001uub.1
chr13	31828632	31828634	BRCA2	CM012590	Breast and/or ovarian cancer	DM	y	uc001uub.1
chr13	31828672	31828674	BRCA2	CM994287	Breast and/or ovarian cancer ?	DM	y	uc001uub.1
chr13	31835487	31835489	BRCA2	CM043984	Breast and/or ovarian cancer	DM	y	uc001uub.1
chr13	31835520	31835522	BRCA2	CM004715	Breast cancer	DM	y	uc001uub.1

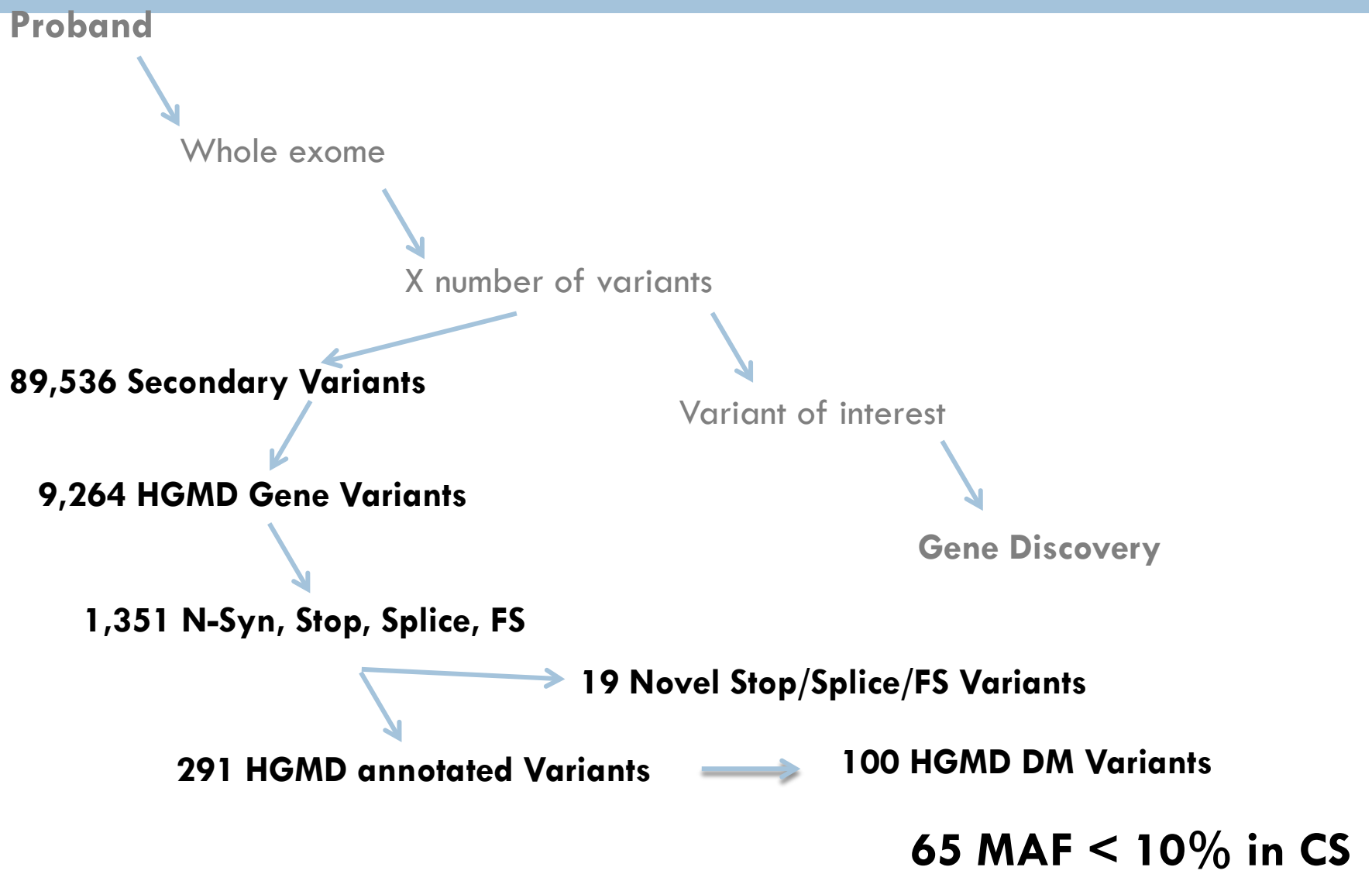
W04 Secondary Variant



W04 Secondary Variant



W04 Secondary Variant



Secondary Variant Paradox

- Exome/WGS sequencing can uncover life altering predictive information

- Value can only be appreciated if research practitioners annotate exomes for secondary variants

What is the future?

- Secondary annotation is a burden (for researchers)
- It is important
- NIH and others need to improve resources for this
  - Databases
  - Interpretation tools & services