Maintaining Accurate Information in Variant Databases

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CENTER FOR PERSONALIZED GENETIC MEDICINE



Laboratory for Molecular Medicine at PCPGM

- CLIA accreditation in 2003
- LMM offers >150 tests in cardiovascular disease, cancer, hearing loss, pharmacogenetics and genetic syndromes

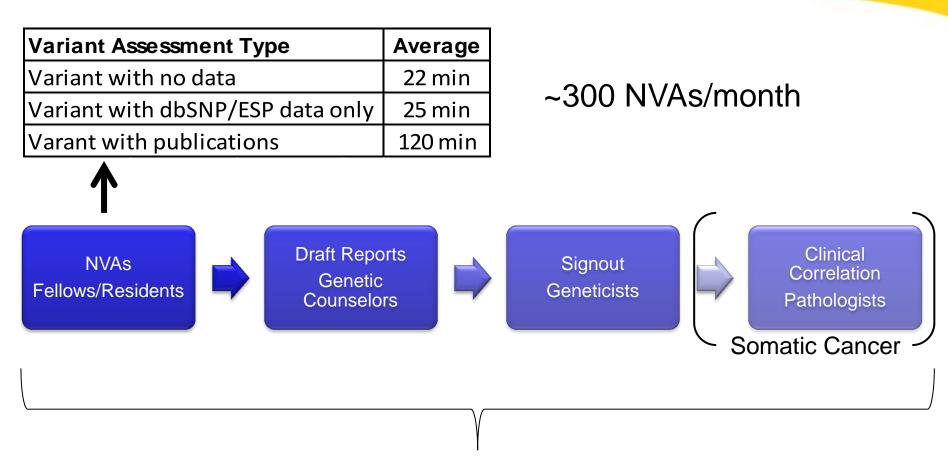
25% of testing is from Partners' patients 75% is from other US and International patients



- Main focus of testing is large multi-gene panels using sequencing technologies (Sanger, chip-based, NGS)
- Other technologies include TaqMan, Luminex, allele-specific PCR, MLPA, PNAs, STRs, droplet PCR
- WGS interpretation service will launch in Oct 2012



Average Time to Assess a Novel Variant



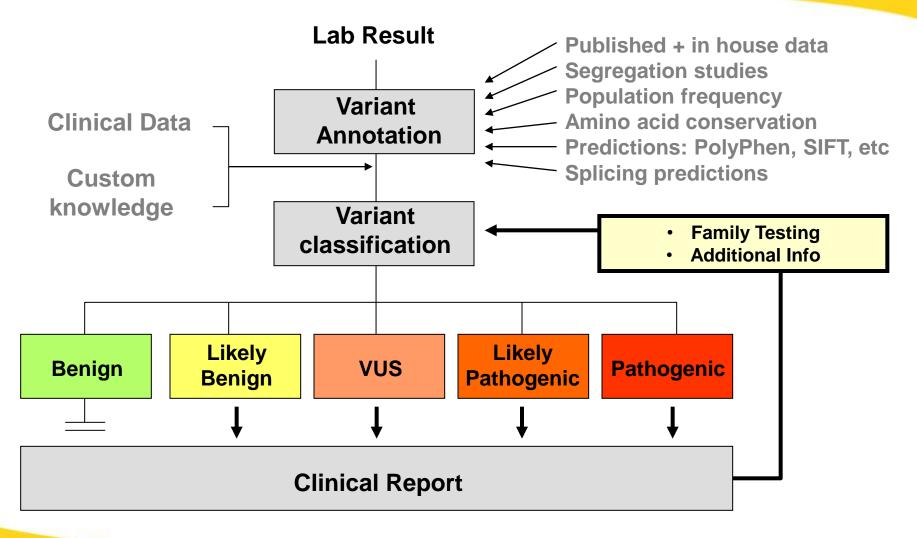
~25,000 variants curated to date



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Clinical Grade Variant Assessment



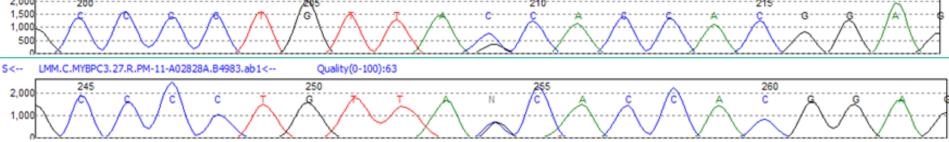
Courtesy of Birgit Funke



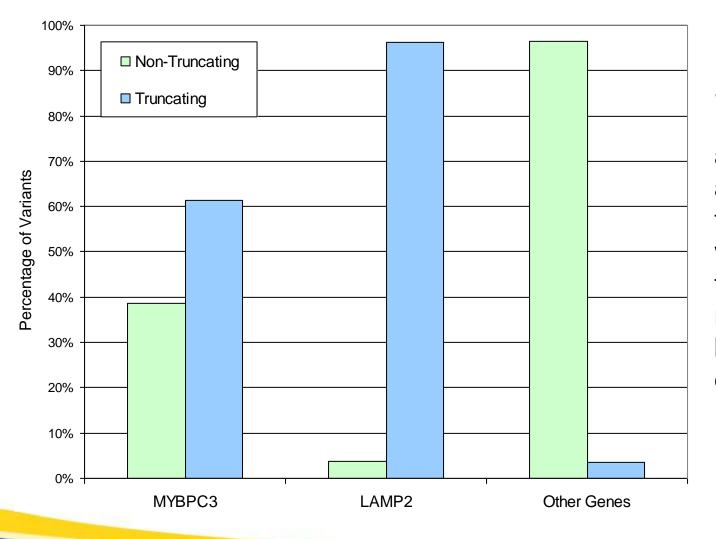


NVA: Gene Characteristics and Variant Spectrum

15 VARIANT INFO	Variant Folders link	Alamut link	Link to NVA form			
16		Transcript	Protein	HGNCID	GI Name	_
17 Gene	MYBPC3	NM 000256.3	NP 000247	7551	MVDDC2	
18 Genome build, coordinates	GRCh37	chr11:47356628-47	356628		Variant	spectrum by Variant Type
20 Exon/Intron	27	of 33 total exons			vanant	spectrum by variant Type
22 Zygosity	Het					
23 Nucleotide Change	2870C>G	Wildtype AA	Position	²⁵⁰		Pathogenic
24 Amino Acid change	Thr957Ser	Т	957			Likely Pathogenic
25 Variant Type	Missense			200		
26 Distance from exon/intron junction	36			200		Unknown Significance
27 Protein Domains	Fibronectin, type III, F	ïbronectin, type III-lik	ke fold	ts		Likely Benign
23 Gene-specific warning	None			Number of Variants		Benign
30 Exon-specific warning & link	None			Var		
31				÷.		
32 VARIANT SPECTRUM & ALTERNATE	VARIANTS			ੁੱ 100 	_	
		Missense	LOF	ě		
33 LMM Variant Classification	s Mis		(FS, Nonronro, +/-1,2	Lin I		
33 LMM Variant Classification:	5 MIS		splico)	Z 50	_	
39 Tota	l 217	217	120			
40 % of type that are pathogenic or likely path.	36%	36%	99%	0		
41 % of type that are VUS	53%	53%	1%		-	
42 % of all pathogenic or likely pathogenic	37%	37%	56%		ense v	of male monie
43 Variant type pathogenic in this gene?	Y	GeneBeviews	Cardio Guide link		sense C	Ariant Type Silenul from
44 Same as change from different variant?	N	Genelnsight link		42		. In Martin
45 Different as change at this codon?	N				\	/ariant Type 🔗
46						
> LMM.C.MYBPC3.27.F.PM-11-A02828A.B4983.ab 000[200	1> Quality(0-:	100):64		0		215



Gene-specific distribution of mutation types in HCM



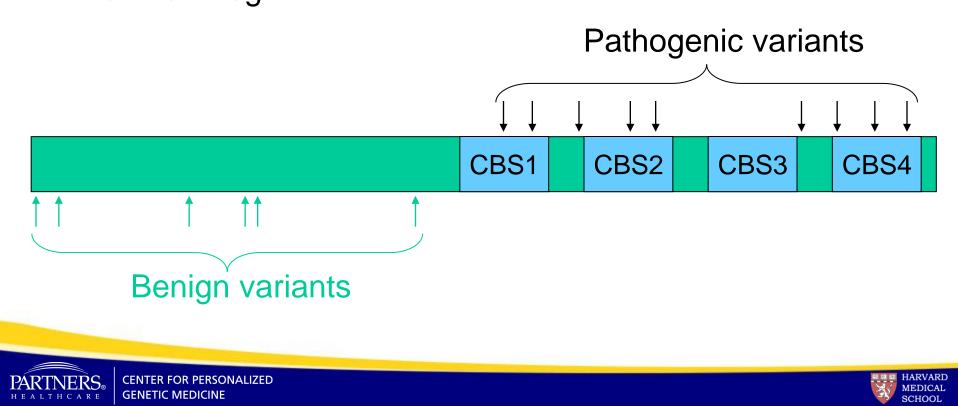
Silent and intronic variants are largely assumed benign for genes in which no truncating mutations have been observed or reported.



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PRKAG2 – AMP-activated protein kinase

All pathogenic mutations are missense and occur in or very close to the CBS domains. All benign missense variants occur outside the CBS domain region.



NVA Form: Google, PubMed and LSDB Searches

LOCUS SPECIFIC AND OTHER DATABASES																																				
Disease Gene	db\$NP locus ID	<u>GeneInsight</u>	HGMD	<u>GET-Evidence</u>	PubMed	Google (IE) (non-IE)	UMD	Cardio Genomics	<u>Interm Fil. DB</u>	<u>Leiden Muscular</u> Dystrophy	<u>Barth</u>	Inherited Arrhythmias DB	ARVC DB	Cardio genomics SNPs	TTR database	PTPN11-Base	RAS-MAPK Syndromes	LMM Noonan-CS-CFC DB	COSMIC	IARC TP53 DB	MUTp53LOAD	LMM Germline p53 DB	MutDB	MMR DB	MMRUV DB	InSiGHT Colon Cancer DB	Zhejiang Colon Cancer DB	TCA Cycle Gene Mut DB	ARUP MEN2 DB	LMM Usher DB	TOVD	Connexin-deafness	MitoMap	mtDB	Pendred-BOR DB	LMM Ohio Deafness DB
JUP	3728																					Ĺ														
LAMA4	3910																																			
LAMP2	3920																																			
LDB3	11155																																			
LMNA	4000																																			
MAP2K1	5604																																			
MAP2K2	5605																																			
MYBPC3	4607	Y	Y	N	Y	Y		N																												
MYH6	4624																																			
MYH7	4625																																			
MYL2	4633																																			
MYL3	4634																																			
MYLK2	85366																																			
MYOZ2	51778																																			
NEXN	91624																																			
NKX2-5	1482																																			
PKP2	5318																																			
HEARING L	.oss																																			
CDH23	64072																																			
CLRN1	7401																																			
COCH	1690																																			
DFNB31	25861																																			
DFNB59	494513																																			
EYA1	2138																																			
GJB2	2706																																			
GJB6	10804																																			
GPR98	84059																																			
MTRNR1	4549																																			
MTTS	4574																																			
MYO6	4646																																			
MYO7A	4647																																			
OTOF	9381																																			
PCDH15	65217																																			
POU3F4	5456																																			
SLC26A4	5172																																			
TMC1	117531																																			
TMIE	259236																																			
TMPRSS3	64699																																			

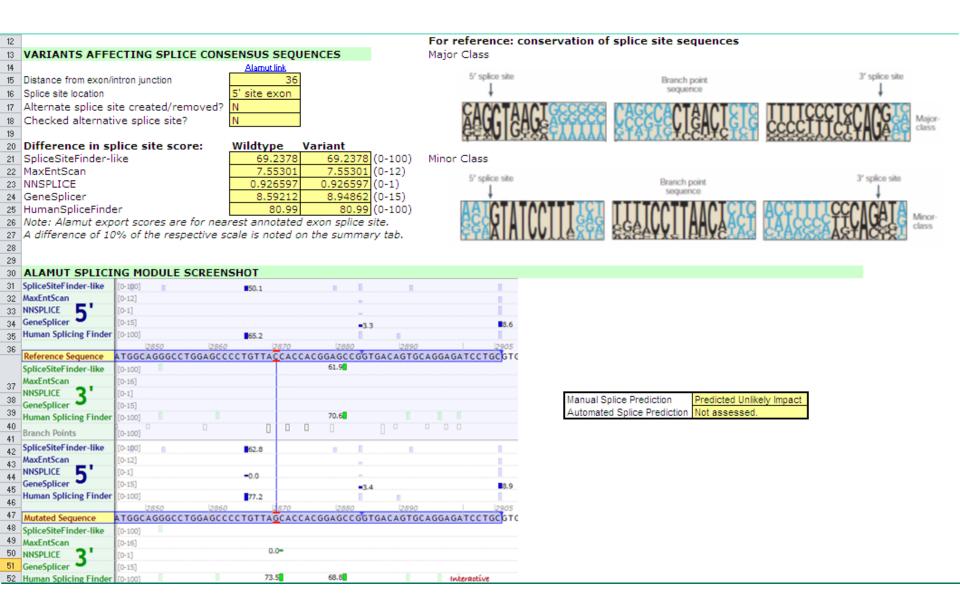
NVA: Case/Control Data from Literature and Databases

																			1
	A	В	С	D	E	F	G	H	I	J	К	L	M	N	0	P	Q	R	
8		MYBPC3				al LMM				8/4006			Segreg				0		-
9	Exon	27			Autom	ated LN	/M Prol	band C	hr	7/4006			Non-Se				0		
10	DNA	2870C>G											Coupo	und/Do	uble He	ets	5		
11	Protein	Thr957Ser			Manua	al Lit Pr	oband (Chr		2/576			De nov	/o varia	ants		0		
12	Link to current variant fo	older		1	Autom	ated Lit	t Proba	nd Chr		2/576			Seen ii	n differ	ent dise	eases?	Y		-
15								ROBAND)S				SEG	REG		CONT	ROL DA	ТА	
16	LMM data or Literature Reference	PMID	Family History?	# proband tested	# proband chrom tested	# positive HET probands	# positive HOM probands	positive proband phenotype(s)	Race matches case?	Allele frequency	# comp/double hets	# de novo variants	# informative meioses	# non-segregations	# control chrom tested	# positive control chrom	Control race matches case?	Allele frequency	Com (population details, fa e
	American Indian or Alaska		<u> </u>	16	32					4	-12	-75	-12 - 2	-42	4 4		0 5	4	
	American Indian or Alaska		-	16 193	32				N N										
19	Asian		-																
20	Black or African American			279	558				N			_							
21	Hispanic or Latino			106	212				N										
23	Native Hawaiian or Other Pacific			6	12	_			N										
26	White and Caucasian			2003	4006	7	0	HCM/	Y	0.002	4	0	0	0	0	0	0		The compound/double h
27	LMM race matched		 	2003	4006	7	0			0.002	4	0	0	0	0	0			
28	LMM all races		 	3297	6594	8	0			0.001	5	0	0	0	0	0			
29	Ehlermann, 2008	18957093	N	158	316	1	0	нсм	Unkno	0.003	0	0	0	0	860	0	Unkn own	0.000	Variant listed as novel a further information is giv proband.
30	Rodriguez-Garcia, 2010	20433692		130	260	1	0	нсм	Unkno	0.004	0	0	0	0	400	0	Unkn own	0.000	The pathogenicity of this uncertain and no further
31	Lit. race matched			0	0	0	0				0	0	0	0	0	0			
32	Literature all races			288	576	2	0			0.003	0	0	0	0	1260	0		0.000	
33																			
34	TOTAL CHROM race matched			2003	4006	7	0			0.002	4	0	0	0	0	0			
35	TOTAL CHROM all races			3585	7170	10	0			0.001	5	0	0	0	1260	0		0.000	
36	GraphPad Fisher's Exact Test link		-				-				_	-	-	-					
14 4	Variant / DB Publ+LMM	1 data 🗐	MM I	Family	Contr	ol Freq	/ Cons	erv Bio	hem /	Splicing	/ 0	OSMIC(car	ncer only	v) / S	ummary	/ One-	-liner 🖌	CLASSIFIC	ATION RULES 052611
	ol Frquency data			21111/ 1						- phone g				11 4 -		A			
	t in dbSNP? N					HapMap link													
RS Nu Clinica	mber Illy associated SNP?		<u>dbs</u>	SNP rs reco	rd link	All	ele 1		Allele	2	E								
Valida	ted?						С		G										
FREQ	UENCY INFORMATION Population 1	ation	Ch	roms test	ted F	requency	# identif	ied Freq	uency #i	dentified		M	anual	Contr	rol Fre	auen	cy 9/6	5692	
	Population 1 Population 2										1 1		mated				-		Rule: Take pop with hig
	Population 3										1 -	Autor	nateu	CONU	orrie	quen	cy 9/6	0092	Rule. Take pop with hig
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					5692.00 3208.00	0.0013449 (9 0	9 0.99	1 1	6683 3208	6								Note, excel only allowe

NVA: In Silico Analysis

12 AlignGVGD C0 89.28 57.75 (GV, GD) Prediction ranges from C0-benign to C65-pathogenic (Class [same as Prediction], Probability of damaging) 13 PolyPhen-2 Benign Benign 0.002 (Class [same as Prediction], Probability of damaging) 14 SIFT Tolerated 0.008 3.93 (Weight, Median) 15 SarcomerePolyPhen Benign (reliability, accuracy) 100 15 SIFT Tolerated 0.002 (reliability, accuracy) 100 16 SiFT Gomments (reliability, accuracy) 100 100 17 More as a strain and strain as a strain as strain as a strain as a str	15	COMPUTATIONAL PATHOGENICITY P	REDICTIONS						
18 PolyPhen-2. Behan Behan 0.002 (Class (same as Prediction], Probability of damaging) 9 SIT Toterated 0.08 3.93 (Vedath, Median) 25 Six/P Warnings: (Pelability, accuracy) (Pelability, accuracy) 22 Six/P Warnings: (Pelability, accuracy) (Pelability, accuracy) 23 Six/P Warnings: (Pelability, accuracy) (Pelability, accuracy) 24 BOCHEMICAL PROPERTIES (AUTOLOCKUP) Relatedness Amino Acid Biochemistry Wildtype Variant 25 BOCHEMICAL PROPERTIES (AUTOLOCKUP) Relatedness Amino Acid Biochemistry Wildtype Variant 26 BOCHEMICAL PROPERTIES (AUTOLOCKUP) Relatedness Amino Acid Biochemistry Wildtype Variant 27 Amino Acid Biochemistry Relatedness Amino Acid Biochemistry 0.731 1.422 (P-2.75) ratio of carbon/non-carbon weil 26 BOCSUM49 1 standard Molecular volume 6.8 0.23 2.45.9.13) 27 Substitutions >D occur frequently (clorated), <0 ar are (cloteratod), <0 ar are (cloteratod), <0 ar are (cloteratod), <0 ar an are (16		Prediction	Score 1	Score 2	Score 1 & 2 (lescriptions		
19 SIFT TOLERAGE 0.68 3.93 (Weaht, Median) SarcomerPolyPhen Benan (Pelability, accuracy) (Weaht, Median) (Pelability, accuracy) (Weaht, Median) (Pelability, accuracy) (Pelability, acc	17	AlignGVGD	C0	89.28	57.75	(GV, GD) Pred	iction ranges fi	rom CO-benign to	C65-pathogenic
19 SIFT TOLERAGE 0.68 3.93 (Weaht, Median) SarcomerPolyPhen Benan (Pelability, accuracy) (Weaht, Median) (Pelability, accuracy) (Weaht, Median) (Pelability, accuracy) (Pelability, acc	18	PolyPhen-2	Benign	Benign	0.002	(Class [same a	as Prediction],	Probability of dam	naging)
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34 VPLUTIONARY CONSERVATION SUMMARY Image: construction of the system of the syst		Substitutions >0 occur inequently (colorat		lecenousy	Granthann unterence ranges	are. 0-30 Low,	50-150 Milarai	ige, >150 mign	
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40 Patient variant AA in other species? N Invertebrates & Fungl 0 0 0 0 41 CCTGCCACGGGGGCCCGGCTGCTTTTCCGAGTGCGGGCACACAATATGGCAGGGCCTGGAGCCCCTGTTACCACCACGGAGGCCGGTGACAGTGCAGGAGATCTGCGCGTGAGGCCCCCTTT P P C T T P P V V Q E I Q 935 940 945 950 955 960 955 960 965 969 VOrthologues (Source: Interactive Biosoftware) HumanP T G A R L F R V A H N M A G P V T T E P V V Q E I Q 969 VOrthologues (Source: Interactive Biosoftware) HumanP T G A P V T T T E P V V Q E I Q Q I Q Q I Q Q Q I L Q Q I L Q Q I							-		
41 Total 9 4 0 Control of the construction of the constrelating cond the constructin construction of the cons									
CCTGCCACGGGGGCCCGGCTGCTTTTCCGAGTGCGGGGCACACTATATGGCAGGGCCTGGAGGCCCTGTACCACCACGGAGCCCGGTGACAGTGCAGGAGATCCTGCGTGAGTGCCCCTTT L P T G A R L L F R V R A H N M A G P G A P V T T T E P V T V Q E I L Q 935 940 945 950 955 960 965 969 V Orthologues (Source: Interactive Biosoftware) Human P T G A R L L F R V R A H N M A G P G A P V T T T E P V T V Q E I L Q Chimp P T G A R L L F R V R A H N M A G P G A P V T T T E P V T V Q E I L Q Mouse P T G A R L L F R V R A H N V A G P G G P I V T K E P V T V Q E I L Q Mouse P T G A R L L F R V R A H N V A G P G G P I V T K E P V T V Q E I L Q Mouse P T G A R L L F R V R A H N V A G P G G P I V T K E P V T V Q E I L Q Cow P T G A R L L F R V R A H N V A G P G A P V T T K E P V T V Q E I L Q Cow P T G A R L L F R V R A H N V A G P G A P V T T K E P V T V Q E I L Q Cow P T G A R L L F R V R A H N L A G A G P G A P V T T K E P V T V Q E I L Q Cow P T G A R L L F R V R A H N L A G A G P G A P V T T K E P V T V Q E I L Q Cow P T G A R L L F R V R A H N L A G A G P G A P V T T K E P V T V Q E I L Q Cow P T G A R L L F R V R A H N L A G A G P G A P V T T K E P V T V Q E I L Q Cow P T G A R L L F R V R A H N M A G P G A P V T T R E P V T V Q E I L Q Com P T G A R L Q F R V R A H N M A G P G A P V T T R E P V T V Q E I L Q Com P T G A R L Q F R V R A H N M A G P S A P A T M K E P V T V Q E I M Q African clawed frog E R L A F R V R A I N L A G E S G A A I I K E P V T V Q E I M Q Zebrafish T G E K M Q F R V R A Y N M A G P S A P A T L Q Q A V T I R E I M Q Vascular endothelial growth factor receptor, VEGFR, N-terminal Fibronectin, type III		Patient variant AA in other species?	N				-	_	
L P T G A R V R A H N M A G P G A P V T T T E P V T V Q E I L Q 935 940 945 950 955 955 960 965 969 965 969 ♥ Orthologues (Source: Interactive Biosoftware) Human P T G A R L L F R V R A H N M A G P V T T T E P V T V Q E I L Q Mouse P T G A R L F R V R A H N A G P Q T T T E P V T V Q E I L Q Mouse P T G <th< th=""><th>41</th><th></th><th></th><th></th><th>Tota</th><th>9</th><th>4</th><th>0</th><th></th></th<>	41				Tota	9	4	0	
-Fibronectin, type III-subdomain- Vascular endothelial growth factor receptor, VEGFR, N-terminal Fibronectin, type III		L P T G A R L L F 935 940 ♥Orthologues (Source: Interactive Bioso Human P T G A R L L F Chimp P T G A R L L F Mouse P T G A R L L F Rad, P T G A R L L F Rad, P T G A R L L F Horse P T G A R L Q F Chickent T G D K L Y F African clawed frog E R L A F	R V R A H 945 945 945 945 945 R V R A H H R V R A H R V R A H R V R H R V R A H R V R H R V R A H R V R H R V R A H R V R H R V R A H R V R A H R V R A I I I I I I I	N M A 950 N M A N V A N V A N V A N V A N V A N L A N L A	G P G A P V 1 955 955 G P G A P V 1 G P G A P V 1 G P G A P V 1 G P G G P I 1 G P G G P V 1 G P G A P V 1 G P G A P V 1 G P G A P V 1 G P G A P V 1 G P S G A A 1 G P S E P C 1	T T E 960 T T T E 960 T T T E 7 T T K E 7 T K E 7 K E T K E 7 K E T K E 7 K E T T Q E E T K E T Q E T K E T K E T M K E T K E	P V T V P V T V P V T V P V T V P V T V P V T V P V T V P V T V P V T V P V T V P V T V P V T V	Q E I I 965 965 1 1 Q E I 1 Q E I 1 Q E I 1 Q E I 1 Q E I 1 Q E I 1 Q E I 1 Q E I 1 Q E I 1 Q E I 1 Q E I 1 Q E I 1 Q E I 1 Q E I 1 R E I 1	
Fibronectin, type III		Fibronectin, type III subdomain		22					
			tor, veork, w-cermi	Idi					
-Immunoglobulin-like					1				
		-Immunoglobulin-like							

NVA: Splicing Analysis



EXPRESSION

Instructions: For each orga	in system,	indicate whether the gen	e is express	sed (H f				.ow). If no dat	ta is available, leave	e blank			
	<u>BioGPS</u>	Gene Expression Atla	<u>GeneNote</u>	<u>Tiger</u>	Testis	Seminifero TestisL Testis	Pancreas usTubule eydigCell						■ Ge
Auditory/Eye: inner, outer, and middle ear, eye Circulatory: heart, blood					Colore Bro	ctaladenoc nchialEpith Smoo	Testis- arcinoma- elialCells-	_	1000		ſ	n	
vessels, arteries, veins, blood, etc. Digestive: mouth,			м		Leukemialym Leukemia_chronic Lymph Leukemia_p Lymp		busK-562- ts(Daudi)- tic-HL-60- kitts/Rai)-		100 -	L & Ø _Ø			B
esophagus, stomach, intestines, liver, etc. Endocrine:	М	н		н		071+ Early Small	Thyroid Prostate Lung Placenta Erythroid- intestine		Normalized intensity				
hypothalamus, pituitary, thyroid, pancreas, adrenals. etc.			м				Colon- Liver Heart usCorpus Appendix Ovary			And Sher Dod	od ^a Brain Cord	Care to the training to the tr	P HORT DO THE OF
Excretory: kidney, uterus, bladder, urethra, Immune: bone marrow,			м			DorsalRool Ciliary Atrioventric Trigemina riorCervical	Ganglion ularNode Skin Ganglion Ganglion		Bone	A Anole mer	Some	Caret	State Suite L
lymph nodes and vessels, WBCs, T- and B-cells, etc.			м			Skelet Pin Pi W	Tongue talMuscle- Retina- eal night- noeal day- holebrain wygdala-	-	Uterus Tongue Thymus	-		1	I
Muscular: skeletal and smooth muscle, etc. Nervous: brain, spinal					-	Pretron S Hypo	talCortex pinalcord thalamus etalbraim fhalamus- tenucleus		Testis Stomach Spleen	-			
cord, peripheral nerves, etc.	H	н	н	Н		Par MedullaC Cingula Occi Temp	ietalLobe Oblongata ateCortex- pitalLobe- soralLobe		Soft tissue Small intestine Skin				
Respiratory: lungs, trachea, nasal, etc.	м					C	Pons- IsPallidus Prebellum		Prostate Placenta				
Reproduction: ovaries, uterus, cervix, vagina, testes, seminal vesicles, penis. etc.			м	L	CD19	erebellumP CD105+ Er 21 B lým)+ BCells(r CA4+ Den	eduncies CD34+ ndothelial- phoblasts- neg. sel.)-		PNS Pancreas Ovary Muscle				
					Gene Wiki				Mammary gland Lymph node	-			
Is gene universally expressed?	N								Lung Liver				
Is gene predominantly expressed in a certain tissue?	Y	If yes, enter tissue(s):	Digestive, N	lervous	system				Larynx Kidney Heart				
How many databases support tissue expression?	4								Eye Colon Cervix Brain				
<u>Key</u> High Medium									Bone marrow Bone Blood	-			
Low									Bladder 0	0.5	5	1	1.5
Paste Screenshots B	elow												Enrichment
♦ ▶ ■ Intro / Gene	Expre	ssion / Pathways /	Disease A	ssociat	ions 🖉 M	lodel (Organisms	s / Literat	ure Search 📝 A	Assessment	Classific	ation Rule	s / NGAers /

PATHWAYS			
	Pathway List	Pathways Relevant to Current Case	Comments
KEGG	Arginine and proline metabolism	N/A	
Biocarta			No entires
GO	ATP binding, kinase activity		
Pathway Interaction Database			No entires
Reactome	Creatine metabolism		
List of all pathways relevant to current case:	Creatine metabolism		
How many databases support these pathways?	3		
25 21.3.11 Q3.5.1.16 Q I.44133 2.722 0nething 21.3.9 21.3.9 D-Arg & D-Orn netholizm 3416 Quatamoyl.P 0nething 0nething D-Arg & D-Orn netholizm 14 N-Acetyl- 26.1.11 23.5.1.16 Un 14 N-Acetyl- 26.1.11 23.5.1.16 Un 14 N-Acetyl- 26.1.11 23.5.1.6 Un 15 15.1.2 N-Acetyl- 21.1.13 Peptide 15 15.1.2 L-Obstample- 15.1.2 15.1.2 15 15.1.2 1.4.9 yrrola- 15.1.2 15.1.2 15 1.1.9 yrrola- 2.5.0 isoco- 1.4.9 yrrola- 15.1.2 1.1.9 yrrola- 2.5.0 isoco- 1.4.9 yrrola- 1.5.1.2 1.5.1.2 1.4.33 cis-4.4 lythcoxy- 2.5.1.13 1.4.9 yrrola- 1.1.9 yrrola- 2.5.0 isoco- 1.4.9 yrrola- 2.5.1.13 1.4.9 yrrola- 1.5.1.13 1.4.9 yrrola- 2.4.9 yrrola- 1.4.9 yrrola-	1 1	3.5.4.1 3.5.4.21 5.2.14 N-Methyl- hydroxy-N6,N8; N6-trimethyl-L- hysine 1141339 23.1.109 1141339 23.1.109 1141339 23.1.109 4- Trimethylammoniobut anal Nkhr NADH Nithe oxide 0 ALDH9A1 tet 02 1 1 1 02 1	AMP (PP)

DISEASE ASSO	CIATIONS					
		Disease List		Diseases Commo	on to Current Cas	Comments
KEGG						No entries
OMIM		None		N/A		
Genetic Associati	<u>on Database</u>	None		N/A		
HuGE Navigator						No entries
Gene Atlas		None		N/A		
<u>HGMD</u>		None		N/A		
		List of all diseases common to		e:		
		How many databases support	these			
		disease associations?				
MODEL ORGAN						
Mouse Genome Inf	formatics Datab	base				
N	C-4		C1	D'	Commente	
Model	Category	Affected Anatomical Systems	Similar Huma	an Diseases	Comments	-
Ckmt1tm2Bew creatine kinase, mitochondrial 1, ubiquitous; targeted mutation 2, Be Wieringa	Targeted KO (exons 7-8)	behavior, hearing/vestibular/ear, nervous system, digestive/alimentary			It is unclear if th surrounding gen affected by this especially given region is repetit	nes were targeted KO, that this
▶ Intro ∠Gene ∠ Expres	ision / Pathways) Di	sease Associations / Model Organisms / Litera	iture Search / Asses	ssment / Classification Rules	s / NGAers / 🐄 🕕 🖣	

1	LITERATURE SE	EARCH				· ·	
2	Pubmed						
	GeneCards						
4	Gene Reviews						
5	Literature Reference	PMID	Disease Association	Mutation Type(s) Implicated	Functional Data	Comments	
6	Zhang 2007	17098888				100Kb deletion at 15q15.3 (encompassing KIAA0377/PPIP5K1, CKMT1B, STRC, and CATSPER2) identified in three families with deafness infertility syndrome (DIS). asthenoteratozoospermia). In addition to DIS, congenital dyserythropoietic anaemia type I (CDAI) has also been described in patients with these deletions. The authors suggest that deletion of STRC is responsible for deafness, while deletion of CATSPER2 is responsible for infertility.	
7	Cimino_2008	18561318				Upregulation of CKMT1B was associated with disease-free and overall survival in patients with breast cancer.	
8	Zhang 2009	21686705				Basically same info as in Zhang 2007. A nearly identical copy of CKMT1B, designated CKMT1A (613415), is telomeric to CKMT1B on chromosome 15, and contains only two mismatches that predict synonymous mutations in the coding region in addition to two mismatches in the 3' UTR, suggesting CKMT1A is functional. This would suggest that CKMT1B and CKMT1A are genetically redundant and that deletion of one of these genes is unlikely to have functional consequences.	
9							
	Zhang 2009						
11 12 13	Normal allele	CEN	UNNTIB CAT	Ster.	WKARO3	NIA RCAISPER	
14 15				//			
16 17	Deletion scope		D1557	84		Transcribed gene	
17	Family D_S	SM e					
10	Family L70					Pseudo gene	
20	Family L10					High homology	
21	· · · · ·					J	
22	SNM		and the second s				
23							
24							IARVARD
25							IEDICAL
1	🕩 🕨 🛛 Intro 🖉 Gene	Expression	/ Pathways	/ Disease As	ssociations 🖉 M	odel Organisms 🚶 Literature Search 🖉 Assessment 🦯 Classification Rules 🛒 NGAers 🦯 😤	CHOOL

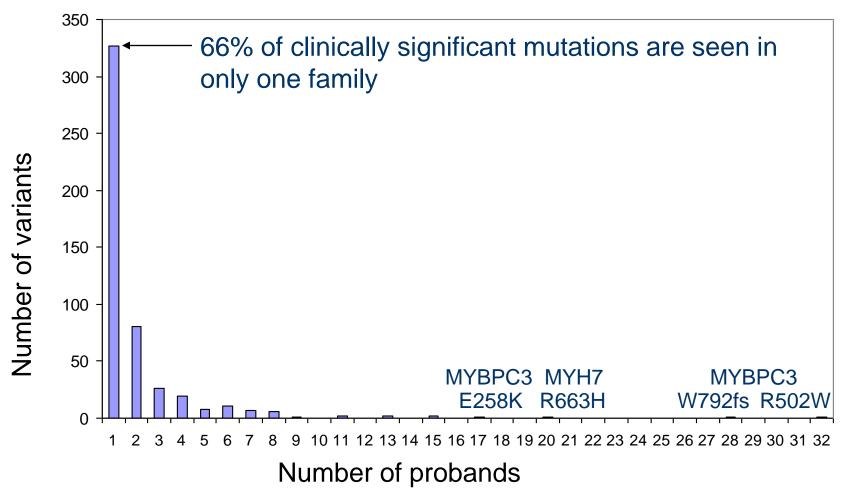
Final Variant Interpretation

Form completed by: Will Accession#
Patient Name Image: Constraint of the second of the se
Family Black Race Black Ethnicity Ethnicity Disease POU3F4 Gene POU3F4 Exon 1 DNA 964G>A Protein Val322Met Interpretation from Classification Rules Unknown Significance. Fellow Interpretation & Rationale Interpretation Variant Interpretation Unknown significance-4 Rationale Statements The Val322Met variant in POU3F4 has not been reported in the literature, but has been previously detected in 1 of the 38 probands tested by our laboratory. However, this mutation occurs within the POU homeodomain (amino acids 279-336) where almost all pathogenic missense mutations have been identified. Valine (Val) at position 322 is conserved across mammals and other species, increasing the likelihood that the change is pathogenic. In addition, computational analyses (AlignGVGD, SIT) suggest that the Val322Met variant may impact the protein. However, this information is not predictive enough to assume pathogenic role but additional studies are necessary to determine the clinical significance of the Val322Met variant with certainty. Entered Variant & Category in GeneInsight? Updated GeneInsight. link
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Gene POU3F4 Image: Second
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certainty. Entered Variant & Category in GeneInsight? Updated GeneInsight link
Entered Variant & Category in GeneInsight? Updated
Geneticist Final Review
Comments on Interpretations All your points are fine, we just reworded for consistency with our templates. I also bumped to VUS-5. I think this variant is
probably pathogenic given the very close phenotype match to POU3F4 HL subtype and location of variant.
Final Novel Variant Assessment Unknown significance-5
Final Variant Interpretation (blurb) The Val322Met variant in POU3F4 has not been reported in the literature nor previously identified by our laboratory in any other families.
However, this mutation occurs within the POU homeodomain (amino acids 279-336) where almost all pathogenic missense mutations have been
identified. In addition, this residue is conserved across mammals and lower species and computational analyses (PolyPhen2, SIFT, AlignGVGD,
MAPP) suggest that the Val322Met variant may impact the protein. However, this information is not predictive enough to assume pathogenicity.
In summary, the clinical significance of this variant cannot be determined with certainty at this time; however based upon the arguments
described above, we would lean towards a more likely pathogenic role.
Entered Interpretation in GeneInsight?
🕨 📕 🗛 🗛 Alamut.Common 🧹 Alamut.Orthologues 🖉 Variant 🖉 DB 🖉 Publ+LMM_data 🧹 Conserv_Biochem 🧹 Splicing 🦯 COSMIC(cancer_only) 🖉 Classification_Rules 🔵 Summary 🖉 Links 🗶 RaceAnd Tail 🖣 💷 🕨

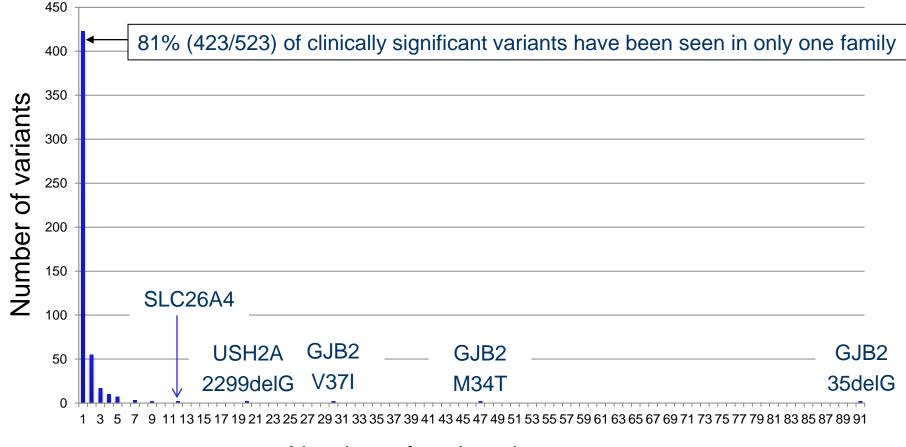


HCM Gene Mutations – 3000 cases tested

>500 clinically significant mutations identified



Hearing Loss Gene Mutations – 2000 Cases Tested

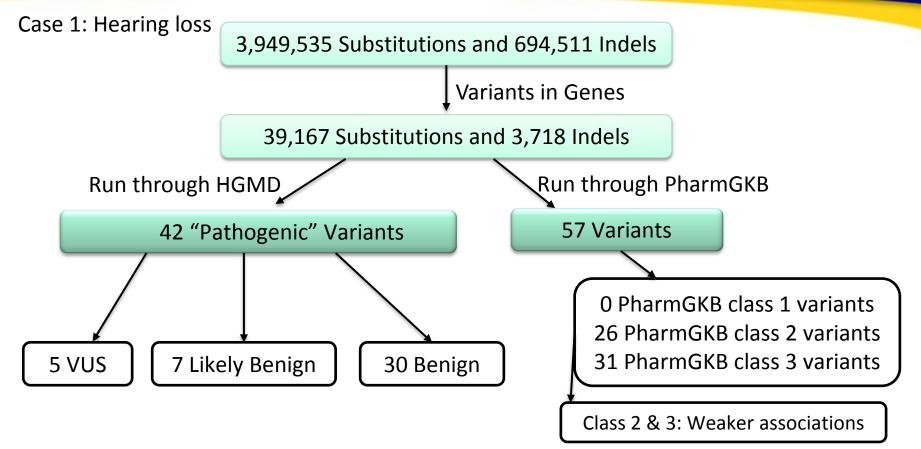


Number of probands

Human Gene Mutation Database

CM023927	CGC-CTC	Arg32Leu	c.95G>T	p.R32L	Deafness	<u>Wu (2002) Genet Med 4, 279</u>	DM G
CM108184	tCGC-AGC	Arg32Ser	c.94C>A	p.R32S	Sensorineural hearing loss	Hayashi (2010) Int J Pediatr Otorhinolaryngol 75, 211	DM G
CM106662	ATT-AAT	Ile33Asn	c.98T>A	p.I33N	Hearing loss	<u>Tsukada (2010) Clin Genet 78, 464</u>	DM7 G
CM091631	ATT-ACT	Ile33Thr	c.98T>C	p.I33T	Deafness	Mani (2009) Eur J Hum Genet 17, 502	DM G
CM077555	ATG-AGG	Met34Arg	c.101T>G	p.M34R	Sensorineural hearing loss	Putcha (2007) Genet Med 9, 413	DM G
CM057547	ATGa-ATA	Met34Ile	c.102G>A	p.M34I	Deafness	<u>Snoeckx (2005) Am J Hum Genet</u> <u>77, 945</u>	Срм G
CM014357	tATG-TTG	Met34Leu	c.100A>T	p.M34L	Deafness	Kudo (2001) Otol Neurotol 22, 858	DM G
QM970679	ATG-ACG	Met34Thr	c.101T>C	p.M34T	Deafness, autosomal dominant 3	Kelsell (1997) Nature 387 , 30 Martin (1999) <i>Hum Mol Genet</i> 8 : 2369 [Functional characterisation] <u>Houseman (2001) <i>J Med Genet</i> 38: 20 [Additional phenotype] 5 more reference(s).</u>	DM2 sLSNP FALQ
CM098251	tATG-GTG	Met34Val	c.100A>G	p.M34V	Hearing impairment, nonsyndromic	<u>Yilmaz (2009) Biochem Genet 48,</u> <u>248</u>	DM G
CM014708	ATC-AGC	Ile35Ser	c.104T>G	p.I35S	Deafness	Dahl (2001) Med J Aust 175 , 191 Mani (2009) Eur J Hum Genet 17 : 502 [Functional characterisation]	DM G
CM065234	CTC-CCC	Leu36Pro	c.107T>C	p.L36P	Deafness	Propst (2006) Laryngoscope 116 , <u>317</u>	DM G
CM042707	GTT-GCT	Val37Ala	c.110T>C	p.V37A	Deafness	Azaiez (2004) Hum Mutat 24, 305	
CM000016	cGTT-ATT	Val37Ile	c.109G>A	p.V37I	Deafness, autosomal recessive 1	Abe (2000) J Med Genet 37 , 41 Wilcox (2000) Hum Genet 106 : 399 [Additional report] Bruzzone (2003) FEBS Lett 533 : 79 [Functional characterisation]	DM7 HEGHP FREQ G

Variant Analysis for General Genome Report

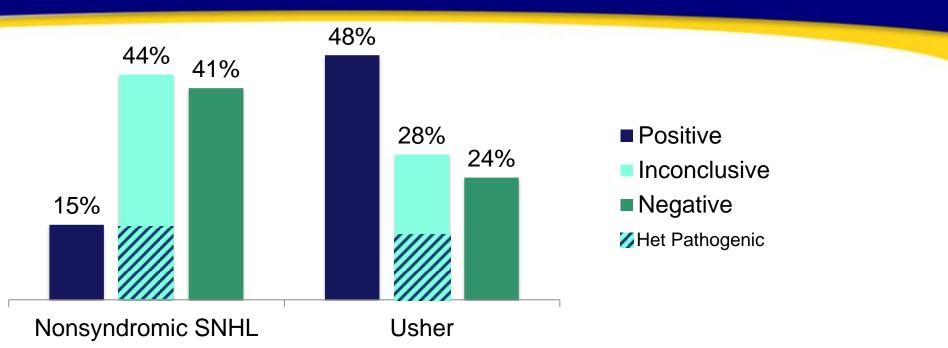


Take home:

No variants met criteria for return as secondary findings in this patient HGMD (representing published literature, has many incorrect classifications)



OtoChip Results – 293 Cases Analyzed



15/186 (8%) of early childhood (≤10yr) HL cases tested positive for an Usher gene mutation





Fumbled DNA Tests Mean Peril for Breast-Cancer Patients

By Robert Langreth - Sep 10, 2012 12:00 AM ET



Debbie McCarron was prepared to get both of her breasts taken off if a blood test in December 2006 revealed she carried a gene that vastly increases the risk of breast cancer. Having survived the disease five years earlier, she didn't want to risk getting it again.

To her relief, her oncologist told her the test, done by Myriad Genetics Inc. (MYGN), had come back negative, "just like I knew it would," McCarron recalls her doctor saying.

🔍 Enlarge image



Debbie McCarron, right, with her genetic counselor, Mariana Niell. Source: Bloomberg



He was wrong. The results, in fact, were positive. McCarron didn't learn this, though, until July 2009, more than two years later, when a genetic counselor reviewed the test following McCarron's surgery to remove a new malignant breast tumor. Since then, her oncologist, Haresh Jhangiani, told Bloomberg he isn't clear about what happened.

"I don't think she was positive. Was she positive?" the doctor said. "I would not tell her it was negative if the test was positive, there must be something more to it."

McCarron now 50 was devastated when she found out "The

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•

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Gorman Call to Pandit Said to Spark Sn Deal **⊡**

Prince Harry's Naked Escapades Expo Vegas Remake ⊡

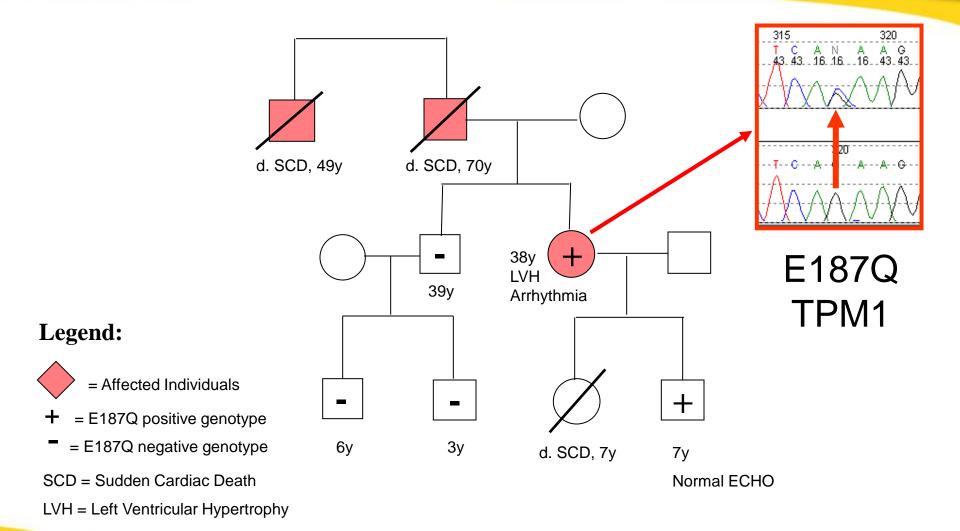
More New	S	>>
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Advertisement



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HCM Family 90







\$\extstyle\$ 125%



Disease-causing mutations in the human <u>beta-cardiac Myosin Heavy</u> <u>Chain</u> gene

- <u>194 hypertrophic cardiomyopathy mutations</u>
- <u>13 dilated cardiomyopathy mutations</u>
- <u>7 other mutations</u>
- <u>7 variants of uncertain effect</u>
- <u>15 polymorphisms</u>

Mutation	Disease	position in <u>M57965.1</u>	UCSC hg17 position	exon/intron
<u>Gly10del</u>	НСМ	57485750	2297275422972752	3
<u>Ala26Val</u>	НСМ	5797	22972705	3
Val39Met	НСМ	5835	22972667	3
Val59Ile	НСМ	5895	22972607	3
Tyr115His	НСМ	6366	22972135	4
Thr124Ile	НСМ	6682	22971819	5
Arg143Gly	НСМ	6738		5
Arg143Trp	НСМ	6738	22971763	5
Arg143Gln	НСМ	6739	22971762	5
1 4464	Цем	C740	22074752	_
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hypertrophic cardiomyopathy mutations

Documenting Logic

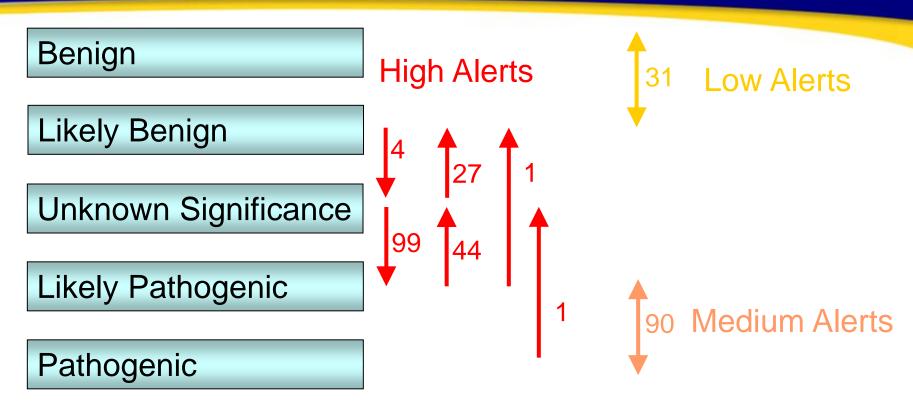
The Ala26Val variant has been reported in 10 HCM probands of Asian descent and was absent from 832 race-matched control chromosomes (Konno 2005, Liu 2005, Song 2005, Wang 2009). However, one of the probands had another pathogenic HCM variant on the same copy of the gene which segregated with all 8 affected family members (Wang 2009). Although segregation in 3 family members was observed in one other family, an additional 5 individuals had the variant without disease including three over age 70 (Liu 2005). Our laboratory has observed this variant in one HCM proband and one DCM proband, neither with a family history of disease, out of over 3500 cases tested (1/215 Asian probands). Across all published and internal studies, this leads to a cumulative allele frequency of 1% (7/652) in Asian HCM probands or 0.1% (8/7848) across all probands. This variant has been observed at a frequency of 0.3% (7/2177) in the 1000 Genomes project with a sub-population frequency of 1.5% (6/388) in the Chinese population. Computational analyses (biochemical amino acid properties, conservation, AlignGVGD, PolyPhen2, and SIFT) suggest that the Ala26Val variant is less likely to impact the protein, particularly given the lack of conservation of the alanine residue in mammals (horse has an aspartic acid) and minimal biochemical change of the alanine to valine substitution. In summary, although additional data is necessary to conclusively determine the clinical significance of this variant, based upon the higher frequency in a race-matched control population (1.5% vs. 1%), the absence of statistically significant segregation data, the lack of a predictive effect from computational algorithms, observations in both HCM and DCM which have different mutational mechanisms, and presence on the background of another pathogenic mutation, this variant is more likely benign.

Variant Interpretations Maintained in GeneInsight

	Tests	Genes	Variants	Disease	References
PÁ	CENTER FOR PERSONAL GENETIC MEDICINE	LIZED GeneInsight [®] I Lab	Users GeneIn	-	& Rehm, Heidi Log Out Guide Support Change Password
	/ariant List 🖾 🛛 p.Ala26Val	(🛛		Variant	Accession
١	/ariant Details: MYH7 c.770	C>T (p.Ala26Val)			>>
			=		
F	ull Details Frequency Not	tes References Interpretat	tion Interp. Hist Asse	essments Seq. Alignm	
	Proposed Interpretation			Edit Appr	ove Reject <<
	Approve Content				A
	Reason(s) For Update New Evidence				
	Interpretation				
	Category/Inher./Excl. Likely Benign				
	Variant Interpretation				
	chromosomes (Konno 2003 the same copy of the gene was observed in one other laboratory has observed th 3500 cases tested (1/215 (7/652) in Asian HCM proba the 1000 Genomes project (biochemical amino acid pro- impact the protein, particul biochemical change of the clinical significance of this v statistically significant segr	een reported in 10 HCM proba 5, Liu 2005, Song 2005, Wang which segregated with all 8 a r family, an additional 5 individu his variant in one HCM proband Asian probands). Across all pu ands or 0.1% (8/7848) across t with a sub-population frequen operties, conservation, AlignGV larly given the lack of conserva alanine to valine substitution. variant, based upon the higher regation data, the lack of a pre- tional mechanisms, and preser	2009). However, one of the affected family members (Wa uals had the variant withou d and one DCM proband, ne blished an internal studies, all probands. This variant h ncy of 1.5% (6/388) in the C /GD, PolyPhen2, and SIFT) s ation of the alanine residue In summary, although addit r frequency in a race-matche edictive effect from computa	probands had another pat ang 2009). Although segreg t disease including three ov ither with a family history o this leads to a cumulative a as been observed at a freq Chinese population. Comput suggest that the Ala26Val va in mammals (horse has a a cional data is necessary to c ed control population (1.5% tional algorithms, observati	hogenic HCM variant on lation in 3 family members ver age 70 (Liu 2005). Our f disease, out of over allele frequency of 1% uency of 0.3% (7/2177) in cational analyses ariant is less likely to spartic acid) and minimal conclusively determine the vs. 1%), the absence of ons in both HCM and DCM

benign.

Variant Classification Changes – HCM Data



~300 category changes over 5 year (~4% of reports/yr)

Aronson SJ, Clark EH, Varugheese M, Baxter S, Babb LJ, Rehm HL. Communicating new knowledge on previously reported genetic variants. *Genet Med 2012:14(8):713-719*.





GeneInsight ClinicSM Interface

	_						l	User Guide Sup	port Arc	onson, amuel	Log Out
Patient Sear		ests Users						IMPO	PTANT II	SAGE & DATA L	
George, Curiou	IS 676345(DEMOA	A-MRN) 05/01/1991 (19)	Male								
ccession #	Status	Test		Overall Interpretation	Indicatio	n			Prima Specii		Genomi Source
/-09-3384 iew Report 🚺	FINAL, 04/05/2010 0 PM	HCM CardioChip (11:17 Sequence Confirm	11 Genes Sequenced) nation Test	(Possibly Outdated)		agnosis of cor n-White syndro		l with Wolff-	LMM_B 04/02/2	llood, Peripheral, 2010	Germline
viewed		Variant				Reported	Families	Current Cate	gory*	Reported Cat	egory
	M	Heterozygous c.1030C	T (n His344Tvr) Exon	9 PRKAG2 (Germline)		4	1	Pathogenic		Unknown Signi	ficance
			(p.m.corrigit), excit	10, 11(10,02 (001111110)		I	1	ranogonio		en la cigni	lioanoo
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iseases/drugs are n	field displays the varia	ant significance only within th	e diseases/drugs that hav					-	tations, if p		7

Registered with FDA as a Class I Exempt Medical Device Integrated into EMR at MGH and BWH





Updated Variant Information

ndividual Reported Variant Interpretation History (Variant 1 of 1) IMPORTANT USAGE & DATA LIMITATION											
Warning: This page only li	Varning: This page only lists information on a single variant. This is outside of the patient report context and may be insufficient for										
re-interpretation of the patient report.											
Heterozygous c.1030C>T (p.His344Tyr), Exon 9, PRKAG2 (Germline)											
Report PM-09-3384 (FINAL, 04/05/2010 01:17 PM), HCM CardioChip (11 Genes Sequenced), Sequence Confirmation Test											
Patient	Geo	rge, Curious 676345(DEMOA-MRN) 05/01/1991 (19) Male									
Current Category*		(Reported: Un	(nown Significance)								
Counts	Rep	orts (1), Families (1)									
Alerts											
Status	1	Date	Туре	Message							
Unreviewed	1	04/06/2010 10:27 AM	Non-incidental Level Change	The category for the PRKAG2 c.1030C>T (p.His344Tyr) association to Pathogenic.	to HCM changed from Unknown Significance						
Mark Reviewed)										
Current Knowledge** Approved 04/05/2010 01:22 PM by Matthew Varugheese											
Diseases/Drugs		ategory	Variant Interpretation								
нсм	P	athogenic	not been reported in the literature nor previously identified in our labora nd the His344Tyr variant occurs within the CBS domain region where a n, the presence of concentric HCM and Wolff-Parkinson-White syndror I features consistent with PRKAG2 mutations, as well as follow-up tes this variant being pathogenic.	all pathogenic PRKAG2 variants have been me in the first proband identified with this							

 Physicians receive alerts via email to be notified of variant changes on their patients

Data in this slide should not be used for any clinical purpose.





Documenting Logic

The Ala26Val variant has been reported in 10 HCM probands of Asian descent and was absent from 832 race-matched control chromosomes (Konno 2005, Liu 2005, Song 2005, Wang 2009). However, one of the probands had another pathogenic HCM variant on the same copy of the gene which segregated with all 8 affected family members (Wang 2009). Although segregation in 3 family members was observed in one other family, an additional 5 individuals had the variant without disease including three over age 70 (Liu 2005). Our laboratory has observed this variant in one HCM proband and one DCM proband, neither with a family history of disease, out of over 3500 cases tested (1/215 Asian probands). Across all published and internal studies, this leads to a cumulative allele frequency of 1% (7/652) in Asian HCM probands or 0.1% (8/7848) across all probands. This variant has been observed at a frequency of 0.3% (7/2177) in the 1000 Genomes project with a sub-population frequency of 1.5% (6/388) in the Chinese population. Computational analyses (biochemical amino acid properties, conservation, AlignGVGD, PolyPhen2, and SIFT) suggest that the Ala26Val variant is less likely to impact the protein, particularly given the lack of conservation of the alanine residue in mammals (horse has an aspartic acid) and minimal biochemical change of the alanine to valine substitution. In summary, although additional data is necessary to conclusively determine the clinical significance of this variant, based upon the higher frequency in a race-matched control population (1.5% vs. 1%), the absence of statistically significant segregation data, the lack of a predictive effect from computational algorithms, observations in both HCM and DCM which have different mutational mechanisms, and presence on the background of another pathogenic mutation, this variant is more likely benign.

Control samples, can, and do, have pathogenic variants, particularly for recessive, late-onset, or low-penetrant diseases.



CENTER FOR PERSONALIZED GENETIC MEDICINE



A common *MYBPC3* (cardiac myosin binding protein C) variant associated with cardiomyopathies in South Asia

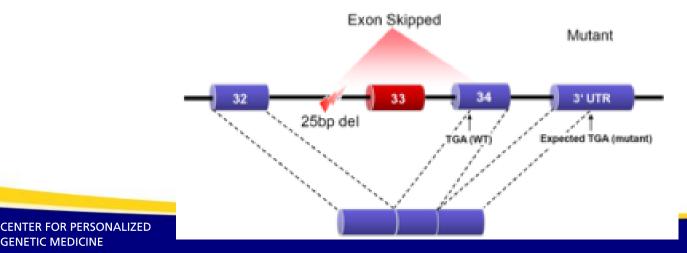


Variant found in 4-8% of South Asians

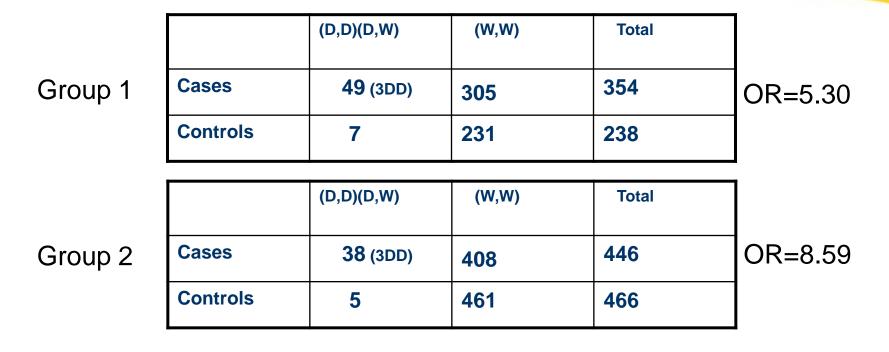
Dhandapany et al. Nat Genet. 2009. 41(2):187-91

IEDICAL

SCHOOL



Case-Control Study

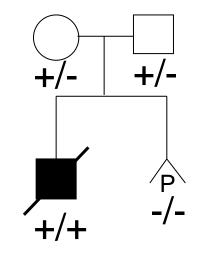


Combined odds ratio from both studied = 6.99

- two homozygotes died as children younger than 3 years
- controls matched for ancestry, age, sex and geography.



MYBPC3 Intron 32 variant in congenital HCM Case





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