CardioGxOne™

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PATIENT INFORMATION	SAMPLE	REFERRING PHYSICIAN
Patient Id	Sample number	Name
Sex	Source	Institution
DOB	Date received	
Reference	Date of report	

Test results of: SAMPLE REPORT

Reason for the study: Dilated cardiomyopathy

Test(s) requested: Dilated cardiomyopathy (81 genes)

RESULT: POSITIVE

We have identified a variant in FLNC considered very likely to be pathogenic, explaining a dilated cardiomyopathy phenotype.

Gene	Variant	Result	Pathogenicity	Population frequency	Number of references
FLNC	**NP_001449.3:p.Ile2150Serfs*25 NM_001458.4:c.6447delC NC_000007.13:g.128493854delC**	Heterozygosis	**Very likely to be pathogenic or disease-causing (++)**	**Mutation (not found in controls)**	0

Clinical interpretation

We consider this mutation in the FLNC gene very likely associated with disease. We have identified several "radical" FLNC mutations in patients referred to our laboratory with common clinical characteristics: potential arrhythmogenic/dilated cardiomyopathy with predominantly left intramyocardial fibrosis and family history of sudden death. These variants cosegregated with the disease in most of the families. Cosegregation studies should be continued to confirm the pathogenicity of this variant. Carrier status could then be used as predictive of disease.

Technical aspects of the study

This sample has been studied by a massive parallel sequencing method using a library that included 81 genes related to dilated cardiomyopathy. Both sensitivity and specificity are above 99% for SNVs and small INDELs (≤ 20 bp).

Signatures

James Dermody

James Dermody, PhD
Laboratory Director
ABMG Certified, Clinical Molecular Genetics
Admera Health LLC

Doc. Lorenzo Monserrat Iglesias
Cardiologist and Scientific Director
Health in Code

DETAILED RESULTS

Gene: FLNC (Encoding the protein: Filamin-C)
NP_001449.3:p.Ile2150Serfs*25/NC_000007.13:g.128493854delC

Heterozygous carrier: mutation occurs in just one copy of the gene.

Next Generation Sequencing stats: Depth of coverage: 1199. Quality of the variant (0-255): 223.

Mutation nomenclature: Nucleotide code: NM_001458.4:c.6447delC, NC_000007.13:g.128493854delC. Amino acid code: NP_001449.3:p.Ile2150Serfs*25. Alternative names at the protein level: NP_001449.3:p.I2150Sfs*25. Located in: Initial exon: 39, Final exon: 39.

Pathogenicity: very likely to be pathogenic or disease-causing (++).

Population frequency: mutation (not found in controls).

Clinical information

To the best of our knowledge, this variant has not been previously described in any scientific publication or in public databases of genotypes of the general population.

- Similar pathogenic variants studied in the *FLNC* gene: In our laboratory, we have identified more than 40 radical mutations in the *FLNC* gene (affecting the splicing mechanism, nonsense-type, or frame-shift mutations). All of them are invariably related to the development of an overlapping dilated/arrhythmogenic cardiomyopathy phenotype (Ortiz *et al.,* J Am Coll Cardiol. 2016;68:2440-2451).
The main findings in carriers are dilation and systolic dysfunction, which predominantly affects the left ventricle (degree of involvement may be low), important presence of fibrosis in left ventricular myocardium, frequent ventricular arrhythmias, and a high rate of familial sudden death (generally occurring around the fourth decade of life). It is worth noting that families with similar mutations identified to date by our group and collaborators do not present with clinical skeletal myopathy, even at an advanced age. Inferolateral negative T waves with low voltages on electrocardiography were observed in one third of the carries. Penetrance was >97% in carriers older than 40. Truncating mutations in FLNC cosegregated with this phenotype with a dominant inheritance pattern.

Survival analysis

Survival curves built by the Kaplan-Meier method showing freedom from cardiovascular death in carriers of truncating variants in FLNC by sex. Events are unusual under the age of 30, and incidence becomes higher after the age of 40. The prognosis seems to be better in females (p=0.003): at the age of 50, 40% of men and only 10% of women had died of a cardiovascular cause. Median survival free of cardiovascular death was 60 years in males and 80 years in females.

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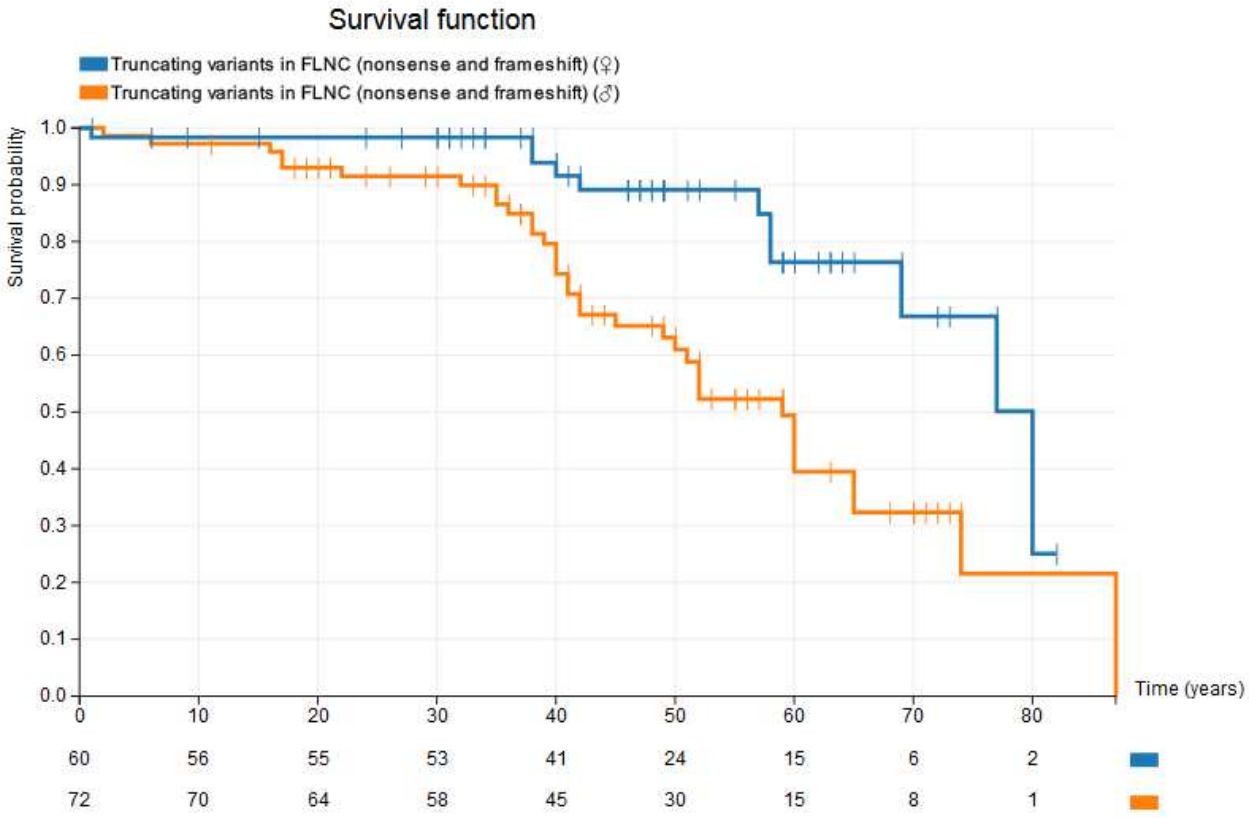

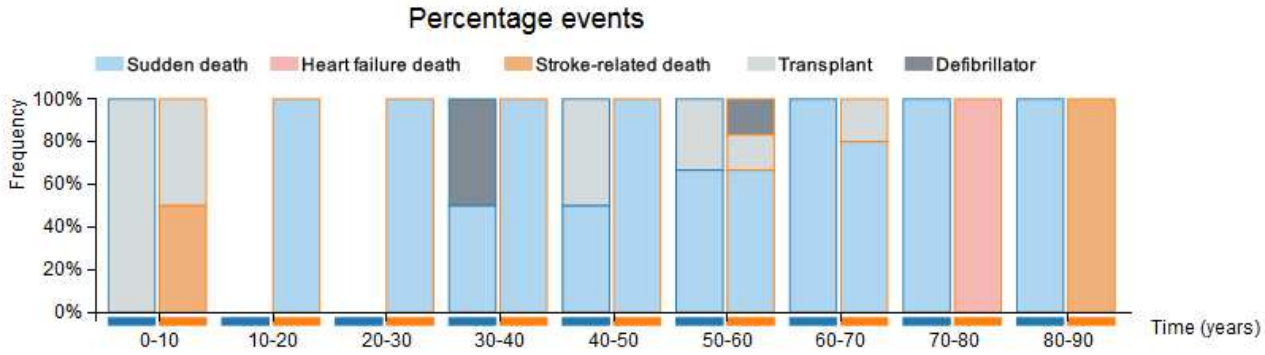

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Bioinformatics study

This deletion leads to a shift in the reading frame at amino acid Ile2150 of the protein, causing the introduction of 24 new amino acids followed by a premature stop codon. Ile2150 belongs to the interdomain insert, which mediates targeting to myofibrillar Z-lines. The presence of this mutation produces an abnormal transcript that could be rapidly degraded before translation by the nonsense-mediated mRNA decay (NMD) pathway. The single functional copy (normal allele) might produce enough protein to fulfil physiological requirements. However, if the amount of protein is not sufficient, disease may appear due to a mechanism called haploinsufficiency. Occasionally, truncated proteins that are not eliminated would result in proteins lacking about 20% of their sequence, missing domain hinge 2 and filamin repeat 24, important for dimerization. They have the potential to interfere with the function of the normal protein in a dominant negative fashion. In the absence of specific functional studies, we cannot determine the actual molecular mechanism set off by this specific variant.

To date, according to the Exome Aggregation Consortium (ExAC), there are only 9 variants (5 nonsense and 4 frameshifts) that lead to truncation in this gene. All of them have been described in only one carrier (MAF <0.01%). In addition, in this database, it is assigned a pLI (probability of LoF intolerance) value of 1.00, indicating that FLNC is very intolerant to haploinsufficiency.

Primary functions of protein

FLNC encodes filamin C (or gamma-filamin), one of the three filamin-related proteins. Filamins link actin filaments in orthogonal networks and participate in the anchorage of membrane proteins to the cytoskeleton. In addition to its anchoring and crosslinking functions, FLNC is known to scaffold a wide range of signaling pathways through interactions with signal transduction molecules, receptors, and ion channels. FLNC is mainly expressed in cardiac and skeletal muscle.

Related phenotypes and inheritance patterns

Early reports described mutations in the FLNC gene as causing skeletal myofibrillar myopathy, but later data suggest that the presence of cardiomyopathy could be the main clinical phenotype associated with mutations in this gene. Truncating variants (nonsense, frameshift, and splice-site) are associated with a particular form of arrhythmogenic dilated cardiomyopathy: frequent ventricular arrhythmias with a high incidence of sudden cardiac death, left dominant ventricular involvement with high degrees of fibrosis, and systolic dysfunction. A few missense variants have been described and associated with restrictive cardiomyopathy and hypertrophic cardiomyopathy (with a higher evidence for the first phenotype). The pattern of inheritance is autosomal dominant.

Conclusion

We consider that this mutation in the FLNC gene is very likely associated with disease. We have identified several "radical" FLNC mutations in patients referred to our laboratory with common clinical characteristics: potential arrhythmogenic/dilated cardiomyopathy with predominantly left intramyocardial fibrosis and family history of sudden death. These variants cosegregated with the disease in most of the families, with a very high penetrance after the age of 40.
Cosegregation studies should be continued to confirm the pathogenicity of this variant. Carrier status could then be used as predictive of disease.

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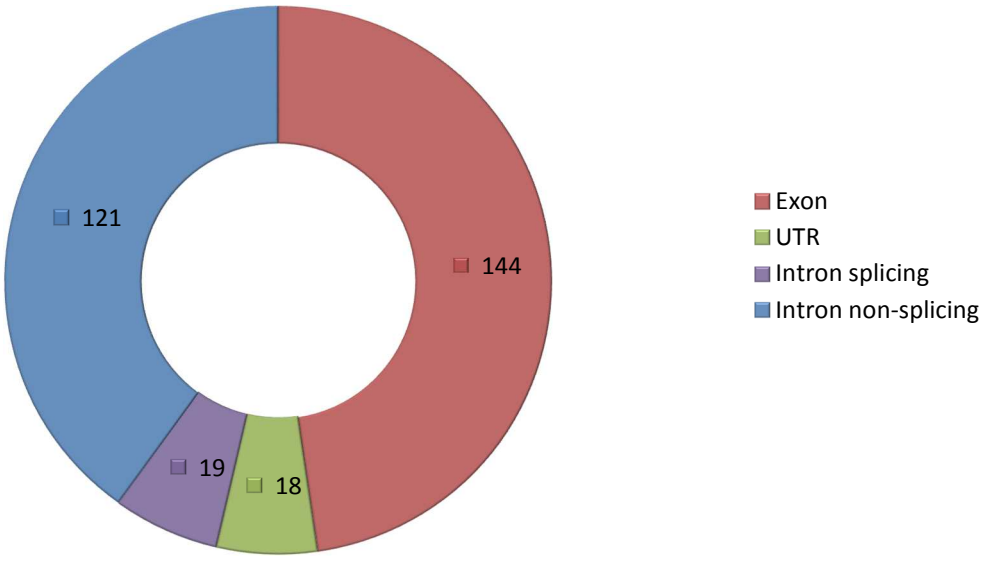
APPENDIX: OTHER IDENTIFIED VARIANTS

AVAILABLE INFORMATION ON OTHER IDENTIFIED VARIANTS

We have identified other genetic variants that we consider not to be associated with disease development either because they have been reported in healthy controls or because they do not affect protein structure nor function.

Region	Variants found
Exonic	**144**
Synonymous	85
Nonsynonymous	56
Nonsense	1
Insertion	1
Frameshift	1
Intronic	**140**
Intronic splicing	19
UTR	**18**
Total	**302**



Only good quality variants were included (QUAL ≥170)

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DETAILED INFORMATION ON OTHER EVALUATED VARIANTS

Gene	Variant	Result	Pathogenicity	Population frequency	Number of references
BRAF	**NP_004324.2:p.Arg360* NM_004333.4:c.1078C>T NC_000007.13:g.140494170G>A**	Heterozygosis	**Unknown clinical significance (?)**	**Mutation (not found in controls)**	0

Gene: BRAF (Encoding the protein: Serine/threonine-protein kinase B-raf)
NP_004324.2:p.Arg360*/NC_000007.13:g.140494170G>A

Heterozygous carrier: mutation occurs in just one copy of the gene.

Next Generation Sequencing stats: Depth of coverage: 985. Quality of the variant (0-255): 223.

Mutation nomenclature: Nucleotide code: NM_004333.4:c.1078C>T, NC_000007.13:g.140494170G>A. Amino acid code: NP_004324.2:p.Arg360*. Alternative names at the protein level: NP_004324.2:p.R360*. Located in: exon 8.

Pathogenicity: unknown clinical significance (?).

Population frequency: mutation (not found in controls).

Clinical information

To the best of our knowledge, this variant has not been previously described in any scientific publication or in public databases of genotypes of the general population.

BRAF is a member of a small family of protein kinases that are effectors of the RAS/MAPK pathway. Mutations in this gene have been associated with the cardiofaciocutaneous syndrome (50-75% of patients) and, less frequently, with Noonan and Leopard syndromes (about 2% of cases). The majority of the mutations are missense variants, probably associated with a gain of function, located in the highly conserved functional domains CR1 to CR3. Truncating variants have not been associated with disease to date; on the other hand, according to the Exome Aggregation Consortium (ExAC), there are only two variants in the control population that lead to truncation in this gene with a MAF <0.01%. In addition, in this database, BRAF is assigned with a pLI (probability of LoF intolerance) value of 1.00, indicating that could be intolerant to haploinsufficiency.

Bioinformatics study

This variant produces a premature stop codon at amino acid p.Arg360. The presence of this mutation produces an abnormal transcript that could be rapidly degraded before translation by the nonsense-mediated mRNA decay (NMD) pathway. The single functional copy (normal allele) might produce enough protein to fulfil physiological requirements. However, if the amount of protein is not sufficient, disease may appear due to a mechanism called haploinsufficiency. Occasionally, truncated proteins that are not eliminated could result in proteins lacking about 53% of the sequence, missing the protein kinase domain. They have the potential to interfere with the function of the normal protein in a dominant negative fashion. In the absence of specific functional studies, we cannot determine the actual molecular mechanism set off by this specific variant.

Primary functions of protein

This protein plays a role in regulating the MAP kinase/ERKs signaling pathway, which affects cell division, differentiation, and secretion.

Conclusions

With the available information we cannot establish the pathogenicity of this variant with certainty. Germinal mutations in this gene have been clearly associated with RASopathies, but most of them are missense ones located in relevant functional domains of the protein (the mechanism would be a gain of function). No truncating variants have been associated with disease to date, but they are also very rare in the control population. Inclusion of the variant could be considered only in a research context, especially if a RASopathy is suspected in the patient or his/her family.

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List of probably non-disease causing exonic genetic variants (excluding synonymous)

Gene	Variant	Function	Exonic function	dbSNP	dbSNP freq.	1000G MAF	5000G MAF	HiC freq.	AF1	DP Qual	Qual	Freq. alt.
ABCC9	NP_005682.2:p.Val734Ile; NM_005691.3:c.2200G>A; NC_000012.11:g.22017410C>T	Exon splicing	Nonsynonymous	rs61688134	0.69	0.4	0.92	3.71	Het.	932	223	48.8
ALMS1	NP_055935.4:p.Ser524_Leu525insPro; NM_015120.4:c.1573_1574insCTC; NC_000002.11:g.73675230_73675231insCTC	Exon	Insertion	rs587621330	63.69	60.04	44.92	87.24	Het.	1388	255	46
ALMS1	NP_055935.4:p.Val671Gly; NM_015120.4:c.2012T>G; NC_000002.11:g.73675669T>G	Exon	Nonsynonymous	rs2037814		87.22	11.94	98.74	Het.	1437	223	51.4
ALMS1	NP_055935.4:p.Ser2574Asn; NM_015120.4:c.7721G>A; NC_000002.11:g.73716810G>A	Exon	Nonsynonymous	rs3820700		12.68	12.01	21.31	Het.	1558	223	48.7
ALMS1	NP_055935.4:p.Asp2672His; NM_015120.4:c.8014G>C; NC_000002.11:g.73717103G>C	Exon	Nonsynonymous	rs2017116	85.92	13.6	12.89	22.05	Het.	1632	223	48.4
ALMS1	NP_055935.4:p.Arg4029Lys; NM_015120.4:c.12086G>A; NC_000002.11:g.73828538G>A	Exon	Nonsynonymous	rs1052161		58.23	46.34	86.86	Het.	1064	223	48.1
DMD	NP_003997.1:p.Arg2937Gln; NM_004006.2:c.8810G>A; NC_000023.10:g.31496350C>T	Exon	Nonsynonymous	rs1800280		88.19	4.32	95.9	Hom.	800	255	100
DMD	NP_003997.1:p.Arg1745His; NM_004006.2:c.5234G>A; NC_000023.10:g.32380996C>T	Exon	Nonsynonymous	rs1801187		46.52	33.31	51.01	Het.	1270	223	47.2
DMD	NP_003997.1:p.Asp882Gly; NM_004006.2:c.2645A>G; NC_000023.10:g.32503194T>C	Exon	Nonsynonymous	rs228406	72.22	74.83	34.98	72.89	Hom.	1121	255	100
DSG2	NP_001934.2:p.Arg773Lys; NM_001943.3:c.2318G>A; NC_000018.9:g.29122799G>A	Exon	Nonsynonymous	rs2278792	26.64	24	19.73	39.04	Het.	551	191	52.3
EYA4	NP_004091.3:p.Gly277Ser; NM_004100.4:c.829G>A; NC_000006.11:g.133789728G>A	Exon	Nonsynonymous	rs9493627	34.44	40.95	38.3	56.63	Het.	1556	223	49
FKTN	NP_001073270.1:p.Arg56Cys; NM_001079802.1:c.166C>T; NC_000009.11:g.108363426C>T	Exon splicing	Nonsynonymous	rs41277797	2.28	1.04	2.06	6.7	Het.	1157	223	47.9
FKTN	NP_001073270.1:p.Arg203Gln; NM_001079802.1:c.608G>A; NC_000009.11:g.108366734G>A	Exon	Nonsynonymous	rs34787999	24.12	15.81	26.46	49.38	Het.	953	223	45.7
FOXD4	NP_997188.2:p.*440Tyr; NM_207305.4:c.1320G>C; NC_000009.11:g.116800C>G	Exon	Nonsynonymous	rs79220013	26.18		20.31	47.78	Het.	594	223	48.8
FOXD4	NP_997188.2:p.Ser430Gly; NM_207305.4:c.1288A>G; NC_000009.11:g.116832T>C	Exon	Nonsynonymous	rs4742632	27.39		23.56	49.49	Het.	821	223	46.9
GAA	NP_000143.2:p.Asp91Asn; NM_000152.3:c.271G>A; NC_000017.10:g.78078656G>A	Exon	Nonsynonymous	rs1800299	2.07	1.16	2.31	5.37	Het.	1282	223	47.9

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Gene	Variant	Function	Exonic function	dbSNP	dbSNP freq.	1000G MAF	5000G MAF	HiC freq.	AF1	DP Qual	Qual	Freq. alt.
GAA	NP_000143.2:p.His199Arg; NM_000152.3:c.596A>G; NC_000017.10:g.78079597A>G	Exon	Nonsynonymous	rs1042393	67.16	60.08	32.75	92.98	Hom.	669	255	100
GAA	NP_000143.2:p.Arg223His; NM_000152.3:c.668G>A; NC_000017.10:g.78079669G>A	Exon	Nonsynonymous	rs1042395	67.3	60.24	32.76	92.91	Hom.	703	255	100
GAA	NP_000143.2:p.Glu689Lys; NM_000152.3:c.2065G>A; NC_000017.10:g.78087041G>A	Exon	Nonsynonymous	rs1800309	8.72	7.81	2.96	7.18	Het.	934	223	44.9
GAA	NP_000143.2:p.Val780Ile; NM_000152.3:c.2338G>A; NC_000017.10:g.78091405G>A	Exon splicing	Nonsynonymous	rs1126690	72.89	71.19	26.68	93.97	Hom.	840	255	100
GLB1	NP_000395.2:p.Cys521Arg; NM_000404.2:c.1561T>C; NC_000003.11:g.33055721A>G	Exon	Nonsynonymous	rs4302331	97.79	92.71	6.74	99.97	Hom.	517	255	100
GLB1	NP_000395.2:p.Pro10Leu; NM_000404.2:c.29C>T; NC_000003.11:g.33138549G>A	Exon	Nonsynonymous	rs7637099	55.72	43.19	47.4	87.39	Het.	1536	223	44.8
HFE	NP_000401.1:p.His63Asp; NM_000410.3:c.187C>G; NC_000006.11:g.26091179C>G	Exon	Nonsynonymous	rs1799945	10.53	7.31	11.07	32.05	Het.	992	223	48.8
JUP	NP_068831.1:p.Met697Leu; NM_021991.2:c.2089A>T; NC_000017.10:g.39912145T>A	Exon splicing	Nonsynonymous	rs1126821	34.37	58.73	30.3	92	Hom.	629	255	100
LAMA4	NP_001098676.2:p.Gly1117Ser; NM_001105206.2:c.3349G>A; NC_000006.11:g.112457390C>T	Exon	Nonsynonymous	rs2032567	77.2	83.99	24.87	92.3	Het.	1030	223	48.9
LAMA4	NP_001098676.2:p.Tyr498His; NM_001105206.2:c.1492T>C; NC_000006.11:g.112493872A>G	Exon	Nonsynonymous	rs1050348		75.84	35.12	87.18	Het.	529	223	46.9
LAMA4	NP_001098676.2:p.Ala283Glu; NM_001105206.2:c.848C>A; NC_000006.11:g.112508770G>T	Exon	Nonsynonymous	rs9400522	100	100		99.79	Hom.	1080	255	100
MYBPC3	NP_000247.2:p.Ser236Gly; NM_000256.3:c.706A>G; NC_000011.9:g.47370041T>C	Exon	Nonsynonymous	rs3729989		6.71	10.34	22.7	Hom.	695	255	100
MYH6	NP_002462.2:p.Ala1130Thr; NM_002471.3:c.3388G>A; NC_000014.8:g.23859610C>T	Exon	Nonsynonymous	rs28730771		7.29		27.42	Het.	1737	223	36.4
MYOT	NP_006781.1:p.Lys74Gln; NM_006790.2:c.220A>C; NC_000005.9:g.137206560A>C	Exon	Nonsynonymous	rs6890689	99.59		1.48	99.98	Hom.	1351	255	99.9
MYPN	NP_115967.2:p.Phe628Leu; NM_032578.3:c.1884C>G; NC_000010.10:g.69926334C>G	Exon	Nonsynonymous	rs10823148	43.34	31.65	39.6	70.28	Het.	1203	223	48.3
MYPN	NP_115967.2:p.Ser691Asn; NM_032578.3:c.2072G>A; NC_000010.10:g.69933921G>A	Exon	Nonsynonymous	rs10997975	42.7	33.59	39.24	67.82	Het.	1585	223	48.1
MYPN	NP_115967.2:p.Ser707Asn; NM_032578.3:c.2120G>A; NC_000010.10:g.69933969G>A	Exon	Nonsynonymous	rs7916821	42.18	32.51	39.24	67.66	Het.	1418	223	46.6

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Gene	Variant	Function	Exonic function	dbSNP	dbSNP freq.	1000G MAF	5000G MAF	HiC freq.	AF1	DP Qual	Qual	Freq. alt.
MYPN	NP_115967.2:p.Ser803Arg; NM_032578.3:c.2409C>G; NC_000010.10:g.69934258C>G	Exon	Nonsynonymous	rs3814182	51.85	47.14	47.7	78.68	Hom.	1317	255	99.9
MYPN	NP_115967.2:p.Pro1135Thr; NM_032578.3:c.3403C>A; NC_000010.10:g.69959242C>A	Exon	Nonsynonymous	rs7079481	42.86	34.03	40.59	67.54	Het.	1193	223	47.4
NEBL	NP_006384.1:p.Asp378His; NM_006393.2:c.1132G>C; NC_000010.10:g.21134282C>G	Exon	Nonsynonymous	rs41277370	6.41	3.85	6.1	16.03	Het.	878	223	43.6
NEBL	NP_006384.1:p.Met351Val; NM_006393.2:c.1051A>G; NC_000010.10:g.21139389T>C	Exon	Nonsynonymous	rs4025981	6.4	3.87	6.11	16	Het.	1264	223	49.1
PRDM16	NP_071397.3:p.Ser533Pro; NM_022114.3:c.1597T>C; NC_000001.10:g.3328358T>C	Exon	Nonsynonymous	rs870124		94.51	12.23	97.41	Hom.	1758	255	100
RBM20	NP_001127835.2:p.Trp768Ser; NM_001134363.2:c.2303G>C; NC_000010.10:g.112572458G>C	Exon	Nonsynonymous	rs1417635	0.17	99.1		99.66	Hom.	1245	255	100
RBM20	NP_001127835.2:p.Glu1223Gln; NM_001134363.2:c.3667G>C; NC_000010.10:g.112595719G>C	Exon	Nonsynonymous	rs942077	74.98	69.71	24.16	96.44	Hom.	716	255	99.9
TMPO	NP_003267.1:p.Gln599Glu; NM_003276.2:c.1795C>G; NC_000012.11:g.98927830C>G	Exon	Nonsynonymous	rs17459334	8.64	5.89	7.11	16.19	Het.	618	223	50
TTN	NP_003310.4:p.Arg22110His; NM_003319.4:c.66329G>A; NC_000002.11:g.179412829C>T	Exon	Nonsynonymous	rs72648251			0.07	0.05	Het.	1291	223	48.9
TTN	NP_003310.4:p.Ala13351Pro; NM_003319.4:c.40051G>C; NC_000002.11:g.179444768C>G	Exon	Nonsynonymous	rs4145333	99.85	99.46	0.44	99.69	Hom.	1602	255	100
TTN	NP_003310.4:p.Arg13140Lys; NM_003319.4:c.39419G>A; NC_000002.11:g.179446381C>T	Exon	Nonsynonymous	rs72646869		0.58	1.62	4.1	Het.	1416	223	48.5
TTN	NP_596870.2:p.Arg4915His; NM_133379.4:c.14744G>A; NC_000002.11:g.179612383C>T	Exon	Nonsynonymous	rs72648907		1.46	4.24	10.17	Het.	1451	223	50
TTN	NP_596870.2:p.Asp3747Gly; NM_133379.4:c.11240A>G; NC_000002.11:g.179615887T>C	Exon	Nonsynonymous	rs922984	82.33	73.3	18.19	97.86	Hom.	1300	255	99.9
TTN	NP_596870.2:p.Leu3732Phe; NM_133379.4:c.11196G>C; NC_000002.11:g.179615931C>G	Exon	Nonsynonymous	rs922985	99.19	97.5	2.75	99.55	Hom.	1357	255	99.8
TTN	NP_001254479.2:p.Gly3751Asp; NM_001267550.2:c.11252G>A; NC_000002.11:g.179620951C>T	Exon splicing	Nonsynonymous	rs7585334	84.89	80.45	9.72	99.01	Hom.	996	255	100
TTN	NP_001254479.2:p.Ala3576Thr; NM_001267550.2:c.10726G>A; NC_000002.11:g.179621477C>T	Exon	Nonsynonymous	rs6433728	99.97	99.9	0.08	99.98	Hom.	947	255	100
TTN	NP_003310.4:p.Ser3373Asn; NM_003319.4:c.10118G>A; NC_000002.11:g.179623758C>T	Exon	Nonsynonymous	rs2291310	84.95	80.89	9.76	98.82	Hom.	1306	255	100

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Gene	Variant	Function	Exonic function	dbSNP	dbSNP freq.	1000G MAF	5000G MAF	HiC freq.	AF1	DP Qual	Qual	Freq. alt.
TTN	NP_003310.4:p.Val3215Met; NM_003319.4:c.9643G>A; NC_000002.11:g.179629461C>T	Exon	Nonsynonymous	rs2291311	84.82	80.49	10.35	99.01	Hom.	871	255	99.9
TTN	NP_003310.4:p.Ser1249Leu; NM_003319.4:c.3746C>T; NC_000002.11:g.179644035G>A	Exon	Nonsynonymous	rs1552280		92.01	4.91	99.57	Hom.	1171	255	100
TTN	NP_003310.4:p.Lys1155Glu; NM_003319.4:c.3463A>G; NC_000002.11:g.179644855T>C	Exon	Nonsynonymous	rs10497520	68.67	50.02	26.51	96.19	Hom.	1353	255	99.8
TTN	NP_003310.4:p.Thr765Ile; NM_003319.4:c.2294C>T; NC_000002.11:g.179650408G>A	Exon	Nonsynonymous	rs35813871		10.04	18.97	42.76	Hom.	1067	255	100
TXNRD2	NP_006431.2:p.Ile370Thr; NM_006440.4:c.1109T>C; NC_000022.10:g.19868218A>G	Exon	Nonsynonymous	rs1139793	70.19	71.83	20.39	93.44	Het.	886	223	44
TXNRD2	NP_006431.2:p.Ser299Arg; NM_006440.4:c.895A>C; NC_000022.10:g.19882984T>G	Exon	Nonsynonymous	rs5992495	19.04	25.12	27.09	34.17	Het.	1201	223	49.5
TXNRD2	NP_006431.2:p.Ala66Ser; NM_006440.4:c.196G>T; NC_000022.10:g.19907099C>A	Exon	Nonsynonymous	rs5748469	44.99	48.3	29.56	58.81	Het.	739	223	50.2

Function: location of the variant according to RefSeq annotation database: exonic, intronic, splicing, UTR. dbSNP: identification of the Single Nucleotide Polymorphism Database. dbSNP freq.: variant frequency taken from dbSNP (%). 1000G MAF: minor allele frequency taken from the 1000 Genomes Project (%). 5000G MAF: minor allele frequency taken from the 5000 Genomes Project (%). HiC freq.: variant frequency taken from our HiC database (%). AF1: heterozygous, hemizygous or homozygous. DP Qual: depth of coverage after filtering low quality bases or low quality alignments. Qual: quality of the variant reported by SAMtools (maximum value is 255 and means that the variant has a high probability of being different from homozygous wild type. Low values indicate that it has a high probability of being homozygous for wild type and thus having a low probability of being a true variant). Freq. alt.: the frequency of alternative allele in high quality fragments (%). Only good quality variants were included (QUAL ≥170).

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List of probably non-disease causing intronic genetic variants in splicing zones

Gene	Variant	dbSNP	dbSNP freq.	1000G MAF	5000G MAF	HiC freq.	AF1	DP Qual	Qual	Freq. alt.
ABCC9	NM_005691.3:c.574-5C>A; NC_000012.11:g.22068849G>T	rs3759236	61.51	64.48	41.21	86.16	Het.	603	223	48.8
ACTN2	NM_001103.3:c.877-8C>G; NC_000001.10:g.236902594C>G	rs2288601	77.5	76.76	22.25	94.81	Hom.	665	255	100
CRYAB	NM_001885.2:c.324+4T>G; NC_000011.9:g.111781047A>C	rs11603779	26.84	23.98	27.24	54.53	Het.	1385	223	48.3
DMD	NM_004006.2:c.94-9_94-8insT; NC_000023.10:g.32867953_32867954insA	rs79920566	86.82	7.63	9.37	15.53	Het.	1405	191	48.7
GAA	NM_000152.3:c.547-4C>G; NC_000017.10:g.78079544C>G	rs3816256	32.7	60.28	32.73	92.98	Hom.	564	255	99.7
GAA	NM_000152.3:c.858+7_858+8insAGCGGGC; NC_000017.10:g.78081528_78081529insAGCGGGC	rs3071247				92.3	Hom.	660	255	100
GATA6	NM_005257.5:c.1620+7A>G; NC_000018.9:g.19763011A>G	rs3764962		22.4	21.67	6.97	Het.	964	223	51.8
GLB1	NM_000404.2:c.1233+8T>C; NC_000003.11:g.33063050A>G	rs13093698	20.65	16.35	21.25	35.78	Het.	540	223	47.6
HFE	NM_000410.3:c.340+4T>C; NC_000006.11:g.26091336T>C	rs2071303		42.67	34.58	61.81	Het.	829	223	46.9
LAMA2	NM_000426.3:c.8548-10T>C; NC_000006.11:g.129826335T>C	rs113644365	0.86	0.36	0.92	3.36	Het.	702	223	49
MYH7	NM_000257.3:c.3337-3_3337-2insC; NC_000014.8:g.23889447_23889448insG	rs564923630	0.41	4.03		16.77	Het.	1147	223	33
NEBL	NM_006393.2:c.1671+9T>C; NC_000010.10:g.21120116A>G	rs10491056	39.25	46.9	43.02	64.44	Hom.	1005	255	100
NEBL	NM_006393.2:c.1008+5A>G; NC_000010.10:g.21141469T>C	rs703089	97.02	95.25	0.72	100	Hom.	470	255	100
RBM20	NM_001134363.2:c.3452-9G>C; NC_000010.10:g.112590810G>C	rs7070640	98.43	96.96	2.87	99.73	Hom.	487	255	100
SLC22A5	NM_003060.3:c.652+6A>G; NC_000005.9:g.131719999A>G	rs4551059	100	100		100	Hom.	853	255	100
TNNI3	NM_000363.4:c.373-10T>G; NC_000019.9:g.55665584A>C	rs7252610	100	100		99.98	Hom.	294	255	99.3
TNNI3	NM_000363.4:c.25-8T>A; NC_000019.9:g.55668509A>T	rs3729836	63.99	45.81	31.62	43.37	Het.	676	223	50.6
TNNT2	NM_001001430.2:c.53-11_53-7delCTTCT; NC_000001.10:g.201341181_201341185delAGAAG	rs45533739		52.04		81.9	Het.	982	255	49.7
TTN	NM_003319.4:c.4342+6C>T; NC_000002.11:g.179642425G>A	rs719201	95.69	91.15	5.35	99.73	Hom.	1017	255	100

dbSNP: identification of the Single Nucleotide Polymorphism Database. dbSNP freq.: variant frequency taken from dbSNP (%). 1000G MAF: minor allele frequency taken from the 1000 Genomes Project (%). 5000G MAF: minor allele frequency taken from the 5000 Genomes Project (%). HiC freq.: variant frequency taken from our HiC database (%). AF1: heterozygous, hemizygous or homozygous. DP Qual: depth of coverage after filtering low quality bases or low quality alignments. Qual: quality of the variant reported by SAMtools (maximum value is 255 and means that the variant has a high probability of being different from homozygous wild type. Low values indicate that it has a high probability of being homozygous for wild type and thus having a low probability of being a true variant). Freq. alt.: the frequency of alternative allele in high quality fragments (%). Only good quality variants were included (QUAL ≥170).

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APPENDIX: TECHNICAL ASPECTS

Detailed technical aspects

CardioGxOne was performed using a multiple approach based on targeted Next Generation Sequencing (NGS) combined with the gold standard Sanger technique. Patient specimens (blood, saliva, tissue) are subjected to automatic genomic DNA purification (QIAsymphony SP®, Qiagen), and sample preparation is carried out using the Agilent SureSelectXT Target Enrichment technology for Illumina paired-end multiplexed sequencing method. Enrichment is performed using a custom SureSelect library (Agilent) for the coding regions and adjacent intronic areas for the selected genes. After cluster generation on a cBot (Illumina), captured DNA is sequenced on either Illumina HiSeq 1500, MiSeq or NextSeq platform. Clinically relevant variants and low-coverage regions are tested in parallel by standard Sanger sequencing. The analytical sensitivity and accuracy of this assay are greater than 99% for single nucleotide variants (SNVs) and small insertions/deletions (INDELs).

CardioGxOne was developed and assessed for accuracy and precision by Admera Health. The design of the custom capture library is property of Health in Code and includes the following 81 genes related to dilated cardiomyopathy:

ABCC9, ACTA1, ACTC1, ACTN2, ALMS1, ANKRD1, BAG3, BRAF, CAV3, CRYAB, CSRP3, CTF1, DES, DMD, DNAJC19, DOLK, DSC2, DSG2, DSP, EMD, EYA4, FHL2, FHOD3, FKRP, FKTN, FLNC, FOXD4, GAA, GATA4, GATA6, GATAD1, GLA, GLB1, HFE, JUP, KCNJ2, KCNJ8, LAMA2, LAMA4, LAMP2, LDB3, LMNA, MURC, MYBPC3, MYH6, MYH7, MYL2, MYL3, MYOT, MYPN, NEBL, NEXN, NKX2-5, PDLIM3, PKP2, PLN, PRDM16, PSEN1, PSEN2, PTPN11, RAF1, RBM20, RYR2, SCN5A, SGCA, SGCB, SGCD, SLC22A5, TAZ, TBX20, TCAP, TMEM43, TMPO, TNNC1, TNNI3, TNNT2, TPM1, TTN, TTR, TXNRD2, VCL.

The genes included in this test have been selected on a clinical basis according to their relation with a particular phenotype and classified taking in consideration the level of evidence of this relation (priority genes, secondary genes, candidate genes).

Probes were designed to cover all coding exons and 30 bp at intronic or UTR flanking regions. Those regions with suboptimal quality coverage were sequenced by dideoxy Sanger technique. This test is not able to identify genetic variants located at deep intronic/UTR regions.

CardioGxOne is aimed at identifying single nucleotide variants (SNVs) and small insertions/deletions (INDELs) up to 20 bp. Genetics variants are described following the Human Genome Variation Society (HGVS) recommendations (www.hgvs.org).

Those selected genetic variants that were considered potentially associated with the patient's phenotype or constitute relevant incidental findings are reported in the main table of the report on the first page. Please note that clinical interpretation of variants could be subject to changes as new scientific evidence appears.

Confirmation by dideoxy Sanger sequencing will be performed in those selected variants included in the main table that meet the following conditions:

- Point mutations identified with suboptimal quality parameters: coverage \<30x, alternative allele frequency different from 40%-60% / 80%-100%, or quality score \<170
- Point mutations affecting regions/genes with high homology with other genomic regions (i.e., pseudogenes)
- Insertions or deletions

We have also developed an alternative bioinformatics pipeline that is able to identify gross deletions/insertions affecting one or more exons of a gene/s included in the panel (CNVs: Copy Number Variations). This complementary analysis is

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possible when bioinformatics data is adequate and might not be available in some cases. An alternative method is used to confirm this kind of variants.

Frequently, our test is not able to identify the phase (same/different alleles) of more than one variant affecting the same gene. This limitation should be considered in cases of recessive disorders that need both alleles of the gene to be mutated.

Although CardioGxOne has an analytical sensitivity and specificity of over 99%, some genotyping errors could occur in specific situations:

- Pre-arrival contamination of samples
- Mosaic mutations
- Monosomies and trisomies
- Genetic paternity problems
- Genetic variants producing allelic drop-outs
- Studies performed on paraffin-embedded tissues
- Presence of pseudogenes
- Incorrect identification of variants in homo-polymers or high GC-content zones
- Errors in the reference sequence

We have developed an efficient method, which ensures tracking of samples after arrival, guaranteeing their proper identification once they arrive at our lab. However, we cannot take responsibility for labeling errors in samples prior to their arrival.

The clinical report: Admera, powered by Health in Code, provides a detailed report with all relevant existing clinical data on the detected mutations. This information has been evaluated by experts on the disease and includes a description of all families with reported cases of each mutation along with information from our own research and existing information on *in vitro* and *in vivo* (animal models) studies for the different mutations. To handle all this information, Health in Code has developed a computerized database that includes records of more than 113,400 individuals from the existing literature on inherited cardiovascular diseases and from our own research.

Comments, recommendations and disclaimers

It is highly recommended that the interpretation of this genetic report is done with the help/counseling from a physician with enough expertise in genetic conditions. Our test is not designed in a direct-to-consumer fashion. The results of this test must be interpreted in the clinical context of each patient. This test does not replace clinical assessment of patients and must not to be used as the only tool to decide on treatment, diagnosis, and/or pre-implantation/pre-natal studies.

When the genetic study identifies one or more genetic variants potentially associated with the development of pathology, family screening is recommended. All first-degree relatives (parents, siblings, children; whether or not clinically affected) should be considered for inclusion in this screening due to variable penetrance and age of onset associated with the majority of these genetic alterations. Genetic diagnosis can identify those family members who are at risk of disease development and need periodical clinical assessment. Moreover, testing in family members can be useful in determining the cosegregation of the identified variants with the phenotype and the associated prognosis in carriers.

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This test has not been cleared or approved by the U.S. Food and Drug Administration (FDA) but the FDA has determined that such clearance or approval is not necessary. The CardioGxOne test is used for clinical purposes. It should not be regarded as investigational or for research. This laboratory is certified under the Clinical Laboratory Improvement Amendments (CLIA) as qualified to perform high complexity clinical laboratory testing. Health in Code provided the professional component of clinical interpretation of the CardioGxOne results.

For additional information or comments, please contact us at ClientCare@admerahealth.com.

Resource references

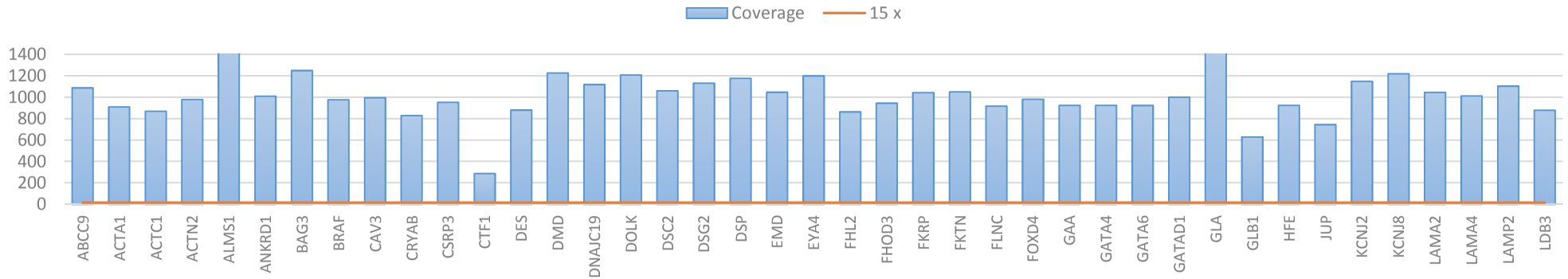
Population databases:

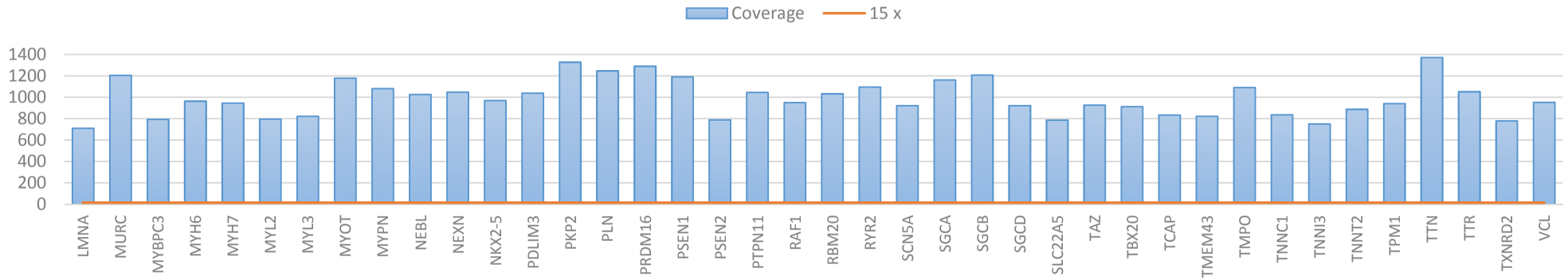
- Exome Aggregation Consortium (ExAC), Cambridge, MA (URL: http://exac.broadinstitute.org) [version 0.3.1]
- Exome Variant Server, NHLBI GO Exome Sequencing Project (ESP), Seattle, WA (URL: http://evs.gs.washington.edu/EVS/) [ESP6500SI-V2-SSA137]
- 1000 Genomes Project, An integrated map of genetic variation from 1,092 human genomes, McVean et Al, Nature 491, 56–65 (01 November 2012) doi:10.1038/nature11632 (www.1000genomes.org/)
- Database of Single Nucleotide Polymorphisms (dbSNP) [Internet]. Bethesda (MD): National Center for Biotechnology Information, National Library of Medicine (dbSNP Build ID:135) Available from: www.ncbi.nlm.nih.gov/SNP.
- HGMD® [Internet]: Stenson PD etal. Genome Med. 2009;1(1):13 www.hgmd.cf.ac.uk.
- ClinVar: Landrum MJ, Lee JM, Riley GR, Jang W, Rubinstein WS, Church DM, Maglott DR. ClinVar: public archive of relationships among sequence variation and human phenotype. Nucleic Acids Res. 2014 Jan 1;42(1):D980-5. doi: 10.1093/nar/gkt1113. PubMed PMID: 24234437.
- Health in Code proprietary database

Functional studies:

- POLYPHEN: A method and server for predicting damaging missense mutations. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, Kondrashov AS, Sunyaev SR. Nat Methods. 2010 Apr;7(4):248-9.
- SIFT: Predicting the effects of coding nonsynonymous variants on protein function using the SIFT algorithm. Kumar P, Henikoff S, Ng PC. Nat Protoc. 2009;4(7):1073-81.
- MUTATION TASTER: MutationTaster2: mutation prediction for the deep-sequencing age. Schwarz JM, Cooper DN, Schuelke M, Seelow D. Nat Methods. 2014 Apr;11(4):361-2.
- NNSplice: Eeckman, FH, Kulp, D, Haussler, D, 1997. Improved Splice Site Detection in Genie. J Comp Biol 4(3), 311-23.
- HSFHuman Splicing Finder: FO Desmet, Hamroun D, Lalande M, Collod-Beroud G, Claustres M, Beroud C. Human Splicing Finder: an online bioinformatics tool to predict splicing signals. Nucleic Acid Research, 2009.
- MaxEntScan: Yeo G, Burge CB. Maximum entropy modeling of short sequence motifs with applications to RNA splicing signals. J Comput Biol. 2004;11(2-3):377-94.

Average coverage per gene





Coverage stats

Stats	Studied genes	Priority genes
Average coverage	1162 x	1260 x
Bases sequenced	353026	183617
% Bp with coverage ≥15	100%	100%
% Bp with coverage ≥30	100%	100%

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