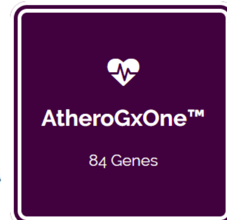
HELICE
GENOMIC SCIENCES, INC.

Third Floor CVMC Building
3434 Ramon Magsaysay Boulevard
Sta. Mesa, Manila, Philippines
+632 624 9684 · +63917 865 8743
wecare@helicegenomics.com

PATIENT INFORMATION	SAMPLE	REFERRING PHYSICIAN
Name: **JOHN SMITH**	Sample Number: **22B54321**	Name: **Physician, M.D.**
Gender: **Male**	Source: **Saliva**	Institution: **Any Hospital**
Birthday: **04/22/1973**	Date Received: **01/16/2016**	Address: **21 Any Street Any City, Philippines**
Age: **43**	Date of Report: **02/08/2016**	Contact: **+632 123-4567**
Address: **14 Any Street, Any Village Any City, Philippines**		
Reference: **A-0987654321**		



Test results of: John Smith

Reason for the study: Homozygotic familial hypercholesterolemia

Test(s) requested: Dyslipidemia and premature atherosclerosis panel

RESULT: POSITIVE

We have identified a mutation in homozygosis in the LDLR gene. This mutation has been previously described in association with familial hypercholesterolemia. This result is in agreement with the informed clinical picture of the submitted patient. We suggest performing the familial screening of the mutation. Heterozygous family carriers would also be affected.

Gene	Variant	Result	Pathogenicity	Population frequency	Number of references
LDLR	NP_000518.1:p.Pro105_Gly314delinsArg NM_000527.4:c.314-1121_941-1446del NC_000019.9:g.11214785_11219892del	Homozygosis	Pathogenic or disease-causing	Mutation (not found in controls)	11

Clinical interpretation

The identified mutation has been extensively described and is known as FH-Valencia 4 and FH Vancouver-6. It consists of the in frame deletion of 3 exons, producing the loss of a crucial region for the protein function: the LDL-binding site. All of the described carriers presented a definite diagnosis of familial hypercholesterolemia. No other homozygous carriers have been previously described.

Technical aspects of the study

This sample has been studied by massive parallel sequencing method using a library that included genes related to dyslipidemia and premature atherosclerosis.

Signatures

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

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

HELICE
GENOMIC SCIENCES, INC.

Third Floor CVMC Building
3434 Ramon Magsaysay Boulevard
Sta. Mesa, Manila, Philippines
+632 624 9684 · +63917 865 8743
wecare@helicegenomics.com

DETAILED RESULTS

Gene: LDLR (Encoding the protein: Low density lipoprotein receptor) NP_000518.1:p.Pro105_Gly314delinsArg/NC_000019.9:g.11214785_11219892del

Homozygous carrier: mutation occurs in both copies of the gene.

Mutation nomenclature: Nucleotide code:NM_000527.4:c.314-1121_941-1446del, NC_000019.9:g.11214785_11219892del. Amino acid code: NP_000518.1:p.Pro105_Gly314delinsArg. Alternative names at the DNA level: NM_000527.4:IVS3-1121_IVS6-1446del; characterized LDLR Ex3_6del. Alternative names at the protein level: NP_000518.1:p.P105_G314delinsR. Located in: Initial intron: 3, Final intron: 6.

Pathogenicity: pathogenic or disease-causing.

Population frequency: mutation (not found in controls).

Clinical information

The identified mutation has been widely described in association with heterozygous familial hypercholesterolemia (FH). In previous years, mutations in LDLR were named according to the site where they were first identified. In this case, the mutation is called FH Valencia-4 and FH Vancouver-6. Both mutations are very similar and consist of an in-frame deletion of exons 4 to 6. This means that the mutation leads to the synthesis of a shortened protein lacking the sequence encoded by these exons. Deleted exons include the binding site of lipoprotein LDL receptor, thereby altering hepatic LDL uptake for metabolism and increasing the value of this lipoprotein in plasma.

As the name suggests, this mutation was firstly identified in Spanish and Canadian families. It has also been identified in Dutch individuals. In some Spanish HF cohorts, this mutation presented a relatively high frequency, representing up to 5% of cases. It cannot be determined whether it is due to a founder effect. It is also possible that mutation arose *de novo* in different populations as a result of the mechanism that causes the mutation (see details in bioinformatics section). As far as we know, no homozygous carriers have been described.

In a paper published by Chaves et al. (2001), carriers of this variant had a poorer response to simvastatin treatment compared to carriers of other defective missense mutations.

At least 6 similar mutations have been previously published, consisting of in-frame deletions of exons encoding the LDL ligand site. All reported cases had clinical criteria for heterozygous familial hypercholesterolemia, with no carriers presenting normal or borderline lipid profile.

Bioinformatics study

A deletion of exons 4 to 6 of the LDL gene has been identified in this sample. As the rest of protein synthesis is not affected, this is an in-frame mutation (p. Pro105_Gly314delinsArg). The deletion causes a loss of the protein segment located between regions LDL-receptor Class A3 and LDL-receptor Class A7. The molecular mechanism leading to the deletion of these exons is known as "microhomology-mediated break-induced replication". It is based on homologous recombination between two Alu sequences, in this case between AluSq in intron 3 and AluSc in intron 6 (see figures 1 and 2 at the end of the report).

HELICE GENOMIC SCIENCES, INC.

Third Floor CVMC Building
3434 Ramon Magsaysay Boulevard
Sta. Mesa, Manila, Philippines
+632 624 9684 · +63917 865 8743
wecare@helicegenomics.com

Gene comment

The low-density lipoprotein receptor (LDLR) gene is located in chromosome 19 and encodes the LDLR protein. LDLR is a cell surface receptor predominantly present in the liver and also found in most other tissues. It binds to particles containing apoB-100 and ApoE (mostly LDL, chylomicron remnants, and IDL), removing them from the blood via endocytosis. Lipoprotein particles are degraded in lysosomes and cholesterol is released. An increased amount of cholesterol in cells inhibits HMGCoA reductase activity and inner cholesterol synthesis and also decreases the LDLR activity. LDLR may be targeted for degradation by PCSK9 in lysosome or recycled for the cell surface. LDLR in the liver plays a major role in determining plasma LDL levels: a low number of LDLR is associated with high plasma LDL levels, while a high number of hepatic LDLRs is associated with low plasma LDL levels.

Mutations in the LDLR gene lead to familial hypercholesterolemia (FH), a disease with an autosomal dominant pattern of inheritance. Over 1,700 mutations have been identified in the LDLR gene, of which 79% are probably expressed as a hypercholesterolaemic phenotype. Mutations in the LDLR gene comprise small deletions, insertions, duplications, and missense mutations, as well as large splicing defects. Pathogenic variants can occur in the promoter or in introns or exons. The majority of pathogenic variants fall within the ligand-binding (40%) or epidermal growth factor precursor-like (47%) domains, with the highest frequency of pathogenic variants reported in exon 4 (20%) [Leigh et al., 2008; Usifo et al., 2012]. In heterozygotes for the LDLR pathogenic variant, penetrance for FH approaches 90%.

Familial hypercholesterolemia (FH) is a result of the absence or dysfunction of LDLR on the surface of hepatocytes due to a mutation in the LDLR gene, leading to a dramatic increase in LDL cholesterol and total cholesterol blood levels and to an early onset of atherosclerosis and cardiovascular complications. Clinical features of the disease are arcus corneae, xanthelasmas, xanthomas, and premature atherosclerosis, which are more severe in homozygous cases compared to heterozygotes. Individuals with heterozygous FH may have no visible signs of the disease, especially in children and in individuals being in lipid lowering treatment.

Conclusions

This mutation has been clearly associated with familial hypercholesterolemia. Its presence in homozygosis is in agreement with the clinical picture reported in the case. Heterozygous carriers of the family would also be affected, therefore we suggest completing the familial genetic and clinical screening.

HELICE
GENOMIC SCIENCES, INC.

Third Floor CVMC Building
3434 Ramon Magsaysay Boulevard
Sta. Mesa, Manila, Philippines
+632 624 9684 · +63917 865 8743
wecare@helicegenomics.com

References (FH-Valencia, FH-Vancouver)

1. Langlois S, Kastelein JJ, Hayden MR. Characterization of six partial deletions in the low-density-lipoprotein (LDL) receptor gene causing familial hypercholesterolemia (FH). Am J Hum Genet. 1988 ; 43(1):60-8.
http://www.ncbi.nlm.nih.gov/pubmed/?term=2837085

2. Chaves FJ, Real JT, Garcia-Garcia AB, Puig O, Ordovas JM, Ascaso JF, et al.. Large rearrangements of the LDL receptor gene and lipid profile in a FH Spanish population. Eur J Clin Invest. 2001 ;31(4):309-17.
http://www.ncbi.nlm.nih.gov/pubmed/11298777

3. 1. Chaves FJ, Real JT, Garcia-Garcia AB, Civera M, Armengod ME, Ascaso JF, et al. Genetic diagnosis of familial hypercholesterolemia in a South European outbreed population: influence of low-density lipoprotein (LDL) receptor gene mutations on treatment response to simvastatin in total, LDL, and high-density lipoprotein cholesterol. J Clin Endocrinol Metab. 2001;86(10):4926-32.
http://www.ncbi.nlm.nih.gov/pubmed/?term=11600564

4. Garcia-Garcia AB, Real JT, Puig O, Cebolla E, Marin-Garcia P, Martinez Ferrandis JI, et al. Molecular genetics of familial hypercholesterolemia in Spain: Ten novel LDLR mutations and population analysis. Hum Mutat. 2001;18(5):458-9.
http://www.ncbi.nlm.nih.gov/pubmed/11668640

5. Blesa S, Garcia-Garcia AB, Martinez-Hervas S, Mansego ML, Gonzalez-Albert V, Ascaso JF, et al. Analysis of sequence variations in the LDL receptor gene in Spain: general gene screening or search for specific alterations?. Clin Chem. 2006;52(6):1021-5.
http://www.ncbi.nlm.nih.gov/pubmed/16627557

6. Alonso R, Defesche JC, Tejedor D, Castillo S, Stef M, Mata N, et al. Genetic diagnosis of familial hypercholesterolemia using a DNA-array based platform. Clin Biochem. 2009;42(9):899-903.
http://www.ncbi.nlm.nih.gov/pubmed/19318025

7. Hobbs HH, Brown MS, Goldstein JL. Molecular genetics of the LDL receptor gene in familial hypercholesterolemia. Hum Mutat. 1992;1(6):445-66.
http://www.ncbi.nlm.nih.gov/pubmed/1301956

8. Fouchier SW, Defesche JC, Umans-Eckenhausen MW, Kastelein JP. The molecular basis of familial hypercholesterolemia in The Netherlands. Hum Genet. 2001 ;109(6):602-15.
http://www.ncbi.nlm.nih.gov/pubmed/11810272

9. Real JT, Ascaso JF, Chaves FJ, GonzÃ¡lez C, Puig Ã, Armengod ME, et al. Asociación de factores lipídicos, genotipo de APOE y tipos de mutación del gen del receptor de LDL con el infarto agudo de miocardio en sujetos con hipercolesterolemia familiar heterocigota. Med Clin. 2002;118(18):681-5.
http://www.ncbi.nlm.nih.gov/pubmed/12042130

10. Leigh SE, Foster AH, Whittall RA, Hubbart CS, Humphries SE. Update and analysis of the University College London low density lipoprotein receptor familial hypercholesterolemia database. Ann Hum Genet. 2008 Jul;72(Pt 4):485-98.
http://www.ncbi.nlm.nih.gov/pubmed/18325082

11. Ahmad Z, Adams-Huet B, Chen C, Garg A. Low Prevalence of Mutations in Known Loci for Autosomal Dominant Hypercholesterolemia in a Multiethnic Patient Cohort. Circ Cardiovasc Genet. 2012 Dec;5(6):666-75.
http://www.ncbi.nlm.nih.gov/pubmed/23064986

Third Floor CVMC Building
3434 Ramon Magsaysay Boulevard
Sta. Mesa, Manila, Philippines
+632 624 9684 · +63917 865 8743
wecare@helicegenomics.com

APPENDIX

Detailed technical aspects

AtheroGxOne™ 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 SureSelect XT Target Enrichment technology for Illumina paired-end multiplexed sequencing method (Agilent). 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 is greater than 99% for single nucleotide variants (SNVs) and small insertions/deletions (INDELs).

AtheroGxOne™ 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 84 genes related to dyslipidemia and premature atherosclerosis:

ABCA1, ABCB1, ABCG1, ABCG5, ABCG8, AGPAT2, AKT2, AMPD1, ANGPTL3, APOA1, APOA5, APOB, APOC2, APOC3, APOE, BLK, BSCL2, CAV1, CEL, CETP, CH25H, CIDEC, COQ2, CPT2, CYP2D6, CYP3A4, CYP3A5, EIF2AK3, FOXP3, GATA6, GCK, GLIS3, GPD1, GPIHBP1, HNF1A, HNF1B, HNF4A, IER3IP1, INS, INSIG2, INSR, KCNJ11, KLF11, LCAT, LDLR, LDLRAP1, LEP, LIPA, LIPC, LMF1, LMNA, LPA, LPL, LRP6, MEF2A, MTTP, MYLIP, NEUROD1, NEUROG3, NPC1L1, PAX4, PCDH15, PCSK9, PDX1, PLIN1, PLTP, PNPLA2, PPARA, PPARG, PTF1A, PTRF, PYGM, RFX6, RYR1, SAR1B, SCARB1, SLC22A8, SLC25A40, SLC2A2, SLCO1B1, TBC1D4, TRIB1, WFS1, ZMPSTE24.

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.

AtheroGxOne™ 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 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.

HELICE
GENOMIC SCIENCES, INC.

Third Floor CVMC Building
3434 Ramon Magsaysay Boulevard
Sta. Mesa, Manila, Philippines
+632 624 9684 · +63917 865 8743
wecare@helicegenomics.com

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 AtheroGxOne™ 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 98800 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.

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 AtheroGxOne™ 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 AtheroGxOne™ results.

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

HELICE
GENOMIC SCIENCES, INC.

Third Floor CVMC Building
3434 Ramon Magsaysay Boulevard
Sta. Mesa, Manila, Philippines
+632 624 9684 · +63917 865 8743
wecare@helicegenomics.com

Resource references

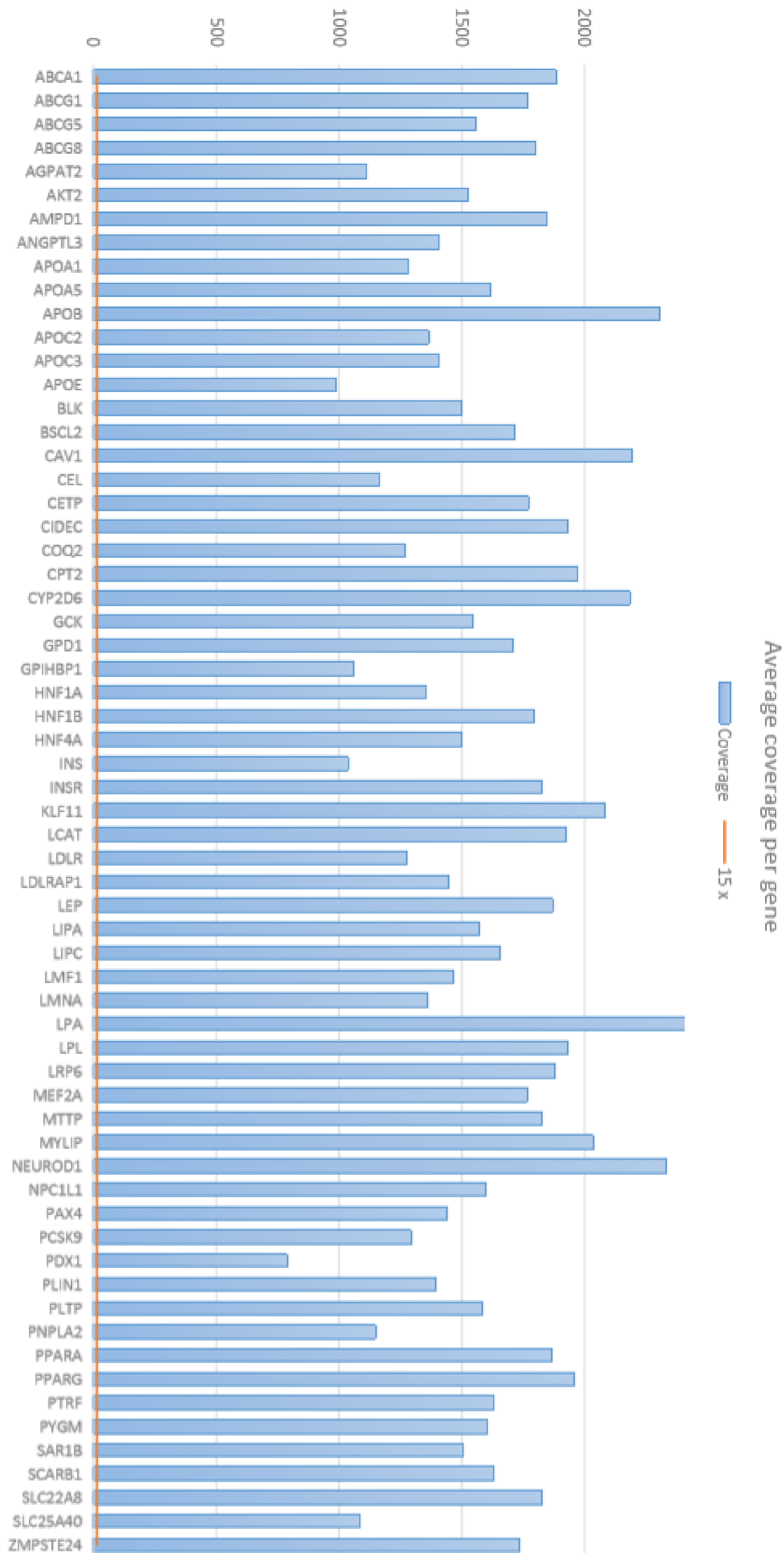
Population databases:

- Exome Aggregation Consortium (ExAC), Cambridge, MA (URL: http://exac.broadinstitute.org) [version 0.3]
- 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
- Splice-site Finder (SSF)
- HSF
- MaxEnt

Third Floor CVMC Building
3434 Ramon Magsaysay Boulevard
Sta. Mesa, Manila, Philippines
+632 624 9684 · +63917 865 8743
wecare@helicegenomics.com



Third Floor CVMC Building
3434 Ramon Magsaysay Boulevard
Sta. Mesa, Manila, Philippines
+632 624 9684 · +63917 865 8743
wecare@helicegenomics.com

Coverage stats

Stats	Studied genes	Priority genes
Average coverage	1770 x	1770 x
Bases sequenced	133930	133930
% Bp with coverage ≥15	99.46%	99.46%
% Bp with coverage ≥30	99.44%	99.44%

Third Floor CVMC Building
3434 Ramon Magsaysay Boulevard
Sta. Mesa, Manila, Philippines
+632 624 9684 · +63917 865 8743
wecare@helicegenomics.com

Bioinformatics analysis of the study.

Figure 1. Screen capture of IGV (integrate genomic viewer) showing the deletion.

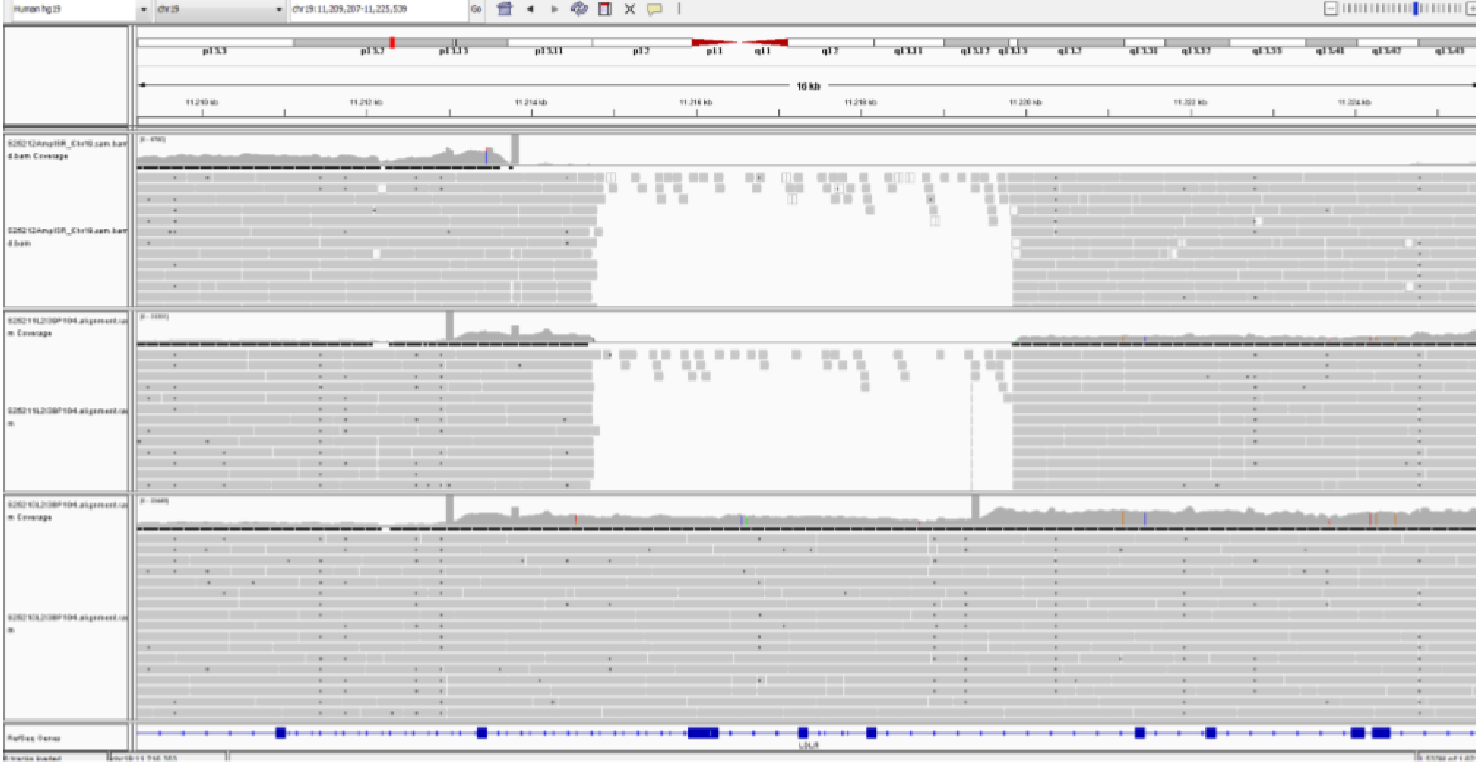
Figure2 depicts the mutagenic mechanism. Genome Browser visualization. A) Region of LDLR containing exons 4-6. B) Repeat masker of LDLR region of interest. C) Repetitive sequences causing deletion by the mechanism "microhomology mediated break induced replication". In bold showed the homologous regions between 5' and 3' flanking sequences of the deletion, which are underlined.

Figure 1

Sample 1. Proband

Sample 2. Relative of the carrier

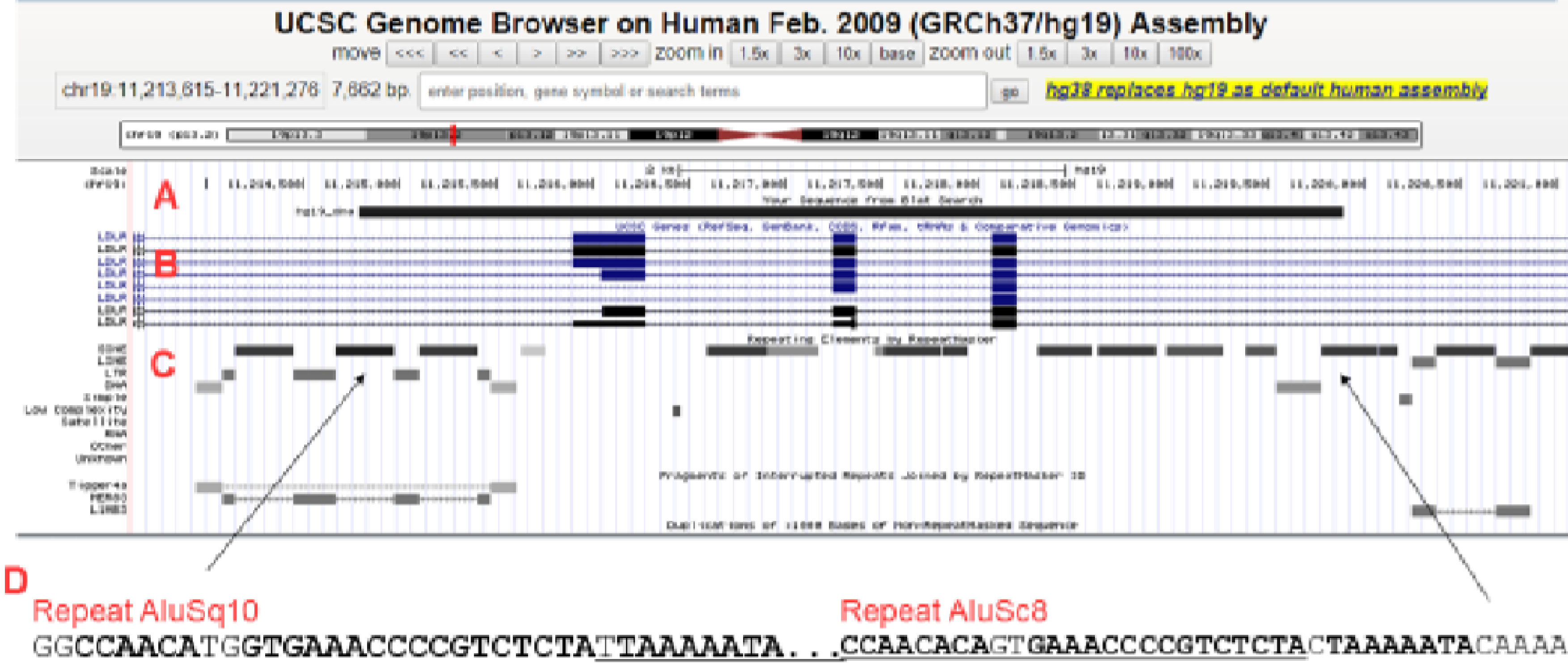
Sample 3. Control



HELICE
GENOMIC SCIENCES, INC.

Third Floor CVMC Building
3434 Ramon Magsaysay Boulevard
Sta. Mesa, Manila, Philippines
+632 624 9684 · +63917 865 8743
wecare@helicegenomics.com

Figure 2.



HELICE
GENOMIC SCIENCES, INC.

Third Floor CVMC Building
3434 Ramon Magsaysay Boulevard
Sta. Mesa, Manila, Philippines
+632 624 9684 · +63917 865 8743
wecare@helicegenomics.com

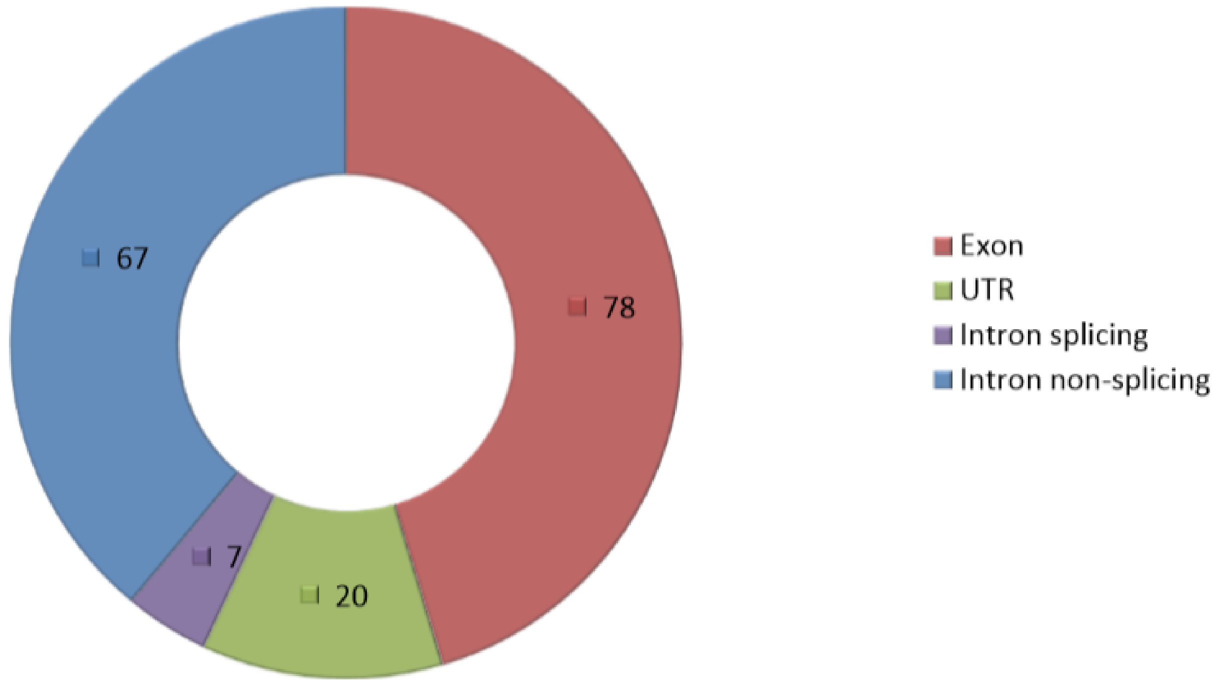
SUPPLEMENTAL MATERIAL

AVAILABLE INFORMATION ON OTHER IDENTIFIED VARIANTS

We have identified genetic variants that we consider not to be associated with disease development either because they have been identified in healthy controls or because they do not affect protein structure and function.

Region	Variants found
Exonic	**78**
Synonymous	39
Nonsynonymous	38
Nonsense	1
Intronic	**74**
Intronic splicing	7
UTR	**20**
Total	**172**

Third Floor CVMC Building
3434 Ramon Magsaysay Boulevard
Sta. Mesa, Manila, Philippines
+632 624 9684 · +63917 865 8743
wecare@helicegenomics.com



Only good quality variants were included (QUAL ≥170)

Third Floor CVMC Building
3434 Ramon Magsaysay Boulevard
Sta. Mesa, Manila, Philippines
+632 624 9684 · +63917 865 8743
wecare@helicegenomics.com

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.
ABCA1	NP_005493.2:p.Lys1587Arg; NM_005502.3:c.4760A>G; NC_000009.11:g.107562804T>C	Exon	Nonsynonymous	rs2230808		46.17	41.5	100	Het.	1715	255	47.5
ABCG5	NP_071881.1:p.Gln604Glu; NM_022436.2:c.1810C>G; NC_000002.11:g.44040401G>C	Exon	Nonsynonymous	rs6720173	20.74	24	21.03	43.33	Het.	1759	255	45.9
ABCG8	NP_071882.1:p.Tyr54Cys; NM_022437.2:c.161A>G; NC_000002.11:g.44071743A>G	Exon splicing	Nonsynonymous	rs4148211	37.44	43.47	32.09	76.67	Het.	1153	255	45.9
ABCG8	NP_071882.1:p.Val632Ala; NM_022437.2:c.1895T>C; NC_000002.11:g.44104925T>C	Exon splicing	Nonsynonymous	rs6544718	86.7	7.71	16.28	100	Hom.	2284	255	99.9
AMPD1	NP_000027.2:p.Lys320Ile; NM_000036.2:c.959A>T; NC_000001.10:g.115222237T>A	Exon	Nonsynonymous	rs34526199	2.17	1.1	2.41	6.67	Het.	1407	255	44.3
ANGPTL3	NP_055310.1:p.Arg332Gln; NM_014495.3:c.995G>A; NC_000001.10:g.63069703G>A	Exon	Nonsynonymous	rs192778191	0.07	0.04	0.01	3.33	Het.	1666	255	45.6
APOB	NP_000375.2:p.Ser4338Asn; NM_000384.2:c.13013G>A; NC_000002.11:g.21225281C>T	Exon	Nonsynonymous	rs1042034		37.04	19.7	94.85	Hom.	1476	255	100
APOB	NP_000375.2:p.Glu4181Lys; NM_000384.2:c.12541G>A; NC_000002.11:g.21225753C>T	Exon	Nonsynonymous	rs1042031		12.78	16.61	34.22	Het.	2292	255	48.3
APOB	NP_000375.2:p.Ile2313Val; NM_000384.2:c.6937A>G; NC_000002.11:g.21232803T>C	Exon	Nonsynonymous	rs584542	97.74	1.38	1.84	99.65	Hom.	1982	170	100
APOB	NP_000375.2:p.Tyr1422Cys; NM_000384.2:c.4265A>G; NC_000002.11:g.21235475T>C	Exon	Nonsynonymous	rs568413	99.91	0	0.03	99.94	Hom.	1857	255	99.9
APOB	NP_000375.2:p.Val730Ile; NM_000384.2:c.2188G>A; NC_000002.11:g.21249716C>T	Exon	Nonsynonymous	rs12691202		1.42	2.55	9.08	Het.	1706	255	42.2
APOB	NP_000375.2:p.Ala618Val; NM_000384.2:c.1853C>T; NC_000002.11:g.21250914G>A	Exon	Nonsynonymous	rs679899	42.25	48.5	36.55	68.03	Het.	1827	255	50.7
APOB	NP_000375.2:p.Thr98Ile; NM_000384.2:c.293C>T; NC_000002.11:g.21263900G>A	Exon	Nonsynonymous	rs1367117	21.65	16.93	25.05	48.06	Het.	1679	255	50.5
CETP	NP_000069.2:p.Val422Ile; NM_000078.2:c.1264G>A; NC_000016.9:g.57016092G>A	Exon	Nonsynonymous	rs5882	57.45	46.61	40.88	88.5	Het.	1717	255	46.9
COQ2	NP_056512.5:p.Val66Leu; NM_015697.7:c.196G>T; NC_000004.11:g.84205872C>A	Exon	Nonsynonymous	rs6818847	67.59	35.02	31.48	91.45	Hom.	569	255	100
CPT2	NP_000089.1:p.Val368Ile; NM_000098.2:c.1102G>A; NC_000001.10:g.53676448G>A	Exon	Nonsynonymous	rs1799821	47.67	41.31	45.64	80	Hom.	1945	255	100

HELICE GENOMIC SCIENCES, INC.

Third Floor CVMC Building
3434 Ramon Magsaysay Boulevard
Sta. Mesa, Manila, Philippines
+632 624 9684 · +63917 865 8743
wecare@helicegenomics.com

Gene	Variant	Function	Exonic function	dbSNP	dbSNP freq.	1000G MAF	5000G MAF	HiC freq.	AF1	DP Qual	Qual	Freq. alt.
CPT2	NP_000089.1:p.Met647Val; NM_000098.2:c.1939A>G; NC_000001.10:g.53679229A>G	Exon	Nonsynonymous	rs1799822	14.59	10.04	16.74	30	Hom.	1453	255	100
CYP2D6	NP_000097.3:p.Thr486Ser; NM_000106.5:c.1457C>G; NC_000022.10:g.42522613G>C	Exon	Nonsynonymous	rs1135840	53.94	40.12	40.83	63.33	Het.	2139	191	29.7
CYP2D6	NP_000097.3:p.Cys296Arg; NM_000106.5:c.886T>C; NC_000022.10:g.42523943A>G	Exon	Nonsynonymous	rs16947	62.22	35.92	40.04	93.33	Het.	2441	191	28.2
GPIHBP1	NP_835466.2:p.Cys14Phe; NM_178172.4:c.41G>T; NC_000008.10:g.144295183G>T	Exon	Nonsynonymous	rs11538389	11.47	15.87	8.93	20	Het.	1118	255	46.9
HNF1A	NP_000536.5:p.Ser574Gly; NM_000545.5:c.1720A>G; NC_000012.11:g.121437382A>G	Exon	Nonsynonymous	rs1169305	29.57	1.48	1.31	100	Hom.	504	255	99.8
LIPC	NP_000227.2:p.Asn215Ser; NM_000236.2:c.644A>G; NC_000015.9:g.58838010A>G	Exon	Nonsynonymous	rs6083	54.27	39.14	46.97	63.33	Het.	1912	170	44.3
LIPC	NP_000227.2:p.Phe356Leu; NM_000236.2:c.1068C>A; NC_000015.9:g.58853079C>A	Exon	Nonsynonymous	rs3829462		6.39	5.47	100	Hom.	767	170	99.9
LMF1	NP_073610.2:p.Pro562Arg; NM_022773.2:c.1685C>G; NC_000016.9:g.904551G>C	Exon	Nonsynonymous	rs4984948	7.81	5.27	0.82	13.33	Het.	1199	255	49.1
LMF1	NP_073610.2:p.Gly36Asp; NM_022773.2:c.107G>A; NC_000016.9:g.1020874C>T	Exon	Nonsynonymous	rs111980103	5.4	7.41	6.38	20	Het.	997	255	51.7
LPA	NP_005568.2:p.Leu1961Pro; NM_005577.2:c.5882T>C; NC_000006.11:g.160953642A>G	Exon	Nonsynonymous	rs41267809	1.25	1.22	1.53	16.67	Het.	1779	255	45.9
LPA	NP_005568.2:p.Ile1891Met; NM_005577.2:c.5673A>G; NC_000006.11:g.160961137T>C	Exon	Nonsynonymous	rs3798220	4.96	5.13	1.58	10	Het.	2067	255	46.3
LPA	NP_005568.2:p.Met1679Thr; NM_005577.2:c.5036T>C; NC_000006.11:g.160969629A>G	Exon	Nonsynonymous	rs1801693		35.18	25.37	93.33	Het.	1429	255	45.8
LPA	NP_005568.2:p.Leu1372Val; NM_005577.2:c.4114C>G; NC_000006.11:g.161007496G>C	Exon	Nonsynonymous	rs7765781	41.72	41.49	41.17	53.33	Het.	2108	255	48.3
LPA	NP_005568.2:p.Leu1358Val; NM_005577.2:c.4072C>G; NC_000006.11:g.161007538G>C	Exon	Nonsynonymous	rs7765803	43.03	40.91	40.67	53.33	Het.	2246	255	48
LPL	NP_000228.1:p.Ser474*; NM_000237.2:c.1421C>G; NC_000008.10:g.19819724C>G	Exon splicing	Nonsense	rs328	8.6	9.25	8.63	23.33	Het.	2082	255	48.3
LRP6	NP_002327.2:p.Val1062Ile; NM_002336.2:c.3184G>A; NC_000012.11:g.12301898C>T	Exon	Nonsynonymous	rs2302685	85.12	11.44	16	96.89	Hom.	1678	255	100
MYLIP	NP_037394.2:p.Asn342Ser; NM_013262.3:c.1025A>G; NC_000006.11:g.16145325A>G	Exon	Nonsynonymous	rs9370867	68.67	23	38.49	73.33	Hom.	1997	255	100

Third Floor CVMC Building
3434 Ramon Magsaysay Boulevard
Sta. Mesa, Manila, Philippines
+632 624 9684 · +63917 865 8743
wecare@helicegenomics.com

Gene	Variant	Function	Exonic function	dbSNP	dbSNP freq.	1000G MAF	5000G MAF	HiC freq.	AF1	DP Qual	Qual	Freq. alt.
PAX4	NP_006184.2:p.His321Pro; NM_006193.2:c.962A>C; NC_000007.13:g.127251188T>G	Exon	Nonsynonymous	rs712701		32.95	23.11	100	Het.	1472	255	50.6
PCSK9	NP_777596.2:p.Val474Ile; NM_174936.3:c.1420G>A; NC_000001.10:g.55524237G>A	Exon	Nonsynonymous	rs562556	83.02	13.1	18.22	96.83	Hom.	994	255	100
PCSK9	NP_777596.2:p.Gly670Glu; NM_174936.3:c.2009G>A; NC_000001.10:g.55529187G>A	Exon	Nonsynonymous	rs505151	88.33	10.1	11.06	99.69	Hom.	1411	255	100
PLIN1	NP_002657.3:p.Pro194Ala; NM_002666.4:c.580C>G; NC_000015.9:g.90213229G>C	Exon	Nonsynonymous	rs6496589	96.09	10.42	1.06	96.67	Hom.	1265	255	100
PNPLA2	NP_065109.1:p.Leu481Pro; NM_020376.3:c.1442T>C; NC_000011.9:g.824789T>C	Exon	Nonsynonymous	rs1138693	70	34.56	29.32	96.67	Hom.	543	170	99.8
PPARA	NP_005027.2:p.Leu162Val; NM_005036.4:c.484C>G; NC_000022.10:g.46614274C>G	Exon	Nonsynonymous	rs1800206	3.7	2.28	4.56	20	Het.	2058	255	45

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).

HELICE
GENOMIC SCIENCES, INC.

Third Floor CVMC Building
3434 Ramon Magsaysay Boulevard
Sta. Mesa, Manila, Philippines
+632 624 9684 · +63917 865 8743
wecare@helicegenomics.com

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.
ABCG8	NM_022437.2:c.64-7C>T; NC_000002.11:g.44071639C>T	rs4148210	48.61	48.4	39.39	76.67	Het.	1372	255	44.2
ABCG8	NM_022437.2:c.1412-8C>T; NC_000002.11:g.44101538C>T	rs112765285	68.95			86.67	Hom.	1698	255	99.9
GCK	NM_000162.3:c.1253+8C>T; NC_000007.13:g.44185088G>A	rs2908274		41.31	33.49	40	Het.	1531	255	45.9
INS	NM_000207.2:c.-17-6T>A; NC_000011.9:g.2182224A>T	rs689	56.97	35.04	43.29	96.67	Hom.	695	255	99.9
LDLR	NM_000527.4:c.1060+7T>C; NC_000019.9:g.11221454T>C	rs2738442	100	0		99.97	Hom.	1518	170	100
LDLR	NM_000527.4:c.1060+10G>C; NC_000019.9:g.11221457G>C	rs12710260	35.13	27.7	33.77	67.14	Hom.	1508	255	100
LPA	NM_005577.2:c.-45-4T>C; NC_000006.11:g.161085295A>G	rs1853021			21.15	53.33	Het.	1155	191	35.9

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).

Testing and interpretation performed by Admera Health LLC, 126 Corporate Blvd, South Plainfield, NJ 07080 James Dermody Ph.D. Laboratory Director

OncoGxOne™ is a trademark of Admera Health, LLC.