DOI: 10.7860/JCDR/2017/28156.10143

Case Report

Genetics Section

Whole-Exomes Sequencing Delineates Gene Variants Profile in a Young Saudi Male with Familial Hypercholesterolemia: Case Report

EDEM NUGLOZEH

ABSTRACT

Familial hypercholesterolemia is an autosomal dominant genetic disease characterized by earlier elevated Low-Density Lipoprotein (LDL) cholesterol levels and increased risk for premature Myocardial Infarction (MI). Albeit the diagnosis of some medical Familial Hypercholesterolemia (FH) cases are due to mutations in PCSK9, APOB, or LDLR, detection of mutation rate and profiles relies heavily on different gene pools and ethnicity.

We ran exome sequencing on blood genomic DNA (gDNA) from a 26-year-old Saudi patient on Ion Proton Platform (Ion Torrent, Guilford, Connecticut, USA) as part of a pilot study preluding the establishment of the Saudi Human Genome project. The sequencing results were analysed using Ion suit Bioinformatics system. The patient was matched with a lady of lean body mass and Welsh descent, who suffered from hypercholesterolemia.

The first analysis of known FH genes identified five mutations in APOB, 25 mutations of known genes linked to FH, six mutations in LPR2, one mutation in LDLR, and three mutations in PCSK9. Finally, using disease filter algorithms, we filtered out more than 2000 intronic synonymous variants with likely no biological functions. No major new locus was found in FH. However, via variant reduction and TVC protocols we detected 15 new variants, among which 14 genes are linked to hypercholesterolemia, type-I, and type-II diabetes. We also detected three mutations in PCSK9 and confirmed one by Sanger. Taken together, this report suggests that the genetic determinant of FH in this Saudi patient is likely to be heterogeneous, complicating the diagnostic and novel gene discovery process.

Keywords: Cholesterol, Diabetes, Exome sequencing, Hypercholesterolemia

CASE REPORT

During nutritional awareness programs organized by the School of Applied Health Sciences of the University of Hail, we collected random blood samples from 35 individuals. Based on their medical histories, we singled out one patient. This individual is a male Saudi, 26-year-old, who suffered from hypercholesterolemia, diabetes, hypertension, and overweight (BMI 27.5 kg/m^2). He is taking lipitor. His family history stated that his younger brother, as well as his father, suffered from FH and all was taking lipitor. We matched him with a 58-year-old female, a Caucasian of Welsh descent. This second individual is of lean body mass and suffered from hypercholesterolemia [Table/Fig-1].

Exome library preparation and DNA sequencing [Table/Fig-2]

High quality gDNA was purified from whole blood using a Genomic DNA Purification Kit (QIA amp DNA Blood Mini Kit from Qiagen, Hilden, Germany. The library construction was conducted using The Ion AmpliSeq™ Exome RDY Kit 4x2 configuration, which includes the following components for eight exomes (running 2 exomes per Ion PI™ Chip v3):

- 50 ng of gDNA was utilized using transposase-based kit chemistry to create exome libraries;
- The library was quality controlled using the Bioanalyser machine (Agilent, Maryland);
- 2-6 pM libraries were used in a 2 x 76 paired end cycles to perform (+1 cycle to each forward and reverse read to allow for phasing/pre-phasing).

Subjects	Ages (years)	Total Cholesterol*	HDL*	LDL*	VLDL*	TG*
1	58	7.3	0.8	5.7	3.5	1.7
2	26	8.4	0.6	6.1	4.1	1.9
Normal range	NA	1-1.3	1.3	1.42	1.5	0.7

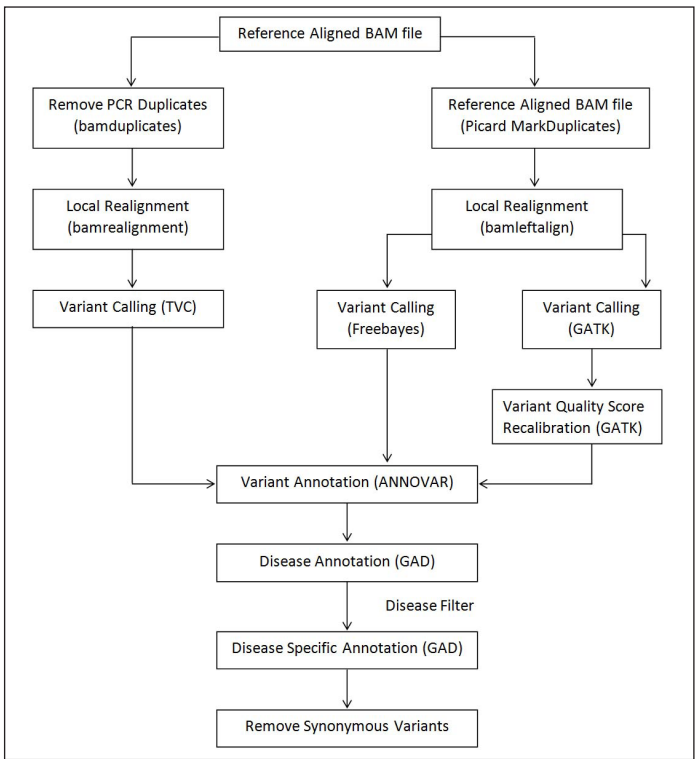
[Table/Fig-1]: Distribution of plasma lipids and cholesterol among the two subjects.
* In mmol/L
HDL: High-Density Lipoprotein, LDL: Low-Density Lipoprotein,
VLDL: Very Low-Density Lipoprotein: triglycerides

Bioinformatics Analyses

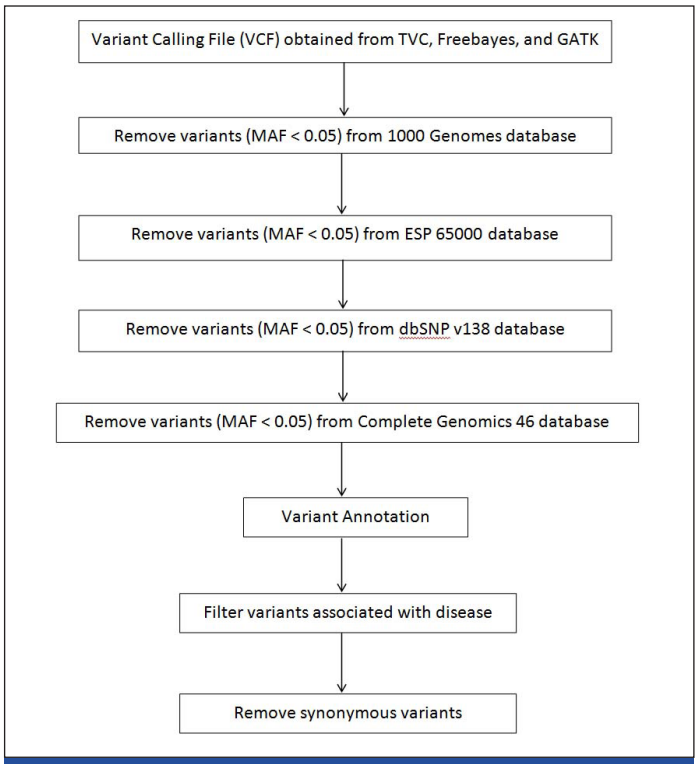
- FastQC files were concatenated from multiple runs;
- Adapter trimming and base quality scores (less than Q30) were removed using Cut adapt (V1.7.1);
- FastQC (V0.11.2) was used to check primary and post trimmed sequences;
- Alignments to the reference human genome (hg19) were conducted using BWA (version 0.7.15);
- The Genome Analysis Tool Kit, GATK (version 3.0.0), was used for base quality score recalibration, variant calling following by hard filtering to identify high-quality variants for downstream analyses;
- SnpEffv4.1 was exploited to determine in silico impacts upon protein function of candidate genes.

Results and Data Analysis for Sample Variant Reduction and Filtering Results

Using the variant filtering protocol, as illustrated in [Table/Fig-3,4], TVC generated 2250 variants from our patient; 12,800 variants from



[Table/Fig-2]: Exome sequencing analysis pipeline listing various steps and tools for processing Ion Torrent data.

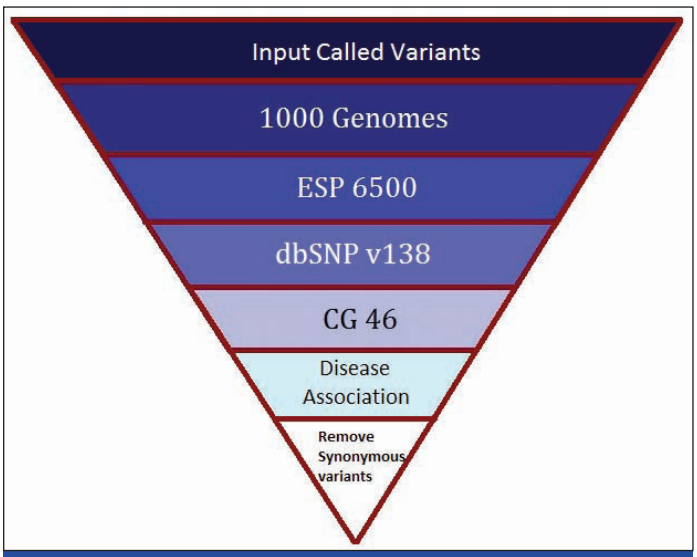


[Table/Fig-3]: Variant reduction pipeline performed using ANNOVAR tool.

Freebayes, and 4089 from GATK. Furthermore, annotation and filtering with GAD for the resulting variants generated 11 variants from TVC (corresponding to seven genes) reported in [Table/Fig-5] (Supplementary Material), 60 variants for Freebayes (corresponding to 29 genes) reported in [Table/Fig-6], and 18 variants for GATK (corresponding to 17 genes) reported in [Table/Fig-7].

From the above filtering analysis, 15 genes from our patient were found to be common in Freebayes and GATK variants, which were found to be highly associated with hypercholesterolemia, viz., ABCA1, ABCG8, ACACA, ACACB, APOA4, APOB, CYBA, GNB3, HMGCR, ITIH4, LDLR, LDLRAP1, LRP2, PCSK9, and SREBF1.

Furthermore, causal variants from our patient were also identified, based on the association of genes with atherosclerosis, cholesterol,



[Table/Fig-4]: Schematic of the steps illustrating variant reduction protocol performed for Sample-1 and Sample-30. (Please note that variants were filtered having MAF lower than 0.05.).

Chr	Gene	Start	End	Ref	Alt	Function	Ref
chr17	ACACA	35478221	35478221	C	-	intronic	
chr12	ACACB	109637188	109637188	G	-	intronic	
chr2	INSIG2	118847182	118847182	-	T	intronic	
chr2	INSIG2	118847183	118847183	G	C	intronic	
chr2	INSIG2	118847184	118847184	T	A	intronic	
chr2	INSIG2	118847182	118847182	T	-	intronic	
chr1	LDLRAP1	25889632	25889632	T	-	exonic	[1]
chr15	LIPC	58838037	58838037	-	G	exonic	[2]
chr2	LRP2	170037992	170037992	G	-	exonic	[3]
chr2	LRP2	170037991	170037992	AG	ACG	exonic	[3]
chr7	NPC1L1	44571892	44571892	A	-	intronic	

[Table/Fig-5]: List of variants obtained from variant reduction protocol associated with hypercholesterolemia in Sample-30 using TVC [1-3].

diabetes, hypercholesterolemia, and obesity. Resulting variants were filtered, which were predicted to have "damaging" impact on the protein function using SIFT and POLYPHEN.

[Table/Fig-8-10] list the variants obtained from the variant reduction protocol, which are associated with diabetes, hypercholesterolemia, cholesterol, and obesity and predicted to be "Damaging." Results in [Table/Fig-8-10] are obtained from TVC, Freebayes, and GATK, respectively. From [Table/Fig-8], five variant (five genes) genes are associated with the diseases. Out of five genes, four (viz., ACAP3, FTCD, HLA-DQA1, and RENBP) are associated with type I diabetes, whereas the NCKAP5 gene is associated with cholesterol formation. On the other hand, Freebayes [Table/Fig-9] gave ten variants (nine genes), of which FTCD, HCFC1, HLA-DQA1, OCA2, and TNXB are associated with type I and II diabetes; ADAM23, IL20RA, and NCKAP5 are associated with cholesterol formation; and TCN2 with atherosclerosis. GATK [Table/Fig-10] gave nine variants (seven genes), of which ACOT11, CPAMD8, FTCD, and JUN are associated with type II diabetes, whereas DOCK6, NCKAP5, and TLE2 are associated with cholesterol formation.

DISCUSSION

Familial hypercholesterolemia (FH (OMIM #143890)) is an autosomal dominant genetic disorder plagued with excessive elevation of Low-Density Lipoprotein Cholesterol (LDL-C) caused, in general, by mutations in three genes: LDLR, APOB, and PCSK9 [14]. A recessive form of FH has also been reported and is characterized by mutations in LDLRAP1 [15].

Chr	Gene	Start	End	Ref	Alt	Function	Ref
chr9	ABCA1	107586838	107586838	G	-	exonic	
chr9	ABCA1	107579565	107579571	TGGGGGA	TGGGGA	intronic	
chr7	ABCB1	87179703	87179710	CTTTTTTA	CTTTTTTTA	intronic	
chr2	ABCG8	44079832	44079832	G	-	exonic	
chr17	ACACA	35479457	35479457	G	-	exonic	
chr17	ACACA	35518723	35518723	G	-	exonic	
chr17	ACACA	35598978	35598985	TAAAAAAG	TAAAAAAAG	intronic	
chr17	ACACA	35597450	35597450	C	-	exonic	
chr17	ACACA	35614782	35614782	T	-	exonic	
chr12	ACACB	109637187	109637189	CGA	CCA	intronic	
chr12	ACACB	109604630	109604630	C	-	intronic	
chr12	ACACB	109604774	109604782	TGGGGGGGA	TGGGGGGA	exonic	
chr12	ACACB	109677804	109677811	AGGGGGGC	AGGGGGC	intronic	
chr17	ACE	61568535	61568541	ACCCCCA	ACCCCA	intronic	
chr17	ACE	61566516	61566516	G	T	intronic	
chr17	ACE	61568448	61568448	G	-	intronic	
chr17	ACE	61561945	61561945	T	C	intronic	
chr17	ACE	61560015	61560015	A	-	exonic	[4]
chr11	APOA4	116691983	116691983	C	A	exonic	
chr11	APOA4	116691848	116691848	G	-	exonic	
chr11	APOA5	116661072	116661074	GTC	GC	exonic	
chr2	APOB	21229253	21229253	G	-	exonic	
chr2	APOB	21234271	21234271	C	-	exonic	
chr2	APOB	21242614	21242614	G	-	exonic	
chr2	APOB	21259995	21259995	G	-	exonic	
chr2	APOB	21229031	21229033	TGG	GGT	exonic	
chr16	CETP	57003311	57003311	C	-	exonic	
chr16	CETP	56997142	56997143	CA	CGA	intronic	
chr16	CYBA	88709633	88709633	C	-	downstream	
chr16	CYBA	88713044	88713050	GAAAAAC	GAAAAC	intronic	
chr10	CYP2C19	96534904	96534904	C	-	exonic	
chr12	GNB3	6952520	6952520	C	-	intronic	
chr5	HMGCR	74647308	74647308	G	-	exonic	
chr5	HMGCR	74655080	74655080	G	-	exonic	
chr3	ITIH4	52863248	52863248	-	G	exonic	
chr19	LDLR	11221471	11221471	C	-	intronic	
chr1	LDLRAP1	25893265	25893266	TG	TCG	intronic	
chr1	LEPR	66075722	66075724	GCT	GT	exonic	
chr1	LEPR	66075946	66075952	GAAAAAG	GAAAAAC	exonic	
chr15	LIPC	58837909	58837911	CTG	CCG	intronic	
chr2	LRP2	169985117	169985124	TAAAAAAT	TAAAAAT	UTR3	
chr2	LRP2	169994045	169994045	A	-	intronic	
chr2	LRP2	170053498	170053498	C	-	exonic	[3]
chr2	LRP2	170053450	170053450	A	-	exonic	[3]
chr2	LRP2	170137020	170137020	G	-	exonic	[3]
chr2	LRP2	170103853	170103861	CAAAAAAAT	CAAAAAAAAT	intronic	[3]
chr11	LRP5	68192646	68192646	G	-	exonic	[5]
chr11	LRP5	68207287	68207294	TGGGGGGC	TGGGGGC	exonic	[5]
chr11	LRP5	68153746	68153746	G	-	intronic	
chr1	MTHFR	11863213	11863219	AGGGGGC	AGGGGC	intronic	
chr7	NPC1L1	44578837	44578843	TGGGGGC	TGGGGC	exonic	[6]
chr7	NPC1L1	44573332	44573332	G	-	intronic	
chr1	PCSK9	55512312	55512319	GCCCCCCG	GCCCCCG	exonic	
chr1	PCSK9	55529187	55529187	G	-	exonic	
chr1	PCSK9	55523200	55523200	C	-	intronic	

chr17	SREBF1	17739987	17739993	GCCCCCT	GCCCCT	intronic	
chr17	SREBF1	17719973	17719973	G	-	exonic	[7]
chr22	SREBF2	42300855	42300855	G	-	intronic	
chr22	SREBF2	42290850	42290850	G	-	exonic	[8]
chr9	TLR4	120476639	120476639	C	-	exonic	[9]

[Table/Fig-6]: List of variants obtained from variant reduction protocol associated with hypercholesterolemia in Sample-30 using Freebayes [4-9].

Chr	Gene	Start	End	Ref	Alt	Function	Ref
chr9	ABCA1	107586837	107586837	G	-	exonic	
chr2	ABCG8	44102403	44102403	G	-	exonic	
chr17	ACACA	35564717	35564717	A	-	intronic	
chr12	ACACB	109702119	109702119	C	-	exonic	
chr12	ACACB	109604629	109604629	C	-	intronic	
chr11	APOA4	116691847	116691847	G	-	exonic	[10]
chr2	APOB	21259994	21259994	G	-	exonic	[11]
chr16	CYBA	88713045	88713045	A	-	intronic	
chr15	CYP1A1	75013852	75013852	G	-	intronic	
chr12	GNB3	6952519	6952519	C	-	intronic	
chr5	HMGCR	74655079	74655079	G	-	exonic	[12]
chr3	ITIH4	52863246	52863246	-	G	exonic	[13]
chr19	LDLR	11221470	11221470	C	-	intronic	
chr1	LDLRAP1	25889632	25889632	T	-	exonic	[1]
chr2	LRP2	169985118	169985118	A	-	UTR3	[3]
chr1	PCSK9	55512313	55512313	C	-	exonic	
chr17	SREBF1	17718197	17718197	C	-	exonic	[7]
chr22	SREBF2	42300854	42300854	G	-	intronic	

[Table/Fig-7]: List of variants obtained from variant reduction protocol associated with hypercholesterolemia in Sample-30 using GATK [10-13].

Chr	Gene	Start	End	Ref	Alt	DISEASE
chr1	ACAP3	1233962	1233962	A	G	Type 2 Diabetes| oedema | rosiglitazone
chr21	FTCD	47574116	47574116	G	T	Type 2 Diabetes| oedema | rosiglitazone
chr6	HLA-DQA1	32609800	32609800	C	A	Diabetes mellitus type II| Diabetes Mellitus, Type 2
chr2	NCKAP5	133489586	133489586	C	T	Cholesterol
chrX	RENBP	153209587	153209587	C	A	Type 2 Diabetes| oedema | rosiglitazone

[Table/Fig-8]: List of disease causal non-synonymous variants obtained from variant reduction protocol using TVC, which are predicted to be damaging in our patient.

Chr	Gene	Start	End	Ref	Alt	DISEASE
chr2	ADAM23	207429779	207429779	A	G	Cholesterol
chr21	FTCD	47574116	47574116	G	T	Type 2 Diabetes| oedema | rosiglitazone
chr21	FTCD	47574117	47574117	C	T	Type 2 Diabetes| oedema | rosiglitazone
chrX	HCFC1	153220581	153220581	G	C	Type 2 Diabetes| oedema | rosiglitazone
chr6	HLA-DQA1	32609800	32609800	C	A	Diabetes Mellitus, Insulin-Dependent|Diabetes Mellitus, Type 1
chr6	IL20RA	137323444	137323444	C	T	Cholesterol, LDL
chr2	NCKAP5	133489586	133489586	C	T	Cholesterol
chr15	OCA2	28234812	28234812	T	C	Type 2 Diabetes| oedema | rosiglitazone
chr22	TCN2	31013431	31013431	G	T	atherosclerosis
chr6	TNXB	32064062	32064062	C	T	Diabetes Mellitus, Type 1

[Table/Fig-9]: List of disease causal non-synonymous variants obtained from variant reduction protocol using Freebayes, which are predicted to be damaging in Sample-30.

Chr	Gene	Start	End	Ref	Alt	DISEASE
chr1	ACOT11	55070857	55070857	A	G	Type 2 Diabetes| oedema | rosiglitazone
chr19	CPAMD8	17015101	17015101	C	G	Type 2 Diabetes| oedema | rosiglitazone
chr19	DOCK6	11358800	11358800	G	A	Cholesterol, HDL
chr21	FTCD	47574116	47574116	G	T	Type 2 Diabetes| oedema | rosiglitazone
chr21	FTCD	47574117	47574117	C	T	Type 2 Diabetes| oedema | rosiglitazone
chr1	JUN	59247799	59247799	T	C	Type 2 Diabetes| oedema | rosiglitazone
chr1	JUN	59247800	59247800	G	C	Type 2 Diabetes| oedema | rosiglitazone
chr2	NCKAP5	133489586	133489586	C	T	Cholesterol
chr19	TLE2	3005827	3005827	G	T	Cholesterol

[Table/Fig-10]: List of disease causal non-synonymous variants obtained from variant reduction protocol using GATK, which are predicted to be damaging in Sample-1and -30.

In Saudi Arabia, no National Registry really exists for FH. However, CHD constitutes the number one killer [16]. The importance of FH in the U.K. recommended the use DNA information for diagnostic purpose [17]. A 93% of patients with FH bear their mutations on the LDLR gene [18]. The APOB variant (c.10580G>A, p. (Arg3527Gln)) accounts for ~5% of U.K. FH, whereas a gain-of-function mutation in PCSK9 {c.1120G>T, p. (Asp374Tyr)} can be found in roughly 1.7% of FH patients [10,19].

The genes annotation, the SNPs and INDELS characterized in this study are scattered on different chromosomes. Most of them are linked to cardiovascular diseases, hypercholesterolemia, and diabetes [Table/Fig-11]. Our patient was hypercholesterolemic, ascertained by the multiple gene variants we observed in exonic position, as assessed by our bioinformatics suite analysis using TVC, Freebayes, and GATK. These observations were also reported by other authors, which we summarized in [Table/Fig-11]. We also reported, via disease association algorithms, some genes in [Table/Fig-8-10].

Most of these variants deal with type 2 diabetes, cholesterol metabolism, atherosclerosis, and oedema, attributes that are characteristic of our patient from the phenotype point of view. Representative studies from 13,745 U.S. men and women found

Chr	Gene	Start	End	Ref	Alt	Function	Ref
chr1	LDLRAP1	25889632	25889632	T	-	exonic	[1]
chr2	LRP2	170037992	170037992	G	-	exonic	[3]
chr2	LRP2	170037991	170037992	AG	ACG	exonic	[3]
chr9	ABCA1	107586838	107586838	G	-	exonic	
chr2	ABCG8	44079832	44079832	G	-	exonic	
chr17	ACE	61560015	61560015	A	-	exonic	[4]
chr2	LRP2	170053498	170053498	C	-	exonic	[3]
chr2	LRP2	170053450	170053450	A	-	exonic	[3]
chr2	LRP2	170137020	170137020	G	-	exonic	[3]
chr11	LRP5	68192646	68192646	G	-	exonic	[5]
chr11	LRP5	68207287	68207294	TGG-GGG-GC	TGG-GG-GC	exonic	[5]
chr7	NPC1L1	44578837	44578843	TGG-GG-GC	TGG-GGC	exonic	[6]
chr1	PCSK9	55512312	55512319	GCC-CCC-CG	GC-CC-CCG	exonic	
chr1	PCSK9	55529187	55529187	G	-	exonic	
chr17	SREBF1	17719973	17719973	G	-	exonic	[7]
chr22	SREBF2	42290850	42290850	G	-	exonic	[8]
chr9	TLR4	120476639	120476639	C	-	exonic	[9]
chr9	ABCA1	107586837	107586837	G	-	exonic	
chr2	ABCG8	44102403	44102403	G	-	exonic	
chr11	APOA4	116691847	116691847	G	-	exonic	[10]
chr2	APOB	21259994	21259994	G	-	exonic	[11]
chr5	HMGCR	74655079	74655079	G	-	exonic	[12]
chr3	ITIH4	52863246	52863246	-	G	exonic	[13]
chr1	LDLRAP1	25889632	25889632	T	-	exonic	[1]
chr2	LRP2	169985118	169985118	A	-	UTR3	[3]
chr17	SREBF1	17718197	17718197	C	-	exonic	[7]

[Table/Fig-11]: Summary list of variants obtained from variant reduction protocol associated with hypercholesterolemia in Sample-1and -30 using TVC, Freebayes, and GATK.

that high prevalence of hypertension, diabetes, dyslipidemia, and metabolic syndrome substantially rise with increasing body mass index. Our findings in this young Saudi patient, with an association of his metabolic state and a plethora of mutated genes (reported in [Table/Fig-11]) linked to hypercholesterolemia, diabetes, oedema, and hypertension, are nothing new. These authors have demonstrated in exhaustive studies that obesity, hypercholesterolemia, and hypertension coexist in conglomerate.

Aside from canonical genes observed linked to FH, like APOB, LDLR, and HMGCR (which are also characterized in our study), we also observed in [Table/Fig-5-7] the presence of LDLRAP1 and PCSK9, both located on chromosome1. LDLRAP1 (also known as ARH), whose gene product binds (PTD) domain, interacts with the cytoplasmic tail of the LDL receptor. Mutations in this gene lead to LDL receptor malfunction and cause the disorder of Autosomal Recessive Hypercholesterolemia (ARH).

From this Saudi patient, we observed two types of SNPs in the LDLRAP1 gene: one exonic consisting of the deletion of T, at the position 2588932 [Table/Fig-5,7]; and one intronic consisting of insertion of C, at the position 25893265 [Table/Fig-6]. Whether or not this patient suffers from a form of autosomal recessive FH remains to be clarified by further family-wide genetic studies aimed at developing a family pedigree tree. We identified two variants in our patient on the LDLRAP1 genomic sequence. Taken together in the genetic point of view, our patient presents a heterozygocity form of FH as ascertained by the multiple variants we uncovered in this study.

Our data also points out the identification of multiple variants of the PCSK9 gene, characterized by gain of function as ascertained by the ANNOVAR algorithm [Table/Fig-6,7]. These variants are characterized by the deletion of C, G, C, and C at exonic and intronic positions respectively at {55512312-55512319}, 555291871, 55523200, and 55512313. PCSK9 is an important gene involved in cholesterol metabolism and accounts for 1.7% of FH. Its major function is to down-regulate the circulating cholesterol uptake by the liver. Elevated plasma levels of PCSK9 or increased activity of PCSK9 reduces cholesterol uptake and causes hypercholesterolemia. Conversely, a decrease in plasma levels of PCSK9 or decreased activity of PCSK9 increases cholesterol uptake and causes hypocholesterolemia [20].

In our study, we mapped PCSK9 to chromosome 1 and characterized with two different damageable variants in our Saudi patient. PCSK9 is widely known to be involved in the metabolism of cholesterol [20]. PCSK9's main known function is to degrade the receptor of LDL.

Our patient harbors two SNPs: one on exon 9 R474I (G-A) and one on exon 12 G670E (GLY-GLU). Both of these two variants on PCSK9 are characterized by Gain of Function (GOF)—deleterious for cholesterol clearance, as they accelerate LDL receptors' degradation. These have been observed in previous studies as well in patients characterized by a hypercholesterolemic state.

Administration of PCSK9-inhibiting humanized monoclonal antibodies has proven to be a very efficient way of reducing hypercholesterolemia [21]. Studies with humans, including phase III clinical trials have demonstrated that PCSK9 inhibition actually does lower those diseases.

A number of monoclonal antibodies that bind to PCSK9 near the catalytic domain that interacts with the LDLR and hence inhibits the function of PCSK9, are currently in clinical trials. From Amgen, mAb-AMG145 in phase II and III clinical trial levels can lower plasma LDL-C by 43-55% [22]. From RegeneronmAb-REGN727 / SAR236553 in the phase II clinical trial level can lower plasma LDL-C by 40-72% [23].

Subsequent studies we undertook led us to uncover other PCSK9 SNPs characterized by loss of function and a Leucine 11 allelic variant in signal peptide of PCSK9 (Leucine-Zipper stretch), all in exon 1. Interestingly, these two motifs cosegregate together and are linked to people with low plasma cholesterol [24] (data not shown and this will be reported separately).

CONCLUSION

In summary, we have run the whole exome sequencing on a young Saudi blood genomic DNA using ion proton platform. Analysis identified 15 disease-causing variants in already known FH loci, as well as six previously reported APOB variants. Variants filtration using SIFT and POLYPHEN algorithms identified causal damageable genes associated with atherosclerosis, cholesterol, diabetes, hypercholesterolemia, and obesity. Of importance is the identification of two SNPs in the LDLRAP1 gene and four variants in the PCSK9 gene, among which two are highly damageable, imparting a polygenic characterization for this patient afflicted with FH. The two major PCSK9 variants uncovered constitute an excellent clinical tool to genotype this population and ultimately to design monoclonal antibodies treatments.

REFERENCES

[1] Fellin R, Arca M, Zuliani G, Calandra S, Bertolini S. The history of Autosomal Recessive Hypercholesterolemia (ARH). From clinical observations to gene identification. Gene. 2015;555:23-32.
[2] Kondkar AA, Nair KG, Ashavaid TF. Genetic analysis of Indian subjects with clinical features of possible type IIa hypercholesterolemia. JCLA. 2007;21(6):375-81.
[3] Corella D, Ordovas JM. Single nucleotide polymorphisms that influence lipid metabolism: interaction with dietary factors. Annu Rev Nutr. 2005;25:341–90.
[4] Dhar S, Ray S, Dutta A, Sengupta B, Chakrabarti S. Polymorphism of ACE gene as the genetic predisposition of coronary artery disease in Eastern India. Indian Heart Journal. 2012;64:576-81.

[5] Saarinen A, Saukkonen T, Kivelä T, Lahtinen U, Laine C, Somer M, et al. Low density lipoprotein receptor-related protein 5 (LRP5) mutations and osteoporosis, impaired glucose metabolism and ypercholesterolemia. Clin Endocrinol (Oxf). 2010;72(4):481-88.
[6] Villa F. Mutations in NPC1L1 and coronary heart disease. N Engl J Med. 2015;372(9):882-83.
[7] `Laaksonen R, Thelen KM, Paiv H, Matinheikki J, Vesalainen R, Janatuinen T, et al. Genetic variant of the SREBF-1 gene is significantly related to cholesterol synthesis in man. Atherosclerosis. 2006;185:206–09.
[8] Kim Y, Nam YJ, Lee C. Analysis of the SREBF2 gene as a genetic risk factor for vascular dementia. AJMG. 2005;1:19–22.
[9] Kiechl S, Lorenz E, Reindl M, Wiedermann CJ, Oberhollenzer F, Bonora E, et al. Toll-like Receptor 4 Polymorphisms and Atherogenesis. N Engl J Med. 2002;343:185-92.
[10] Kamboh MI, Friedlaender JS, Ahn YI, Ferrell RE. A common deletion polymorphism in the apolipoprotein a4 gene and its significance in lipid metabolism. Arterioscler Thromb. 1994;14:656-62.
[11] Tremblay AJ, Lamarche B, Ruel IL, Hogue JC, Bergeron J, Gagné C, et al. Increased production of VLDL apoB-100 in subjects with familial hypercholesterolemia carrying the same null LDL receptor gene mutation. Journal of Lipid Research. 2004;45:866-72.
[12] Sun C, Huang F, Liu X, Xiao X, Yang M, Hu G, et al. MiR-21 regulates triglyceride and cholesterol metabolism in non-alcoholic fatty liver disease by targeting HMGCR. International Journal of Molecular Medicine. 2015;35:847-53.
[13] Fujita Y , Ezura Y, Emi M, Takada KSD, Iino Y, Takahashi YKK, et al. Hypercholesterolemia associated with splice-junction variation of inter-a-trypsin inhibitor heavy chain 4 (ITIH4) gene. J Hum Genet. 2004;49:24–28.
[14] Marks D, Thorogood M, Neil HA, Humphries SE. A review on the diagnosis, natural history, and treatment of familial hypercholesterolaemia. Atherosclerosis. 2003;168:1–14.
[15] Ciccarese M, Pacifico A, Tonolo G, Pintus P, Nikoshkov A, Zuliani G, et al. A new locus for autosomal recessive hypercholesterolemia maps to human chromosome 15q25-q26. Am J Hum Genet. 2000;66:453–60.
[16] El-Hazmi MA, Warsy AS. Prevalence of obesity in the Saudi population. Ann Saudi Med. 1997;17(3):302-06.
[17] Wierzbicki AS, Humphries SE, Minhas R. Familial hypercholesterolaemia: summary of NICE guidance. BMJ. 2008;337:a1095.
[18] Usifo E, Leigh SE, Whittall RA, Lench N, Taylor A, Yeats C, et al. Low-density lipoprotein receptor gene familial hypercholesterolemia variant database: update and pathological assessment. Ann Hum Genet. 2012;76:387–401.
[19] Graham CA, McIlhatton BP, Kirk CW, Beattie ED, Lyttle K, Hart P, et al. Genetic screening protocol for familial hypercholesterolemia which includes splicing defects gives an improved mutation detection rate. Atherosclerosis. 2005;182:331–40.
[20] Mbikay M, Mayne J, Chretien M. Proprotein convertases subtilisin/kexin type 9, an enzyme turned escort protein: hepatic and extra hepatic functions. Journal of Diabetes. 2013;5:391-405.
[21] Zhang XL, Zhu QQ, Zhu L, Chen JZ, Chen QH, Li GN, et al. Safety and efficacy of anti-PCSK9 antibodies: a meta-analysis of 25 randomized, controlled trials. BMC Medicine. 2015;13:123.
[22] Sullivan D, Olsson AG, Scott R, et al. Effect of a monoclonal antibody to PCSK9 on low-density lipoprotein cholesterol levels in statin-intolerant patients: the gauss randomized trial. JAMA. 2012;81:1-10.
[23] McKenney JM, Koren MJ, Kereiakes DJ, Hanotin C, Ferrand AC, Stein EA. Safety and efficacy of a monoclonal antibody to proprotein convertase subtilisin/kexin type 9 serine protease, SAR236553/REGN727, in patients with primary hypercholesterolemia receiving ongoing stable atorvastatin therapy. J Am Coll Cardiol. 2012;59:2344-53.
[24] Pisciotta L, Sallo R, Rabacchi C, Wunsch A, Calandra S, Bertolini S. Leucine 10 allelic variant in signal peptide of PCSK9 increases the LDL cholesterol-lowering effect of statins in patients with familial hypercholesterolaemia. Nutrition, Metabolism, and Cardiovascular Diseases. 2012;22:831-35.

PARTICULARS OF CONTRIBUTORS:
1. Assistant Professor, Department of Biochemistry, University of Hail, School of Medicine and Center for Molecular Diagnostic, Personalized and Therapeutic Unit, Hail, Saudi Arabia.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Edem Nuglozeh,
Assistant Professor, Department of Biochemistry, University of Hail, School of Medicine and Center for Molecular Diagnostic, Personalized and Therapeutic Unit, Hail, Saudi Arabia.
E-mail: nuglozeh@gmail.com

FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Mar 09, 2017**
Date of Peer Review: **Apr 18, 2017**
Date of Acceptance: **May 11, 2017**
Date of Publishing: **Jun 01, 2017**