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Regular paper

Compound heterozygous LDLR variant in severely affected familial hypercholesterolemia patient

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Familial hypercholesterolemia (FH) is most commonly caused by mutations in the LDL receptor (LDLR), which is responsible for hepatic clearance of LDL from the blood circulation. We described a severely affected FH proband and their first-degree blood relatives; the proband was resistant to statin therapy and was managed on an LDL apheresis program. In order to find the causative genetic variant in this family, direct exon sequencing of the *LDLR*, *APOB* and *PCSK9* genes was performed. We identified a compound heterozygous mutation in the proband with missense p.(W577C) and frameshift p.(G676Afs*33) variants at exons 12 and 14 of the *LDLR* gene respectively. DNA sequencing of *LDLR* gene from the parents demonstrated that the missense variant was inherited from the mother and frameshift variant was inherited from the father. The frameshift variant resulted in a stop signal 33 codons downstream of the deletion, which most likely led to a truncated protein that lacks important functional domains, including the trans-membrane domain and the cytoplasmic tail domain. The missense variant is also predicted to be likely pathogenic and affect EGF-precursor homology domain of the LDLR protein. The segregation pattern of the variants was consistent with the lipid profile, suggesting a more severe FH phenotype when the variants are in the compound heterozygous state. The finding of a compound heterozygous mutation causing severe FH phenotype is important for the genotype-phenotype correlation and also enlarges the spectrum of FH-causative *LDLR* variants in the Arab population, including the Saudi population.

Key words: familial hypercholesterolemia (FH), low-density lipoprotein receptor (LDLR), compound heterozygous, missense variant, frameshift variant, sequencing, Arab, coronary artery disease (CAD), cholesterol, genetics

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Abbreviations: CAD, Coronary artery disease; CHD, Coronary heart diseases; FH, Familial hypercholesterolemia; LDLR, low-density lipoprotein receptor; APOB, apolipoprotein B; PCSK9, Pro-protein convertase subtilisin/kexin type 9; DNA, Deoxyribonucleic acid; LDL, low-density lipoprotein; LDL-C, low-density lipoprotein-cholesterol; HDL, High-density lipoprotein; HDL-C, High-density-lipoprotein-cholesterol; TC, Total cholesterol; TG, Triglycerides; EDTA, Ethylenediaminetetraacetic acid; PCR, Polymerase chain reaction; EGF, Epidermal growth factor.

SUPPLEMENTAL MATERIAL

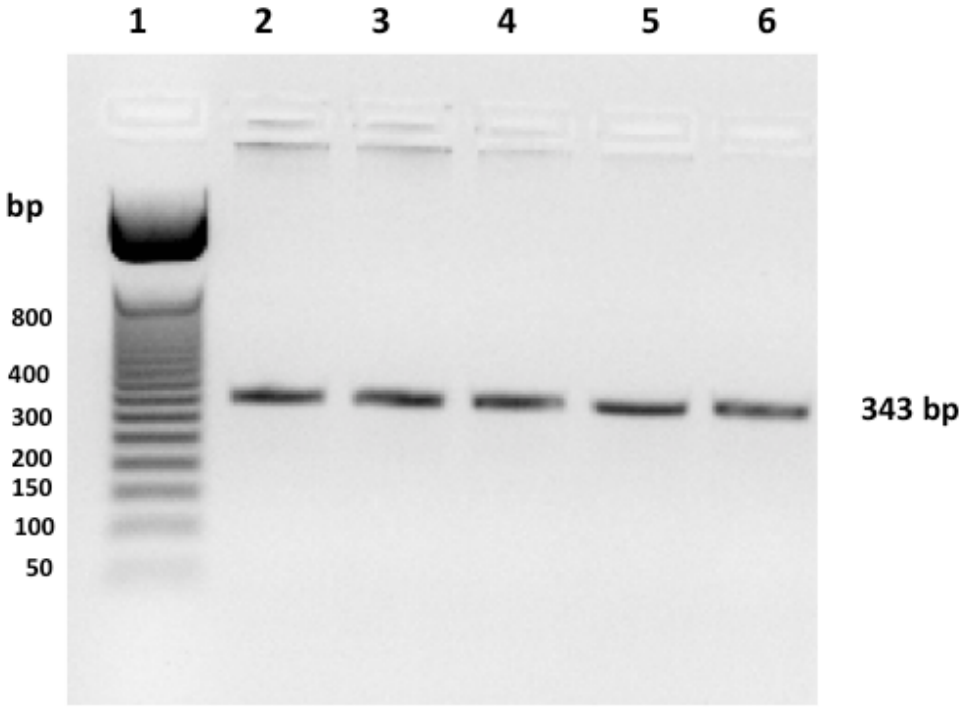
Appendix A

The *LDLR*, *APOB* and *PCSK9* gene primers used in both PCR amplification and capillary sequencing.

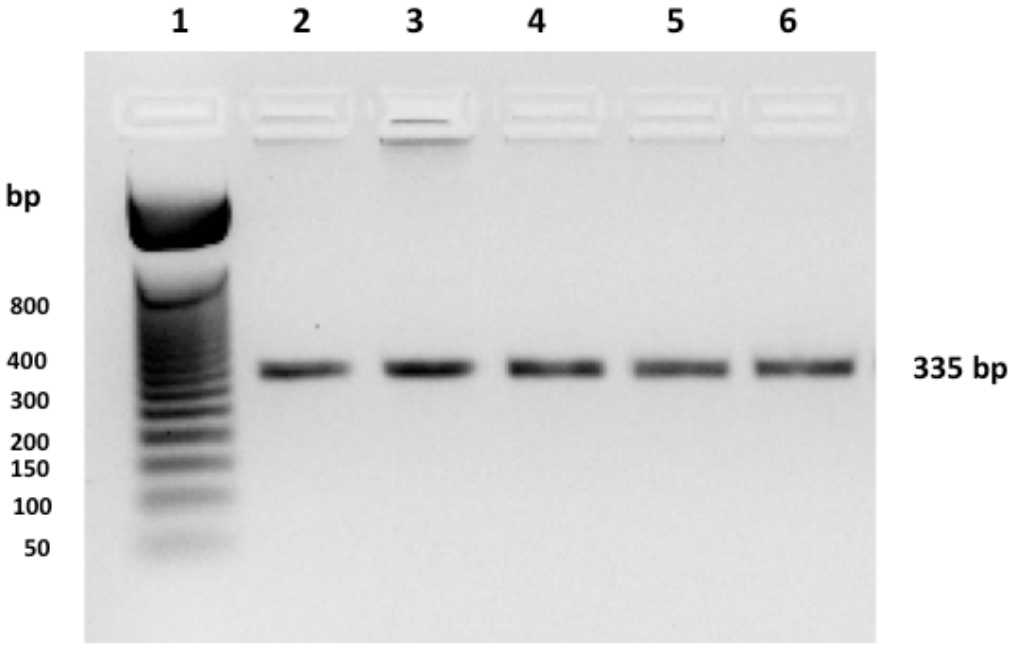
	Forward primers (5'-3')	Tm (°C)	Reverse primers (5'-3')	Tm (°C)	PCR Product size
LDLR					
Exon 1	tcctcctcttgcagtgaggtgaag	58.6	tccctctcaacctattctggcg	57.1	298
Exon 2	cttgtgcttgcttaattccctg	53.3	caacatggcgagaccctgtc	55.1	339
Exon 3	ggtcttgaacccctgacctcac	56.2	aacactccccaggactcagatag	53.1	311
Exon 4-1	atgaggaaactgaggcaccgag	57	tgtccccttggaacacgtaaag	55.5	443
Exon 4-2	tccagtgcaacagctccacc	55.8	gttggaaatccacttcggcac	55.8	341
Exon 5	gactcttcttgagaaaatcaac	56.7	ggcacagagaatgggggatg	55.3	210
Exon 6	ttacaggcacaaaccaccgtg	55.8	accctacagcactcatgtctcagtc	55.4	350
Exon 7	ggcgaagggatgggtagggg	56.5	gttgccatgtcaggaagcgc	55.7	236
Exon 8	gtttccttgattacatctcccgag	55	gagtctgtgcaaagttcagaggatg	56.1	359
Exon 9	tccatcgacgggtcccctctgaccc	61.2	agccctcatctcacctgcgggccaa	60.8	273
Exon 10	tgacctgtcccagagaatgatctg	56.9	cccactaaccagttcctgaagctc	57.2	415
Exon 11	ttcgttgcacgcattggc	56.3	gggaaaccttcagggagcag	55	300
Exon 12	aggtgcttttctgctaggtccctg	58.9	tgcgttcatcttggcttgagtg	57.6	343
Exon 13	ggcaacccccgtgaaactctgtctg	65.4	gcaggaacgagatcatcagctattc	57.3	398
Exon 14	cttgaaacctccttgtggaaactc	55.1	tgacagatgagcagagagaggctc	56.7	335
Exon 15	tggtattttgccatgttgaccag	56.3	aggacgacacctggactccatc	56.6	439
Exon 16	tgggaagttctccaagtgtccag	56.9	tcacatagcgggaggctgtg	55.9	262
Exon 17	ggcgatctctaaacaaacataaaag	52.9	tgtcctcgatctggagggc	54.7	402
Exon 18	actcaccgtctccctctggc	55.4	aaaggaagaaaccaaaatcccaac	58.8	384
ApoB					
Exon 26	ggagcagttgaccacaagcttagcttgg	62.9	agagttccagggtggctttgcttgtatg	61.4	352
PCSK9					
Exon 1	ctgcacagtcctccccac	54.9	aggcccgagaggaaacag	52.6	382
Exon 2	tacacctagggtttgctggg	53.8	cagtgtatatgcagtggccc	53.8	313
Exon 3	gctgtgtttgctgctgtcc	53.2	gcagagcaaatggattcagc	51.8	244
Exon 4	ggatgtgctctgtagtttctgtg	55.3	ccaggatggggatatggg	52.6	273
Exon 5	gttcatccatccagccacc	53.2	acctcccaggccaggtc	54.3	279
Exon 6	ctctgtgcctgtaagggagg	55.9	cccacgtgccacaagaag	52.6	373
Exon 7	tgagtctgcctctgcaacc	53.2	acagaccctgactgccaaag	53.8	316
Exon 8	gtgtgcactggcaggagtc	55.4	cacacagacctcccaagcc	55.4	307
Exon 9	ctgtggaggtcccctcactc	57.9	gcccagcactgacacacac	55.4	287
Exon 10	tgtgttttcaaagcccattc	47.7	acttgtgaggaccaagcctc	53.8	318
Exon 11	agagagagggtctgatgggg	55.9	gctgtgcaggagagacacg	55.4	320
Exon 12	agaaatgaagtgtgggtggg	51.8	ccattttaaagctcagcccc	51.8	348

Appendix B

A) Gel image showing the size and quality of PCR amplified product of *LDLR* exon 12.



B) Gel image showing the size and quality of PCR amplified product of *LDLR* exon 14.



Well 1: 50 bp DNA ladder, Well 2: Father, Well 3: Mother, Well 4: Proband, Well 5: Brother, Well 6: Sister