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Identification of a recurrent frameshift mutation at the *LDLR* exon 14 (c.2027delG, p.(G676Afs*33)) causing familial hypercholesterolemia in Saudi Arab homozygous children

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ABSTRACT

Familial hypercholesterolemia (FH) is an autosomal dominant disease, predominantly caused by variants in the low-density lipoprotein (LDL) receptor gene (*LDLR*). Herein, we describe genetic analysis of severely affected homozygous FH patients who were mostly resistant to statin therapy and were managed on an apheresis program. We identified a recurrent frameshift mutation p.(G676Afs*33) in exon 14 of the *LDLR* gene in 9 probands and their relatives in an apparently unrelated Saudi families. We also describe a three dimensional homology model of the LDL receptor protein (LDLR) structure and examine the consequence of the frameshift mutation p.(G676Afs*33), as this could affect the LDLR structure in a region involved in dimer formation, and protein stability. This finding of a recurrent mutation causing FH in the Saudi population could serve to develop a rapid genetic screening procedure for FH, and the 3D-structure analysis of the mutant LDLR, may provide tools to develop a mechanistic model of the LDLR function.

1. Introduction

Familial hypercholesterolemia (FH) is a lipid metabolism disorder that is genetically inherited in an autosomal dominant manner, which clinically results in high concentrations of plasma cholesterol bound to low-density lipoprotein (LDL). If untreated, persistent hypercholesterolemia may cause xanthomata on the tendon, hands, and feet, cutaneous planar, corneal arcus, and atheroma. Because the disease is initially asymptomatic and painless, the majority of patients may not be aware of their illness until a severe myocardial infarction occurring in the fourth or fifth decade of life due to instant atheroma, which often leads to sudden cardiac death or other severe cardiovascular events [4]. FH is predominantly caused by mutations in the LDL receptor (*LDLR*) gene, which is responsible for hepatic clearance of LDL from the blood circulation. To date, more than 1700 different *LDLR* variants have been reported in FH patients ([26]; www.ucl.ac.uk/ldlr), making genetic screening very laborious. FH can also be caused by certain mutations in the apolipoprotein B (*APOB*) gene, which encodes the ligand for LDLR [45]. The pro-protein convertase subtilisin/kexin type 9 (*PCSK9*) gene has been proposed to be the third gene with pathogenic mutations accounting for some FH cases [1,41].

Mutations in the *LDLR* gene have been divided into five classes based on biochemical and functional studies on *LDLR* variants; such that Class 1 mutations include null alleles that lack any LDLR protein product.

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Class 2 mutations encode LDLR proteins that have abnormal transport from the endoplasmic reticulum to the Golgi apparatus. In Class 3 mutations, LDLR protein shows defect in binding the LDL ligand, and Class 4 mutation genes encode LDLR that is defective in the internalization/endocytosis of LDL. Class 5 mutations in the *LDLR* gene encode a recycling defective LDL receptor protein [25].

In the majority of studied populations, the heterozygous form occurs in less than 1:500 and the homozygous form is one in one million [19,39]. The highest frequency of heterozygosity with an incidence of less than 1:80 is found in the Afrikaner population in South Africa [40]. Studies on the French Canadian population where five common mutations have been reported show a frequency of 1:270 [20,28,48]. This unusually high frequency is due to founder effects and/or consanguinity and there no heterozygote advantage has been identified. There is a lack of epidemiological genetic studies examining the frequency of FH in the Arab and/or Saudi population. However, the high incidence of consanguineous marriages (>54% in Saudi population), suggests that the incidence of FH might be high [22], as we estimated that at least 46,000 (this number can be as high as 230,000) Saudis are affected by FH and related disorders (Al-Allaf et al., unpublished data).

Recently we have reported two novel mutations causing FH in severely affected patients. The first was a novel nonsense [2] and the second was a novel frameshift variant [3]. In continuation to our further mutation screening for FH patients, studies described here were focused on patients who were therapeutic recruited in apheresis program to manage hypercholesterolemia in Saudi children. Patients were recruited from a referral hospital center for such apheresis program in the country and thus allow access to FH patients who may be affected with the LDLR function, and thus allows a continuing study in the targeted patient population. From such a selected group of homozygous patients, herein, we describe a high frequency frameshift mutation p.(G676Afs*33) located in the exon 14 of *LDLR* gene and its probable impact on the structure and function of the LDLR receptor protein in Saudi Arab FH families with severe risk of premature CHD. Associations between the frameshift mutation p.(G676Afs*33) and phenotypic behavior of the LDLR protein were also analyzed using the predicted 3D protein structure as well as model format and molecular dynamic (MD) simulations.

2. Materials and methods

2.1. Subjects

All samples were approved by the Institutional Review Board and the Research ethics committees (REC) at King Faisal Specialist Hospital and Research Center (KFSHRC), Riyadh, Saudi Arabia for the use in the research reported here. Blood samples were obtained from the subjects after obtaining informed written consent from the patient or their representative. Eighteen samples including 9 probands and 9 first-degree blood relatives from 8 unrelated Saudi families were analyzed. One family (named B) has provided two proband individuals. All probands were children with a clinical diagnosis of homozygous familial hypercholesterolemia. Two additional samples were analyzed from two unrelated Saudi families, Family H, and Family I (Tables 1 & 2), with clinical diagnosis of heterozygous familial hypercholesterolemia also included. Since the frequency of homozygous familial hypercholesterolemia in the majority of studied population worldwide is one in a million, the collected 9 homozygous probands across Saudi Arabia assumed to cover 1/3rd Saudi population. Patients were recruited from a referral hospital LDL apheresis center (KFSHRC), Riyadh, Saudi Arabia. At the time of this study this is the only center in Saudi Arabia for LDL apheresis, and our previously published articles on FH causative mutations used patient's samples from the same center. The collected specimens represent families from the central, northern and western regions of Saudi Arabia. Enrollment criteria for the patients genetic screening were based on the Simon Broome register [37]. The homozygous FH patients are managed on an apheresis program and are resistant to statin therapy. The name of Tribes and Families described in this report is decoded (to keep the identities of subjects anonymous).

2.2. DNA sequencing

Genomic DNA was isolated from EDTA treated whole blood with the MagNA Pure Compact Nucleic Acid Isolation Kit I (Roche) according to the manufacturer's instructions. Polymerase chain reaction (PCR) amplification of the *LDLR* gene (including the 18 coding exons and flanking intron regions), *APOB* gene (exon 26 of the *APOB* gene containing codons 3475–3592, which harbors three known pathogenic mutation

Table 1
Frameshift and silent mutations identified in familial hypercholesterolemia Saudi families.

Tribe	Family	Family members	Code	Frame-shift mutation in *LDLR* exon 14	Silent variant in *LDLR* exon 10, c.1413A>G/*APOB* exon 26, c.10701G>A/*PCSK9* exon 1, c.141C>T
Ela	A	Father	FA01	c.2027delG, p.(G676Afs*33), Htz	c.1413A > G, p.(=), Htz
		Mother	FA02	c.2027delG, p.(G676Afs*33), Htz	–
		Brother	FA03	c.2027delG, p.(G676Afs*33), Htz	–
		Proband 1	FA04[a]	c.2027delG, p.(G676Afs*33), Hmz	–
		Sister	FA05	c.2027delG, p.(G676Afs*33), Htz	–
		Sister	FA06	c.2027delG, p.(G676Afs*33), Htz	–
Qla	B	Father	FB01	c.2027delG, p.(G676Afs*33), Htz	c.1413A>G, p.(=), Htz
		Mother	FB02	c.2027delG, p.(G676Afs*33), Htz	c.1413A>G, p.(=), Htz
		Proband 2	FB03[a]	c.2027delG, p.(G676Afs*33), Hmz	c.10701G>A, p.(=), Htz
		Proband 3	FB04[a]	c.2027delG, p.(G676Afs*33), Hmz	–
Mla	C	Proband 4	FC01[a]	c.2027delG, p.(G676Afs*33), Hmz	c.141C>T, p.(=), Htz
		Sister	FC02	c.2027delG, p.(G676Afs*33), Htz	c.1413A>G, p.(=), Htz; c.141C>T, p.(=), Htz
		Brother	FC03	–	c.1413A>G, p.(=), Hmz
	D	Proband 5	FD01[a]	c.2027delG, p.(G676Afs*33), Hmz	–
	E	Proband 6	FE01[a]	c.2027delG, p.(G676Afs*33), Hmz	c.141C>T, p.(=), Htz
	F	Proband 7	FF01[a]	c.2027delG, p.(G676Afs*33), Hmz	c.141C>T, p.(=), Hmz
	G	Proband 8	FG01[a]	c.2027delG, p.(G676Afs*33), Hmz	c.141C>T, p.(=), Htz
	H	–	FH01	c.2027delG, p.(G676Afs*33), Htz	c.1413A>G, p.(=), Htz
	I	–	FI01	c.2027delG, p.(G676Afs*33), Htz	c.1413A>G, p.(=), Htz
Lla	J	Proband 9	FJ01[a]	c.2027delG, p.(G676Afs*33), Htz	c.1413A>G, p.(=), Htz

Family A belongs to tribe Ela, family B (Qla), family C-I (Mla), and family J (Lla). The name of Tribes and Families described in this report are decoded. Hmz = Homozygous, Htz = Heterozygous.

[a] Index case.