Screening for Familial Hypercholesterolemia in Tunisia using Whole Exome Sequencing: Importance in diagnosis and healthcare management

Dépistage de l'hypercholestérolémie familiale en Tunisie par séquençage de l'exome entier: Importance dans le diagnostic et la gestion des soins de santé

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Abstract

Aim: To determine pathogenic variants linked to Familial Hypercholesterolemia (FH) among a southern Tunisian family using Whole Exome Sequencing (WES).

Methods: Genomic DNA was extracted from whole blood among the index case as well as other affected and unaffected family members. Then, WES was performed only in the proband. The pathogenicity of genetic variation was assessed in a set of 13 genes reported as associated with FH using combined filtering and bioinformatics prediction tools. Finally, sanger sequencing was done to verify the probands' likely pathogenic predicted mutations and to check for familial segregation among all family subjects.

Results: Our results showed the presence of a pathogenic splice site mutation (c.1186+1G>A) in the LDLR gene among the proband and other affected family members. The following up of the family, revealed the effectiveness of the combination of rosuvastatin and ezetimibe with healthy diet to meet the LDL-c treatment goal with approximately 50% of decrease for the proband.

Conclusion: This study is the first of its kind using WES for FH screening and diagnosis in Tunisia. Here, we point up the importance of molecular analysis for a better health care management of FH patients and their families.

Key words: Hypercholesterolemia, LDLR, North Africa, Whole exome sequencing, bioinformatic analysis, Splice site variant.

Résumé

Objectif: Déterminer les variants pathogènes liés à l'hypercholestérolémie familiale (HF) au sein d'une famille du sud de la Tunisie en utilisant le séquençage de l'exome entier (WES).

Méthodes: L'ADN génomique a été extrait du sang total chez le cas index ainsi que chez d'autres membres de la famille, affectés et non affectés. Ensuite, le WES a été réalisé uniquement chez le proband. La pathogénicité des variations génétiques a été évaluée dans un ensemble de 13 gènes rapportés comme associés à l'HF en utilisant des outils de filtrage combiné et de prédiction bioinformatique. Enfin, le séquençage de Sanger a été effectué pour vérifier les mutations probablement pathogènes prédites chez le proband et pour vérifier la ségrégation familiale chez tous les sujets de la famille.

Résultats: Nos résultats ont montré la présence d'une mutation pathogène au niveau du site d'épissage (c.1186+1G>A) dans le gène LDLR chez le proband et d'autres membres affectés de la famille. Le suivi de la famille a révélé l'efficacité de la combinaison de rosuvastatine et d'ézétimibe avec un régime alimentaire sain pour atteindre l'objectif de traitement du LDL-c, avec une diminution d'environ 50 % pour le proband.

Conclusion: Cette étude est la première de son genre à utiliser le WES pour le dépistage et le diagnostic de l'HF en Tunisie. Nous soulignons ici l'importance de l'analyse moléculaire pour une meilleure gestion des soins de santé des patients atteints d'HF et de leurs familles.

Mots clés: Hypercholestérolémie, LDLR, Afrique du Nord, Séquençage de l'exome entier, Analyse bioinformatique, Variant du site d'épissage

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Introduction

Familial Hypercholesterolemia (FH) is a common disorder of lipid metabolism, and the most common inherited metabolic disease with an autosomal dominant inheritance. During the last decades, registries all over the world have shown the prevalence of Heterozygous Familial Hypercholesterolemia (HeFH) estimated as 1 in 50 in the general population, modern estimates are twice as high, typically varying between 1 in 220 to 1 in 310. Estimates for the prevalence of Homozygous Familial Hypercholesterolemia (HoFH) are approximately 1:250,000 (6). However, according to reports, FH is not a rare disease, but it is underdiagnosed. According to recent evidence, the global prevalence of HeFH is estimated as 1 in 200-250 people (7). Contrary to HeFH, HoFH patients have much higher low-density lipoprotein cholesterol (LDL-C) levels and earlier coronary artery disease onset (8). Moreover, these young patients often die of myocardial infarction before the age of 20 years (9). Controversy to untreated men and women with HeFH who are 30% to 50% more likely than healthy individuals to experience a fatal or nonfatal cardiac episode by the ages of 50 and 60, respectively. Actually, FH is an important health problem as the main cause of premature myocardial infarctions (10). Therefore, early diagnosis and effective lipid-lowering treatment are crucial for these patients (11).

From a genetic side , FH is characterized by a large spectrum of mutations in genes encoding proteins involved in lipoprotein metabolism, mainly the low density lipoprotein receptor (LDLR), apolipoprotein B (APOB) and proprotein convertase subtilisin/kexin type 9 (PCSK9) (12). Mutations in these genes lead to an increased level of low-density lipoprotein from the beginning of the patient's life. Approximately 60% to 80% of FH are caused by mutations in the LDLR gene (13). Over than 2300 variants in the LDLR gene causing FH have been identified until now (14). Of pathophysiological way, mutations in the LDLR gene (OMIM#606945), resulting in a lack of functional receptors for LDL on the cell surface, giving rise to increased plasma LDL levels (15). Similarly, mutations in the APOB gene (OMIM#107730) also reduce LDL clearance, resulting in the disorder called Familial Defective Apolipoprotein B (FDB, OMIM#144010), which is clinically indistinguishable from FH. Less frequently mutations including gain-of-function variants occurring in the PCSK9 gene (OMIM #607786) lead to hypercholesterolemia (9). There are also sporadic variants that cause FH, in genes encoding the LDL-receptor adaptor protein 1 (LDLRAP1), apolipoprotein E (APOE), signal-transducing adaptor protein family 1 (STAP1), lysosomal acid lipase (LIPA), and ATP binding cassette subfamily G member 5 and 8 (ABCG5 & ABCG8) (11) (16). Cumulatively, variants in these genes explain around 40% of FH cases. However, none of the FH-related genes have been identified as a causal gene in a significant number of patients with the FH phenotype (17). Added to that , the clinical phenotype caused by mutations in these known genes shows a gradient of clinical severity among FH patients (18). This suggests the involvement of other unknown genes in the pathogenesis of FH, or that polygenic severe hypercholesterolemia may lead to a similar phenotype (19). Moreover, Other genetic factors may also contribute to the phenotypic variation seen in FH, such as small effect genetic variants, gene-gene and gene-environment interactions, and non-Mendelian and epigenetic mechanisms (12). In Tunisia, FH is underdiagnosed and undertreated despite the major advancement in genetic diagnosis. Indeed, the high rate of consanguinity (20) as well as the heterogenous genetic background of the Tunisian population enhance the prevalence of HeFH to one among 165 individual and 1 among 125 000 the frequency of HoFH in Central and Southern Tunisia (21). Until today, eleven FH causative mutations in the LDLR gene in the Tunisian population have been described (22) (23). The main objective of our study was to determine the genetic signature of FH among a southeastern Tunisian family using Next Generation Sequencing (NGS), namely the Whole Exome Sequencing (WES). The specific objectives were to identify pathogenic variants associated with FH and investigate their role in the pathogenesis of the disease in Tunisia. Our study provides a substantial contribution to advancing the clinical diagnosis and treatment of FH, with significant implications for healthcare in our country.

Methods

Subjects

An index patient was examined at the National Institute of Nutrition in Tunis for management of her hypercholesterolemia. The proband was a young girl aged of 9 years old with tuberous xanthomas localized at the extensor surfaces of elbows and knees as well as high level of plasma LDL-C (Figure 1).

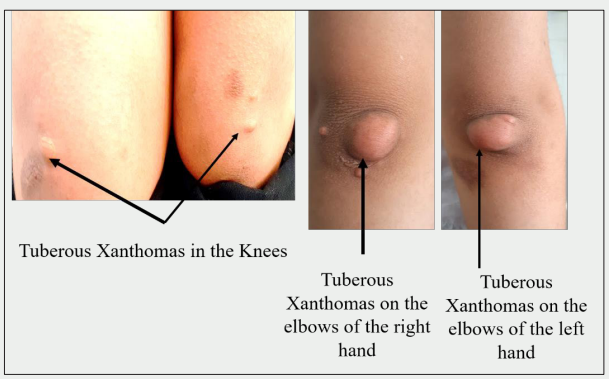

Figure 1. Tuberous xanthomas among the index case with Familial Hypercholesterolemia

No tendinous xanthomas have been observed in the patient. This girl comes from a family of 7 members; parents and 4 siblings. All family members were examined for plasma lipid levels of LDL-C, the presence of extravascular deposits and cardiovascular risk factors. According to the clinical examination and the biochemical measurements of plasma lipid parameters, the patient and all family members were referred for genetic investigation of a clinical suspicion of FH. The diagnosis of FH was based on the "Dutch Lipid Clinic Network

diagnostic criteria for familial hypercholesterolemia" (24). This study was approved by the Ethics Committee on Human Subject Research at Nutrition Institute in Tunisia and conformed to the 1964 Helsinki declaration and its later amendments. The patient and all family members gave their written informed consent for the study.

Biochemical analyses

Total cholesterol (TC), triglycerides (TG), and HDL-cholesterol levels were measured by standard enzymatic methods using commercially available kits. LDL-C was measured directly in the lab or calculated with the Friedewald formula.

LDL-c (g/l) =TC– HDL-c - (TG/5)
*LDL-c: Low Density Lipoprotein Cholesterol
*HDL-c: High Density Lipoprotein Cholesterol
*TG: Triglycerides
** If TG are higher than 4 g/l or 4,6 mmol/l, LDL cholesterol cannot be calculated by this formula and must be measured by assay.

Genetic investigation

DNA extraction
Genomic DNA was extracted from total blood of all family members using the FlexiGene DNA Kit (QIAGEN). Then, DNA quality was assessed through nanodrop spectrophotometer. WES was performed in collaboration with RAN Bio Links SARL (Carthagenomics) using the Twist Core (Twist Biosciences) kit. The captured libraries were sequenced on NovaSeq 6000 System , Illumina.

Bioinformatic analysis

The quality of the sequencing reads in FASTQ files was evaluated using FastQC (https://www.bioinformatics.babraham. ac.uk/projects/fastqc/), which was followed by adapter trimming using BBDuk (https://jgi.doe.gov/data-and-tools/bbtools/bb-tools-user-guide/bbduk-guide/). We aligned reads to the human reference genome hg38, and we subsequently called the genetic variants in a VCF file following the GATK best practices. Variant annotation was processed using ANNOVAR (25).
Prioritization of potential disease-causing variants was carried out in a set of thirteen genes described as associated with FH using Variant Annotation and Filtering Tool (VarAFT) (26). The gene list was prepared through a literature review using PubMed (https://www.ncbi.nlm.nih.gov/pubmed) (Table 1).
In our study, we have retained only variants with Minor Allele Frequency (MAF) < 0.01 in gnomAD (http://gnomad.broadinstitute.org) and in GME database (http://igm.ucsd edu/gme/). As they are more likely to have a functional impact, we kept non-synonymous, non-sense, frameshift, and splice site variants. Non-synonymous variations were filtered to only retain those that were expected to be harmful by the majority of in-silico pathogenicity prediction software tools. In addition, we selected variants predicted to alter splice sites by Human Splicing Finder database (27). Finally, we used Depth and Coverage Analysis (DeCovA) to evaluate the coverage of the genes holding the filtered variations(28). We investigated the prioritized genetic variants in bioinformatics databases such as PubMed (https://www.ncbi.nlm.nih.gov/pubmed), ClinVar, VarSome, and LOVD (https://www.lovd.nl), to see if they had been previously reported and, if so, to learn the clinical traits of the carriers and any genotype-phenotype correlations that had been found. The pathogenicity was assessed according to the classification criteria of the American College of Medical Genetics (ACMG).

Sanger sequencing
Sanger sequencing was performed in order to verify the probands' likely pathogenic predicted mutations and to check for familial segregation. In short, using oligonucleotide primers designed by Primer3 software, genetic variations with exons were amplified from DNA samples using polymerase chain reaction (PCR). The ABI prism Big Dye terminator v3.1 cycle sequencing kit was used to sequence the amplicons produced on the automated ABI3500 (Applied Biosystems, CA, USA). The BioEdit software version 7.1 was used for the sequence analysis (29).

Results

Biochemical results

Clinical characteristics and biochemical measurements of the index case (IV.4p) and other family members at the time of the survey are summarized in table 2.

Table 2. Clinical characteristics and plasma lipid level of all family members

Patient ID	Age (years)	Gender	BMI (kg/m2)	TC (mmol/l)	TG (mmol/l)	HDL-c (mmol/l)	LDL-c (mmol/l) / (g/l)	XT
III.1	54	M	Na	6.55	0.88	1.16	4.99 /1.93	-
III.2	41	F	34.75	7.17	0.78	1.65	5.16 /1.99	-
IV.1	20	F	Na	3.62	Na	Na	Na	-
IV.2	18	M	Na	7.27	1.06	1.18	5.61 /2.17	-
IV.3	13	M	Na	6.33	1.31	1.78	3.95 /1.52	-
IV.4^{p}	9	F	18.0	16.72	0.55	Na	12.9 /4.99	+
IV.5	4	M	Na	4.86	Na	Na	Na	-

BMI: Body Mass Index; **TC**: Total Cholesterol; **TG**: Triglycerides; **HDL-c**: High Density Lipoprotein cholesterol; **LDL-c**: Low Density Lipoprotein; **XT**: Tuberous Xanthoma, **Na**: Non available.

No cardiac problem has been detected in the electrocardiogram of the IV.4p. The cardiac stress test for the index case's parents (III.1 and III.2) shows no cardiac distress. The follow up of the proband since the survey is shown in table 3. Our results showed that the combination of rosuvastatin and Ezetimibe, two lipid lowering treatment with Hygienic and Dietary Measures had the highest rate of LDL-c decrease (48.76%) and improved the lipid plasma level of the patient IV.4p with HoFH. For other affected family members, Atorvastatin was prescribed.

Table 1. Genes associated with Familial Hypercholesterolemia FH

Gene Name	Coding protein	Chromosomal location (number of exons)	OMIM number	Comments
LDLR	Low-Density Lipoprotein Receptor	*19p13.2 (18)*	606945	Most common cause of familial hypercholesterolaemia Mutations
APOA2	Apolipoprotein A2	*1q23.3 (4)*	107741	A SNP of the promoter of the *APOA2* gene (265T-C), influenced the level of total cholesterol and LDL-c in members with a mutation in the LDLR gene causing hypercholesterolemia
EPHX2	Epoxide hydrolase 2, cytosolic	*8p21.2-p21.1*(19)	132811	A significant modification of the phenotype of familial hypercholesterolemia with defective LDLR allele by the EPHX2 R287Q variant.
GHR	Growth hormone receptor	*5p13.1-p12*(10)	600946	Mutation found in the *GHR* gene, resulting in a leu526-to-ile (L526I) substitution, influenced plasma levels of high-density lipoprotein (HDL) cholesterol in affected family members with a mutation in the LDLR gene causing hypercholesterolemia.
APOB	Apolipoprotein B100	2p24.1 (29)	107730	Mutations generally associated with a less severe phenotype than LDLR mutations; common in some populations Gain-of-function
PCSK9	Proprotein convertase subtilisin/ kexin type 9	1p32.3 (13)	607786	Gain-of-function mutations cause familial hypercholesterolemia, whereas loss-of-function mutations cause depressed LDL cholesterol
LDLRAP1	Low Density Lipoprotein Receptor Adaptor Protein 1	*1p36.11 (9)*	605747	Individuals with two mutations have autosomal recessive hypercholesterolemia and often have a milder phenotype than individuals with two LDLR mutations; heterozygous parents of the index case often have no obvious lipid phenotype
PPP1R17	Protein phosphatase 1 regulatory subunit 17	*7p14.3 (5)*	604088	O f341 individuals who carried a mutation in the PPP1R17 gene, approximately 80% had hypercholesterolemia.
CYP7A1	Cholesterol 7-alpha-hydroxylase	*8q12.1 (6)*	118455	Analysis of 150 nuclear families indicated statistically significant linkage between plasma LDL-C concentrations and CYP7. Functional polymorphisms within the CYP7A1 promotor region significantly influence the metabolic pathway for cholesterol elimination from the body.
ABCG8	ATP-Binding Cassette Sub-Family G Member 8	*2p21(13)*	605460	Mutations cause sitosterolaemia; compound heterozygous mutations have been observed in a child with severe hypercholesterolaemia
ABCG5	ATP-Binding Cassette Sub-Family G Member 5 (Sterolin 2)	*2p21 (13)*	605459	
APOE	Apolipoprotein E	19q13.32 (6)	107741	An in-frame deletion of Leu167 has been detected in individuals with familial hypercholesterolaemia phenotypes
LIPA	Lysosomal acid lipase	*10q23.31 (10)*	613497	Mutations cause cholesterol ester storage disease (Wolman disease); homozygous mutations at a splice junction can produce a familial hypercholesterolaemia-like phenotype

Table 3. Follow up of the index case during the different clinical evaluation

Periods	Plasma lipids (mmol/l)				Treatement	Decrease of % LDL-c
	TC	LDL-c	TG	HDL-c		
T0	16.72	12.9	0.55	Na	Life style modifications	
T0+7 months	13.00	11.18	1.06	1.33	Atorvastatin 40 mg (1 pill at night)	13.33
T0+10 months	11.33	9.45	1.18	1.33	Rosuvastatin 20 mg (1pill/day)	26.74
T0+19 months	9.98	8.86	0.52	1.09	Life style modifications not followed + Rosuvastatin 20 mg (1pill/day) + Ezetimibe 10 mg (1cp/day)	31.32
T0+22 months	7.96	6.61	0.55	1.09	Life style modifications well followed + Rosuvastatin 20 (1pill/day) + Ezetimibe 10 (1 pill/day)	48.76

T0: Time of the first examination; **TC**: Total Cholesterol; **TG**: Triglycerides; **HDL-c**: High Density Lipoprotein cholesterol; **LDL-c**: Low Density Lipoprotein; % ↘ **LDL-c**: the rate of LDL-c decrease, **Na**: Non available.

Genetic investigation

The WES analysis revealed the presence of a splice site mutation in the LDLR gene NM_000527.5: c.1186+1G>A among the index case. It is a substitution of the first base of intron 8, guanine (G) by adenine (A). The study of the complementary DNA of homozygous patients carrying this mutation shows a deletion of 51 final nucleotides of exon 8, with preservation of the reading frame. At the protein level, it corresponds to a deletion of 17 amino acids encoded by exon 8: 380 to 396. The truncated protein synthesized has a residual activity of less than 10%. This mutation was confirmed by sanger sequencing among the proband at the homozygous state. The same variant was found at the heterozygous state among parents and three siblings. Only the elder sister is homozygous for the reference allele. The figure 2 and table 4 show all the results.

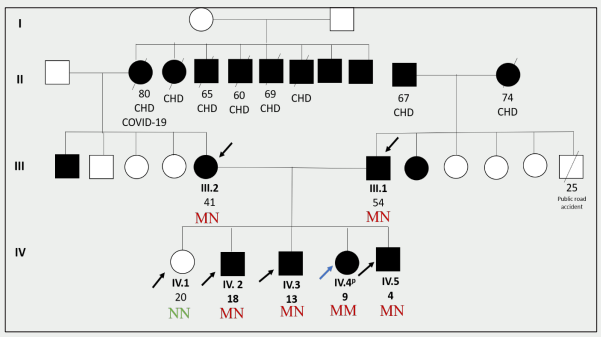

Figure 2. Family Pedigree with variants in LDLR gene associated to FH

Filled black symbols, and empty symbols represent ADH patients and healthy individuals, respectively. The present age of the individuals is shown in black under squares and circles. Genotypes are expressed by homozygous normal allele (NN) in green and heterozygous mutated allele (MN) in red. Oblique lines through symbols represent deceased individuals. The arrows indicate family members, whose DNA samples are available in the present study, the blue one indicates the proband. CHD refers to coronary heart disease.

Table 4. Genetic results among the seven members of the studied family

Family members	Gene & exon	Ref seq	Genetic variant	dbSNP	Genotype
III.1	*LDLR* exon 8	NM_000527.5	c.1186+1G>A	rs730880131	G//A
III.2					G//A
IV.1					G//G
IV.2					G//A
IV.3					G//A
IV.4^{p}					A//A
IV.5					G//A

Discussion

In this study we described the molecular investigation of a family from the South East of Tunisia (Mednine) with a clinical suspicion of FH. We identified a splice site mutation (c.1186+1G>A) located in the exon 8 of the LDLR gene in the index case (IV4p). The proband was homozygous for this variant. However, parents (III.1 and III.2) and siblings (IV.2, IV.3 and IV.5) were heterozygous. Only the eldest sister does not carry the mutation.

Genetic of LDLR worldwide and in Tunisia

Mutations in this gene cause the autosomal dominant disorder of FH. More than 2,000 mutations in this gene have been reported worldwide. These mutations are represented in the database LOVD (Leiden Open Variation database, https://databases.lovd.nl/shared/genes/LDLR). Some of these genetic changes reduce the number of low-density lipoprotein receptors produced within cells. Other mutations disrupt the receptor's ability to remove LDLs from the blood. As a result, people with mutations in the LDLR gene have very high blood cholesterol levels. As the excess cholesterol circulates through the bloodstream, it is deposited abnormally in tissues such as the skin, tendons, and arteries that supply blood to the heart (coronary arteries). A buildup of cholesterol in the walls of coronary arteries greatly increases a person's risk of having a heart attack.

To date, 11 mutations affecting the LDLR gene have been reported as associated with FH in Tunisia. Actually, five substitution mutations have been described: The c.443G>C and the c.796G>A were identified respectively among FH-Kairouan (Central Tunisia) and FH-Tozeur (Southern Tunisia) patients (30). The other three mutations ((c.1027G> T ; c.2446A> T & c.267C> G) have been discovered by Jelassi et al in 2009 (31). Two splice site mutation: the first was identified by Jelassi et al (c.1186+1 G>A) and our study (3). The second mutation (c.1845+1 G>A) described by Jelassi et al in 2008 as the most frequent FH-causing mutation in Tunisia called also FH-Tunis. (31). Added to that, a frameshift mutation (c.2299delA) located in exon 15 of the LDLR gene have been described in one Tunisian family(23). One minor rearrangements variation in exon 10 (1477-1479 delinsAGAGACA) have been identified resulted in a truncated protein. This frameshift mutation was found in two families from central Tunisia and named FH-Souassi. It seems to be a founder mutation in our population associated with a mild phenotype of FH (32). All these mutations have been identified using direct sequencing and enzyme restriction methods. Moreover, two large rearrangements in the LDLR gene have been identified by MLPA analysis among two Tunisian family (22).

In addition, in 2012 mutations in PCSK9 and APOE genes have been reported for the first time to be linked to FH in Tunisia. In fact, the novel c.520C > T variant in the PCSK9 gene seems to reduce the severity of FH, acting as a loss-of-function variant. Slimani et al suggested that genetic variations in the PCSK9 and APOE genes may contribute to the variability in clinical expression seen in Tunisian patients with FH and LDLR gene mutations (23).In opposition to our study , the screening of pathogenic mutations in several genes including PCSK9 and APOE (Table 1) revealed only the LDLR mutation.

LDLR splice site variant: NM_000527.5: c.1186+1G>A

From genetic point of view, the LDL receptor is encoded by the LDLR gene, which is 45 kb long, has 18 exons and

is located on the short arm of chromosome 19p13.1-13.3. This gene has ubiquitous expression, but the liver concentrates 70% of the receptors in the human body (33). This receptor plays a crucial role in the homeostasis of cholesterol. Dysfunction of the activity of this receptor leads to accumulation of LDL in the circulation resulting in FH (34).

At the structural level, the LDLR protein has different domains. Indeed, exon 1 codes for the signal sequence. The region from exons 2 to 6 encodes the ligand docking domain. The sequence from exons 7 to 14 encodes the epidermal growth factor (EGF) homology domain. The exon 15 encodes the glycosylation domain while the exon 16 and the beginning of exon 17 encode the transmembrane domain. Finally, the end of exon 17 and exon 18 encode the cytoplasmic domain (35).

According to the literature , our study is in agreement with Jelassi et al who identified this variant for the first time also in a single southern Tunisian family with FH in 2008 (3). It has not been reported in other populations. This mutation in the splice site does not lead to premature termination, indeed, the deletion of 51 bp preserves the reading frame, and consequently results in a protein with a deletion of 17 amino acids in the EGF homology domain of the LDL receptor (from 380 to 396). This domain is also required to keep the proper conformation needed for the specific binding of ApoB (36). Besides , a recent functional study characterized 13 rare missense variants as associated with FH among the Portuguese population, out of them eight were found in the EGF and classified likely pathogenic or as a variant with uncertain significance (VUS) (37). The study on cultured skin fibroblasts by Jelassi et al, showed that this splice site variant is associated with a decrease in the LDL receptor activity of 10% among homozygous patients compared to control subject (3).

The c.1186+1G>A mutation in the LDLR gene is a pathogenic splice site mutation that leads to improper mRNA splicing and significantly reduced LDL receptor activity. This mutation is associated with familial hypercholesterolemia, characterized by elevated LDL cholesterol levels and an increased risk of cardiovascular disease. Understanding the functional impact of such mutations is crucial for accurate diagnosis and management of FH. This variant has been exclusively described in the Tunisian population and cited in only two studies by Jelassi et al., published in 2008 and 2010 (3) (4). In contrast, a study on the Spanish population identified six LDLR mutations associated with familial hypercholesterolemia (FH), including three affecting donor splice sites of the LDLR mRNA: c.313+2dupT, c.1186+5G>A, and c.1845+1G>C. These splice-site mutations were confirmed to be pathogenic (5)

Near position 1186, a number of mutations that alter intron 8's 5' splice donor location have been described. In fact, Koivisto et al. discovered an uncommon mutation in the Finnish population, a G>A substitution at position 1186 (38) . In one Turkish FH individual, Sözen et al. described a G deletion at position 1186 that resulted in a novel reading frame and the appearance of an early stop codon 30 nucleotides downstream (39). The G>A substitution in the position c.1186+5 was discussed in a French FH condition (40). Damgaard et al. reported the c.1186+10 G>C in one Dannish FH individual (41). One British FH subject reported the c.1186+11 G>A (42). A splicing mutation in intron 7 (c.1061-1G>C) that skips the entirety of exon 8 and linked to partially degraded protein because of the aberrant conformation and weak cell surface expression was described by Yu et al (43).

The identified variant was previously described in seven members of a southern Tunisian family among them only three subjects carried the mutation at the homozygous state. Similarly, to the proband in the present study, homozygous carriers of the LDLR splice site mutation had high level of LDL-c level (12 to 13 mmol/l) and tuberous xanthoma (3). However, our index patient differs from other homozygous cases already reported in the literature by the absence of corneal arcus and CHD (3). Regarding others heterozygotes family members , our results are different from those described by Jelassi et al where one heterozygote carrier of the identified mutation aged of 62 had skin xanthomas, corneal arcus and CHD (44). In contrast to our findings, a recent study in Tunisia reported a 12- year- old girl born to non- consanguineous marriage diagnosed with homozygous FH with arcus cornea and xanthomas (45). Contrary to our study , another study conducted in 56 Tunisian FH patients showed that out of 18 homozygotes subjects , only one patient presented the moderate clinical phenotype without tendon xanthomas at 20 years of age while all other homozygous patients in this study showed tendon xanthomas that appeared before 20 (23).

This discrepancy in the clinical phenotype observed among Tunisians with FH may be explained by the presence of complex interactions with other genes and/ or environmental factors that regulate the circulating lipid levels such as diet and lifestyle habits (32). Indeed, cases of mild phenotype, associated with a mutation in the LDLR gene, have been reported in other population highlighting hence the specific genetic signature of the Tunisian population as admixed population (46). It has been proven in the studied family that the mutant allele (A) cosegregates with the FH phenotype. The quick screening procedure described here using WES and sanger sequencing would also make it possible to identify people who possess this mutation in their families early, allowing for optional patient care to stop the disease's clinical manifestations.

Thanks to advancements in sequencing technology, it is now possible to identify large-scale structural alterations in addition to small mutations (deletions and insertions), taking the example of Maria et al who used the Oxford Nanopore MinION technology to determine the full spectrum of genetic variants in the LDLR gene not only for making the right molecular diagnosis but also for better management of the FH patients (47).

Regarding treatment, the following up of the homozygous index case showed that the combination of rosuvastatin and Ezetimibe with a healthy diet and lifestyle decreased the rate of LDL-c by approximately 50%. Our results are in disagreement with a study conducted in FH Chinese patients showing the effectiveness of rosuvastatin or atorvastatin but not lovastatin, simvastatin, or ezetimibe to meet the LDL-c treatment goal for patients with LDLR

splicing mutation c.1187-2A>G (48). While, Zhang et al results agree in part with our results where heterozygotes family members have shown an improvement in the LDL-c level after treatment with Atorvastatin and the follow up of healthy diet. So, our data provide valuable information for guiding the treatment of Tunisian FH patients. This outcome is consistent with other studies highlighting the importance of personalized treatment plans (49).

The literature reveals a variety of LDLR mutations, including point mutations, nonsense mutations, and splice site mutations, contributing to FH. Splice site mutations, although less common, have been shown to significantly impact LDLR function. For instance, a mutation in intron 14 activates a cryptic splice site, leading to aberrant splicing and impaired LDLR activity (50). Another study identified a splice site mutation that results in a frameshift and premature termination codon, severely decreasing LDLR expression (51). Clinically, these findings underscore the need for early diagnosis and tailored management strategies to prevent long-term cardiovascular complications. They also highlight the potential benefits of establishing a national FH registry and treatment protocol in Tunisia to standardize care and improve outcomes for patients. So, our findings underscore the importance of including non-coding intron splice regions in genetic screening protocols, as they can significantly improve diagnosis for an effective clinical management of FH mutation.

So far, the present study highlighted the presence of the c.1186+1G>A splice site variant for the second time in the south of Tunisia "Mednine". Until today, this variant is reported only in Tunisia. These findings suggest that this could be a founder mutation for three reasons: First, the frequency of heterozygous and homozygous FH is high in this area of south Tunisia, as the result of a founder effect for the FH-Souassi mutation, in agreement with previous reports from other populations with high level of inbreeding (32). Second, although medical records are imperfectly documented, especially in rural areas of Tunisia, it is traditionally known in families that children exhibiting cutaneous xanthomata are prone to sudden premature death. Third, any lifestyle or environmental changes in heterozygous carriers of the mutation could represent a potential risk for cardiovascular disease. Taken into account these arguments, we recommend patients suspected with FH and native of the Mednine region should be screened for this mutation in the LDLR.

Conclusion

This study highlights the presence of the LDLR splice site mutation (c.1186+1G>A) as a likely founder mutation in Southern Tunisia. Our findings demonstrate that whole exome sequencing (WES) is a valuable tool for the diagnosis of FH, enabling the identification of causative mutations and early treatment interventions. For the proband, a combination of rosuvastatin, ezetimibe, and lifestyle modifications led to a significant reduction in LDL-c levels, emphasizing the importance of tailored therapeutic strategies.

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