ORIGINAL ARTICLE

Apolipoprotein B gene mutation related to familial hypercholesterolemia in an Iranian population: With or without hypothyroidism

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Background: Familial hypercholesterolemia (FH) leads to elevated low-density lipoprotein cholesterol (LDL-C) levels in plasma. Mutations of its related gene; apolipoprotein B (APOB) is seen in about two percent of the patient with FH. Thyroid disease is usually part of the exclusion criteria for the detection of FH which alters the lipid profile. We evaluated mutations in the *APOB* gene in patients with high LDL-C levels. **Materials and Methods:** Patients aged between 2 and 80 years with at least one LDL-C level of more than 190 mg/dl were selected (120 patients) from Isfahan Laboratories. Blood samples were obtained from all patients. Genomic DNA was extracted. Primer sequences were designed by Oligo 7.60 to amplify the desired 844 bp region of exon 26 of the *APOB gene* containing *R3500Q* and *R3500W* variants associated with FH. **Results:** Overall, two patients showed a heterozygous form of a common pathogenic variant in exon 26 named c. 10579 C > T (R3500W, cDNA.10707), and one patient was hypothyroidism. We also recognized another nonpathognomonic variant c. 10913G > A (rs1801701, cDNA.11041) in 13 patients, two of them were hypothyroidism. **Conclusion:** This study for the first time shows the coexistence of *APOB* mutation in hypothyroidism, which emphasis screening of patients with hypothyroid for FH detection.

Key words: Apolipoprotein B, hypercholesterolemia, hypothyroidism, Iranian

How to cite this article: Vaseghi G, Malakoutikhah Z, Shafiee Z, Gharipour M, Shariati L, Sadeghian L, *et al.* Apolipoprotein B gene mutation related to familial hypercholesterolemia in an Iranian population: With or without hypothyroidism. J Res Med Sci 2021;26:94.

INTRODUCTION

Familial hypercholesterolemia (FH) is a predominant but common inherited autosomal disorder that is associated with a severe or moderate increase in plasma low-density lipoprotein cholesterol (LDL-C) levels.[1] Heterozygous FH (HeFH) is a common form of the disease and patients usually develop early premature coronary heart disease.[2] Early diagnosis and treatment decrease risk of premature death in such patients.[3]

Access this article online

Quick Response Code:

Website: www.jmsjournal.net

DOI: 10.4103/jrms.JRMS_970_19

It has been proposed that mutations and/or polymorphisms of some genes can increase LDL-C levels.[4] Common genetic mutations in FH are often seen and reported in the following genes: LDL-C receptor (LDLR), apolipoprotein B (APOB) genes, or proportion converts subtilisin/kexin type-9 (PCSK9).[5] Reductions in receptor activities in patients with *LDL R* mutations increase LDL-C plasma levels in FH patients.[6] *PCSK9* reduces the number of hepatic LDLR and therefore increases LDL-C levels.[7]

Mutations of the *APOB* gene, which encodes lipoprotein particles,[5] occur in 2.2% of patients with FH.[8] Three

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Submitted: 21-Jan-2020; **Revised:** 07-Feb-2021; **Accepted:** 24-May-2021; **Published:** 18-Oct-2021

APOB mutations have been described to date: *APOB R3500Q*, *APO B R3531C*, and *APOB R3500W*.[9] Replacement of glutamine with arginine at nucleotide 10708 in exon 26 leads to mutation in *Apo B R3500Q* and decreases the apo B affinity for the LDL receptor.[10] On the other hand, *R3500W* and *R3531C* have been identified in *R3500W* alleles and are found to be associated with diverse haplotypes of chromosomes.[11,12] However, previous studies in Iranian populations failed to report on mutations of the APOB gene.[13-15]

The goal of this study was to identify other potential diseases which could occur concurrently with hypercholesterolemia. Hypothyroidism is the most common thyroid disease and is characterized by elevated levels of thyroid-stimulating hormone (TSH) and reduced thyroid hormone levels. Thyroid hormones regulate metabolic rate, lipogenesis, glucose metabolism, food intake, and heat production. Modulation of all these multiple cellular processes affects body mass index, body weight, and percentage of the adipose tissue.[16] Patients with hypothyroidism showed elevated triglyceride (TG), total cholesterol (TC), and LDL-C levels.[17]

Thyroid disease is usually part of the exclusion criteria for the detection of FH because hypothyroidism is a common health condition which can alter the lipid profile. In the present study, mutations in the *APOB* gene in euthyroid patients and hypothyroidism patients with high levels of LDL have been investigated.

MATERIALS AND METHODS

In this study, 120 patients, males or females aged between 2 and 80 years with minimum LDL-C level >190 mg/dl (LDL-C >150 mg/dl with pharmacological treatments) were selected from Isfahan laboratories and contacted by phone for further evaluation (National Clinical Trial No. 2865694).[18]

We excluded secondary causes of hypercholesterolemia but did not exclude patients who had a history of hypothyroidism. Hypothyroidism was defined as TSH level >4.2 μIU/mL + normal or low FT4.[19] All patients were treated, and their TSH levels were normal at the time of evaluation.

Blood sampling and DNA isolation

Blood samples (3 mL) were obtained from all patients followed by genomic DNA extraction from peripheral blood samples using a High Pure Polymerase Chain Reaction (PCR) Template Preparation Kit (version 20) and the standard salting-out method.[20]

Polymerase *chain reaction*

Primer sequences were designed specifically using Oligo 7.60 software to amplify the 844 bp segment of exon 26 of the *APOB* gene containing *R3500Q* and *R3500W* variants associated with FH. Specificity and affinity of the primers were checked by NCBI primer BLAST.

- Forward primer: ATGGAAGTGTCAGTGGCAAC
- Reverse primer: TGCTGTCTCCTACCAATGCT.

Primers and annealing temperatures

To perform PCR, an Eppendorf gradient type master cycler (Eppendorf, Germany) was used. A volume of 15 μl 2X Master Mix (Biofact®, Korea), 0.8 μl each primer (10 mM), and 1.4 μl genomic DNA were mixed gently, and by adding H_2O, it reached the final volume of 20 μl. The reaction conditions set as follow: Initial denaturation step was carried out at 95°C for 5 min, annealing primers for 30 s at 60.8°C, continued by denaturation 32 cycles each cycle at 95°C for 45 s, and final extension at 72°C for 45 s.

Genotyping

Genotyping was performed using an automated Genetic Analyzer ABI 3130XL (Microsynth, Balgach, Switzerland). Chromatograms were compared with genomic reference sequence, NG_011793.1 using SeqMan software version 5.00© (DNASTAR, Madison, WI, USA).

RESULTS

Genomic DNA was purified, and all the samples showed satisfactory results on agarose gel after PCR step. Demographic and clinical characteristics of the samples are listed in Table 1.

Sequencing results for *APOB* gene

A total of 120 of the 121 samples presented suitable results in molecular studies for inclusion in the analysis. The demographic features and genetic studies of patients are shown in Table 1. Two of the 120 samples showed a heterozygous form of a common pathogenic variant in exon 26 named c. 10579 C > T (R3500W, cDNA.10707) [Figure 1a and b], providing a prevalence of 1.7% for this variant one of them was hypothyroidism. We also recognized another known variant c. 10913G > A (rs1801701, cDNA.11041) in 13 patients [Figure 1c and d]. This variant was shown to be benign in in-silico analysis. Predictions of pathogenicity of identified variants were verified using online software tools [Table 2]. There were no hypothyroid patients in sample without mutations. All the patients with hypothyroidism were on treatment but had at least one previous LDL-C above 190 mg/dl.

DISCUSSION

We report the presence of c. 10579 C > T (*R3500W*), a common pathogenic FH variant in exon 26 in Iran. Another benign variant, c. 10913G > A (rs1801701), was

Table 1: Demographic feature of patients with low-density lipoprotein>190 mg/kg included in this study

Variable	Euthyroid with nonpathognomonic cDNA.11041 mutation (*n*=11)	Hypothyroid with nonpathognomonic cDNA.11041 mutation (*n*=2)	Euthyroid with pathognomonic cDNA.10707 mutation (*n*=1)	Hypothyroid with pathognomonic cDNA.10707 mutation (*n*=1)	Euthyroid without APOB mutation (*n*=105)
Age (mean±SD)	50±4	54±4	60	37	53±3
Gender (female) (%)	36.3	100	100	100	55
Previous LDL-C	198±10	264, 268	219	223	200±14
LDL-C	149±20	150, 140	218	138	159±10
TG	168±8182	162±51	159	122	121
Cholesterol	221±52	206±40	315	224	195±48
HDL-C	37±10	60	44	48	41±7
FBS	98±20	77.5	95	99	103±18
History of premature CVD (%)	16	0	0	0	15
Family history of premature CVD (%)	45.4	0	100	100	55
Diabetes (%)	0	50	0	0	21
Blood pressure (%)	16	50	0	0	25

LDL-C=Low-density lipoprotein cholesterol; SD=Standard deviation; HDL-C=High-density lipoprotein cholesterol; TG=Triglyceride; CVD=Cardiovascular disease; FBS=Fasting blood sugar; APOB=Apolipoprotein B

Table 2: List of variants and their pathogenicity investigation using software prediction tools

Variant / Software	c. 10579 C>T (R3500W)		c. 10913G>A (R3638Q)	
	Score	Prediction	Score	Prediction
SIFT	0	Damaging	1	Tolerated
PROVEAN	−6.30	Deleterious	1.45	Neutral
MutationTaster2	-	Disease causing	-	Polymorphism
Polyphen2.0	1	Probably damaging	0.001	Benign
PANTHER	0.19	Probably benign	0.19	Probably benign
FATHMM	−1.33	Tolerated	6.07	Tolerated

FATHMM=Functional analysis through hidden markov models; PROVEAN=Protein variation effect analyzer

also identified in 13 patients with FH. Previous studies failed to show mutations in Apo B in FH patients.[21] In studies of 130 patients with hypercholesterolemia by Fard-Esfahani *et al*., thirty patients had the criteria for FH; however, the *R3500Q* was not detected in them.[15] Another study in which 16 children with the FH phenotype from five different regions of Iran were screened to detect any possible mutations that may occur in APOB, LDLR, and PCSK9 genes. Of them, 14 had a clinical diagnosis of homozygous FH and 2 were heterozygous FH. They reported no mutations in APOB, but seven different LDLR mutations were identified.[14] The *APOB* gene, which is located in the 2p24.1 region, is used for the molecular diagnosis of FH. The mutation of the *APOB* mutation was discovered in phenotypic FH patients with FH who did not have mutations in *LDLR*.[22]

We identified mutations in two Iranian patients, of which one had a history of hypothyroidism. The patient was receiving treatment with levothyroxine and atorvastatin and her plasma LDL-C was 130 mg/dl. Her fT3 and fT4 levels were normal, indicating that her hypercholesterolemia was not due to hypothyroidism. This finding emphasizes the screening of FH in patients with thyroid diseases. Thyroid hormones modulate the metabolism of glucose and lipid. Hypothyroidism may be due to the decreased 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase activity. Thyroid hormones increase LDL receptors in liver and other tissues, as well as absorption of cholesterol, thereby altering the process of excretion of cholesterol from the intestine by bile acids.

The coexistence of heterozygous FH and hypothyroidism has been reported in previous studies.[23,24] The coexistence of hypothyroidism and heterozygous FH in adolescent girls was estimated to be about 1 in 500,000, which is similar to the prevalence of homozygous FH in patients with *LDL-R* mutations.[25,26]

Another novel finding of our study is of *APOB* gene mutations and polymorphisms in patients with hypothyroidism [Table 1]. Patients with hypothyroidism and high LDL-C levels had greater reductions in LDL-C after treatment, likely because LDLR-independent mechanisms may be involved in reducing serum cholesterol levels, possibly by induction of Cyp7a1 and increased bile acid synthesis.[27]

Limitations

Our study has a small sample size, and we did not perform *LDL-R* and *PCSK9* sequencing. We did not have access to patient's families, we could not screen for the whole *APO B* gene.

CONCLUSION

The present study reported a mutation in the *APO B* gene in patients with high cholesterol level and possible phenotype of FH for the first time. Results showed the importance of

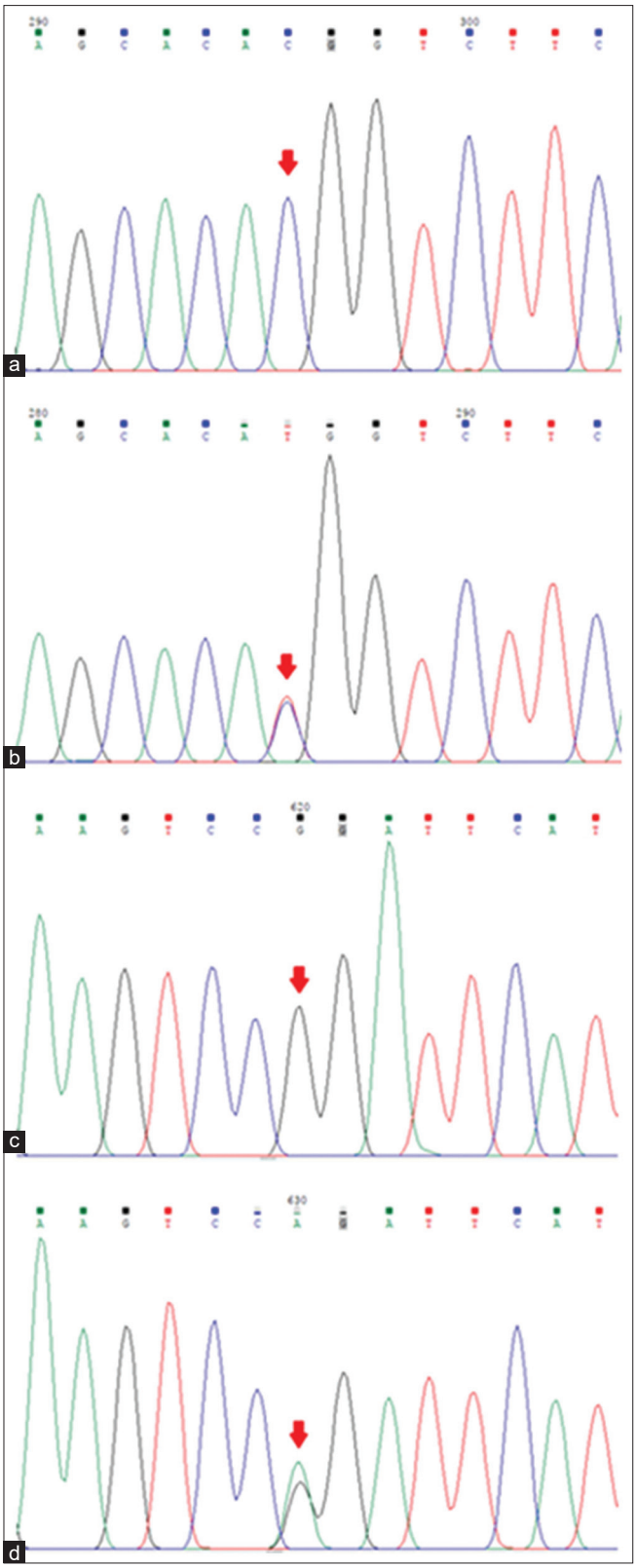

Figure 1: Chromatograms of variants identified in exon 26 of apolipoprotein B gene in patients with familial hypercholestrolemia. (a) Position 10579 of coding DNA (p. R3500W), (b) Position 10579 of coding DNA (p. R3500W), (c) Position 10913 of coding DNA (p. R3638Q) and (d) Position 10913 of coding DNA (p. R3638Q)

including patients with thyroid disease in the FH registry, as these patients may be missed if their LDL-C levels are reduced below 150 mg/kg following thyroid hormone replacement.

Acknowledgments

We gratefully acknowledge from Mahdieh Medical Lab in Isfahan city, for their constant cooperation in referring patients with FH. This project would not have succeeded without the sincere efforts of FH staff, so we would also like to thank the colleagues who were involved in this research project.

Financial support and sponsorship

This work was partly supported by the National Institute for Medical Research Development research grant (No 963548). In addition, this work was partly supported by Behestan Darou Co. and Spadan Heart Health Foundation. The funding agencies have no role in the design of the study. Moreover, they played no role in collection, analysis, and interpretation of the data.

Conflicts of interest

There are no conflicts of interest.

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