

Analysis of the APO B R3500Q Mutation and APOE Polymorphism in Taif Saudi Population using Polymerase Chain Reaction- Reverse Hybridization Technique

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Abstract

This study describes the use of Cardio Vascular Disease (CVD) Strip Assay which based on Polymerase Chain Reaction Reverse Hybridization Technique to study the prevalence of Apo B R3500Q mutation and Apo E genotypes in Taif city Saudi Arabian population. Among 200 unrelated healthy subjects residing in Taif city 5,600 feet above sea level complete absence of Apo B R3500Q mutation was observed. Among six different Apo E genotypes, five genotypes were detected (E3/E3, E4/E4, E2/E3, E2/E4 and E3/E4) with prevalence% (63, 2.5, 8.5, 1 and 25) respectively. The allelic frequencies of Apo E alleles were 0.79 for E3, 0.15 for E4 and 0.048 for E2. Obtained data concluded that, this is the first report about Apo B R3500Q mutation in Saudi Arabia and the absence of it confirmed that this mutation has not reached Saudi Arabia from central Europe which is the common origin of this mutation. Among Apo E genotypes, only E2/E2 genotype was absent while another E2/E3 and E2/E4 genotypes were detected in Taif Saudi population.

Keywords: APO B R3500Q; Mutation; APOE; Polymorphism; Reverse Hybridization; PCR

Introduction

Apolipoprotein B-100 (apo B-100) is a protein molecule consisting of 4560 amino acid residues. It has 500 KD molecular weight and mainly secreted by the liver [1-3]. It is the major protein component of the circulating atherogenic low-density lipoprotein (LDL) particle and play a central role in cholesterol transport by its association to LDL particles as a ligand for LDL receptor [4]. ApoB-100 is encoded by a gene that has been localized to the short arm of chromosome 2. This gene consists of 28 introns and 29 exons. The longest coding sequence within the gene is exon 26. More than one half of the apoB-100 protein molecule is coded by this exon [3,5]. There have been several mutations identified in the apoB-100 gene leading to premature truncation of protein synthesis or to amino acid substitution within the protein [3]. Such changes can influence the metabolism of plasma lipoprotein and may therefore be important in the development of hyperlipidemia and coronary heart disease [3]. One of the most common single site mutations in the human apoB gene, is ApoB-100 R3500Q (apoBR3500Q) mutation, which resulted from a single nucleotide transition, CGG to CAG, in exon 26 at position 10708 of the apoB gene and leads to amino acid substitution of glutamine for arginine at position 3500 [6,7] results in mild to severe hypercholesterolaemia and an increased risk for early onset atherosclerosis [7]. This mutation reduces the affinity to the LDL receptor by at least 95% [8] and is the major cause of familial defective ApoB-100 (FDB). Although it is widely accepted that the average population frequency of the R3500Q mutation is about 1:500-1:700 among white populations [9], other results from population studies, as well as data from studies of high risk groups in Europe, show that frequencies vary ranging between 1:715 and 1:1250 [10]. There is a lack of information about the mutation prevalence in Saudi Arabia population. This is the first study explore this mutation at molecular level among Saudi population.

Apolipoprotein E (apo E) is a structural protein of chylomicrons, very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL) and high-density lipoprotein (HDL) in human plasma [11].

The apo ϵ gene is located at chromosome 19q13.2 and is closely

linked to the apo C-I/C-II gene complex [12]. It consists of four exons and three introns spanning 3,597 nucleotides and produces a 299 amino acid polypeptide [12,13]. It is synthesized primarily in the liver, but other organs and tissues also synthesize apo E, including brain, spleen, kidneys, gonads, adrenals, and macrophages [11].

Apo E plays an important role in the metabolism of cholesterol and triglycerides in human plasma through the uptake of apo E containing lipoprotein by apo B, E (LDL) receptors or by LDL receptor-related protein (LRP) in the liver [11,14-17].

Three common alleles, ϵ 2, ϵ 3 and ϵ 4 at the apo E gene locus code for three protein isoforms, apo E2, E3, and E4, respectively [18]. Six different genotypes are found by different combinations of these three alleles [19]. Accumulating evidence demonstrates that ApoE alleles are associated with both cardiovascular and Alzheimer's diseases [21-25]. The frequency of each APOE isoform varies dramatically with geographical region, race and ethnicity [26]. The aim of the present study was to identify the frequency of (apoBR3500Q) mutation as the first time in Saudi Arabia population and investigate the prevalence of Apolipoprotein E (ApoE) genotypes in Taif Saudi population.

Materials and Methods

Samples collection

Blood samples from 200 unrelated healthy Saudi subjects residing in Taif city 5,600 feet above sea level with no cardiovascular disease

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symptoms were randomly collected into EDTA anticoagulant vacutainer tubes. Verbal consent was obtained from all participants prior to blood samples collection and all institutional requirements were met.

DNA extraction, PCR amplification and reverse hybridization

Analysis of (apoBR3500Q) mutation and Apo E polymorphism was carried out via CVD StripAssay, ViennaLab, Austria (<http://www.viennalab.com>) manufacture protocol. The CVD StripAssay is based on polymerase chain reaction (PCR) and subsequent reverse hybridization. CVD StripAssay includes three steps1) DNA extraction 2) PCR amplification with biotinylated primer pairs and 3) hybridization of amplified products with test strips carrying allele-specific oligonucleotide probes immobilized in parallel lines. Bound biotinylated sequences are detected by streptavidin –alkaline phosphatase and color substrates. Briefly PCR amplifications were carried out in two separate reactions A (amplification mix A) and B (amplification mix B) that differ in primers pairs and 5 µl DNA template for each reaction.PCR reactions A and B were carried out with the same thermal profile as follow. Initial step of 94°C for 2 min., and followed by 35 cycles of 94°C for 15 sec., 58°C for 30 sec. and 72°C for 30 sec, final extension was at 72°C for 3 min. PCR products from reaction A and B were mixed together with hybridization buffer, incubated for 5 min at room temperature and hybridized to the detection test strip. Hybridization was accomplished at 45°C. After series of stringent washes (according to the protocol of provider) the reaction was detected by color development directly on test strip. Results were evaluated from test strips using provided scale included in the kit.

Results

In order to analyze the ApoB R3500Q mutation among 200 subjects included in the present study, CVD StripAssay was utilized. For each subject one of three possible staining patterns may be

obtained as follow;1)Wild type probe only: Normal genotype (GG) ,2) Wild type and mutant probe: Heterozygous genotype(GA) carrier and 3)Mutant type probe only: Homozygous mutant type(AA). Obtained results indicated that, complete absent of R3500Q mutation between studied subjects was observed. According to CVD StripAssay manual, six different APOE genotypes could be detected (E2/ E2, E3/ E3, E4/ E4, E2/ E3, E3/ E4 and E2/ E4). For each individual only one genotype of possible six genotypes will be obtained. Figure 1 demonstrate example for obtained ApoE genotypes during present study.

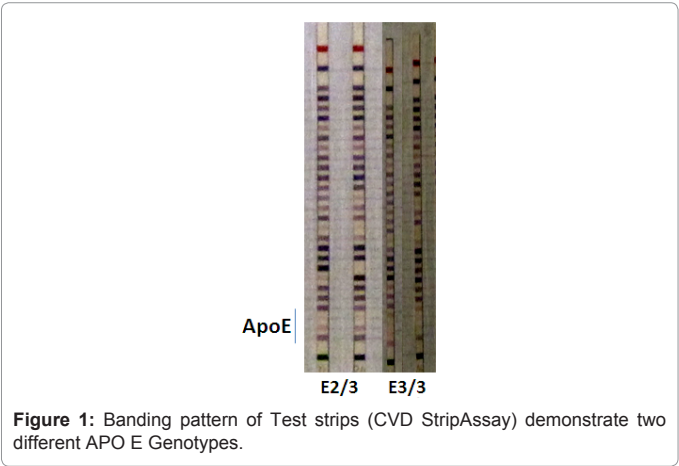
Table (1) illustrates resulted genotypes, number and % prevalence of each genotype in addition allele frequency of three alleles (E2, E3and E4) respectively in Saudi population under this study. Based on obtained staining banding pattern for each test strip of CVD StripAssay, five ApoE genotypes were detected (E3/ E3, E4/ E4, E2/ E3, E3/ E4 and E2/ E4). However homozygous ApoE2/E2 was absent in this studied Saudi population. Among 200 studied individuals, there were 400 ApoE alleles. Out of which 319 (0.79) were E3, 62 (0.156) E4 and 19(0.048) E2. Five genotypes were resulted. The most frequent genotype was E3/ E3 with prevalence % 63%, while, the lowest frequent genotype was E2/ E4 1%. E3/E4, E2/E3 and E4/E4 genotypes were detected and theirs Prevalence was 25%, 8.5% and 2.5% respectively.

Discussion

After association between R3500Q and FDB and association between apoE polymorphism with cardiovascular disease and cardiovascular disease risk factors was reported, several studies worldwide have been carried out to study the prevalence of apoB100R3500Q mutation and apoE genotypes in different populations and their geographical distributions [7,27-30]. Molecular screening of the ApoE alleles and genotypes using DNA amplification was carried out by CVD StrippAssay. Test strips carrying allele-specific oligonucleotide probe, immobilized in parallel lines, allow hybridization of PCR amplification products and immediate recognition of ApoB100 R3500Q mutation as well as ApoE alleles and genotypes that could be detected after a colour development reaction directly on the test strip. For one patient, one strip is needed [31].

The obtained results indicated that complete absence of apoB R3500Q among studied Saudi population. This result is in agreement with the previous reports which indicated the high frequency of the apoB R3500Q mutation are reported to be clustered in central Europe, and the mutation frequency decreases as one moves east, north, and south west [32]. In Germany, UK and USA, prevalence ranges from 1/700 to 1/500 [32,33].The rate of mutation in California was found to be 0.08% [34]. The mutation could not be found in Turkish [11,35-36]; Spain [37]; Israel and Japan [38]; Lebanon [39] and Iranian [40]. The absence of the apoBR3500Q mutation among studied Saudi population also supports the hypothesis of the origin of this mutation which hypothesized that this mutation arose within the Central European region from a common ancestor approximately 7000 years ago and spread across Europe [36,41-42].

In the present study high frequency of E3 allele (79%) was observed and followed by 15.6% for E4 and 4.8% for E2. These results in the same trend with previous results collected from different 12 populations [28,43]. Among the resulted five genotypes E3/E3 was the most prevalent genotype (63%) and the lowest was E2/E4 (1%) while the prevalence of E3/E4, E2/E3 and E4/E4 genotypes were 25%,8.5% and 2.5% respectively. These genotypes prevalence's are concordant with the results among different population such as Nigerians(44); African Americans [45]; American Indian [46]; Caucasians [47]; Germany



Genotype	No. of subjects	Prevalence	Allele Frequency		
			E2	E3	E4
E3/ E3	126	63		252	
E4/ E4	5	2.5			10
E2/ E3	17	8.5	17	17	
E2/ E4	2	1	2		2
E3/ E4	50	25		50	50
Total	200		19 (0.048)	319 (0.79)	62 (0.156)

Table1: Prevalence % of Apo E genotypes and allele frequency.

[48]; Finland [49]; France [50]; Italy [51]; Chinese [52]; Japanese [53] and Swedish [18]. In comparison with previously published Saudi studies, there are three published studies demonstrate apoE genotypes prevalence and allele frequencies with general population over 65 years [54]; with normal individuals [55] and with primary glaucoma Saudi patients [19]. All of these studies in agreement with the present study that E3/E3 genotype was the highest prevalent genotype and E3 was the more frequent allele. On the contrary the last studies reported that E2 allele and its different genotypes were absent while in the present study document the detection of this allele and its genotypes. These differences may be due to geographical distribution gradients, high altitude at Taif city, race and/or ethnicity of studied population. The frequency of each APOE isoform varies dramatically with geographical region, race and ethnicity [26-27,56-57]; In Europe [58] found a clear north to south decline in E4 allele frequency but for E3 they found an inverse south to north decreasing gradient and in China (59) when they concluded that there is a conspicuous south to north, but not an east to west gradient for the apoE4 allele in China. Results of the present study similar with those found in other Arabian country such as Gulf Arab population in Kuwait [26]; Tunisia population (60) and Lebanese [43].

In conclusion, among 200 unrelated healthy subjects residing in Taif city in Kingdom Saudi Arabia (KSA) complete absent of ApoB3500Q was observed. Five genotypes of Apo E (E3/ E3, E4/ E4, E2/ E3, E3/ E4 and E2/ E4) were detected. E3 was the highest frequent allele (0.79) followed by E4 (.156). E2 allele was detected with allele frequency (.048). Further detailed studies are needed to 1) estimate the prevalence of apoB3500Q mutation in different KSA locations 2) assess the correlation between presence of E2 allele and geographical distribution, high altitude and/or race and ethnicity background.

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