International Journal of Biomedical Science & Bioinformatics
Volume 3 : Issue 2 *[ISSN 2475 - 2290]*
Publication Date : 31 August, 2016

Detection and haplotype analysis of defective Apolipoprotein B-100 R3500Q mutation in Familial hypercholesterolemia in Vietnamese patients by AS-PCR (Allele specific PCR)

Truong Kim Phuong, Lao Duc Thuan, Nguyen Hoang Anh Tuan, Le Huyen Ai Thuy*

***Abstract* – Familial defective Apolipoprotein B-100 (Apo B-100) was caused by the R3500Q mutation of the Apo B gene resulting in a glutamine substitution for the arginine residue, consequently, decreased binding of LDL to the LDL receptor. In current study, a total of 40 blood samples were collected from hyperlipidemia, which were confirmed by the concentration of cholesterol over 5.2 mmol/L. AS PCR (allele specific PCR) was carried to analyze the R3500Q mutation, then, confirmed by PCR sequencing. As the results, 27 of 40 (counting for 67.50%) cases were identified being R3500Q mutation. In which, the prevalence of heterozygote and homozygous in this selected population was 25 of 27 (counting for 92.59%), and 2 of 27 (counting for 7.41%), respectively. By PCR sequencing, results were totally according to results of AS PCR analyzation. Giving clearly evidence, two peaks were observed corresponding to two alleles, one allele sequence is G and another is A, that concluded as heterozygote (G→A transition). In the case of homozygote, only one peak corresponding to a sequence allele A. Therefore, our data supported that AS-PCR method that we have applied in current study proved consistent, rapidly, correctly identified R3500Q mutation in Vietnamese population.**

***Keywords* – ApoB, ApoB-100, familial hypercholesterolemia, haplotype R3500Q, Vietnamese population.**

I. Introduction

Familial Hypercholesterolemia (FH; MIM#143890) is a common dominant disorder characterized by elevation of serum cholesterol bound to low density lipoprotein (LDL), which promotes deposition of cholesterol in the skin (xanthelasma), tendons (xanthomas) and coronary arteries (atherosclerosis) [6]. The disorder occurs in two clinical forms: homozygous familial hypercholesterolemia and heterozygous familial hypercholesterolemia [12]. Mutation in genes, such as *LDLR*, *ApoB*, *PCSK9*, counting for 60 – 80%, have to be proved as the risk causes of FH [2, 6, 12]. In current study, we focused on the mutation in *ApoB-100* gene (Apolipoprotein B-100, MIM 107730), one of the two main forms *ApoB-100, ApoB-48* of *ApoB* (Apolipoprotein B) gene, have been considered as the second cause of FH [6]. *ApoB* gene is located on chromosome 2p24.1 that codes for ApoB protein, known as the main Apolipoprotein on chylomicrons, low density lipoprotein (LDLs) and functions as the ligand for LDLR (Low density lipoprotein receptor) [6, 21]. Therefore, mutation in *ApoB-100*, even in case of heterozygous mutation, will drastically effect on its functional activity, subsequently, decreasing its binding activity, so-called familial ligand-defective Apolipoprotein B or type B familial hypercholesterolemia (MIM#144010).

Previous studies indicated that many functional mutations in *ApoB-100* have been identified such as R3500Q [1, 7, 10, 14, 15, 17, 23, 17], R3500W [5, 8, 18], R3531C [16], T3540T [20], T3552T [20], etc. Among them, the first to be described, and the most characterized, is R3500Q mutation. R3500Q was firstly reported by Vega *et al.* (1986) by extensive sequence analysis of 2 alleles of *ApoB* gene in case of moderate hypercholesterolemia. Then, it was found to be heterozygous for familial ligand-defective Apolipoprotein B by Innerarity *et al.* (1987) and Soria *et al.* (1989). They demonstrated that there is a mutation in the codon for amino acid 3500 that results in the substitution of Glutamine (CAG) for Arginine (CGG), named *ApoB* R3500Q (MIM#107730.0009; ClinVar: RCV000019479.28). The mutations all occur in Arginine codons and result in an Apo B-100 molecule that exhibits defective binding to the LDL receptor, leading to impaired uptake of LDL into the cell and consequently, hypercholesterolemia (Henderson *et al.*, 1997). According to Rauh *et al.* (1992), *ApoB* R3500Q had been found to be approximately 1:500 to 1:700 in North American and European population. However, they pointed out that mutation has also not been found in Finland [22], Japan [4], etc. However, notably, there are very limited data regarding *ApoB* gene mutations in Vietnamese population. Therefore, this current study was aimed to characterize the ApoB-100 mutation and its associated haplotype in subjects with hypercholesterolemia from Vietnamese population by AS-PCR (Allele –Specific PCR) assay.

II. Materials and methods

A. *Samples collection, DNA extraction*

A total of 40 blood samples were collected from hyperlipidemic patients, which cholesterol concentration was over 5.2 mmol/L (ranged from 5.33 to 17.46 mmol/L) without tendon xanthomas, retrieved from Xuyen A Hospital and Thu Duc hospital, Vietnam. The procedures followed were in accordance with the current revision of the Helsinki Declaration of 1975.

DNA was extracted from clinical sample by means of an enzyme digestion using 700 µl lysis buffer (NaCl 5M,

Truong Kim Phuong, Lao Duc Thuan, Le Huyen Ai Thuy
Faculty of Biotechnology
Ho Chi Minh City Open University
Ho Chi Minh City, Vietnam

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Tris-HCl 1M, EDTA 0.5M, SDS 10% and Proteinase K 1 mg/ml). The samples were incubated at 56°C overnight. Then, DNA obtained and purified by Phenol/Chloroform extraction and ethanol precipitation. The quality and purity of DNA extraction was measured by the proportion of A_{260}/A_{280}. Then, the DNA solution was stored at EDTA 0.5M, -20°C for further used.

B. *Detection of R3500Q in clinical samples*

R3500Q detection was carried out by AS-PCR within two sets of primers (Table I). The procedure of detection was according to follow stages: (1) PCR was carried out within set primer of APOB-F and APOB-R; (2) Then, the PCR product of stage 1 was continuously amplified by set primer of APOB-F and APOB-RM (for allelic mutation detected) or APOB-F and APOB-RW (for wide type allele detected). For PCR assay, the amplification was done in a total volume of 15 µl, containing 1 µg DNA template. PCR reaction was subjected to initial at 95°C for 5 minutes, followed by 35 cycles at 95°C for 30 seconds, 54°C for 30 seconds, 72°C for 30 seconds, and finally 72°C for 10 minutes. Each PCR product was directly loaded onto a 2.0% agarose gel, stained with Ethidium bromide, and directly visualized under UV illumination. Then, PCR product was sequenced to confirm target gene and mutation analysis.

TABLE I. PRIMERS' SEQUENCES USED IN R3500Q DETECTION

Primer	Sequence (5'-3')	P
APOB-F	GACCACAAGCTTAGCTTGG	334
APOB-R	GGGTGGCTTTGCTTGTATG	
APOB-RM	TGCAGCTTCACTGAAGACT	167
APOB-RW	TGCAGCTTCACTGAAGACC	167

C. *Results and discussion*

Initially, total DNA of all samples were extracted by phenol/chloroform method, then, measured by the proportion of A_{260}/A_{280}. As the result, the ratio of A_{260}/A_{280} ranged from 1.8 to 2.0 (Data not shown), indicated pure DNA preparations were done. Firstly, the amplification was carried out by using the APOB-F and APOB-R. PCR products, 334 bp, were observed by electrophoresis in distinctly different sizes and easily identified (Fig. 1).

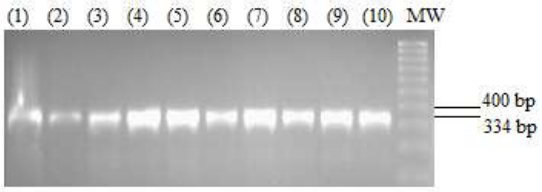

Figure 1. Agarose gel electrophoresis showing the presence of PCR product amplified by APOB-F and APOB-R. *(1) → (10): Clinical blood samples.*

Then, the PCR product was 10-fold diluted, then, 5 µl DNA was enrolled into R3500Q detection by PCR assay within APOB-F and APOB-RM (for allelic mutation detected) or APOB-F and APOB-RW (for wide type allele detected). As the result, only one 167-bp length band was easily identified and observed (Fig. 2). According to Fig. 2, there were two bands observed, thus, it could be concluded that sample 1, 2, 3 and 4 were heterozygous allelic mutation.

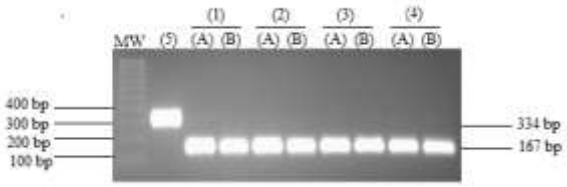

Figure 2. Agarose gel electrophoresis showing the presence of PCR product amplified by (A) APOB-F and APOB-RM; (B) APOB-F and APOB-RW. *(1) (2) (3) (4): Clinical blood samples.*

In current report, we pointed out the 344-bp length DNA sequencing of representative samples, which amplified by APOB-F and APOB-R, for analyzed allelic mutation detected by AS-PCR. The homozygous and heterozygous allelic mutation were shown in Fig. 3 and Fig. 4, respectively. According Fig. 3 and Fig. 4, the signal of peaks in PCR product sequencing was quite good for reading nucleotide. Based on the DNA sequences, there was only one clear peak on nucleotide 117 (arrowed, Fig. 3), indicated homozygous allelic mutation, and double clear peaks on nucleotide 121 (arrowed, Fig. 4), indicated heterozygous allelic mutation or haplotype defective Apolipoprotein B-100. Thus, based on these results, it could be included that, AS-PCR was successful in detection of R3500Q Apolipoprotein B-100, additionally, homozygous and heterozygous were easily and rapidly distinguished.

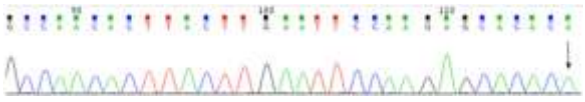

Figure 3. DNA sequencing of clinical sample 22, indicated homozygous allelic mutation (arrowed)

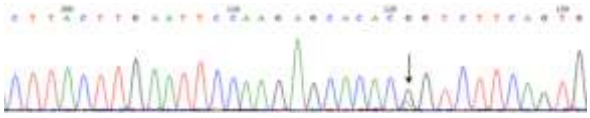

Figure 4. DNA sequencing of clinical sample 4, indicated heterozygous allelic mutation (arrowed)

In current initial study, total of 40 blood samples were enrolled into R3500Q detection by AS-PCR. The frequency of R3500Q was 68.0% (27 of 40 cases), compared to other previous researches, the frequency of R3500Q was higher. Giving examples, it was widely accepted that the average population frequency was ranged from 1:700 to 1:500 among white population [11], from 3% to 5% in most countries in Europe [3], and not be found in Russia [9], Turkey [19]. It was expected to the geographic distribution of R3500Q mutation, and this mutation could be a significant characteristic to Vietnamese population. Moreover, in 27 samples, which was positive to R3500Q mutation, the frequency of heterozygous was 92.59% (25 of 27 cases), only 7.41% (2 of 27 cases). In heterozygous familial hypercholesterolemia, an individual inherits a mutation for FH from one parent. Therefore, heterozygous carriers, indicated familial ligand-defective Apolipoprotein B, towards higher in Vietnamese population. In further study, amount of samples will be increased to be tested to reveal

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the contribution of this mutation in causing familial ligand-defective Apolipoprotein B in Vietnamese population, therefore, applied to early diagnosis and prognosis of this disease.

III. Conclusion

In summary, 27 of 40, counting for 68.0% familial ligand-defective Apolipoprotein B blood samples were detected within R3500Q mutation by AS-PCR method. In Vietnamese population, this R3500Q mutation is linked to the higher frequency of haplotype, which reached to 92.59% (25 of 27 R3500Q mutation samples), than other countries. It could be inferred that, the hypothesis of heterozygous familial ligand-defective Apolipoprotein B is the significant characteristic in Vietnamese population, further study, amount of samples will be expanded and screened of R3500Q to support our hypothesis. Notably, our finding supported that AS-PCR was consistent, easy, correct and rapid in identification of R3500Q mutation. Therefore, this method will be easily in clinical application for screening and detection of R3500Q, revealed to familial ligand-defective Apolipoprotein B, in Vietnamese population.

Acknowledge

We would like to thank all the individuals assistance in Xuyen A Hospital and Thu Duc hospital, Vietnam. This study was supported by Ho Chi Minh City Open University fund, Vietnam.

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About author (s):

Le Huyen Ai Thuy, PhD, Associate Professor.
Research field: Molecular oncology, Cancer epigenetics, etc.

Truong Kim Phuong, MSc, PhD student
Research field: Molecular of Cancer, Cancer epigenetics, etc.

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