FAMILIAL DEFECTIVE APOLIPORROTEIN B 100: FREQUENCY OF R3500Q MUTATION OF APOLIPOROTEIN B GENE IN IRANIAN HYPERCHOLESTEROLEMIC PATIENTS

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Abstract: Familial defective apolipoprotein (apo) B 100 (FDB) causes early-onset coronary heart diseases (CHD). It is produced by R3500Q mutation of the apoB gene resulting in decreased binding of LDL to LDL receptor. We screened the apo B gene for R3500Q mutation in 130 hypercholesterolemic patients, among whom 30 patients met criteria of familial hypercholesterolemia (FH). The prevalence of R3500Q allele in this patient population was 0%. To obtain better estimation of mutation frequency, a broad survey is needed.

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INTRODUCTION

Familial defective apolipoprotein (apo) B 100 (FDB), together with familial hypercholesterolemia (FH), belong to the type II/a primary hyperlipidemia group based on Fredrickson's classification (1). FDB is an autosomal dominant trait resulting in hypercholesterolemia (2). Clinical manifestations of FDB are explained by plasma accumulation of low density lipoproteins (LDL) bearing defective apoB100. This change lowers the protein affinity for LDL receptor (LDLR) which is responsible for 80% of its clearance from plasma. The consequences are hypercholesterolemia, tendinous xanthomata and premature atherosclerosis, which cause early onset of cardio- and cerebrovascular disease and early death (3-5).

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FDB is caused by mutation in apoB 100 gene which results in an amino acid change in receptor binding domain of apoB 100 protein and reduction of affinity to its ligand, LDLR. The apoB gene is localized to chromosome 2p24. It is composed of 29 exons and 28 introns. Four mutations in exon 26 are described to cause FDB. The most common mutation is G10699A, which results in substitution of Arg for Gln (R3500Q) (6). The LDL particles from carriers of the R3500Q mutation posses only 32% of the average binding affinity for the LDLR in cultured fibroblasts (7).

FDB is one of the few genetic problems which can be treated phenotypically by lipid lowering drugs and diet regimens, and thus its early diagnosis and treatment is greatly valuable. Treatment prevents atherosclerotic plaque formation or increase in their size (8, 9). Indeed atherosclerotic plaque formation begins early in childhood and intervention should be considered as soon as possible after diagnosis.

Similar to FH, the clinical diagnosis of FDB is based on Simon Broome criteria: total cholesterol (TC) > 7.5 mmol/L (290 mg/dl), with either

xanthomata in a first or second degree relative, family history of myocardial infarction under age 60 in a first degree relative, family history of myocardial infarction under age 50 in a second degree relative, or family history of TC > 7.5 mmol/L (290 mg/dl) in a first or second degree relative. In fact it is very difficult to distinguish between FH and FDB by clinical appearance, as the phenotype of the two diseases is very similar (10).

The definite diagnosis of FDB is based on detection of mutation in apoB gene. As the most prevalent mutation is R3500Q, a relatively simple genetic diagnosis method can be selected. The relevant techniques will be accessible in developing countries. In addition, the test will be done usually once in the life which reduces the expenses. Also after finding a patient, FDB can be sought easily in proband's relatives.

MATERIALS AND METHODS

Patient Selection

A total of 130 adult patients were evaluated at Labafi-Nejad and Shariati Hospital (Shahid-Bheshti and Tehran University of Medical Sciences, respectively) based on the following laboratory criteria: TC > 260 mg/dl and FBS and TG in normal range; 30 patients met Simon Broome criteria for clinical diagnosis of FH.

DNA preparation and PCR amplification

Whole blood samples were collected in EDTA tubes and genomic DNA were extracted by GenomicPrep Blood DNA Isolation Kit (Amersham Biosciences). The apoB R3500Q mutation was examined by allele-specific PCR technique using primers introduced before, UOL: 5' GAC CAC AAG CTT AGC TTG G 3', LOL: 5' GGG TGG CTT TGC TTG TAT G 3' and ASO: 5' TGC AGC TTC ACT GAA GAC T 3' (11). PCR conditions consisted of 2 mM $MgCl_2$, 96^o C 5 min × 1, (96^o C 1 min, 54^o C 1 min, 72^o C 1 min) × 30, and 72^o C 5 min. The 25 microliter reactions contained the 3 above mentioned primers. PCR amplified products were separated on 2.5% agarose gel containing 0.5 micrograms Ethidium Bromide (Amersham Biosciences) in each milliliter.

RESULTS

A region of 335 bp in exon 26 of the apoB gene was amplified; simultaneously a region of 167 bp allele specific PCR took place if the sample carried R3500Q mutation (Fig.1). None of the 130 samples showed additional 168 bp bands. Even in the group of 30 patients clinically diagnosed as FH, no R3500Q mutation was found.

DISCUSSION

In 1998 Soria *et al.* announced the main genetic cause of FDB as a change of the codon CGG triplet to CAG in apoB gene (12). Since then several researches have been done to find prevalence of the R3500Q in different populations. The frequency of heterozygous FDB is estimated as 1/200 in Central Europe (*e.g.* Switzerland), decreasing gradually in Mediterranean or Northern European populations. In Germany, UK and USA, prevalence ranges from 1/700 to 1/500 (13, 14).

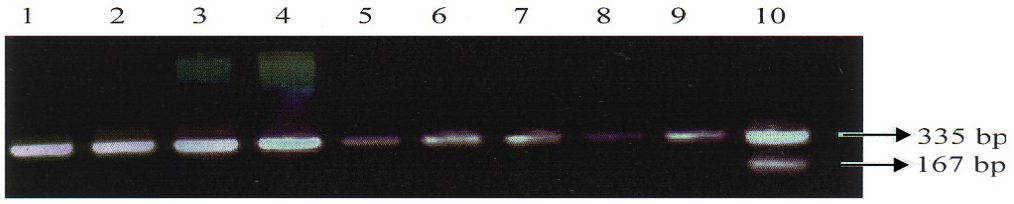

Fig. 1. Allele-specific PCR for detection of R3500Q mutation in apoB gene. Lane 1-9, samples without mutation. Lane 10, individual heterozygous for known R3500Q mutation.

The rate of mutation in California was found to be 0.08% (15). The mutation could not be found in Israel or Japan (16). So far there has been no registered data for frequency of R3500Q mutation in Iran. In our present study, not finding any mutation may be due to its low frequency rate, or small number of patients engaged in the survey. On the other hand, some patients with FDB may show moderately elevated or even normal total cholesterol level due to elimination of VLDL and IDL (precursors of LDL) by LDL receptor via apolipoprotein E (17). To evaluate this, we recommend a broad study to find a more exact estimation of R3500Q mutation rate in Iran.

The method mentioned in this study is fast, cheap and convenient; it may be very valuable for screening the hypercholesterolemic patients as after finding a FDB patient, seeking other involved relatives, including their children is simple and subsequent therapy may extend patients' life expectancy for 10 to 30 years.

In conclusion, this method is sensitive, fast, convenient, and relatively cheap. Considering many patients carrying this mutation die in their productive ages, using this method as a screening test in hypercholesterolemic patients may be cost effective.

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