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Common polymorphisms of ATP binding cassette transporter A1, including a functional promoter polymorphism, associated with plasma high density lipoprotein cholesterol levels in Turks

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Abstract

The role of high levels of high density lipoprotein cholesterol (HDL-C) in protection against development of atherosclerosis is generally attributed to its role in reverse cholesterol transport, and the ATP binding cassette transporter A1 (ABCA1) is a key element of this process. We examined polymorphisms in ABCA1 in Turks, a population characterized by very low HDL-C levels. We discovered 36 variations in ABCA1 and genotyped informative polymorphisms in over 2300 subjects. The rare alleles of C–14T and V771M polymorphisms were associated with higher HDL-C levels in men and, in combination with the rare alleles of R219K and I883M, respectively, with higher HDL-C in both sexes. Rare alleles of the C–14T and V771M polymorphisms were more frequent in the high HDL-C ($\geq$40 mg/dl) than in the low HDL-C group ($\leq$30 mg/dl) in men ($P < 0.05$). Moreover, the T allele of C–14T had more in vitro transcriptional activity than the C allele (20–88%), depending on the cell line ($P < 0.05$), suggesting its functionality. Haplotype construction and haplotype association with phenotype were performed in the promoter and coding region of ABCA1 separately. Analysis of the promoter haplotype block supported the association with the C–14T polymorphism. The C–14T and R219K polymorphisms were on different haplotype blocks. Analysis of the coding region structure revealed that the rare M allele of V771M was distributed predominantly among three common haplotypes, but the sum of their frequencies comprise only two-thirds of the frequency of the M allele. The rare alleles of the V771M and the I883M polymorphisms do not exist together on any of the common haplotypes. In conclusion, we describe a functional promoter polymorphism (C–14T) and a coding sequence variant (V771M) of ABCA1 and their interactions with two other variants (R219K and I883M) on plasma HDL-C levels in Turks.

Keywords: ATP binding cassette transporter A1; HDL cholesterol; Triglyceride; Turkish population; Polymorphism

1. Introduction

Atherosclerotic cardiovascular disease is a leading cause of death worldwide [1], and a low level of high density lipoprotein cholesterol (HDL-C) is a major independent risk factor for atherosclerosis [2,3]. The protective role of HDL-C is generally attributed to its participation in reverse cholesterol transport, a process in which excess cholesterol is transported from peripheral cells to HDL particles for delivery to the liver and excretion. The ATP binding cassette transporter A1 (ABCA1) participates in apolipoprotein-mediated efflux of cholesterol and phospholipid from peripheral cells, especially macrophages, that is crucial for the initial step of reverse cholesterol transport. The identification of mutations in the ABCA1 gene in patients with Tangier disease, who have very low HDL-C, elevated triglyceride levels, and increased risk of premature coronary atherosclerosis, suggested a major role for ABCA1 in regulating plasma HDL-C levels [4–8]. Some common polymorphisms of ABCA1, including R219K

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[9] and I883M [9–11], are associated with elevated HDL-C levels, although not in all studies [10,12–17]. Interestingly, common polymorphisms of ABCA1 may significantly alter the severity of atherosclerosis, apparently without influencing plasma lipid levels [12,13,15,18,19]. Recently, it was shown that some polymorphisms of ABCA1 were associated with increases (V771M and V825I) or decreases (R1587K) in HDL-C in women and with some consistent trends in men in a large random Danish population [17].

Haplotype analysis of the ABCA1 gene with respect to plasma HDL-C levels [20,21] and plasma apolipoprotein (apo) AI levels and myocardial infarction [19] have been reported. Haplotype analysis revealed that ABCA1 accounted for about 10% of HDL-C variation [21], but no haplotype effect on apoAI variability or on the risk of myocardial infarction was detected [21]. Certain haplotypes were more frequent among coronary artery disease (CAD) patients than controls in the Malay population, but not in the Chinese and Indian populations [20].

Although cardiovascular risk factor profiles and the frequency of coronary events differ by gender, the mechanisms for the differences remain to be resolved. Gender differences were observed in other lipid-related association studies [22–27], suggesting that gender-related mechanisms or factors might interact differently with the variants of a particular gene. This phenomenon was also observed with ABCA1. In one study, where males and females were analyzed separately, R219K and I883M were associated with elevated HDL-C levels in females only [9].

We hypothesized that polymorphisms in ABCA1 are important for determining plasma lipid levels and that gender may modulate the role of these polymorphisms. To test this hypothesis, we studied male and female subjects from the Turkish Heart Study [28], a large, random epidemiological survey of the Turkish population. The main characteristic of this population is a very low level of plasma HDL-C, making this an ideal population in which to study genes that influence HDL-C levels [28–31]. We screened the promoter region and the exons and exon/intron splice junctions of ABCA1 with denaturing high-performance liquid chromatography (dHPLC) to detect polymorphisms, and subsequently analyzed their associations with plasma lipid levels.

2. Methods

2.1. Study population

The study population ($n = 2700$) was randomly selected from the Turkish Heart Study database of more than 9000 volunteers from six regions of Turkey [28]. Detailed biodata were obtained from each participant. The protocol was approved by the Committee on Human Research of the University of California, San Francisco, and was in accordance with the Helsinki Declaration. Subjects who were taking any lipid-lowering medication or had a history of diabetes mellitus were excluded.

2.2. Biochemical analyses

Blood samples were obtained after an overnight fast. Total cholesterol and triglyceride levels were determined by enzymatic colorimetric methods, and the HDL-C levels were determined with the CHOD-PAP method with precipitation of very low density lipoproteins and low density lipoproteins (LDL) [28]. LDL cholesterol (LDL-C) was calculated by the Friedewald formula [32] for participants with triglyceride levels <400 mg/dl. Plasma total apoAI levels were measured with Hydragel ApoAI kits (Sebia, Norcross, GA, USA) in a subset of the study population [33].

2.3. Detection of polymorphisms by dHPLC

DNA was screened to identify variations in the ABCA1 gene among subjects whose HDL-C levels were in the lowest and highest fifth percentiles. These DNAs were randomly plated and screened ($n = 95$–240). Primers were designed to amplify the ABCA1 promoter, the 5′ untranslated region, and all exons, including intron/exon splicing boundaries if possible. The amplified DNA was denatured and slowly reannealed to form homo- and heteroduplex DNA. Subjects who were heterozygous in any region on the amplified product formed heteroduplex DNA. The amplified DNA (10–15 μl) was loaded onto the dHPLC apparatus (WAVE DNA fragment analysis system, Transgenomic, Omaha, NE) and run under conditions determined by the WAVE software for dHPLC for the given DNA sequence. Representative genomic DNA samples that displayed heterozygous profiles were sequenced to confirm the mutation or polymorphism. DNA sequences were aligned and analyzed with Sequencher DNA analysis software (Gene Codes, Ann Arbor, MI, USA). Because not every heterozygous profile was sequenced, it is possible that some single nucleotide polymorphisms were not discovered using this method. No other method was used to detect polymorphisms in this study.

2.4. Genotyping

After polymerase chain reaction amplification, each polymorphism was genotyped by restriction fragment length polymorphism or allele-specific oligonucleotide hybridization [34]. The conditions of all assays are described in Supplemental Table I. The accuracy of the genotyping was evaluated by randomly inserting duplicate DNA samples in the assays (~6% replication). Genotyping discrepancies were found in less than 1% of the samples and were resolved by rescoring or eliminating the data.

2.5. Cloning the ABCA1 promoter into a reporter vector

Although multiple transcriptional start sites have been suggested for ABCA1, the base numbering used in this study is relative to the transcriptional start in the published sequence by Santamarina-Fojo et al. (AF275948) [35].