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Common genetic variation in the ATP-binding cassette transporter A1, plasma lipids, and risk of coronary heart disease☆

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Abstract

The ATP-binding cassette transporter A-1 (ABCA1) regulates cholesterol efflux from cells and is involved in high-density lipoprotein (HDL) metabolism and atherogenesis. We investigated whether common *ABCA1* variants, previously reported to have phenotypic effects in humans, were associated with plasma lipids and CHD in a prospective study of coronary heart disease (CHD) in healthy women.

Three polymorphisms in the promoter region (−565C/T, −191G/C, and −17C/G) and two in the coding region (I883M and R1587K) were genotyped in the Nurses' Health Study. During 8 years of follow-up, 249 incident cases of CHD were identified and matched to controls (1:2) on age and smoking.

The I883M variant was associated with higher HDL-cholesterol levels among younger women. Nearly complete linkage disequilibrium was observed between −565C/T and −191G/C and their less common alleles predicted a lower risk of CHD (odds ratio of CHD per −191C allele: 0.8; 95% CI, 0.6–1.0). Neither the −17C/G SNP nor the 2 the coding polymorphisms were associated with risk of CHD. The −565C/T and the −191G/C variants were inversely associated with risk of CHD among healthy women, without pronounced effects on plasma lipids.

Keywords: Reverse cholesterol transport; Genetic epidemiology; ATP-binding cassette transporter A1; Coronary heart disease; Plasma lipids

1. Introduction

Concentration of high-density lipoprotein cholesterol (HDL-C) is inversely associated with risk of coronary heart disease (CHD) [1]. An important mechanism underlying the anti-atherogenic properties of HDL-C is its role in reverse cholesterol transport, the pathway that facilitates the transfer of cholesterol from peripheral tissues back to the liver [2]. The ATP-binding cassette transporter A1 (ABCA1) is a large trans-membrane protein that mediates the cellular efflux of cholesterol and phospholipids to lipid poor HDL apolipoproteins [3]. Homozygosity for mutations in *ABCA1* causes Tangier disease, a rare disorder characterised by HDL-C deficiency and increased susceptibility for atherosclerosis [4]. The gene encoding ABCA1 encompasses 50 exons and more than 100 mutations and single nucleotide polymorphisms (SNPs) have been identified [5].

The relation of more common genetic variants in *ABCA1* with lipid concentrations or risk of CHD remains less clear. Common polymorphisms in *ABCA1* have been inconsistently

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related to plasma lipids or cardiovascular disease [6–16]. In one screening study, 26 SNPs in the promoter and coding region were identified and subsequently genotyped in cases and controls of myocardial infarction [7]. Only the R1587K and the −565C/T promoter variant were found associated with apolipoprotein AI levels, the R219K SNP was associated with risk of myocardial infarction, and none of the 16 identified promoter SNPs were significantly associated with risk [7]. However, this study did not include two common promoter variants (−191G/C and −17C/G) that may influence risk of recurrent cardiovascular events [12]. As the majority of studies that have included a clinical CHD endpoint have been carried out in populations with existing cardiovascular disease [8,9,12–15], the prospective associations of known, common variants in *ABCA1* on plasma lipid concentrations and risk of CHD in generally healthy populations remains unclear.

Furthermore, interactions of environmental factors with *ABCA1* polymorphisms have not been thoroughly explored. For example, an interaction with cigarette smoking has been reported for some *ABCA1* variants [7,13], but most studies have not addressed the potential interaction between *ABCA1* polymorphisms and other factors that are known to influence HDL-levels and risk of CHD, such as age, overweight, alcohol, smoking, postmenopausal hormone use, and dietary fat intake.

The objective of our study was to investigate common SNPs in the coding and promoter regions of *ABCA1* (MAF > 10%) that have been inconsistently related to cardiovascular disease endpoints in previous studies. In total, three polymorphisms in the promoter: −565C/T (also referred to as −477C/T) [13,14], −191G/C (also known as C1176G) [17], and −17C/G (G1355C) [17], and two polymorphisms in the coding region: I883M in exon 18 (A2589G and I823M) [7,9], and R1587K in exon 35 [7] were genotyped in two well-characterized populations of female health professionals; the Nurses Health Studies I and II. We also sought to examine interactions with potentially important environmental exposures.

2. Material and methods

2.1. Study design and population

The Nurses' Health Studies I and II (NHSI and NHSII) are prospective cohort studies among U.S. registered female nurses, respectively, involving 121,700 participants 30–55 years of age at baseline in 1976 and 116,671 participants between 25 and 42 years old at baseline in 1989. The women have received follow-up questionnaires biennially to update information on health and disease and information about diet is obtained through a food frequency questionnaire every 4 years [18]. The validity and reproducibility of the collected data have been reported previously [19]. Between 1989 and 1990, a blood sample was requested from all active participants in NHSI and collected from 32,826 women. Similarly, blood samples were obtained between 1996 and 1998 from 29,614 participants in NHSII. Women who gave blood were similar to those in the overall cohorts.

Among NHSI participants who provided blood samples and who were without cardiovascular disease or cancer in 1990, we performed a nested case–control study of CHD risk. Study physicians blinded to participants' exposure status confirmed nonfatal myocardial infarction if it met World Health Organization criteria (symptoms and either diagnostic electrocardiographic changes or elevated cardiac enzymes). Fatal CHD was confirmed by hospital records or on autopsy, or if CHD was the underlying and most plausible cause, and if evidence of previous CHD was available. We confirmed 212 women with incident nonfatal myocardial infarction (MI) and 37 with fatal CHD between blood draw and June 30, 1998. We randomly selected controls in a 2:1 ratio using risk-set sampling [20], and matching on age, smoking, fasting status, and month of blood return.

In cross-sectional analyses of the NHSII, blood samples were selected from premenopausal women who collected their blood during the luteal phase of their menstrual cycle and who were not using exogenous hormones. We randomly selected 473 participants free of cardiovascular disease, diabetes, gastrointestinal illness, or malignancy within strata of different patterns of self-reported alcohol consumption (the original selection of this subset was to investigate alcohol drinking patterns and novel biomarkers of CHD) [21,22].

2.2. Information on genetic variants, plasma lipids and markers of inflammation

Details on methods for genotyping and measurement of biochemical markers have been submitted as an online supplement (see Supplement I). Plasma lipids available in both study populations included total cholesterol, HDL-C, low-density lipoprotein cholesterol (LDL-C), and triglycerides. Measured markers of inflammation included: interleukin-6, C-reactive protein, soluble tumor necrosis factor-α receptors-1 and -2, and fibrinogen.

Primers and probes used were designed by Applied Biosystems: −565C/T (rs2422493), −191G/C (rs1800976), −17C/G (rs2740483), I883M (rs4149313), and R1587K (rs2230808). Replicate quality control samples were included and genotyped with 100% concordance. A few subjects could not be genotyped with this platform; genotype data were available for 465 women from NHSII and 745 women from NHSI (249 cases and 496 controls).

2.3. Statistical analysis

Allele frequencies were estimated and departure from Hardy-Weinberg equilibrium was tested among NHSI-controls and the NHSII participants separately.

We first evaluated *ABCA1* and plasma lipids. The associations between each *ABCA1* polymorphism and plasma