Association of ATP-Binding Cassette Transporter A1 (ABCA1)-565 C/T Gene Polymorphism with Hypoalphalipoproteinemia and Serum Lipids, IL-6 and CRP Levels

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Abstract

Background: ATP-binding cassette transporter A1 (ABCA1) is a membrane integral protein which plays a vital role in High Density Lipoprotein (HDL) metabolism and exerts a protective effect against Hypoalphalipoproteinemia (HA) by mediation of rate-limiting step in HDL biogenesis. In addition, this protein possesses anti-inflammatory effects by inhibiting the production of some inflammatory cytokines in macrophages. This study investigated the association of ABCA1-565 C/T gene polymorphism with HA and serum lipids, IL-6 and CRP levels.

Methods: A population which consisted of 101 HA and 95 normal subjects were genotyped for ABCA1-565C/T polymorphism by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP). The serum concentrations of lipids, IL-6 and high sensitive-CRP (hs-CRP) were measured by the relevant methods.

Results: The frequency of T allele was significantly higher in the HA group than the controls (31.7 vs. 19.5%, p=0.002). Thus, carriers of the T allele (CT and TT genotypes) had a higher risk for HA (p=0.016, OR=2.04, 95% CI=1.14-3.63). T allele carriers demonstrated decreased HDL-C and increased triglyceride, IL-6 and CRP levels than those with the CC genotype.

Conclusion: This study suggests that the-565 C/T polymorphism of ABCA1 gene is associated with an increased risk of HA, decreased HDL-C and increased TG, IL-6 and CRP.

Keywords: -565 C/T polymorphism, ABCA1, CRP, Hypoalphalipoproteinemia; IL-6; Lipids

Introduction

Hypoalphalipoproteinemia (HA) is a dyslipidemia which is characterized by HDL-C concentration below 1.04 mmol/L (40 mg/dL) 1. High Density Lipoprotein (HDL) is a multifunctional lipoprotein particle which plays a vital role in the metabolism of lipids and protection against a number of diseases 2,3 by I) removing excess cholesterol from tissues and transporting them to liver for elimination; II) protecting the endothelial cell function; III) inhibiting the LDL oxidation and platelet aggregation and IV) participating in lipid metabolism by transferring the lipase cofactor and receptor. HA is associated with an increased risk of Cardiovascular Disease (CVD), type 2 diabetes mellitus, dementia, lung cancer and lymphoma 4-6. Its occurrence may be due to environmental factors and lifestyle (e.g. smoking, medications, overweight, physical inactivity) as well as genetic defects 6.

The most severe form of HA is the Tangier Disease (TD), a disorder which is characterized by near zero level of HDL. Homozygosity for mutations in ABCA1 gene leads to TD 3. ABCA1 gene is located in the q31 region of chromosome 9 and consists of 50 exons that encode a 2261 amino acid integral membrane protein 7. ABCA1 mediates the rate-limiting step in HDL biogenesis by transporting cellular excess free cholesterol and phospholipids to an apolipoprotein acceptor 8. It is broadly expressed in macrophages, hepatocytes, intestinal cells, lung cells, adrenal gland and placental trophoblast 9. This expression is highly induced by sterols, through nuclear Liver X Receptor (LXR)/Retinoid X Receptor (RXR) pathway 10. The accumulation of sterol-rich lipoproteins, togeth-
er with their oxidation in the artery wall, results in the recruitment of macrophages and the production of inflammatory molecules. A study by Tang et al indicated that the macrophage ABCA1, in addition to cholesterol efflux, functioned as an anti-inflammatory receptor. The interaction of apolipoprotein (apo) A-I with ABCA1-expressing cells stimulates autophosphorylation (activation) of Janus kinase2 (JAK2) by an ABCA1-dependent mechanism. Activated JAK2 has two independent effects: I) it enhances the apo A-I binding activity of ABCA1 which is essential for lipid export; II) Phosphorylation and activation of Signal Transducer and Activator of Transcription3 (STAT3). The activated STAT3 has anti-inflammatory function in macrophages. The phosphorylated STAT3 migrates to the nucleus and regulates the transcription of proteins which repress the production of inflammatory cytokines IL-1β, IL-6, and TNF-α. Regarding the increase of the mentioned inflammatory cytokines, especially IL-6 and to a lesser degree TNF-α and IL-1β, the liver and aortic endothelial cells produces C-Reactive Protein (CRP) 7,8.

Although the TD is rare, common Single Nucleotide Polymorphisms (SNPs) in the ABCA1 gene are present in the general population 9. Consequently, much attention has been paid to the relationship between ABCA1 gene polymorphisms with different phenotypes, including lipid variables and clinical endpoints. One of the common SNPs is -565C/T (rs2422493), which serves as a cytosine to thymine substitution in the promoter region of the ABCA1 gene, which affects expression, resulting in the formation of promoter low-activity (C/T, T/T) and high-activity (C/C) genotypes. The previous association studies on this polymorphism were inconsistent. As HA is the most common dyslipidemia in Iranian population, and moreover there exists no data yet on the association of the ABCA1 gene polymorphisms with serum inflammatory markers, the present study investigated the association of ABCA1-565 C/T polymorphism with HA and serum lipids, IL-6 and CRP levels in an Iranian population.

Materials and Methods

Subjects

This research was approved by the Ethics Committee of Babol University of Medical Sciences and the researches undertook Helsinki treaty. Participants in the study were selected among a large number of individuals who referred to the laboratories of Babol city hospitals from January 2013 to August 2014. HA subjects were selected among the individuals with HDL-C <40 mg/dL and the control group among the individuals with HDL-C ≥40 mg/dL who were accepted to participate in the study. Written, informed consent was obtained from the volunteers. Their personal data including age, residence, past medical history, drugs, cigarette and alcohol consumption were collected through an interview. Anthropometric characteristics of the included individuals were measured and the Body Mass Index (BMI) was calculated for each individual (weight in kilograms/height in meters squared). Individuals with a BMI > 30, consumers of cigarettes, alcohol and those with diabetes, rheumatic fever, tuberculosis, infectious diseases accompanied by fever, diseases of liver and thyroid, pregnant and patients treated with lipid-lowering agents, anti-inflammatory and anti-cancer drugs were excluded from the study. The remaining 343 subjects were referred to the Cardiology department of Rouhani Hospital (Babol, Iran). Eventually, 196 subjects enrolled in the study and based on HDL-C levels, were divided into two groups: HA (HDL-C <40 mg/dL) and normal (HDL-C ≥40 mg/dL).

Sample collection

3 ml of blood sample was taken from each subject after overnight fasting. 1.5 ml of blood was transferred to a tube containing Ethylene Diamine Tetra Acetic acid (EDTA) for DNA extraction and 1.5 ml was transferred to a tube without any anticoagulant agent to obtain serum for biochemical analysis.

Biochemical analysis

Lipid profile analysis: The serum total cholesterol, LDL-cholesterol (LDL-C) and triglycerides were measured by enzymatic colorimetric methods. HDL-C was determined after precipitation of apoB-containing lipoproteins with dextran sulfate and magnesium. All measurements were done using Pars Azmun diagnostic kits (Pars Azmun, Iran).

Inflammatory factors analysis: CRP was determined by turbidimetric immunoassay method with auto-analyzer (Hitachi 902, Japan), using hs-CRP diagnostic kit (Pars Azmun, Iran). IL-6 level was determined using enzyme-linked immunosorbent assay (ELISA) method (Pelikine Compact human IL-6 ELISA kit, CLB, Amsterdam, the Netherlands).

Genotyping of ABCA1-565 C/T polymorphism analysis: Genomic DNA was extracted from peripheral blood leukocytes using salting out method. Genotyping was accomplished using PCR-RFLP technique. For amplification of fragment containing-565 C/T polymorphism, a pair of forward primer 5'-CTCGGTTCC TCTGAGGGACCT-3/ and reverse primer 5 /-CCGCA TCTGAGGGACCT-3/ (CinnaGen Co, Iran) was used. PCR reaction mixture, having a final volume of 50 μl, contained 2 μl DNA, 2 μl (10 μM) of each primers, 4 μl (2.5 mM) of dNTPs, 3 μl (25 mM) of MgCl2, 5 μl of 10x buffer, 2.5 U of Taq DNA polymerase (Fermentas, Burlington, USA) and the remaining was DDW. The reaction mixture was denatured at 95°C for 2 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 60 s and extension at 72°C for 60 s, with a final extension at 72°C for 10 min. After amplification, aliquots of the PCR products...
were digested with 2U AciI restriction enzyme (Thermo scientific, USA) at 37°C overnight. Separation of the fragments obtained after digestion was done using 12% Polyacrylamide Gel Electrophoresis (PAGE) for 3 hr, under constant voltage (200 V). The gel was stained with silver nitrate and the bands were visualized. The AciI restriction enzyme has the ability to recognize the (C/CGC) sites. After digestion of 351 bp fragment, three possible genotypes were distinguished: homozygous CC (148, 130 and 73 bp), heterozygous CT (278, 148, 130 and 73 bp), and homozygous TT (278 and 73 bp).

**Statistical analysis**

Data were analyzed statistically using the SPSS 18 software (SPSS Inc., Chicago, IL, USA), and were expressed as mean±standard deviation (M±SD). hs-CRP and IL-6 concentrations were natural log transformed to improve normality. An independent T-test was used for comparison of the demographic and biochemical variables between the two groups of HA and control. Allele frequencies were calculated by the gene counting method and differences in the frequency distributions of the alleles and genotypes between HA and control groups were evaluated by Chi-square ($\chi^2$) test. Hardy-Weinberg equilibrium (HWE) for both of the groups with the HWE was assessed by the $\chi^2$ test. Multivariate logistic regression was performed to analyze the relationship between -565 C/T polymorphism and HA and control groups was assessed by the $\chi^2$ test. Multivariate logistic regression was performed to analyze the relationship between -565 C/T polymorphism and HA and control groups were evaluated by Chi-square ($\chi^2$) test. Hardy-Weinberg equilibrium (HWE) for both of the cases and control groups was assessed by the $\chi^2$ test.

**Results**

Table 1 presents the demographic and biochemical data of HA and control groups. There existed a significant difference in total cholesterol, triglycerides, HDL-C, LDL-C, IL-6 and hs-CRP between the two groups in sex, age and BMI ($p>0.05$). However, there were no significant differences between the two groups in sex, age and BMI ($p>0.05$). The frequency distribution of the alleles and genotypes in ABCA1-565 C/T polymorphism between HA and control groups, are shown in table 2.

There was consistency in the frequency distribution of -565 C/T polymorphism in the HA and control groups with the HWE. It also showed that the frequency of T allele was significantly higher among the HA group in comparison with the controls. In addition, the results illustrated that the distribution of genotypes between the two groups was significantly different.

A multivariate logistic regression was performed to evaluate the association of -565 C/T polymorphism with HA. When the CC genotype was used as a reference, the TT and CT genotypes were both significantly associated with HA risk. So that, a significant association was observed for T allele carrier genotypes when compared with CC genotype for HA risk (Table 3).

The analysis of biochemical parameters, based on genotypes, demonstrated that the T allele carriers exhibited significantly lower HDL-C levels and significantly higher TG, IL-6 and hs-CRP levels than those with the CC genotype ($p<0.05$), while the total cholesterol and LDL-C were not significantly different among genotypes ($p>0.05$, Table 4).

**Discussion**

The results of the current study showed an association between -565 C/T polymorphism and the risk of HA. Based on the results, individuals carrying the T allele were more susceptible to HA.

Previous studies investigated the effect of -565 C/T polymorphism on HA-related diseases, such as various types of CVD. The results of these studies have been
The differences between means were analyzed by multivariate ANOVA and adjusted for age, sex and BMI. * p<0.05.

controversial. Lutacuta et al showed that the presence of the T allele in -565 C/T locus was associated with the severity of the atherosclerosis in patients with established Coronary Artery Disease (CAD) 18. Benton et al in a Multi-Ethnic Study of Atherosclerosis (MESA) reported a borderline association between the presence of T allele and higher prevalence of the coronary artery calcification 19. In contrast, Jensen et al reported an inverse relationship between the -565 C/T variant with the risk of Coronary Heart Disease (CHD) among healthy women 21.

Of course, there existed no association in a number of studies. For example, in a study of 316 Tunisian patients who underwent coronary angiography, according to Rejeb et al there was no significant association between -565 C/T polymorphism with the stenosis risk 17. In addition, Takagi et al found no relationship between -565 C/T with myocardial infarction or angina pectoris in Japanese patients 22.

The reason for these conflicting results obtained in various studies is still unclear. Racial difference of different populations might be one of the major reasons for disputes. In addition, the presence or absence of an observed association in any race, ethnicity or geographic populations may be related to a number of other factors, such as gene-gene interactions and environmental factors.

Also, there have been contradictory results regarding the association between -565 C/T variant and lipid profile. While some investigators did not find any significant association between the polymorphism and lipid profile 7,14,18, Liu et al found a significant decrease of HDL-C concentrations in TT genotype compared to those with the CC genotype 23. In the present study, the assessment of lipid profile based on genotype showed that T allele carriers had significantly lower HDL-C and higher TG levels than those with the CC genotype.

In conclusion, this study suggests that the ABCA1-565 C/T polymorphism is associated with an increased risk of HA, decreased HDL-C and increased TG, IL-6 and CRP. Due to the association of HA with a number of diseases, the identification of genetic risk factors associated with HA is an opportunity to identify individuals that are susceptible to this disorder, and also for pre-emptive measures to be taken in order to prevent HA, and consequently the related diseases. On the other hand, an evaluation of the ABCA1 variants association with the inflammatory factors can lead to
the recognition of the agent involved in progression of inflammation. Hence, further study with larger sample sizes is recommended in order to confirm the findings of this study.

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References


