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Relationship between the G75A polymorphism in the apolipoprotein A1 (*ApoA1*) gene and the lipid regulatory effects of pravastatin in patients with hyperlipidemia

T.N. Liu[1], C.T. Wu[1], F. He[1], W. Yuan[1], S.X. Li[1], H.W. Li[1], H.Y. Yu[1] and M. Wu[2]

[1]Cardiovascular Division,
Affiliated Hospital of the North China University of Technology,
Tangshan, Hebei, China
[2]The People's Hospital of Tangshan, Tangshan, China

Corresponding author: M. Wu
E-mail: wuman_l@163.com

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ABSTRACT. In this study, we investigated the relationship between the G75A polymorphism in the apolipoprotein A1 (*ApoA1*) gene and the lipid regulatory effect of pravastatin in patients with hyperlipidemia. A total of 179 patients were divided into two groups: the pravastatin (N = 97) and policosanol (N = 82) treatment groups. The total cholesterol (TC), triglyceride, low-density lipoprotein (LDL-c), high-density lipoprotein, ApoA, and ApoB concentrations in the serum were measured using an automatic biochemical analyzer before and after treatment for 12 weeks. The genotypes of the *ApoA1* G75A SNP were detected by polymerase chain reaction-restriction fragment length polymorphism, and were subsequently statistically analyzed. Pravastatin treatment induced a significant decrease in the TC, LDL-c, and ApoB levels in patients expressing the *ApoA1* AA+GA genotype ($P < 0.05$), and not in those expressing the GG genotype ($P > 0.05$). However, policosanol

treatment induced a non-significant decrease in the serum TC levels ($P > 0.05$) and a significant decrease in the ApoB levels ($P < 0.05$), and did not induce a decrease in the LDL-c ($P > 0.05$) levels in patients with the AA+GA genotype. Policosanol also induced a significant decrease in the TC and LDL-c levels in patients with the GG genotype ($P < 0.05$). The various genotypes of the *ApoA1* G75A SNP influence the efficacy of lipid regulation by pravastatin and policosanol in patients with hyperlipidemia.

Key words: Hyperlipidemia; Apolipoprotein A1; Total cholesterol; Pravastatin; Policosanol; Gene polymorphism

INTRODUCTION

Hyperlipidemia (HLP) is a common crinose metabolic disease; it is a lipid abnormality wherein the levels of total cholesterol (TC), triglycerides (TG), low density lipoprotein cholesterol (LDL-c), and apolipoprotein B (ApoB) are abnormally high, while those of high density lipoprotein- cholesterol (HDL-c) and apolipoprotein A1 (ApoA1) are significantly low (Marsh and Drabkin, 1960). Moreover, HLP is the primary cause of death in coronary heart disease patients and an important risk factor for chronic congestive heart failure, cerebral infarction, and senile dementia. Statins are the first drugs developed to efficiently and safely treat lipid abnormalities, decrease cardio-cerebrovascular disease, and reduce coronary death rate (Fan et al., 2010); therefore, these drugs have been extensively studied worldwide.

3-Hydroxy-3-methylglutaryl-coenzyme A (HMG-coA) reductase is the key rate-limiting enzyme of cholesterol synthesis and metabolism. Statins can reduce the synthesis of cholesterol in the liver, as well as decrease the level of cholesterol in the blood, via competitive inhibition (Cannioto, 2008). Moreover, statins can be used to reduce the LDL-c and TG levels, and increase the HDL-c levels, in the body (Nordøy et al., 2001).

APO A1, C3, and A4 are key players in the metabolism of major plasma lipoproteins and are potential candidates for the plasma modulation of LDL particle size. Defects or variations in these lipoproteins are also associated with altered concentrations of lipids and lipoproteins in the plasma (Gomez et al., 2010).

A common G-to-A substitution in the promoter area (-75 base pairs) of *APOA1* has been extensively studied. The expression of the minor A allele, which occurs at a frequency of 0.15-0.20 in Caucasian populations, has been associated with increased promoter activity *in vitro* and higher plasma HDL-c or ApoA1 levels (Jeenah et al., 1990; Pagani et al., 1990). Other studies, however, yielded conflicting results (Matsunaga et al., 1995). These inconsistencies could be the result of interactions between environmental factors that modulate the effect of this genetic polymorphism.

Based on the available evidence, we hypothesized that genetic variations affecting the LDL particle size could be influenced by dietary fat content. Therefore, in this study, we attempted to identify the possible association between a G75A single nucleotide polymorphism (SNP) in *APOA1* and the lipid regulatory effect on pravastatin in patients with hyperlipidemia.

MATERIAL AND METHODS

Subjects and sample collection

A total of 179 subjects were recruited to this study from the in- and out-patient medical wards of the Affiliated Hospital of the North China University of Technology between January 2014 and September 2015. Informed consent was obtained from all recruited subjects. The subjects were divided into the pravastatin (N = 97) and policosanol (N = 82) groups. The study design was approved by the Ethics Committee of the hospital. The detailed medical history of all cases and controls was obtained, with special emphasis on the risk factors of cardiovascular disease; this was followed by a thorough clinical examination. Patients were treated with 10 mg pravastatin or policosanol at the same time every day for 12 weeks. Venous blood was collected from the antecubital vein of all patients after overnight fasting under sterile conditions.

Methods

Reagents and instruments

The total blood DNA extraction kit was obtained from Tiangen Biotechnology (Shanghai, China). Taq DNA polymerase, 10X PCR mix, and restriction endonuclease were obtained from Thermo Fisher Scientific (Waltham, MA, USA). A Beckman Lx20 automatic biochemical analyzer was obtained from Beckman Coulter (Brea, CA, USA).

Sample preparation

Blood samples were obtained from all patients after an overnight fast. Venous blood (5 mL) was drawn from all subjects and stored in Vacutainer tubes containing an anticoagulant (EDTA). The blood samples were centrifuged at 3000 *g* for 10 min, in order to separate the serum for biochemical detections. The buffy coat and red blood cell pellet were used for DNA extraction using the standard kit.

Genotyping

DNA was extracted and the purity was measured by determining the ratio of absorbance at 260 nm to that at 280 nm (A_{260}/A_{280}). Only DNA samples with a purity range of 1.5 to 1.8 was used for polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP) amplification.

Genomic DNA was amplified using primers specific for *APOA*: forward primer: 5'-AGG GAC AGA GCT GAT CCT TGA ACT CTT AAG-3', and reverse primer: 5'-TTA GGG GAC ACC TAC CCG TCA GGA AGA GCA-3'. The reaction conditions were set as follows: initial denaturation at 96°C for 6 min; 30 cycles of denaturation at 94°C for 1 min, annealing at 58°C for 1 min, extension at 72°C for 30 s; and a final extension at 72°C for 7 min. PCR products were separated on a 4% agarose gel by electrophoresis, and the bands visualized with ethidium bromide.

The 433-bp amplified fragment was digested using the restriction enzyme *Msp*I The fragments were analyzed using 2-2.5% agarose gels.

Biochemical analysis

Serum blood glucose, triglycerides, total cholesterol, low-density lipoprotein cholesterol (LDL-c), and high-density lipoprotein cholesterol (HDL-c) levels were measured by the standard enzymatic method using the automatic biochemical analyzer.

Statistical analysis

All data was analyzed using the SPSS v.20.0 software platform (IBM, Armonk, NY, USA). SNP data was evaluated for conformance with the Hardy-Weinberg equilibrium. Data are reported as means ± standard deviation (means ± SD). The data was analyzed using the chi-square test and Fisher's exact test. Logistic regression analysis was used to compare gene polymorphism frequencies between groups. The Student *t*-test was used to compare the different serum lipid levels. A P value < 0.05 was considered statistically significant.

RESULTS

Analysis of the G75A polymorphism in *ApoA1*

The frequency of distribution of the various genotypes, AA, GA, and GG, of the G75A SNP in *ApoA1* was 26/179 (14.53%), 71/179 (39.66%) and 82/179 (45.81%), respectively. The allele frequencies of G and A were 34.36% and 65.64%, respectively. The genotype and allele distributions were in accordance with the Hardy-Weinberg equilibrium ($P > 0.05$).

Association between the G75A SNP in the *ApoA1* and serum lipoprotein levels

The general medical status and various indices did not differ significantly among the study subjects, allowing for better comparability.

The TC levels differed significantly in patients with the AA+GA genotype before and after treatment with pravastatin (for 12 weeks) ($P < 0.05$), while no significant differences were observed in patients with the GG genotype pre- and post-treatment ($P > 0.05$). On the other hand, treatment with policosanol for 12 weeks induced a significant difference in the TC levels in patients with the GG genotype ($P < 0.05$), as opposed to those with the AA+GA genotype ($P > 0.05$). Pravastatin treatment also induced a significant difference in the ApoB level in patients with the AA+GA genotype ($P < 0.05$), and not in those exhibiting the GG genotype ($P > 0.05$). Alternately, policosanol treatment induced a significant difference in the ApoB levels in patients with the GG genotype ($P < 0.05$), and not in those expressing the AA+GA genotype ($P > 0.05$). None of the other indices was significantly different between patients with the AA+GA and GG genotype before and after pravastatin and policosanol treatment (Tables 1 and 2).

Table 1. Lipoprotein levels in patients with the AA+GA and GG genotypes of the *ApoA1* G75A polymorphism before and after pravastatin treatment (means ± SD, mM).

Genotype		N	TC	TG	HDL-C	LDL-C	ApoA	ApoB
AA+GA	Pre-treatment	47	6.65 ± 0.96	1.86 ± 0.85	1.43 ± 0.24	4.32 ± 1.14	1.47 ± 0.45	1.36 ± 0.25
	Post-treatment	47	6.05 ± 1.03	1.76 ± 0.81	1.32 ± 0.29	3.89 ± 1.08	1.57 ± 0.32	1.17 ± 0.31
	P value		0.002*	0.612	0.087	0.001*	0.446	0.014*
GG	Pre-treatment	50	6.57 ± 0.74	1.86 ± 0.77	1.38 ± 0.29	4.42 ± 0.83	1.35 ± 0.24	1.34 ± 0.21
	Post-treatment	50	6.18 ± 0.76	1.79 ± 0.71	1.36 ± 0.32	3.92 ± 0.71	1.48 ± 0.31	1.23 ± 0.32
	P value		0.082	0.605	0.812	0.018*	0.087	0.215

TC = total cholesterol; TG = triglyceride; HDL-c = high density lipoprotein cholesterol; LDL-c = low density lipoprotein cholesterol; ApoA = apolipoprotein A; ApoB = apolipoprotein B. *P < 0.05 indicates a significant difference.

Table 2. Biochemical analyses of patients with the AA+GA and GG genotypes of the *ApoA1* G75A polymorphism pre- and post-policosanol treatment (means ± SD, mM).

Genotype		N	TC	TG	HDL-C	LDL-C	ApoA	ApoB
AA+GA	Pre-treatment	35	6.61 ± 0.86	1.46 ± 0.82	1.63 ± 0.54	4.31 ± 0.72	1.57 ± 0.35	1.33 ± 0.23
	Post-treatment	35	6.33 ± 1.31	1.73 ± 0.87	1.57 ± 0.28	3.92 ± 1.21	1.63 ± 0.42	1.06 ± 0.34
	P value		0.246	0.267	0.491	0.072	0.572	0.004*
GG	Pre-treatment	47	6.53 ± 0.54	1.63 ± 0.67	1.54 ± 0.31	4.12 ± 0.85	1.48 ± 0.24	1.21 ± 0.19
	Post-treatment	47	6.13 ± 0.73	1.83 ± 1.07	1.55 ± 0.32	3.57 ± 0.73	1.62 ± 0.30	1.14 ± 0.28
	P value		0.003*	0.362	0.862	0.000*	0.079	0.384

TC = total cholesterol; TG = triglyceride; HDL-c = high density lipoprotein cholesterol; LDL-c = low density lipoprotein cholesterol; ApoA = apolipoprotein A; ApoB = apolipoprotein B. *P < 0.05 indicates a statistically significant difference.

DISCUSSION

The HMG-coA reductase inhibitor (Statins) might effectively decrease the LDL, TC, and TG levels, and simultaneously upregulate HDL production. Statins are structurally similar to HMG-coA reductase; therefore, they can be used to inhibit the activity of HMG-coA reductase, thereby downregulating cholesterol synthesis (Vaughan and Delanty, 1999). Statins have therefore seen wide clinical application; however, the efficacy and reactivity of statins are dependent on the individuals themselves.

Jeenah et al. (1990) reported that patients expressing the A allele of the G75A SNP in *ApoA1* showed significantly higher serum ApoA1 and HDL-c levels compared to those expressing the G allele. On the contrary, Smith et al. (1992) reported that the G/A mutation did not affect the serum ApoA1 and HDL levels. This was validated by Lahoz et al. (2003), who reported that pravastatin therapy for 16 weeks induced a 4.9% increase in the HDL-c levels in patients with the GG genotype, and no significant changes in those expressing the A allele.

In this study, we observed that patients with the AA+GA and GG genotypes displayed different reactivity to pravastatin treatment; for example, AA+GA patients displayed a significant decrease in the serum TC levels, compared to patients with the GG genotype. Policosanol treatment, on the other hand, induced the opposite effect in these patients. This suggested that ApoA1 influenced the pravastatin reactivity of patients with hyperlipidemia, and that this mechanism might be different from that of policosanol. We also observed that the ApoB reactivity of patients with the AA+GA genotype of *ApoA1* G75A to pravastatin, as well as policosanol, was much higher (significant decrease in ApoB levels) than that of patients with the GG genotype. Stancu and Sima (2001) reported that statins could inhibit ApoB-100 synthesis by inducing a decrease in the TC, LDL-c, and TG levels as well as a decrease in

the TG levels. McCarty (2002) suggested that policosanol induces a decrease in the HMG-CoA synthesis, similar to pravastatin; additionally, policosanol has been reported to inhibit cholesterol synthesis by controlling the consumption of acetate (Gámez et al., 2001).

The prevention of hyperlipidemia is the primary factor responsible for the decrease in the risk of cardio-cerebrovascular disease, including coronary heart disease and stroke. Changes in blood lipid levels are directly correlated with the interaction between multiple genes and factors. Therefore, the combined effect of statins, multiple genes and genomic sites, and the environment on the blood lipid levels, and its mechanism of action must be elucidated in the future for therapeutic and prognostic purposes.

Conflicts of interest

The authors declare no conflict of interest.

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