

Original Article

Relationship between genetic polymorphisms related to HMG-CoA reductase inhibitors and lipid-lowering efficacy of statins

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Abstract: Objective: This study aimed to analyze the distribution of SLC01B1 and APOE gene polymorphisms in Asian population in Shenzhen and its clinical significance to statin therapy. Methods: A total of 3006 human peripheral blood samples were collected. The SLC01B1 and ApoE genes in the whole blood genome were detected and analyzed by PCR-fluorescence probe technique, and the polymorphism distribution was analyzed. Results: The frequency of SLC01B1 388A>G gene was 75.27%, the frequency of SLC01B1 521T>C gene was 12.38%, and the percentages of *1b/*1b (34.5%) and *1a/*1b (32.24%) in haplotypes were the highest, accounting for 66.7%, followed by *1b/*15 (19.05%), 15/*15 (2.03%), 1a/*15 (4.59%), 1a/*1a (4.66%) and *1a/*15 (0.40%). Of the six phenotypes of APOE genes, ε3/ε3 accounted for 76.08%, followed by ε3/ε4 (11.48%), ε2/ε3 (8.42%), ε2/ε4 (2.79%), and ε4/ε4 (1.03%). Among them, ε3 was the highest in frequency (86.06%), followed by ε4 (8.17%) and ε2 (5.81%). In addition, through the case investigation, it was found that the subjects for SLC01B 521 (CC) had no history of related adverse reactions. Conclusion: SLC01B1 and APOE gene polymorphisms in Asian population in Shenzhen were analyzed in this study, providing an important reference for personalized medications of statin in Shenzhen.

Keywords: HMG-CoA, genetic polymorphisms, Shenzhen, APOE

Introduction

The effect of lipid metabolism is a severe risk factor for cardiovascular diseases, and dyslipidemia is mainly manifested as hyperlipidemia, which can be further divided into high- and low-density lipoprotein, hypertriglyceridemia, mixed hyperlipoproteinemia and hypercholesterolemia according to the difference of specific indices [1]. Statins (also known as HMG-CoA reductase inhibitors) are the most widely used medications to lower lipid levels clinically, which have significant inhibitory effects on cardiovascular diseases by regulating lipid metabolism. Musculotoxicity, hepatotoxicity and gastrointestinal reaction are relatively common side effects of these drugs, accounting for about 1-5% according to statistics [2, 3].

Currently, empirical studies reveal that SLC01B1 gene mutation can lead to changes in the

optimal blood concentration of statins and elevate the risk of side effects. It regulates cell sodium uptake by encoding SLC01B1 carriers, and 388A>G (rs2306283) and 521T>C (rs4149056) are the two common gene polymorphism types [4]. Serum apolipoprotein E (ApoE) is an important factor affecting the blood lipid levels of human body. A study has indicated that ApoE polymorphism is related to cardiovascular diseases, Alzheimer's disease and other diseases [5]. ApoE polymorphism is related to the therapeutic effects of statins, and is also an important indicator of evaluated SLC01B1 and ApoE genotypes of different ethnic groups [6-10]. Shenzhen is located between Guangzhou and Hong Kong, and there are no reports of SLC01B1 and ApoE gene polymorphisms. Single nucleotide polymorphism (SNP) provides an ideal typing model for complex gene detection. Similar to those in western countries, statins are widely used in Asia to

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treat cardiovascular diseases and ischemic strokes. The incidence rate of coronary heart disease (CHD) in Asian population is lower than that in Caucasians, while the incidence rate of stroke in Asians is higher.

In this study, SLC01B1 and APOE gene polymorphism sites of Asian population in Shenzhen were analyzed simultaneously using polymerase chain reaction (PCR)-fluorescence probe method, and the distribution of SLC01B1 and APOE gene polymorphism in Shenzhen was analyzed based on the molecular level, providing a theoretical basis for prevention and individualized treatment of cardiovascular and cerebrovascular diseases in Shenzhen.

Materials and methods

Study subjects

A total of 3006 samples of EDTA-K anticoagulated venous blood were collected from the subjects who underwent SLC01B1 and ApoE gene polymorphism detection respectively in the clinic from May 2017 to June 2019, and the polymorphic loci of SLC01B1 and ApoE genes were analyzed using multiplex PCR-probe method. All the subjects were Asians who had lived in Shenzhen for a long time. All the subjects signed and provided written informed consent. The present study had been approved by the ethics committee of our hospital. A total of 1826 males and 1180 females were enrolled in this study, ranging from 10 to 87 years old, with an average age of 65.95 ± 11.73 years for males and 67.60 ± 11.57 years for females.

Blood lipid level measurement

3 ml of venous blood sample was drawn from the subjects, and the plasma was separated and stored at -80°C . Plasma triglyceride (TG), total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL), apolipoprotein A1 (Apo-A1) and apolipoprotein B (Apo-B) levels were determined by the physical and chemical analysis.

DNA extraction

2 ml of blood sample was collected. Ethylenediaminetetraacetic acid dipotassium anticoagulant was adopted. Whole blood genomic nucleic acid extraction was performed and

genomic DNA was extracted using purification reagents (Shanghai Xingyao Medical Science and Technology Development Co., Ltd., Shanghai, China). All the operations were performed according to instructions. The Smart Lab-Assist-32 was used for automatic nucleic acid purification and extraction (Taiwan Dot Nanotechnology Development Co., Ltd., Taiwan, China). The concentration and purity of DNA were measured using a Nanodrop2000 ultramicro spectrophotometer (NanoDrop Company, Waltham, Massachusetts, United States). The genomic DNA stock solution was stored at -20°C .

PCR amplification

Before PCR amplification and detection, the DNA sample was diluted to 5-15 ng/ μL according to the quantitative concentration. The amplification procedure was: 37°C for 10 min, pre-denaturation at 95°C for 5 min; at the amplification stage, denaturation at 95°C for 15 s and 60°C for 60 s; there were totally 40 cycles with a reaction system of 25 μL . The signal acquisition device acquired fluorescence signals at the end of the extension phase, and VIC channels (SLC01B1*1b 388G, SLC01B1*5 521C, ApoE2 526T and ApoE4 388C), FAM channels (SLC01B1*1b 388A, SLC01B1*5 521T, ApoE2 526C and ApoE4 388T) and ROX channels (internal standard genes) were used. The internal reference genes used in the experiment were produced by Wuhan Youzhiyou Medical Technology Co., Ltd.

Statistical analysis

SPSS.23.0 was used for the statistical analysis, and Graphpad 8.0 was used to draw relevant statistical charts. The data were expressed as mean \pm standard deviation (mean \pm SD), and chi-square test was used for analysis of enumeration data. ANOVA was used to analyze the difference of SLO1B1 and APOE genotypes. $P < 0.05$ indicated a statistical significance.

Results

General data and clinical features

Among the subjects investigated, there were 1826 males and 1180 females, with an average age of 65.95 ± 11.73 years for males and 67.60 ± 11.57 years for females. There was

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Table 1. Clinical characteristics of patients

	Male	Female	P values
No. of subjects	1826	1180	
Age, year	65.95 ± 11.73	67.60 ± 11.57	.001
TG, mmol/L	1.845 ± 1.645	2.014 ± 1.777	.005
TC, mmol/L	4.893 ± 1.303	5.238 ± 1.325	<.001
HDL, mmol/L	1.237 ± 0.359	1.360 ± 0.382	<.001
LDL, mmol/L	2.829 ± 0.945	2.938 ± 0.960	.001
Apo-A1, g/L	1.100 ± 0.281	1.215 ± 0.310	<.001
Apo-B, g/L	0.901 ± 0.294	0.935 ± 0.294	.001
Apo-A1/Apo-B	1.335 ± 0.555	1.405 ± 0.542	<.001

Note: TG, triglyceride; TC, total cholesterol; HDL, high density lipoprotein; LDL, low density lipoprotein; Apo-A1, lipoprotein A1; Apo-B, lipoprotein B.

statistically significant difference in blood lipid levels between males and females, as shown in **Table 1**.

Genotype and gene frequency

According to the results, the frequency of SLC01B1 388A>G gene was 75.27%, and the frequency of SLC01B1 521T>C gene was 12.38%, determining the corresponding allele frequency, as shown in **Table 2**.

According to the haplotype test results, the haplotype proportions of *1b/*1b (34.5%) and *1a/*1b (32.24%) were the highest, accounting for 66.7% of the population, followed by *1b/*15 (19.05%), while the proportions of other types were relatively small, namely *15/*15 (2.03%), *1a/*15 (4.59%), *1a/*1a (4.66%), and *1a/*15 (0.40%). The remaining types were not detected (**Table 3**). In addition, there was certain difference between males and females. As shown in **Figure 1**, the distribution of *1b/*1b in females was significantly higher than that in males.

Regarding the subjects investigated, $\epsilon 3/\epsilon 3$ occupied 76.08%. The frequencies of other gene types were as follows: $\epsilon 3/\epsilon 4$ (11.48%), $\epsilon 2/\epsilon 3$ (8.42%), $\epsilon 2/\epsilon 4$ (2.79%), and $\epsilon 4/\epsilon 4$ (1.03%) (See **Table 4** for specific data). APOE polymorphism revealed no significant difference in gender (**Figure 2**). The high proportion of $\epsilon 3/\epsilon 3$ genotype resulted in the highest allele frequency of $\epsilon 3$ (**Table 5**).

Based on the gene polymorphisms of different genders in Shenzhen, it was found that there

was no significant difference in other gene polymorphisms except *1b/*1b (**Figure 3**).

Effects of SLC01B1 521 (CC) polymorphism on side effects of statins

SLC01B1 521C mutation type is the site most closely related to statin muscle-related adverse reactions. In this study, there were 41 SLC01B1 521 (CC) subjects, including 26 males and 15 females. No statin-induced myopathy was observed in these populations through a case review of statin drug use and adverse reactions (**Table 6**).

Discussion

Statins have become the most widely used medications to lower lipid levels in clinical practice since its advent. Currently, the primary reason affecting the taking of statins is the symptoms related to adverse reactions (statin associated symptom, SAS), including muscle symptoms, liver injury and central issues. Additionally, previous reports have indicated that long-term use of statins may lead to cognitive impairment [11-13]. It is worth noting that numerous reports have shown that there are remarkable individual differences in the therapeutic effects of statins, with many patients failing to achieve the ideal lipid-lowering effects after treatment.

SLC01B1 and ApoE genes are important factors affecting statin drugs, and their mutations are an important molecular basis for individual differences [14, 15]. Among them, SLC01B1 gene is located on chromosome 12, and its polymorphism is mainly shown in 388A>G and 521T>C, which leads to the functional increase of statin metabolic pathway, thus causing the change in statin efficacy and the increase in toxic and side effects. 388GG can improve the transport rate of statins to increase the drug efficacy. Meanwhile, it can decrease the drug metabolism rate, resulting in a rise in the risk of rhabdomyolysis (e.g. taking additional simvastatin) [16-18]. However, 521C can decrease the drug affinity, thus leading to a long-acting drug.

There are significant differences in genes of different ethnic groups, and the two genes are highly unbalanced in distribution among different ethnic groups. Relevant studies have demonstrated that Asians need lower doses of

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Table 2. SLC01B1 genotype and gene frequency of subjects

Genotypes and alleles	Male (n = 1826, alleles = 3652)		Female (n = 1180, alleles = 2216)	
	No. of individuals	Relative frequency (%)	No. of individuals	Relative frequency (%)
SLC01B1 388A>G AA	103	5.64%	58	4.92%
AG	697	38.17%	433	36.69%
GG	1026	56.19%	689	58.39%
A (AF)	903	24.73%	549	23.26%
G (AF)	2749	75.27%	1811	76.74%
TT	1384	76.82	946	81.14
TC	416	21.59	219	17.64
CC	26	1.58	15	1.22
T (AF)	3184	87.62	2111	89.45%
C (AF)	468	12.38	249	10.55%

Note: AF indicates allele frequency.

Table 3. SLC01B1 gene haplotypes

	SLC01B1 gene haplotypes							Total
	*15/*15	*1a/*15	*1a/*1a	*1a/*1b	*1a/*5	*1b/*15	*1b/*15	
Males	42	98	91	592	9	375	619	1826
Females	19	42	59	387	3	222	448	1180
Total	61	138	140	969	12	577	1037	3006

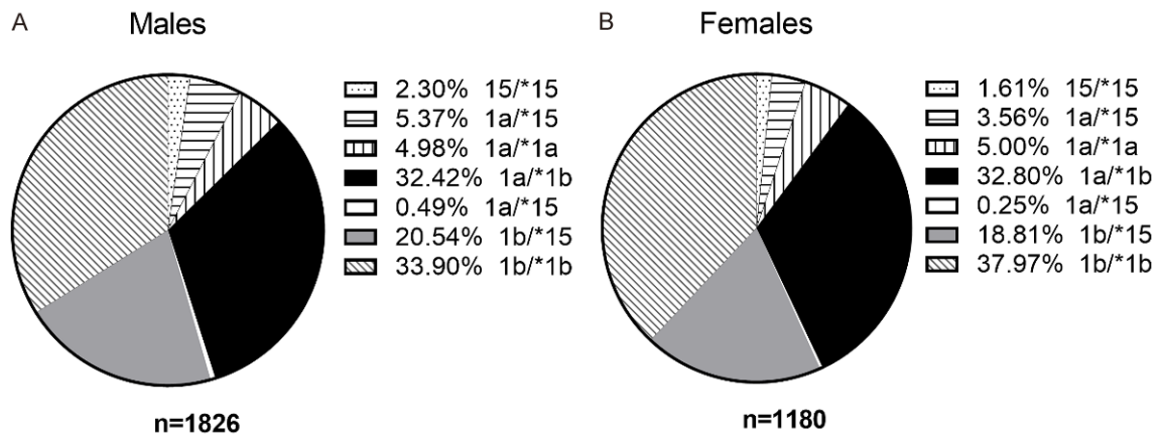


Figure 1. Haplotype ratio of SLC01B1 gene of males and females.

Table 4. Genotypes and alleles of ApoE gene

Genotypes	APOE gene polymorphisms					
	$\epsilon 2/\epsilon 2$	$\epsilon 2/\epsilon 3$	$\epsilon 2/\epsilon 4$	$\epsilon 3/\epsilon 3$	$\epsilon 3/\epsilon 4$	$\epsilon 4/\epsilon 4$
Males	4	153	51	1389	210	19
Females	2	99	33	898	136	12
Total	6	253	84	2287	345	31

statins than Caucasians to achieve the same lipid-lowering effects, which are subject to the differences in related enzymes and receptors

affected by the genes. It is reported that the mutation rate of SLC01B1 388A>G is 25-30% in Caucasians, while the mutation rate of 521T>C is 15-20% in Caucasians. However, the allele frequency of SLC01B1 388A>G in Han ethnicity in China is higher than that in Caucasians, while the allele frequency of 521T>C in Han ethnicity in China is lower [19]. The results of this study suggest that the frequency of SLC01B1 388A>G gene is 75.27%, and the frequency of SLC01B1 521T>C gene is 12.38% in Asian population in Shenzhen. In

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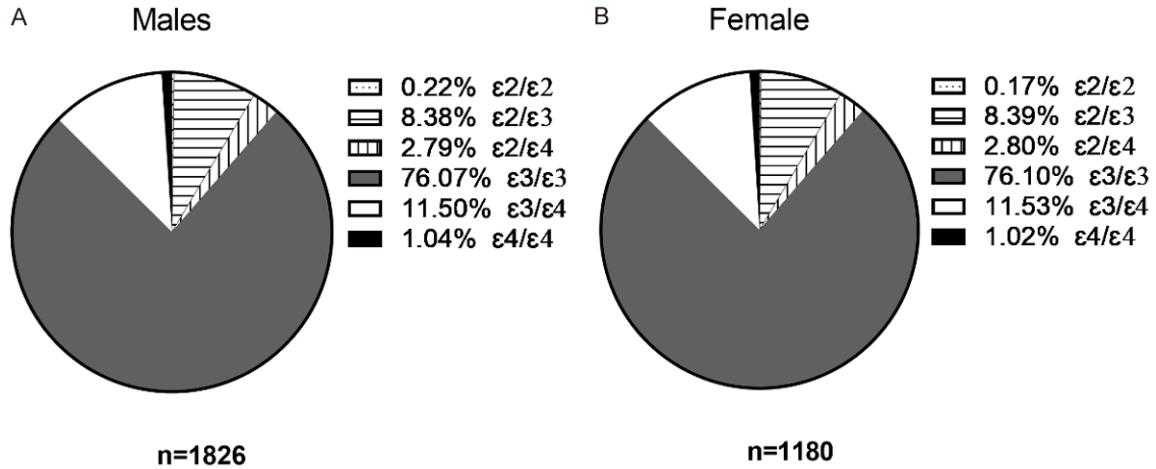


Figure 2. APOE gene polymorphism of males and females.

Table 5. Allele distribution of ApoE gene

Alleles	$\epsilon 2$	$\epsilon 3$	$\epsilon 4$
Males	212 (5.81%)	3141 (86.01%)	299 (8.19%)
Females	136 (5.76%)	2031 (86.06%)	193 (8.18%)
Total	349 (5.81%)	5172 (86.03%)	491 (8.17%)
Males vs females P values	0.621	0.946	0.731

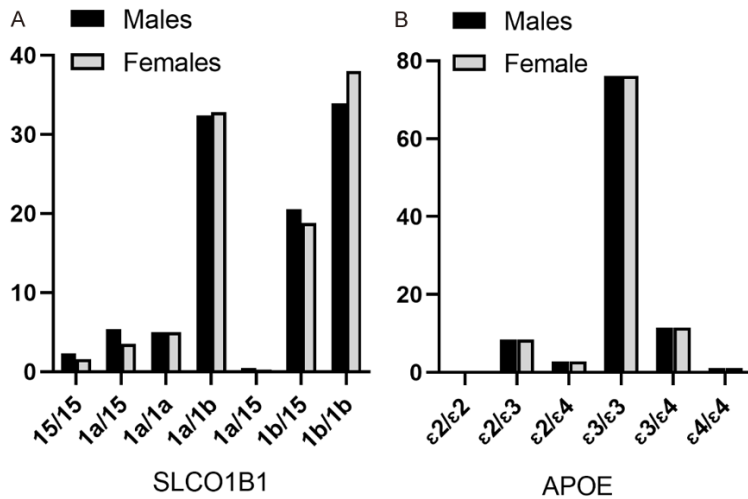


Figure 3. Genetic polymorphism difference between males and females.

In addition, the SLCO1B1 genes in Asian population in Shenzhen are primarily *1a/*1b and *1b/*1b genotypes, accounting for 66.7%, exhibiting that most Asian population in Shenzhen can tolerate large doses of statins, and there are some differences in SLCO1B1 between males and females.

The distribution trend of APOE genotype is the same as that of the general population. In most

regions of China, $\epsilon 3/\epsilon 3$ genotypes are the most common ones, and $\epsilon 3/\epsilon 3$ is the predominant genotype in APOE, accounting for 76.07%, while the other genotypes are $\epsilon 3/\epsilon 4$ (11.48%), $\epsilon 2/\epsilon 3$ (8.42%), $\epsilon 2/\epsilon 4$ (2.79%), and $\epsilon 4/\epsilon 4$ (1.03%), indicating that most Asians in Shenzhen are relatively safe to take statins, and can achieve the therapeutic purpose of controlling blood lipid through taking statins continuously. Compared with that of the population in other regions, the gene frequency of Asians in Shenzhen is similar to that in many regions such as north-east China, Jiangsu, Kunming of Yunnan [20-22]. In 2016, a study of 390 healthy elderly people in Chongqing showed that $\epsilon 3/\epsilon 3$ (64.19%) and $\epsilon 4/\epsilon 4$ (0.59%) had basically the same distribution as this study [23]. This is related to the primitive type of $\epsilon 3$ gene. In most regions and populations, the proportion of $\epsilon 3/\epsilon 3$ often exceeds 60%, while the frequency of $\epsilon 4$ genes in Asian populations is significantly lower than that in European and American populations. The results of this study reveal that statin therapy is highly sensitive in Asian population in Shenzhen. Therefore, medium-intensity statins can be used to achieve good therapeutic purposes, and people in this region should be prevented

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Table 6. SLC01B1 521(CC) phenotype and subjects who have used statins

	Drugs and doses of statins					Total
	Atorvastatin (20 mg/d)	Atorvastatin (10 mg/d)	Pravastatin (2 mg/d)	Rosuvastatin (10 mg/d)	Rosuvastatin (5 mg/d)	
Males	9	5	3	11	0	28
Females	4	5	1	2	1	13
Total	13	10	4	13	1	41

from using high-intensity and large-dose statins [24, 25].

The shortcoming of this study is that the sample size is limited, which is also the same problem of similar studies. High throughput techniques can be used in large scale studies. In addition, due to the limited data and samples in this study, the corresponding standardized treatment model could not be further accurately established in this region, which requires further in-depth study.

It is of great significance to strengthen the guidance of clinical medication and health management. The relevant studies on gene mutation and the efficacy and safety of statins are widely performed to provide important references for clinical application. Detection of SLC01B1 and APOE gene polymorphisms is of great significance to achieve individualized treatment. Detection of SLC01B1 and ApoE gene polymorphisms should be performed for patients who are taking or considering statin drugs, and individualized medication and safe medication should be carried out. In addition, with the extensive exploration of high-throughput sequencing technology (NGS), more convenient and sensitive detection methods are provided for disease-related gene detection, which will be extensively investigated in the future studies [26-28].

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Disclosure of conflict of interest

None.

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