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Correlation between the *Xba* Ⅰ polymorphism of *apoB* gene and serum lipid profiles in Li ethnic group

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ABSTRACT

Objective: To study correlation between the *Xba* Ⅰ polymorphism of *apoB* gene and plasma lipid profiles in Li ethnic group. **Methods:** Total 151 cases of healthy Li people were recruited randomly by cluster sampling and 200 Han people were recruited as control; blood was drawn to analyze *Xba* Ⅰ polymorphism distribution of *apoB* gene and serum lipid levels. **Results:** There were lower serum total cholesterol (TC) and low density lipoprotein cholesterol (LDL-C) levels in serum of Li people; while, high density lipoprotein cholesterol (HDL-C), X^-/X^+ genotype and X^+ allele frequencies exhibited higher levels than Han people. Interestingly, HDL-C level was reduced, while LDL-C level was enhanced in subjects carrying heterozygous (X^-/X^+) genotype compared to homozygous (X^-/X^-) genotype. Additionally, there were no difference in serum level of triglyceride, TC, apoprotein A (apo A) and apoprotein B (apo B) between Li and Han people, the same results were showed between X^-/X^+ and X^-/X^- genotype carriers. **Conclusions:** *Xba* Ⅰ polymorphism of *apoB* gene is correlated to the profiles of serum lipid level, X^-/X^+ genotype carriers are phenotyped with higher LDL-C level and lower level of HDL-C in Li ethnic group.

1. Introduction

Apolipoprotein B (apo B) is an important protein subunit contributing to the formation of very low (VLDL), intermediate (ILDL), low density lipoprotein (LDL) particles, and is responsible for clearance of LDL in blood. Apart from this, it works as ligand of LDL receptors and contributes to cellular uptake of cholesterol[1]. Recently, restriction fragment length polymorphism was observed in several typical sites of gene region coding *aop B*, and *Xba* Ⅰ polymorphism as a kind of silent mutation at Thr^{2488}, which is correlated to serum lipid levels and the incidence of coronary heart disease[2-4]. Li ethnic group is the smallest ethnic group among 55 minority ethnic groups in China. Till now, less information is known about *Xba* Ⅰ polymorphism of *apoB* gene and its association with lipid profiles in Li ethnic group, and this study is aimed to elucidate it.

2. Materials and methods

2.1. Study population

This study protocol was approved by the ethnic committee of the people's hospital of Sanya, China. All participants involved in the study have signed consent form. A total of 151 (93 men and 58 women, aged 20-84 years) unrelated

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healthy Li people were selected randomly by cluster sampling as study group. A total of 200 (113 men and 87 women, aged 22-83 years) unrelated healthy Han people were recruited as control group. All participants reside in Changjiang or Lingshui counties of Hainan province without special dietary habits. They were screened on the basis of no history in heart, nervous and hereditary diseases, hyperlipemia secondary to diabetes, hyperthyroidism, chronic hepatic or nephritic diseases and other diseases affecting lipid metabolism.

2.2. Blood sample collection and process

Whole blood (10 mL) was collected from forearms vein in a sitting position post 12 h's fasting. A part of sample (5 mL) without anticoagulation was used to isolated serum within 4 h and stored at -20 ℃ for lipid and lipoprotein assay. The remaining sample was transferred into EDTA-containing tubes for genotyping. Genomic DNA was extracted according to standard protocols[5].

2.3. Lipid and lipoprotein determination

Blood total cholesterol (TC), total triglyceride (TG), high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) were assessed by enzymatic methods with commercial available kits. Apoprotein A (apo A) and apoprotein B (apo B) were determined by immunoturbidimetric immunoassay, using a commercial kit. All the determinations were completed with an autonomic biochemistry analyzer (Type LX-20; Backmen Ltd., USA) in the Clinical Science Experiment Center of the Affiliated Hospital, Hainan Medical College.

2.4. Genetic analysis

Xba Ⅰ polymorphism of *apo B* gene was determined by PCR amplification in a total volume of 50 μL, composed of 5 μL template DNA, 4 μL primers, 5 μL 10×GC buffer, 4 μL dNTPs, 0.2 μL *Taq* polymerase, and 31.8 μL double distilled water. The primers were 5′-GGAGACTATTCAGAAGCTAA-3′and 5′-GAAGAGCCTGAAGACTGACT-3′[6,7]. Cycling was performed with conditions consist of an initial melting temperature of 95 ℃ for 5 min, followed by 30 cycles of melting (95 ℃, 60 s), annealing (50 ℃, 50 s), extension (72 ℃, 50 s) with sequent extension for 7 min. The product (30 μL) was subject to *Xba* Ⅰ restriction enzymatic analysis according to manufacture's instruction, and then isolated by electrophoresis with fine resolution agarose gel for 30 min, and visualized under UV lamp. X^-/X^- genotype produced a 710 bp fragment, X^-/X^+ genotype produced 710 bp, 277 bp and 433 bp fragments, while X^+/X^+ genotype yielded 277 bp and 433 bp fragments[8].

2.5. Statistical analysis

Statistical analysis was carried out using the SPSS statistical package version 13. Values were present with mean±SD, or percentage. Allelic frequency was calculated by gene counting, Hardy-Weinberg equilibrium was employed to compare observed and expected genotypes. Student's test and one-way ANOVA were used to analyze differences of serum lipid and lipoprotein levels among genotypes and alleles. The χ^2 test was used to compare genotypic and allelic frequencies between study and control groups. The *P* value of less than 0.05 was considered to be statistically significant.

3. Results

3.1. Apo B genotyping

Products of PCR amplification and *Xba* Ⅰ restriction enzyme digested product were shown in Figure 1 and 2; those were in accordance with sequencing results performed by TIANGEN BIOTECH (BEIJING) co., Ltd.

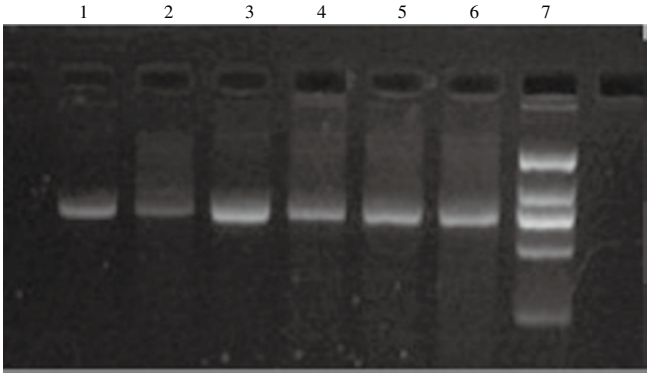

Figure 1. Product of PCR amplification of *apoB* gene.
No 1-6 are from samples, No 7 is from marker.

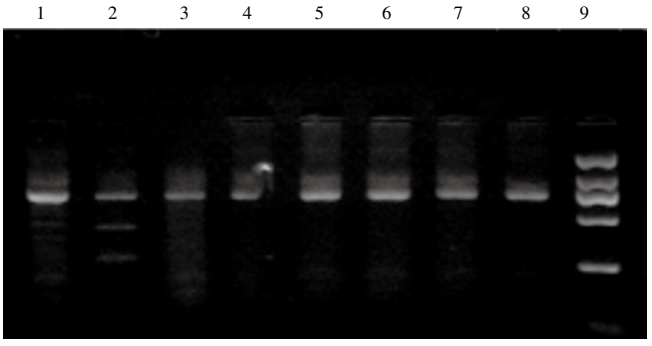

Figure 2. *Xba* Ⅰ restriction enzyme digested product of *apoB* gene.
No 2 is for X^-/X^+ genotype, No 9 is marker, the left is for X^-/X^- genotype.

3.2. Genotypic and allelic frequency distribution of Xba Ⅰ sites in apoB gene

Genotypes of *apoB Xba* Ⅰ of Li (χ^2=1.06, *P*=0.60) and Han people were in Hardy-Weinberg equilibrium (χ^2=0.00, *P*=1.00) (Table 1). Table 2 shows the distributions of allele frequency and genotype of *Xba* Ⅰ sites in *apoB* gene in study and control groups. The heterozygotes X^-/X^+ genotype frequency in Li people was significantly higher than Han people (χ^2=5.61, *P*=0.02), while X^+/X^+ genotype wasn't detected in both Li and Han people. X^+ allele was more frequent in Li people than Han people (χ^2=5.38, *P*=0.02).

Table 1

Hardy-Weinberg equilibrium of genotypes of *Xba* Ⅰ polymorphism in *apoB* gene in Li and Han people.

Group		Frequency of genotypes (*n*, %)		
		X^+/X^-	X^-/X^-	X^+/X^+
Li people	Actual frequency	138	13	0
	Theoretical frequency	16.77	133.71	0.52
Han people	Actual frequency	10	190	0
	Theoretical frequency	9.75	190.12	0.12

Table 2

Genotypes and allele frequencies of *Xba* Ⅰ polymorphism in *apoB* gene in Li and Han people.

Group	Frequency of genotypes (*n*, %)			Frequency of allele (*n*, %)	
	X^+/X^-	X^-/X^-	X^+/X^+	X^+	X^-
Li people	18(0.12)*	133(0.89)	0(0.00)	18(0.06)*	284(0.94)
Han people	10(0.05)	190(0.95)	0(0.00)	10(0.02)	390(0.97)

* *P*<0.05 , as compared to Han people.

3.3.Serum lipids levels and its correlation to Xba Ⅰ polymorphism of apoB gene

As shown in Table 3 and 4, serum TC and LDL-C levels in Li people were lower (*P*<0.05 *P*<0.01), while HDL-C level was significantly higher compared to Han people (*P*<0.01). HDL-C level was reduced while LDL-C level was enhanced (*P*<0.05), and serum levels of TC, TG, apoA, apoB were identical in X^-/X^+ genotype carriers compared to X^-/X^- carriers both in Li and Han people.

4. Discussion

It is reported that frequencies of *apoB* gene polymorphism vary among different ethnic groups[9], and there is a kind of polymorphism existed in *apoB* gene *Xba* Ⅰ restriction sites, in which allele C is replaced by T at the 2488 nucleotides site in the exon 26; It causes a silent mutation in threonine residue[2]. Indeed, there are some reports shown that X^+ allele carriers have a higher serum LDL than X^- allele carriers[3,10]. Although X^+/X^+ genotype is rare both in Li and Han population, in present study, *apoB* gene *Xba* Ⅰ polymorphism was detected in Li ethnic group, and this is in concordance with previous study performed by Tsunoda K in the Mongolian Buryat[4]. However, X^+ allele frequencies were 0.059 in Li population, higher than Han population, and similar to that in the Mongolian Buryat. But it was dramatically distinct to Mexican patients with coronary artery disease[11]. That is the reason that we need to study *Xba* Ⅰ polymorphism in *apoB* gene in different population.

Our data indicated that serum level of TC and LDL-C were lower, while level of HDL-C was enhanced in Li population compared to those in Han population, and this may be related to their dietary habits. As previously reported by Rantala *et al*[12], *ApoB* gene *Xba* Ⅰ polymorphism is correlated to response of serum TC and LDL-C during baseline, low fat, high fat and switch back diet. Especially, X^-/X^- genotype carriers had the greatest enhancement and

Table 3

Comparison of serum lipids and lipoproteins between Li and Han people.

Group	TG	TC	HDL-C	LDL-C	apo A	apo B
Li people	1.31±0.83	5.13±1.13*	1.62±0.32**	3.07±0.95**	1.36±0.18	0.87±0.36
Han people	1.48±0.76	5.47±0.82	1.42±0.30	3.38±0.66	1.22±0.27	0.90±0.08

* *P*<0.05 , ** *P*<0.01 as compared to Han people.

Table 4

Serum lipid and lipoprotein concentration according to Xba Ⅰ polymorphism in apoB gene in Li and Han people.

Group		*n*	TC (mmol/L)	TG (mmol/L)	HDL-C (mmol/L)	LDL-C(mmol/L)	apo A (g/L)	apo B (g/L)
Li people	X^+/X^-	18	5.13±0.93	1.49±0.77	1.49±0.27*	3.42±0.96*	1.34±0.15	0.93±0.37
	X^-/X^-	133	5.13±1.15	1.29±0.86	1.67±0.32	2.94±0.95	1.36±0.19	0.87±0.36
Han people	X^+/X^-	10	5.49 ±0.98	1.51 ±0.84	1.32±0.31*	3.51±0.93*	1.15 ±0.26	0.93±0.29
	X^-/X^-	190	5.45 ±0.94	1.46 ±0.73	1.45 ±0.33	3.24±0.89	1.27 ±0.24	0.88 ±0.36

* *P*<0.05 , compared to X^-/X^- genotype in the same group.

shrink in their serum TC and LDL-C when they were given high fat and low fat diet respectively, whereas subjects carrying X^-/X^- genotype were more susceptible response to diet than subjects with X^+/X^- and X^+/X^+ genotype. Notably, most Li people reside in rural area in Hainan province, living mainly with vegetable diet. High frequency of X^-/X^- genotype and less dietary lipid intake may explain their greater response to diet and result in lower serum TC and LDL-C in Li people.

X^-/X^+ genotype carriers have suppressed HDL-C and increased LDL-C levels both in Li and Han population. This is in concordance with previous study completed by Hu *et al*[3], but inconsistent from study performed by Han *et al*[10]. According to Han T's study, subjects with X^-/X^+ genotype had a higher serum level of TC, LDL-C and apoB, and X^+ allele was characterized with higher TC and LDL-C. Different sample size and various genetic backgrounds may be responsible for the inconsistence.

X^+ allele is known to be associated with coronary heart disease and dyslipidemia[11,13]. Coronary heart disease is the leading cause of death in developed country, and hypercholesterolemia is the key risk factor for coronary heart disease. Reduction of cholesterol intake and low fat diet are principal treatment for hypercholesterolemia[14-16]. Different genotype carriers respond differently to diet, and have distinct susceptivity of dyslipidemia and coronary heart disease; personalized treatment should be applied to patients according to their genetic background.

Conflict of interest statement

We declare that we have no conflict of interest.

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