



Coalition of DNA polymorphisms of ApoB and ApoAI genes is related with coronary artery disease in Kazaks

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Abstract

Objective To explore the relationship between polymorphisms of XbaI and MspI loci of apolipoprotein B (ApoB) gene and -75 bp, +83 bp loci of apolipoprotein AI (ApoAI) gene and coronary heart disease (CHD) in Kazaks of Xinjiang Uyghur Autonomous Region, China. **Methods** These loci were analyzed by PCR-restriction fragment length polymorphism (PCR-PFLP). Two hundred and five patients with CHD and two hundred and thirty six controls were involved. **Results** There were significant distinctions among low-density lipoprotein cholesterol (LDL-C), triglyceride (TG) and the ApoAI/ApoB ratio between the two groups, but no significant distinction among the polymorphism frequencies of the four sites between the two groups. The polymorphism coalition frequency of X⁻/Ms⁺⁺/M₁⁺/M₂⁺⁺ (named Coalition 11) was significantly higher in CHD compared to the control group (14.6% vs. 7.2%, $P < 0.05$). The level of total cholesterol (TC) in Coalition 11 was significantly higher and the level of the ApoAI/ApoB ratio in Coalition 11 was significantly lower than Coalition 1~10 in CHD patients. The level of the ApoAI/ApoB ratio of Coalition 11 was significantly lower than the Coalition 1~10 in control group. The levels of ApoAI/ApoB ratio of Coalition 3 were significantly higher compared to Coalition 11 in the two groups, respectively. The level of LDL-C of Coalition 3 was significantly lower than in the Coalition 11 in control group. The level of TC of Coalition 5 was significantly higher than Coalition 3 in the CHD group. The level of the ApoAI/ApoB ratio of Coalition 5 was significantly lower than in Coalition 3 or Coalition 1~10 of the two groups, respectively. The level of LDL-C of Coalition 5 was significantly higher than in Coalition 3 in control group. The ratio of ApoAI/ApoB was negatively related to TC, LDL-C and was positively related to HDL-C, both in CHD and control groups. **Conclusion** Coalition 11 of the 4 loci polymorphisms of the ApoB and ApoAI genes was correlated with CHD in Kazaks, and perhaps the ratio of ApoAI/ApoB was the most diagnostic parameter related with CHD among all lipid parameters. CHD may also be associated with Coalition 5, and, perhaps, Coalition 3 may have been confirmed as a protection factor against CHD, if more samples were enrolled.

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Keywords: ApoB; ApoAI; Gene polymorphism; Coronary heart disease; Kazaks

1 Introduction

In the past years, many linkages and candidate-gene studies have been performed to identify genes characteristic of coronary heart disease (CHD), which is closely related with dyslipidemia. The apolipoprotein B (ApoB) and apolipoprotein AI (ApoAI) genes are thought to be the two

major genes associated with serum lipids. Polymorphisms of XbaI and MspI loci in ApoB and -75 bp and +83 bp loci in ApoAI gene are now found to be related to CHD, however, studies challenging these findings have been reported.^[1–4] Ethnicity and isolated polymorphism locus, perhaps, are reasons for this phenomenon. It has been thought that the Kazak ethnic group has an increased predisposition to CHD^[5], and has led to extensive research to determine the risk factors and pathways which may predispose this subpopulation to the elevated risk of this disease. Important factors among them, include the levels of lipoproteins, homocysteine, lipoprotein (a), proinflammatory cytokines, as well as inheritance and others. The following

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study is undertaken to determine a possible inter-relationship between CHD and inheritance, which is an important risk factor in the atherosclerosis-prone population. Little is known about the genetic factors that may contribute to the increased susceptibility in Kazaks, a minority mainly in north Xinjiang Uyghur Autonomous Region, China. To our knowledge, no study on the association of the coalition of ApoB and ApoAI gene polymorphisms with CHD has been performed in Kazaks.

2 Methods

2.1 Study participants

The study design was approved by the ethics committees of the participating hospitals, and all subjects provided written informed consent and completed a structured questionnaire detailing the classical risk factors, such as family history of CHD, hypertension and smoking, *etc.*

2.2 CHD cases and controls

A total of 205 Kazak CHD cases and 236 controls were recruited in Shihezi and Yili districts. This CHD population comprised acute coronary syndrome (ACS) patients and stable CHD patients diagnosed by selected coronary angiography confirming no less than 70% stenosis in at least one coronary artery. Patients with a history of myocardial infarction were also recruited in the CHD group. Use of lipid-lowering drugs and a history of hepatic or renal disease were excluded from the study group.

Non-CHD controls were required to meet the following criteria: absence of angina (Rose questionnaire), absence of history of any vascular disease (ACS, stroke or intermittent claudication) and a normal resting electrocardiogram.

Total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), apolipoprotein B (ApoB) and apolipoprotein AI (ApoAI) were tested by previously described methods.^[6]

2.3 Genotyping and DNA sequencing

Five milliliters of whole blood samples with EDTA-K2, were collected. DNA was extracted from blood cells using the phenol-chloroform method.^[5] Genotyping of the four single nucleotide polymorphisms (SNPs) in CHD cases and controls was performed by the PCR-restriction fragment length polymorphism (PCR-PFLP) technique.^[5,7,8] The PCR-PFLPs are shown in Figure 1~3. All SNPs were in Hardy-Weinberg equilibrium (HWE) in the controls. DNA sequencing of the four fragments are shown in Figure 4.

2.4 Statistical analyses

All statistical evaluations were performed by a trained statistician using SPASS 13.0. Chi-Square tests were performed for link-disequilibrium tests of these SNPs with the comparison of gene type frequencies between groups when necessary. ANOVA and Spearman's correlation were also used when necessary.

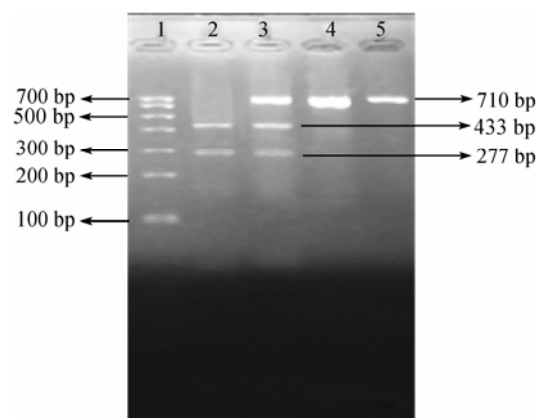


Figure 1. Profile of ApoB gene digested by XbaI. 1: BIOTEKETM 700 bp DNA Mark; 2: X^{++} ; 3: X^{+-} ; 4 and 5: $X^{-+/-}$.

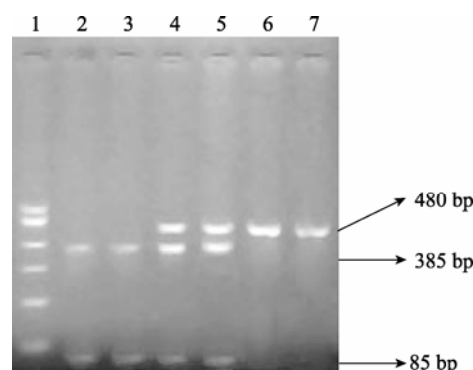


Figure 2. Profile of ApoB gene digested by MspI. 1: BIOTEKETM 600 bp DNA Mark; 2 and 3: M_s^{++} ; 4 and 5: M_s^{+-} ; 6: $M_s^{-/-}$; 7: PCR product.

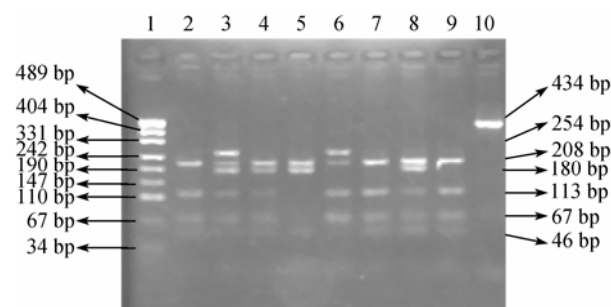


Figure 3. Profile of ApoAI gene -75 bp, +83 bp loci digested by MspI. 1: pUC19/MspI Marker; 2, 7 and 9: $M_1^{++}M_2^{++}$; 3: $M_1^{+-}M_2^{+-}$; 4 and 8: $M_1^{+-}M_2^{++}$; 5: $M_1^{-}M_2^{++}$; 6: $M_1^{++}M_2^{+-}$; 10: PCR product.

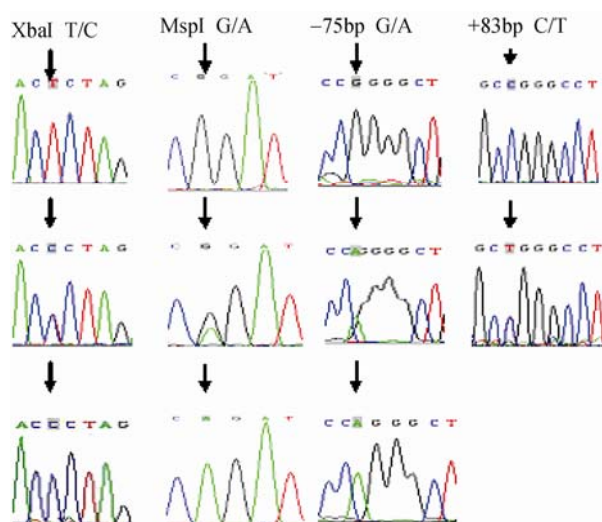


Figure 4. DNA sequencing of the four loci, the arrows indicated the mutational sites.

3 Results

The characteristics of the two groups are shown in Table 1. Significantly elevated levels of LDL-C, TG and a decreased ApoAI/ApoB ratio was observed in patients compared to healthy control subjects (Table 1). Patients with CHD also exhibited higher TC, ApoB and lower HDL-C, ApoAI, however, there was no statistically significant difference among these parameters between the two groups (Table 1).

Table 1. Common characteristics, lipid and apolipoprotein profiles in control subjects and CHD patients.

	Controls, <i>n</i> = 236	CHDs, <i>n</i> = 205
Age (yr)	49.06 ± 8.48	49.28 ± 8.15
Sex (M/F)	148/88	129/76
Hypertension (Y/N)	87/149	90/115
Diabetes (Y/N)	14/222	17/188
BMI (kg/m ²)	25.61 ± 2.83	26.39 ± 3.21
No smoking (<i>n</i> (%))	141 (59.7)	116 (56.6)
Ever smoking (<i>n</i> (%))	18 (7.6)	19 (9.3)
Present smoking (<i>n</i> (%))	77 (32.6)	70 (34.1)
TC (mmol/L)	4.90 ± 1.12	5.40 ± 1.11
TG (mmol/L)	1.50 ± 0.72	2.80 ± 1.18*
LDL-C (mmol/L)	2.30 ± 0.63	3.30 ± 0.71*
HDL-C (mmol/L)	1.00 ± 0.22	0.80 ± 0.19
ApoAI (g/L)	1.31 ± 0.18	1.22 ± 0.14
ApoB (g/L)	0.78 ± 0.15	0.86 ± 0.19
ApoAI/ApoB	1.67 ± 0.44	1.43 ± 0.32*

**P* < 0.05 compared with controls. CHD: coronary heart disease; TC: total cholesterol; TG: triglyceride; BMI: body mass index; HDL: high-density lipoprotein; LDL: low-density lipoprotein; ApoAI: apolipoprotein AI; ApoB: apolipoprotein B; Y: yes; N: no.

There was no significant difference between the two groups in the distributions of genotypes and alleles frequencies of the four loci (Table 2).

No paired linkage disequilibrium was found among the four SNPs analyzed by SHEsis (Table 3), thus, there is no haplotype among the four SNPs, however, the genotypic coalition of these SNPs may play an important role. There are 22 kinds of genotypic coalitions in which 11 coalitions are suitable to be analyzed with no less than five patients, or controls in the two groups, respectively. They are X⁻/Ms⁺⁺/M₁⁺⁺/M₂⁺⁺ (Coalition 1), X⁻/Ms⁺⁺/M₁⁻/M₂⁺⁺ (Coalition 2), X⁺/Ms⁺⁺/M₁⁺/M₂⁺ (Coalition 3), X⁻/Ms⁺/M₁⁺/M₂⁺⁺ (Coalition 4), X⁺/Ms⁺/M₁⁺/M₂⁺⁺ (Coalition 5), X⁻/Ms⁺/M₁⁺/M₂⁺ (Coalition 6), X⁺/Ms⁺/M₁⁺/M₂⁺ (Coalition 7), X⁻/Ms⁺/M₁⁺/M₂⁺ (Coalition 8), X⁻/Ms⁺⁺/M₁⁺⁺/M₂⁺ (Coalition 9), X⁻/Ms⁺⁺/M₁⁺⁺/M₂⁺⁺ (Coalition 10) and X⁻/Ms⁺⁺/M₁⁺/M₂⁺⁺ (Coalition 11).

Table 2. Distribution of frequencies of genotypes and alleles in the two groups.

	Control (<i>n</i> = 236) <i>n</i> (%)	CHD (<i>n</i> = 205) <i>n</i> (%)	<i>P</i>
X ⁻	190 (80.5)	165 (80.5)	NS
X ⁺	42 (17.8)	35 (17.1)	
X ⁺⁺	4 (1.7)	5 (2.4)	
X ⁻	422 (89.4)	365 (89.0)	NS
X ⁺	50 (10.6)	45 (11.0)	
Ms ⁺⁺	162 (68.6)	135 (65.9)	NS
Ms ⁺	67 (28.4)	59 (28.8)	
Ms ⁻	7 (3.0)	11 (5.4)	
Ms ⁺	391 (82.8)	329 (80.2)	NS
Ms ⁻	81 (17.2)	81 (19.8)	
M ₁ ⁺⁺	129 (54.7)	117 (57.1)	NS
M ₁ ⁺	87 (36.9)	72 (35.1)	
M ₁ ⁻	20 (8.5)	16 (7.8)	
M ₁ ⁺	345 (73.1)	306 (74.6)	NS
M ₁ ⁻	127 (26.9)	104 (25.4)	
M ₂ ⁺⁺	152 (64.4)	144 (70.2)	NS
M ₂ ⁺	84 (35.6)	61 (29.8)	
M ₂ ⁺	388 (82.2)	349 (85.1)	NS
M ₂ ⁻	84 (17.8)	61 (14.9)	

NS: not significant; CHD: coronary heart disease.

Table 3. Analysis of pair linkage disequilibrium in these single nucleotide polymorphisms.

	XbaI	MspI
XbaI		0.558
MspI	0.253	
-75bp	-75bp	+83bp
+83bp	0.261	0.769

D' is above the diagonal and *r*² is below the diagonal.

The frequency of Coalition 11 is significantly higher in CHD group compared with control group ($\chi^2 = 6.3$, $P < 0.05$), (Table 4).

The level of TC in Coalition 11 was significantly higher and the level of the ApoAI/ApoB ratio in Coalition 11 was significantly lower than the other polymorphism coalitions

Table 4. Comparison of genotypic Coalition frequencies between the two groups, data are presented as n (%).

	Coa 1	Coa 2	Coa 3	Coa 4	Coa 5	Coa 6
Controls	44 (18.6)	12 (5.1)	17 (7.2)	9 (3.8)	11 (4.7)	10 (4.2)
CHDs	40 (19.5)	10 (4.9)	14 (6.8)	8 (3.9)	9 (4.4)	7 (3.4)
	Coa 7	Coa 8	Coa 9	Coa 10	Coa 11	
Controls	11 (4.7)	15 (6.4)	34 (14.4)	20 (8.5)	17 (7.2)	
CHDs	11 (5.4)	17 (8.3)	29 (14.1)	17 (8.3)	30 (14.6)*	

* $P < 0.05$ compared with controls. Coa: genotypic Coalition. CHD: coronary heart disease.

Table 5. Comparison of apolipoproteins and lipid parameters of different genotypic coalition in the two groups respectively (data only with significant distinction were listed).

		TC (mmol/L)	TG (mmol/L)	LDL-C (mmol/L)	HDL-C (mmol/L)	ApoAI (g/L)	ApoB (g/L)	ApoAI/ApoB
CHD	Coa 3	$5.32 \pm 1.12^{\#}$	1.96 ± 0.24	4.41 ± 1.18	1.14 ± 0.22	1.38 ± 0.21	1.03 ± 0.17	$1.32 \pm 0.18^{*,\#}$
	Coa 5	6.71 ± 1.29	2.46 ± 0.41	4.82 ± 0.89	0.74 ± 0.23	1.11 ± 0.12	1.41 ± 0.22	0.69 ± 0.15
	Coa 1~10	$5.48 \pm 1.15^*$	2.32 ± 0.35	4.45 ± 1.31	0.84 ± 0.23	1.47 ± 0.28	1.21 ± 0.18	$1.23 \pm 0.14^{*,\#}$
	Coa 11	6.52 ± 1.21	2.57 ± 0.41	4.84 ± 1.27	0.76 ± 0.19	1.17 ± 0.13	1.42 ± 0.25	0.82 ± 0.13
Control	Coa 3	4.24 ± 0.68	1.37 ± 0.22	$2.82 \pm 0.35^{\dagger,\S}$	1.16 ± 0.15	1.18 ± 0.16	0.88 ± 0.16	$1.35 \pm 0.26^{\dagger,\S}$
	Coa 5	5.24 ± 0.82	1.44 ± 0.21	4.32 ± 0.52	0.84 ± 0.21	0.93 ± 0.14	1.27 ± 0.20	0.76 ± 0.17
	Coa 1~10	4.79 ± 0.72	1.39 ± 0.24	3.24 ± 0.41	1.19 ± 0.23	1.37 ± 0.19	0.96 ± 0.23	$1.24 \pm 0.21^{\dagger,\S}$
	Coa 11	4.86 ± 0.86	1.47 ± 0.27	4.19 ± 0.50	0.97 ± 0.21	1.11 ± 0.14	0.96 ± 0.18	1.07 ± 0.21

In CHD group: * $P < 0.05$ compared with Coa 11; $^{\#}P < 0.05$ compared with Coa 5; In control group: $^{\dagger}P < 0.05$ compared with Coa 11, $^{\S}P < 0.05$ compared with Coa5. Coa: genotypic Coalition; Coa 1~10: the compound of 10 coalitions of Coalition 1 to Coalition 10. CHD: coronary heart disease; TC: total cholesterol; TG: triglyceride; HDL: high-density lipoprotein; LDL: low-density lipoprotein; ApoAI: apolipoprotein AI; ApoB: apolipoprotein B.

Table 6. Spearman correlation of apolipoproteins with lipid parameters in control subjects and patients with CHD.

	Control ($n = 236$)			CHD ($n = 205$)		
	ApoB	ApoAI	ApoAI/ApoB	ApoB	ApoAI	ApoAI/ApoB
TC	0.149*	-0.117	-0.151*	0.148*	-0.121	-0.180 †
LDL	0.155*	-0.120	-0.141*	0.120	-0.114	-0.175 †
HDL	-0.121	0.161*	0.149*	-0.125	0.106	0.171*
TG	0.116	-0.123	-0.118	0.113	-0.118	-0.124

* $P < 0.05$, $^{\dagger}P < 0.01$. CHD: coronary heart disease; TC: total cholesterol; TG: triglyceride; HDL: high-density lipoprotein; LDL: low-density lipoprotein; ApoAI: apolipoprotein AI; ApoB: apolipoprotein B.

4 Discussion

In our study, the frequency of genotypic Coalition 11 was correlated with CHD, which showed a significantly higher plasma concentration of TG and LDL-C and a significantly lower plasma ratio of ApoAI/ApoB than the control group. It is reasonable to consider that genotypic Coalition 11 caused the apolipoprotein variation, resulting in lipid para-

(Coalition 1~10) in CHD group. The level of the ApoAI/ApoB ratio of Coalition 11 was also significantly lower than Coalition 1~10 in control group. The levels of the ApoAI/ApoB ratio of Coalition 3 were significantly higher compared to Coalition 11 in the two groups. The LDL-C level of Coalition 3 was significantly lower than in the Coalition 11 in control group. The level of TC of Coalition 5 was significantly higher than Coalition 3 in the CHD group. The level of the ApoAI/ApoB ratio of Coalition 5 was significantly lower than in Coalition 3 or Coalition 1~10 in the two groups, respectively. The level of LDL-C of Coalition 5 was significantly higher than Coalition 3 in control group (Table 5).

The ApoAI/ApoB ratio was negatively correlated with TC, LDL-C and was positively correlated with HDL-C, both in CHD and control groups (Table 6).

meters to change into dyslipidemia from which CHD was derived. This viewpoint was conformed by reports of the INTERHEART study and others.^[1,2] Variation of the ApoAI/ApoB ratio perhaps was the most suitable indicator among lipid parameters to reflect the relationship between CHD and the control groups.

Compared with previous studies, the frequencies of X were different from each other ethnicity, Kasaks 89.4%,

Han 99.0%, South Asia 71.0%, and Caucasian 48.0%. The frequencies of M_1^{++} and M_2^{++} were similar between Han and Kasaks ethnicities, 53.85% vs. 54.78% and 66.7% vs. 64.4%.^[9–12] To some extent, these phenomena presumably can account for the different results from different studies.

Our study demonstrated that the genotypic Coalition 11 in Kasaks was correlated with CHD, and we postulate the decreased ApoAI/ApoB ratio primarily connected Coalition 11 with CHD. Furthermore, based on our study, it was not difficult to infer that Coalition 5 may be another risk factor for CHD and that Coalition 3 may be a protective factor from CHD, based on their relationship with lipid parameters.

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References

- 1 Rasouli M, Kiasari AM, Mokheri V. The ratio of ApoB/ApoAI, ApoB and lipoprotein(a) are the best predictors of stable coronary artery disease. *Clin Chem Lab Med* 2006; 44: 1015–1021.
- 2 McQueen MJ, Hawken S, Wang X, *et al.* Lipids, lipoproteins, and apolipoproteins as risk markers of myocardial infarction in 52 countries (the INTERHEART study): a case-control study. *Lancet* 2008; 372: 224–233.
- 3 Sniderman AD, Kiss RS. The strengths and limitations of the ApoB/ApoAI ratio to predict the risk of vascular disease: a Hegelian analysis. *Curr Atheroscler Rep* 2007; 9: 261–265.
- 4 Benn M. Apolipoprotein B levels, ApoB alleles, and risk of ischemic cardiovascular disease in the general population, a review. *Atherosclerosis* 2009; 233: 15.
- 5 Cheng J, An X, Feng WF, *et al.* Relationship between lipoprotein(a) (LP(a)) and coronary heart disease in Kazakhstan patients. *Chin J Cardiovasc Med* 2007; 5: 360–361.
- 6 Huang G, Xie YJ. Association of apolipoprotein B gene EcoRI, XbaI and apolipoprotein AI gene -75bp and +83bp polymorphisms with coronary heart disease. *J Clin Intern Med* 2011; 28: 132–135.
- 7 Benn M, Nordestgaard BG, Jensen JS, *et al.* Polymorphism in ApoB associated with increased low-density lipoprotein levels in both genders in the general population. *J Clin Endocrinol Metab* 2005; 90: 5797–5803.
- 8 Wang LY, Gu Y, Wu GX, *et al.* A case-control study on the distribution of apolipoprotein AI gene polymorphisms in the survivors of atherosclerosis cerebral infarction. *Chin J Epidemiol* 2000; 21: 22–25.
- 9 Zhang AP, Jiang RY, Wu SS. Correlations among ApoB gene's polymorphisms of XbaI and EcoRI loci and coronary heart disease, lipid levels. *Chin J of Med Genetic* 2002; 19: 435–437.
- 10 Pan JP, Chiang AN, Tai JJ, *et al.* Restriction fragment length polymorphisms of Apolipoprotein B gene in Chinese population with coronary heart disease. *Clin Chem* 1995; 41: 424–429.
- 11 Zou YC, Hu DY, Yang XC, *et al.* Relationships among ApoAI gene's polymorphisms and lipid levels, coronary heart disease in Chinese. *Chin J Arterioscler* 2003; 11: 345–348.
- 12 Yan SK, Li XL, Xue H, *et al.* Correlations among ApoB gene's polymorphisms of EcoRI and MspI loci and coronary heart disease. *Chin J Lab Med* 2003; 26: 148–152.