Original Article

Relationship between Dyslipidemia and Gene Polymorphism in Tibetan Population

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Abstract

Objective To investigate the relationship between SNPs reported in previous studies and the blood lipid level in the Tibetan population.

Methods Random cluster sampling was employed in 5 areas (Lhasa, Shigatse, Shannan, Nagqu, and Nyingchi). The levels of cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) from blood samples were determined and DNA was extracted for genotyping and statistical analyses.

Results Among 1 318 subjects aged >18 years enrolled in this study, 367 had dyslipidemia with a prevalence of 27.8%, of whom dyslipidemia males accounted for 33.1% and dyslipidemia females - 24.5%. Results of the correlation analysis between all SNPs and TG showed that the SNPs of rs714052 and rs964184 were related to the serum TG level. Subjects with rs714052 CC genotype had the lowest TG level, and the highest TG level was found in those with rs714052 TT genotype. The serum TG level in individuals with TC genotype lied in between the above two population groups. Subjects with rs964184 CC genotype had the lowest TG level, and the highest serum TG level was noted in those with rs964184 GG genotype.

Conclusion Several SNPs were found to be related to the serum TG level in the Tibetan population. The APOA5 gene and MLXIPL gene may be closely associated with the serum TG level in this ethnic population group.

Key words: Dyslipidemia; SNP; Tibetan Population

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INTRODUCTION

Dyslipidemia is an important risk factor for cardiovascular diseases. Active detection, prevention and treatment of dyslipidemia have become one of the main strategies for prevention of cardiovascular diseases. The Tibet Autonomous Region is located in the Qinghai-Tibet Plateau of China and the diet of Tibetan people is characterized by beef and mutton, dairy products, butter tea and milk-tea due to the special geographical environment. The unique diet and hypoxia environment usually lead to dyslipidemia which has been one of the major conditions threatening the health of the Tibetan population.

The abnormal lipid level is usually associated

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with variations of lipid metabolism-related gene structures and functions. Variations of the gene structure may finally result in alteration of its function affecting the synthesis and metabolism of lipids, thereby causing dyslipidemia. To date, the polymorphism of several lipid metabolism-related genes has been found to be associated with dyslipidemia. Recently, the association between single nucleotide polymorphism (SNP) and dyslipidemia has been investigated in the Genome Wide Association Studies (GWAS) in the context of the whole genome[1-5]. These studies have a large sample size and the results are considered to be reliable. Although such an association is explored in subjects from different races/geographic regions in these studies, it is scarcely studied in the Tibetan population, especially with a large sample size. In the present study, 11 SNPs reported in previous studies (summarized in Table1) with a large sample size were employed, and the association between these SNPs and the blood lipid level was investigated for the Tibetan population.

Table 1. SNPs & Primers for Genotyping

Polymorphism	Loci	Trait	Related Gene	Pre-PCR Primer1	Pre-PCR Primer2	Extended Primer
rs17216525	19p13	TG	NCAN,CILP2	ACGTTGGATGCCCTATG GGTTTTTGACAGG	ACGTTGGATGCCAGGAG GGATAGAAGATAC	GATCCAACCCAACACAC
rs7566605	2q14	TC	INSIG2	ACGTTGGATGAAAACCA CCCTGGTACAGAC	ACGTTGGATGTCATTGCA ATAGCCACTGCC	CAGACCTAAAGGACCAC
rs7557067	2p24	TG	APOB	ACGTTGGATGTGGAATG TGGAGCATCTAGG	ACGTTGGATGGTCCACG TGGGGATCGTTTT	CTCGGAAAAGCCAAGAA
rs1260326	2p23	TG	GCKR	ACGTTGGATGTGCAACT GCCACCTGGGTC	ACGTTGGATGACACAGC ACCGTGGGTCAGA	TCACGGCTGGACTCTCAC C
rs2954029	8q24	TG	TRIB1	ACGTTGGATGTAAACCA GATTTGTTCTGC	ACGTTGGATGCCGTGCC ATTTACAAAGCTG	TCTGCTAATTTGTAGTTG C
rs10889353	1p31	TG	ANGPTL3	ACGTTGGATGCTGGGCA TTTTATTGGGCAC	ACGTTGGATGTAACTACT CTGAGCCTGAGC	TTGCACTCATCTCATTTA AGG
rs964184	11q23	HDL/TG	*	ACGTTGGATGGGAACTT GAAGTCTAGTGGG	ACGTTGGATGCCATGAC ACTAATCACCATC	GGAAAAATGACAATAAA CAGAT
rs714052	7q11	TG	MLXIPL	ACGTTGGATGCAGATGA CTAAAGTTCTGAGC	ACGTTGGATGCCTTGTAA CTATTTTAGTTAG	TGACTAAAGTTCTGAGCC AATCA
rs505151	1q32	TC	PCSK9	ACGTTGGATGAACACGT GTGTAGTCAGGAG	ACGTTGGATGAGCAGAT GGCAACGGCTGTC	GCACTACAGGCAGCACC AGCGAAG
rs12678919	8p21	HDL/TG	LPL	ACGTTGGATGTTGTCTCT CAATCCTCTGTC	ACGTTGGATGTAGGTGG ATATGGGAACTTC	CTCTCCAAAAGTACAAGA TGACACC
rs854560	7q21	TC	PON1	ACGTTGGATGTTTCTGGC AGAAACTGGCTC	ACGTTGGATGGAGCTAA TGAAAGCCAGTCC	TCTGGCAGAAACTGGCT CTGAAGAC

Note. All primers are extended from '3 to 5'. Pre-PCR primer 1 and 2 were used for amplification of SNP sites; primers 1 and 2 were located in the up-stream and down-stream of the SNP site respectively. The anticipated size was about 60-100 bp. Extended primer locates one base pair ahead of SNP site. Extended primer was used to bind to the products from PCR amplification and the DNA sequence was extended by one base pair upstream of the SNP. **APOA1-APOC3-APOA4-APOA5.*

MATERIALS AND METHODS

Materials

Random cluster sampling was employed to select the subjects for investigation in 5 areas (Lhasa, Shigatse, Shannan, Nagqu and Nyingchi). The participants were farmers or herdsmen who were of Tibetan descent living in the above areas. A total of 2 400 subjects were enrolled in the study, of whom 1 318 (54.9%) accepted blood-lipid tests. Those subjects receiving drug treatment or with high altitude erythremia were excluded for the SNP analysis. All the subjects provided written informed consents before the study.

Detection of Blood Lipid

Blood samples were collected and the plasma was separated and stored at -20 °C. The levels of cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were determined by using an automatic biological analyzer (7060, Hitachi, Japan). The reagents and corresponding instructions were provided by the Hitachi, Japan. Dyslipidemia

was diagnosed according to the following criteria[6]: (1) hypertriglyceridemia: serum TG>1.7 mmol/L; (2) hypercholesterolemia: serum TC>5.72 mmol/L; (3) high HDL-C level: HDL-C<1.04 mmol/L (40 mg/dL); (4) mixed hyperlipidemia (MHLP): serum TC and TG levels meeting the above criteria.

DNA extraction and Genotyping

The salting out method was applied to separate blood genomic DNA, the quality of which was determined by electrophoresis. Genotyping was performed on the MassARRAY system (Sequenom, Inc. USA) to detect the SNP. In brief, the target sequence was amplified by PCR (with primers shown in Table 1) and then mixed with SNP primers. The DNA sequence was extended by one base pair upstream of SNP. The prepared samples were added to the single crystal silicon substrate followed by co-crystallization. The crystals were added to the vacuum tube in a mass spectrometer. Then nanosecond laser desorption/femtosecond laser mass spectrometry (10^{-9}s) was performed to sublimate crystals. The nucleic acid was then desorbed and changed into metastable ion filled in the vacuum tube and finally reaching the detector. The smaller the ion mass is, the faster the ion flies. According to the time the ion spent, the type of theextended base pair was determined.

Statistical Analysis

The statistical analysis was performed with SPSS Version 9.0 statistic software package. Allele and genotype frequencies were analyzed by using the gene-counting method and the Hardy–Weinberg equilibrium, and confirmed by the χ^2 test. P<0.01 was defined as significant deviation from the expected Hardy-Weinberg frequency. Samples with the success rate <90% in genotyping were excluded from the analysis. The rare alleles with frequency <5% were not analyzed in the correlation analysis. The analysis of covariance was employed for the correlation analysis. A value of P<0.05 was considered statistically significant.

RESULTS

Epidemiological Survey on Dyslipidemia

A total of 1 318 subjects aged >18 years were recruited into the present study, and 367 subjects had dyslipidemia with a prevalence of 27.8%. Among these subjects, dyslipidemia males accounted for 33.1% and dyslipidemia females -24.5% (Table 2).

Genotyping

Based on the results of the blood lipid test, genotyping was performed in a total of 1 085 subjects aged between18 and 65 years (Table 3).

Genotyping of the rs7566605 SNP failed (with the success rate of genotyping <90%) and thus, this SNP was not included for any further analysis. Genotypes of the remaining SNPs did not deviate significantly from the expected Hardy-Weinberg frequencies (Table 4).

Correlation between SNP and Blood Lipid Level

Results showed that the SNPs of rs714052 and rs964184 were related to the serum TG level. Subjects with rs714052 CC genotype had the lowest TG level, and the highest TG level was found in those with rs714052 TT genotype. The serum TG level in individuals with TC genotype lay in between the above two groups. Subjects with rs964184 CC genotype had the lowest TG level, and the highest serum TG level was noted in those with rs964184 GG genotype. Previous studies have demonstrated that gender, age and BMI are the important factors influencing the lipid level. However, the correlation still existed even after adjustment for gender, age and BMI (Table 5 and 6), which might be reliable.

Table 2. Dyslipidemia in Subjects of Different Ages

Age (yr)	*n*	Hypercholesterolemia		Hypertriglyceridemia		Mixed Dyslipidemia		Low High-Density Lipoprotein Cholesterol	
		n	Prevalence (%)	*n*	Prevalence (%)	*n*	Prevalence (%)	*n*	Prevalence (%)
18-24	175	5	2.86	18	10.29	6	3.43	2	1.14
25-34	277	15	5.42	28	10.11	8	2.89	2	0.72
35-44	382	61	15.97	34	8.90	17	4.45	5	1.31
45-54	269	43	15.99	23	8.55	19	7.06	4	1.49
55-64	133	16	12.03	15	11.28	8	6.02	2	1.50
65-74	64	12	18.75	6	9.38	6	9.38	0	0.00
75-	18	5	27.78	2	11.11	2	11.11	1	5.56
Total	1318	157	11.90	126	9.56	66	5.01	16	1.21

Table 3. Subjects Data Summary

	Total	Mean±SD	Male	Mean±SD	Female	Mean±SD
AGE (years)	1085	37.595±12.738	413	34.938±13.02	672	39.228±12.29
BMI	1048	21.904±3.11	394	21.276±2.755	654	22.283±3.249
TC (mmol/L)	1085	4.594±1.133	413	4.599±1.172	672	4.591±1.109
TG (mmol/L)	1085	1.179±0.594	413	1.225±0.675	672	1.150±0.536
HDL (mmol/L)	1085	1.883±0.597	413	1.849±0.612	672	1.904±0.588
LDL (mmol/L)	1085	3.291±1.103	413	3.317±1.162	672	3.276±1.065

Table 4. SNP Genotyping and Hardy-Weinberg Equilibrium Test

SNP	*n* (GG/GA/AA)	*P* Value
rs505151	3/87/975	0.4480
rs10889353	59/379/647	0.7309
rs7557067	84/416/584	0.3970
rs1260326	211/536/337	0.9510
rs7566605	123/505/457	0.3832
rs714052	63/400/606	0.8690
rs12678919	6/123/956	0.3001
rs2954029	267/531/278	0.6698
rs964184	25/275/780	0.9052
rs17216525	12/172/901	0.2639

Table 5. Relationship between SNP and Serum TG Level

SNP	Genotype	*n*	TG Level (mmol/L)	TG Level after Adjustment for Age and BMI	*P* Value
rs714052	CC	62	1.048±0.475	1.028(a)±0.072	
	TC	390	1.133±0.598	1.137(a)±0.029	0.013
	TT	581	1.215±0.594	1.214(a)±0.024	
rs964184	CC	753	1.116±0.505	1.115(a)±0.02	
	GC	268	1.318±0.761	1.321(a)±0.034	0
	GG	23	1.414±0.595	1.411(a)±0.117	

Table 6. Relationship between SNP and Serum TG Level (by gender)

SNP	Genotype	Female	TG level (mmol/L)	Adjusted TG level	*P* Value	Male	TG level (mmol/L)	Adjusted TG level	*P* Value
rs714052	CC	32	1.051±0.482	1.030[(a)]±0.077		30	1.046±0.476	1.027[(a)]±0.078	
	TC	257	1.138±0.602	1.139[(a)]±0.030	0.011	133	1.130±0.602	1.137[(a)]±0.033	0.014
	TT	373	1.223±0.598	1.221[(a)]±0.028		208	1.209±0.592	1.209[(a)]±0.026	
rs964184	CC	476	1.118±0.511	1.117[(a)]±0.021		277	1.115±0.506	1.112[(a)]±0.022	
	GC	178	1.321±0.771	1.322[(a)]±0.033	0	90	1.313±0.764	1.320[(a)]±0.036	0.002
	GG	15	1.421±0.595	1.419[(a)]±0.121		8	1.411±0.594	1.403[(a)]±0.116	

Note. *P* value: significant was found among the 3 goups. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Nevertheless, the association was not found between SNPs and HDL or LDL even if the analysis was performed by gender (with data not shown).

DISCUSSION

Studies with a large sample size on the blood lipid had never been undertaken in the Tibetan population before. In the present study, a large number of Tibetans were recruited from 5 different areas of the Tibet Autonomous Region, and the incidence of dyslipidemia was obtained in different areas and among subjects of different ages. Especially, the majority of the subjects were from the agricultural and pastoral areas. Our results revealed that the prevalence of dyslipidemia among Tibetans aged <18 years, in both males and females, was significantly higher than the nationwide level. According to the nationwide epidemiological survey in 2002[7], the prevalence of dyslipidemia was 18.6% among the subjects aged >18 years with 22.2% in males and 15.9% in females. The incidence of

dyslipidemia in the subjects of the Tibetan population was significantly higher than the nationwide level. Moreover, the hypercholesterolemia affected 11.9% of the Tibetan population, which was markedly higher than the nationwide incidence (2.9%); the hypertriglyceridemia affected 9.56% of the study population, which was slightly lower than the nationwide level (11.9%); low HDL-C level affected 1.21% of the study population, which was dramatically higher than the nationwide level (7.4%).

The dyslipidemia in China is characterized by hypertriglyceridemia and low HDL-C, which is different from that in the Western population, among whom hypercholesterolemia is the most common type of dyslipidemia. In the present study, the dyslipidemia in the Tibetan population was mainly characterized by hypercholesterolemia, followed by hypertriglyceridemia, and the incidence of low HDL-C was relatively low. The types of dyslipidemia vary among different populations in China[8]. Although the living conditions are relatively poor in vast farming and pastoral areas, the diet of the Tibetan population is still characterized by high protein, high fat and low carbohydrate, and the proportion of food of animal origin is relatively high in their diet. A wealth of studies has demonstrated that dyslipidemia is strongly related to long-term intake of animal fat rich in saturated fatty acids and cholesterol. Diet with rich calorie, fat and saturated fatty acid may promote the synthesis of cholesterol, resulting in increase of the serum cholesterol level[9]. In the study of Ma and his colleagues[10], the prevalence of simple hypercholesterolemia and the TC level in China's Mongolian, Kazakh and Uygur nationalities were significantly higher than those in the Han nationality , and the highest level and incidence were found in the Mongolian nationality. These findings were consistent with the present study. This might be attributed to similar diet habit among these populations. Our results showed a markedly lower prevalence of low HDL-C in the Tibetan subjects than the nationwide level, and this might be associated with the low intake of carbohydrate or other factors which should be clarified in future studies. There is evidence that diet rich in carbohydrate may lead to increased serum VLDL and decreased HDL[11].

The abnormal lipid level may be associated with changes in the structure and/or functions of some lipid-metabolism related genes. The alteration of the gene structure may result in change in the function of these genes, which subsequently affects the synthesis and metabolism of protein, finally resulting in an abnormal lipid level. To date, the SNPs of multiple lipid-metabolism related genes have been found to be associated with blood lipid. In recent years, GWAS have been conducted to investigate the association between SNP and the blood lipid level in the context of the whole genome. These studies had a large sample size and the results were reliable. Although such an association has been investigated in different races and/or regions, it is rarely studied in the Tibetan population of China, especially with a large sample size. To this end, the present study is to some extent an innovative one.

rs964184 is located in the long arm of Chromosome 11 and close to the *APOA1-APOC3-APOA4-APOA5* gene cluster. The distance between *rs964184* and *APOA5* is only about 11.2 kb. *APOA1-APOC3- APOA4-APOA5* gene cluster has been found to be related to the blood lipid level. *APOA5* gene cluster was identified in 2001[12-13]. Evidence has confirmed the relationship between the *APOA1-APOC3-APOA4-APOA5* gene cluster and the serum TG level, but the mechanism is still poorly understood. Studies have revealed that several SNPs of *APOA5* genes might be related to hypertriglyceridemia[14-15]. In the present study, the *rs964184 CC* genotype was a protective genetic factor decreasing the serum TG level by about 0.2 mmol/L, which was consistent with the GWAS study. *rs964184* is located in the far downstream of *APOA5* gene and thus unlikely to directly regulate gene expression. It might be a genetic marker and related to the special changes in the *APOA5* genes.

The *rs714052* locates in the intron of *BAZ1B* gene in the long arm of Chromosome 7. The *BAZ1B* encoded protein belongs to the bromodomain family[16], and is closely related to the gene transcription, regulation and cell cycles. However, no evidence has demonstrated the relationship between this gene and the serum TG level. *MLXIPL* gene is close to this SNP and *MLXIPL* encoded protein belongs to a transcription factor family. This transcription factor can act on the promoter of some TG-synthesis related genes in a glucose dependent manner. Therefore, this gene might play important roles in glycometabolism and lipid metabolism. In a study with a large sample size[17], the *rs3812316* SNP in the *MLXIPL* gene (Gln241His) was strongly associated with the serum TG level. Moreover, three SNPs close to the *rs3812316* were also found to be associated with the serum TG level in this study.

These 4 SNP sites covered 200 kb in the genome and had high linkage disequilibrium. The *rs714052* SNP site as shown in the present study locates in this sequence with linkage disequilibrium. Thus, the influence of *rs714052* SNP on the serum TG level may be related to the *MLXIPL* gene. Similar to the *rs964184* SNP, *rs714052* SNP might also be a genetic marker and related to the changes in the *MLXIPL* gene.

The findings from GWAS were employed and validated in the Tibetan subjects. Among 10 SNPs, 2 SNPs were found to be related to the serum TG level in the Tibetan population, but no correlation was found between these SNPs with the serum TC and HDL-C levels. The *APOA5* gene and *MLXIPL* gene may be closely associated with the serum TG level in the Tibetan population. Our findings were inconsistent with GWAS and other studies with a large sample size. This may be attributable to the special genetic background. Therefore, results from studies on other races are not applicable to the Tibetan population.

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