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Research Paper

Genetic variants at 10q23.33 are associated with plasma lipid levels in a Chinese population

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Abstract

Plasma lipid abnormalities are implicated in the pathogenic process of type 2 diabetes. The *IDE-KIF11-HHEX* gene cluster on chromosome 10q23.33 has been identified as a susceptibility locus for type 2 diabetes. We hypothesized that genetic variants at 10q23.33 may be associated with plasma lipid concentrations. Seven tagging single nucleotide polymorphisms (SNPs: rs7923837, rs2488075, rs947591, rs11187146, rs5015480, rs4646957 and rs1111875) at 10q23.33 were genotyped in 3,281 subjects from a Han Chinese population, using the *Taq*Man OpenArray and Sequenom MassARRAY platforms. Multiple linear regression analyses showed that SNP rs7923837 in the 3´-flanking region of *HHEX* was significantly associated with triglyceride levels (P = 0.019, 0.031 mmol/L average decrease per minor G allele) and that rs2488075 and rs947591 in the downstream region of *HHEX* were significantly associated with total cholesterol levels (P = 0.041, 0.058 mmol/L average decrease per minor C allele and P = 0.018, 0.063 mmol/L average decrease per minor A allele, respectively). However, the other four SNPs (rs11187146, rs5015480, rs4646957 and rs1111875) were not significantly associated with any plasma lipid concentrations in this Chinese population. Our data suggest that genetic variants in the *IDE-KIF11-HHEX* gene cluster at 10q23.33 may partially explain the variation of plasma lipid levels in the Han Chinese population. Further studies are required to confirm these findings in other populations.

Keywords: cholesterol, triglycerides, polymorphism, genetic, iDE-KIF11-HHEX

INTRODUCTION

Plasma lipid abnormalities are associated with risk of type 2 diabetes[1,2]. Some studies have shown that elevated triglyceride (TG) levels and low levels of high-density lipoprotein cholesterol (HDL-C) accelerate the pathogenesis of type 2 diabetes[3-5]. Recently, several studies have investigated the potential effect

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of genetic factors associated with risk of type 2 diabetes on plasma lipid levels. Onuma et al.[6] analyzed the association of polymorphisms in the glucokinase (hexokinase 4) regulator *(GCKR)* gene with type 2 diabetes in a case-control study and with fasting blood glucose and TG levels in the general population. The A allele of SNP rs780094 was found to be associated with reduced risk of type 2 diabetes and lower levels of fasting plasma glucose, but higher levels of TG, in a Japanese population. In the study reported by Chen et al.[7], the authors found that subjects with minor alleles of SNPs rs2283228 and rs2237892 in the KQT-like subfamily member 1 (*KCNQ1*) gene, which were associated with type 2 diabetes, had higher levels of TG. This evidence suggests that genetic variants associated with diabetes risk may also be potential genetic determinants of plasma lipid levels.

A genome-wide association study (GWAS) conducted in a French case-control study identified a novel type 2 diabetes susceptibility locus on chromosome 10q23.33, which is located in a gene cluster including an insulin-degrading enzyme *(IDE)*, a kinesin-interacting factor 11 (*KIF11*), and a hematopoietically expressed homeobox protein *(HHEX)*[8]. Following this discovery, several studies have confirmed this association in British[9], Finnish[10], Japanese[11,12] and Chinese populations[13-15]. Recently, we have also found that SNPs rs7923837 and rs1111875 in the *IDE-KIF11-HHEX* locus at 10q23.33 were independently associated with risk of type 2 diabetes in a Chinese population[16]. However, the relationship between *IDE-KIF11-HHEX* locus and lipid traits in different populations is not clear. Therefore, in an effort to evaluate the influence of the polymorphisms in *IDE-KIF11-HHEX* locus on plasma lipid concentrations, we performed a fine-mapping study by genotyping seven tagging SNPs at 10q23.33 in 3,281 Han Chinese subjects to examine the associations of these variants with plasma levels of total cholesterol (TC), TG, HDL-C and low density lipoprotein cholesterol (LDL-C).

SUBJECTS AND METHODS

Study subjects

The subjects in the current study were selected from a community-based non-communicable diseases screening program comprised of more than 50,000 participants in Jiangsu Province during 2004 and 2008. All subjects were unrelated, ethnic Han Chinese. Subjects were excluded from the study if they had a history of diabetes, hypertension, coronary heart disease or cancer, or fasting plasma glucose $\geqslant$ 5.6 mmol/L. After providing informed consent, all subjects were interviewed face-to-face using a standard questionnaire that included demographic characteristics, risk factors and disease history. Subjects who smoked 1 cigarette per day for over 1 year were defined as smokers, and those who consumed 3 or more alcohol drinks a week for over 6 months were considered as alcohol drinkers. Physical examinations, including measurements of height, weight and blood pressure, as well as laboratory tests to measure TC, TG, HDL-C and fasting plasma glucose concentrations, were performed for each participant. Sitting blood pressure was based on the average of three blood pressure readings measured. Body mass index (BMI) was calculated as weight (in kilograms) divided by the square of height (in meters). Fasting blood samples for routine laboratory examinations were obtained in the early morning after an overnight fast. All biochemical parameters were measured enzymatically on an auto-analyzer (Hitachi 7180 Biochemistry Auto-analyzer, Japan) according to the manufacturer´s instructions. For subjects with TG levels < 4.52 mmol/L, LDL-C levels were estimated indirectly using the Friedewald´s formula. The Institutional Review Board of Nanjing Medical University approved the study.

SNP selection and genotyping

Based on our previous study[16], we used a block-based tagging strategy to select tagging SNPs using Haploview 4.2 software according to the HapMap database [http://www.hapmap.org/, phaseII Nov08, on NCBI B36 assembly, dbSNPb126; population: Han Chinese population (CHB) and Japanese population (JPT)] . The criteria included SNPs with minor allele frequency (MAF) $\geqslant$ 0.10, Hardy-Weinberg equilibrium $P \geqslant 0.05$ and call rate $\geqslant$ 95% when using pairwise linkage disequilibrium (r^2) of 0.8 as the threshold for each block. Seven tagging SNPs (rs7923837, rs2488075, rs947591, rs11187146, rs5015480, rs4646957 and rs1111875) associated with type 2 diabetes in the *IDE-KIF11-HHEX* locus at 10q23.33 were included in the current study.

Genomic DNA was isolated from leucocytes of venous blood by proteinase K digestion and phenol/chloroform extraction. Genotyping was performed using the *Taq*Man OpenArray Genotyping System (Life Technologies, Carlsbad, CA, USA) and the iPLEX Sequenom MassARRAY platform (Sequenom, Inc.). For quality control, two non-template controls were used in each chip or plate. The overall call rates ranged from 98.8% to 99.8% for all SNPs.

Statistical analysis

Associations between the genotypes and plasma lipid

Table 1 **Characteristics of the study population**

Variable	Subjects (*n*= 3,281)
Sex	
Male [*n*(%)]	1,234 (37.61)
Female [*n*(%)]	2,047 (62.39)
Age (years)	
Mean±SD	56.58±9.88
Range	19-95
BMI (kg/m^2)	
Mean±SD	22.12±2.63
Range	14.13-33.73
Systolic blood pressure (mmHg)	
Mean±SD	117.68±14.17
Range	78.00-159.33
Diastolic blood pressure (mmHg)	
Mean±SD	74.14±9.24
Range	45.50-103.00
Total cholesterol (mmol/L)	
Mean±SD	4.40±0.80
Range	2.08-6.22
HDL cholesterol (mmol/L)	
Mean±SD	1.62±0.38
Range	1.03-3.91
LDL cholesterol (mmol/L)	
Mean±SD	2.29±0.74
Range	0.14-4.63
Triglycerides (mmol/L)	
Mean±SD	1.09±0.45
Range	0.13-2.26
Fasting plasma glucose (mmol/L)	
Mean±SD	4.52±0.53
Range	2.80-5.59
Smoking	
Non-smoker [*n*(%)]	2,431 (74.62)
Smoker [*n*(%)]	827 (25.38)
Drinking	
Non-drinker [*n*(%)]	2,619 (80.49)
Drinker [*n*(%)]	635 (19.51)

BMI: body mass index; HDL: high-density lipoprotein cholesterol; LDL: low density lipoprotein cholesterol.

concentrations were determined by multiple linear regression analysis with adjustment for age, sex, smoking status, drinking status and BMI. The Hardy-Weinberg equilibrium was tested by a goodness-of-fit χ^2 test to compare the observed genotype frequencies with the expected ones among the 3,281 subjects. All statistical analyses were performed using Statistical Analysis System software version 9.1.3 (SAS Institute, Cary, NC, USA). All tests were two-sided and the significance level was set at $P < 0.05$.

RESULTS

The demographic and biochemical characteristics of the 3,281 subjects included in this study are shown in ***Table 1***. The mean age was 56.58 (± 9.88) years and the mean BMI value was 22.12 (± 2.63) kg/m^2. The mean values of TC, HDL-C, LDL-C and TG were 4.40 (± 0.80) mmol/L, 1.62 (± 0.38) mmol/L, 2.29 (± 0.74) mmol/L and 1.09 (± 0.45) mmol/L, respectively. Among these subjects, 827 subjects (25.38%) were smokers and 635 subjects (19.51%) were drinkers.

The observed genotype frequencies for the seven SNPs were all consistent with the Hardy-Weinberg equilibrium among 3,281 subjects ($P > 0.05$) ***(Table 2)***. We examined the association between each SNP and TC, TG, HDL-C or LDL-C levels, respectively, in an additive model using a linear regression model with adjustment for age, sex, smoking, drinking and BMI ***(Table 2)***. We found significant associations between rs7923837 and TG ($P = 0.019$), between rs2488075 and rs947591 and TC ($P = 0.041$ and 0.018, respectively). As shown in ***Table 3***, the G allele of rs7923837 was associated with a lower TG levels (0.031 mmol/L average decrease per G allele). Similarly, the C allele of rs2488075 and the A allele of rs947591 were both associated with lower TC levels (0.058 mmol/L average decrease per C allele and 0.063 mmol/L average decrease per A allele, respectively). Conditional analysis indicated that rs2488075 and rs947591 were not significant after adjustment with each other, as the two SNPs were in strong linkage equilibrium (LD) (r^2=0.734). However, no significant associations were observed between the other four SNPs (rs11187146, rs5015480, rs4646957 and rs1111875) and blood lipid concentrations.

We then conducted a stratification analysis for rs7923837, rs2488075 and rs947591 by age, sex, BMI, smoking, and drinking status. As shown in ***Table 4***, the associations between rs7923837 and TG levels were more evident among subjects of the low age group ($P = 0.009$), male subjects ($P = 0.003$), non-drinkers ($P = 0.019$), and subjects with low BMI ($P = 0.003$). The associations between rs2488075, rs947591 and TC levels were more evident among subjects of low age group ($P = 0.021$ and 0.013, respectively), female subjects ($P = 0.048$ and 0.032, respectively), non-smokers ($P = 0.004$ and 0.002, respectively), non-drinkers ($P = 0.007$ and 0.003, respectively) and subjects with low BMI ($P = 0.038$ and 0.037, respectively).

DISCUSSION

To the best of our knowledge, this is the first study to investigate the association between *IDE-KIF11-HHEX* polymorphisms and plasma lipid concentrations in a Chinese population. Of the seven tagging SNPs at the *IDE-KIF11-HHEX* locus, we found that rs7923837 was associated with plasma concentrations of TG, and rs2488075 and rs947591 were associated with plasma concentrations of TC.

Table 2 **Association between lipid concentrations and selected single nucleotide polymorphisms**

SNP	Position	Location	Alleles	MAF	HWE[a]	Total cholesterol	Triglycerides	HDL cholesterol	LDL cholesterol
						P^b	P^b	P^b	P^b
rs7923837	94471897	intergene	A/G	0.198	0.991	0.137	0.019	0.715	0.449
rs2488075	94480154	intergene	T/C	0.140	0.806	0.041	0.081	0.460	0.119
rs947591	94485733	intergene	C/A	0.161	0.413	0.018	0.297	0.056	0.112
rs11187146	94468335	intergene	C/G	0.328	0.422	0.198	0.139	0.201	0.778
rs5015480	94455539	intergene	T/C	0.162	0.950	0.991	0.216	0.753	0.367
rs4646957	94219892	IDE(intron)	C/T	0.228	0.582	0.704	0.945	0.583	0.442
rs1111875	94452862	intergene	T/C	0.258	0.623	0.339	0.579	0.501	0.144

HDL-C: high-density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol; MAF: minor allele frequency; SNP: single nucleotide polymorphism. [a]*P* value for Hardy-Weinberger equilibrium test. [b]*P* value derived from the multiple linear regression with adjustment for sex, age, smoking, drinking and BMI assuming an additive genetic model.

rs7923837 is located in the 3´-flanking region of the *HHEX* gene, which encodes a transcription factor that is involved in Wnt signaling and is critical for hepatic and pancreatic development[17,18]. In addition, *HHEX* may regulate β-cell development and/or function by activating hepatocyte nuclear factor 1α[19]. Several studies reported that the association between rs7923837 and type 2 diabetes is mediated through decreased β-cell secretory capacity or decreased β-cell mass[20-22]. Thus, *HHEX* is critical for insulin signaling and islet function[23]. *HHEX* may influence metabolic phenotypes such as TG and TC because insulin is necessary for the regulation of metabolic phenotypes. On chromosome 10q23.33, genetic variants in *HHEX* have been established as susceptibility loci for type 2 diabetes[8]. In our previous fine-mapping study[16], we reported that rs7923837 and rs1111875 were independently associated with risk of type 2 diabetes in a Chinese population. Several studies have also investigated the relationship between *HHEX* polymorphisms and other metabolic diseases. Zhao et al.[24] found that the type 2 diabetes risk-associated G allele of rs7923837 was associated with higher pediatric BMI in European American children. Cruz et al.[25] analyzed the association between the *HHEX* rs5015480 and risk of metabolic syndrome (MS) in a case-control study from Mexico city and found that rs5015480 was significantly associated with MS. Taken together, this evidence suggests that genetic variants in the *IDE-KIF11-HHEX* gene cluster at 10q23.33 may contribute to metabolism-related traits and diseases, including circulating lipid levels and diabetes risk.

Genetic variants associated with the risk of type 2 diabetes have been found to influence plasma TG levels. The variant rs780094 in *GCKR* was associated with a decreased risk of type 2 diabetes, but with higher TG levels, in a GWAS of European population[26]. Similarly, in the current study, we found that the risk allele of diabetes was associated with lower TG levels. Considering that the subjects included in this study were from a healthy population, the lipid level-related variant might interpret the variation of lipid levels of baseline. The relationships between the same variant and diabetes risk and lipid levels implies

Table 3 **Effects of rs7923837, rs2488075 and rs947591 on plasma lipid concentrations**

SNP	C/C[a]		C/R[a]		R/R[a]		Effect (mmol/L)	P^b
	N	Mean level (mmol/L)	N	Mean level (mmol/L)	N	Mean level (mmol/L)		
SNPs associated with TG								
rs7923837	2098	1.11±0.45	1033	1.05±0.43	127	1.08±0.42	−0.031 (0.013)	0.019
SNPs associated with TC								
rs2488075	2415	4.42±0.80	788	4.36±0.80	62	4.28±0.66	−0.058 (0.028)	0.041
rs947591	2292	4.41±0.80	893	4.38±0.82	78	4.15±0.70	−0.063 (0.027)	0.018

[a]C/C, C/R and R/R represent homozygotes for the common allele, and heterozygotes and homozygotes for the rare allele, respectively. [b]*P* value for the multiple linear regression with adjustment for sex, age, smoking, drinking and BMI assuming an additive genetic model.

Table 4 **Stratification analysis for rs7923837, rs2488075, and rs947591 and lipid levels in an additive genetic model**

Variables	rs7923837 and TG				rs2488075 and TC				rs947591 and TC			
	Subjects[a]	Effect	SEM	*P*[c]	Subjects[a]	Effect	SEM	*P*[c]	Subjects[a]	Effect	SEM	*P*[c]
Age (years)												
⩽ 58	77/587/1,151	-0.046	0.018	0.009	40/461/1,314	-0.086	0.037	0.021	45/520/1,250	-0.089	0.036	0.013
> 58	50/446/947	-0.029	0.020	0.149	22/327/1,101	-0.049	0.043	0.261	33/373/1,042	-0.049	0.040	0.223
Sex												
Female	79/678/1,277	-0.024	0.017	0.159	43/516/1,479	-0.068	0.035	0.048	53/581/1,401	-0.071	0.033	0.032
Male	48/355/821	-0.065	0.022	0.003	19/272/936	-0.078	0.049	0.110	25/312/891	-0.075	0.046	0.100
Smoking												
Non-smoker	91/791/1,530	-0.032	0.015	0.040	47/607/1,765	-0.090	0.032	0.004	58/682/1,675	-0.094	0.030	0.002
Smoker	34/236/553	-0.059	0.027	0.027	14/176/633	-0.015	0.061	0.805	19/207/599	-0.007	0.057	0.899
Drinking												
Non-drinker	103/829/1,667	-0.035	0.015	0.019	48/630/1,929	-0.085	0.031	0.007	61/718/1,825	-0.087	0.029	0.003
Drinker	22/195/415	-0.055	0.029	0.063	13/150/468	-0.014	0.066	0.837	16/168/448	-0.009	0.063	0.882
BMI[b] (kg/cm^2)												
< 24	99/842/1,669	-0.043	0.015	0.003	48/633/1,934	-0.066	0.032	0.038	63/715/1,833	-0.063	0.030	0.037
⩾ 24	28/191/429	-0.017	0.033	0.612	14/155/481	-0.089	0.060	0.139	15/178/459	-0.111	0.058	0.056

[a]Variant homozygote/heterozygote/wild–type homozygote. [b]A BMI of 24 is recommended as the cutoff point for overweight in Chinese. [c]*P* value for the multiple linear regression with adjustment for sex, age, smoking, drinking and BMI assuming an additive genetic model (the stratified factor in each stratum was excluded).

that low lipid baseline levels in some subjects are, in part, genetically determined, causing them to be more susceptible to type 2 diabetes. This was also supported by the results from the stratification analysis, which showed that the associations between genetic variants at 10q23.33 and lipid levels were more evident among young subjects, non-smokers, non-drinkers and subjects with low BMI. However, the underlying mechanism remains unclear, and further studies are needed to elucidate the roles of genetic variants at 10q23.33 in circulating lipid levels.

There is a strong relationship between glucose, cholesterol metabolism and type 2 diabetes[27]. The study by Hao et al.[28] suggested that plasma cholesterol plays a direct role in pancreatic islet dysfunction and may be a key factor underlying the progression of type 2 diabetes. Genetic variants that are associated with the risk of type 2 diabetes have also been found to influence plasma TC levels. Sanghera et al.[29] revealed a significant association between rs10885409 in TCF7L2 with type 2 diabetes and TC levels in Asian Indians. Chen et al.[30] found rs2237895 in KCNQ1, which was thought to be a candidate gene of diabetes that influenced plasma TC levels in the Han Chinese population. They argued that this variant might result in an increased expression of KCNQ1 and a subsequent increase in insulin secretion, which could stimulate lipid synthesis. In this study, we also found that rs2488075 and rs947591 were associated with plasma TC levels. rs2488075 and rs947591 are located downstream of *HHEX*, which may affect metabolic phenotypes. However, the mechanism that allows the CC genotype in rs2488075 and the AA genotype in rs947591 to contribute to lower levels of TC is still unknown.

Our study may be subject to certain limitations. First, the number of subjects in our study was moderate, thus the statistical power was limited. Second, the associations were not strong statistically; none of them passed multiple correction. Third, bias and reverse causation cannot be completely excluded in this observational epidemiological study. A Mendelian randomization approach that uses the random inheritance of genetic variants from parents to offspring may be of benefit in further studies. Thus, larger, well-designed epidemiological studies with ethnically diverse populations are warranted to confirm our findings.

In summary, the results in the current study indicate that genetic variants in the *IDE-KIF11-HHEX* gene cluster at 10q23.33 are associated with plasma lipid levels in the Chinese population. These findings highlight the important correlation between lipid levels and diabetes development at the genetic level. Further studies are needed to replicate our findings in other populations.

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