

Association of a genetic variant of the *ZPR1* zinc finger gene with type 2 diabetes mellitus

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Abstract. Various loci and genes that confer susceptibility to coronary heart disease (CHD) have been identified in Caucasian populations by genome-wide association studies (GWASs). As type 2 diabetes mellitus (DM) is an important risk factor for CHD, we hypothesized that certain polymorphisms may contribute to the genetic susceptibility to CHD through affecting the susceptibility to type 2 DM. The purpose of the present study was to examine a possible association of type 2 DM in Japanese individuals with 29 polymorphisms identified as susceptibility loci for CHD by meta-analyses of the GWASs. The study subjects comprised of 3,757 individuals (1,444 subjects with type 2 DM and 2,313 controls). The polymorphism genotypes were determined by the multiplex bead-based Luminex assay, which combines the polymerase chain reaction and sequence-specific oligonucleotide probes with suspension array technology. To compensate for multiple comparisons of genotypes, the criterion of a false discovery rate (FDR) ≤ 0.05 was adopted for testing the statistical significance of the association. The comparisons of allele frequencies by the χ^2 test revealed that the rs964184 (C→G) of the *ZPR1* zinc finger gene (*ZPR1*) was significantly associated (P=0.0017; FDR=0.050) with type 2 DM. Multivariable logistic regression analysis with adjustment for age, gender

and body mass index revealed that rs964184 of *ZPR1* was significantly associated (P=0.0012; odds ratio, 1.25; dominant model) with type 2 DM with the minor G allele representing a risk factor for this condition. Fasting plasma glucose levels (P=0.0076) and blood glycosylated hemoglobin contents (P=0.0132) significantly differed among *ZPR1* genotypes with the G allele associated with increases in these parameters. *ZPR1* may thus be a susceptibility locus for type 2 DM in Japanese individuals.

Introduction

Type 2 diabetes mellitus (DM) is a major public health issue that affects over one billion people worldwide (1). Type 2 DM is a complex disease that involves genetic and environmental factors and their interactions (2). Due to the high prevalence of type 2 DM, identifying the genes or genetic loci associated with the risk or protection of type 2 DM is important for understanding the mechanisms underlying the disease and for benefiting the patients with personalized prevention and treatment programs.

Genome-wide association studies (GWASs) and subsequent meta-analyses have identified >56 susceptibility loci for type 2 DM (3-11). However, these susceptibility loci have been identified predominantly in Caucasian populations. Differences in allele frequencies and the effect of size among different ethnicity groups yielded the discovery of new loci in different populations (12). Although several single-nucleotide polymorphisms (SNPs) have been identified as susceptibility loci for type 2 DM in Japanese individuals (10,11), the genes that confer susceptibility to this condition remain to be identified definitively.

Previous GWASs have identified various loci and genes that confer susceptibility to coronary heart disease (CHD) for Caucasian populations (13,14). As type 2 DM is an important risk factor for CHD, we hypothesized that certain

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polymorphisms may contribute to the genetic susceptibility to CHD through affecting the susceptibility to type 2 DM. The purpose of the present study was to examine a possible association of type 2 DM in Japanese individuals with 29 SNPs identified as susceptibility loci for CHD by meta-analyses of the GWASs.

Subjects and methods

Study population. The study population comprised of 3,757 Japanese individuals (1,444 subjects with type 2 DM and 2,313 controls) who either visited outpatient clinics or were admitted to the participating hospitals (Gifu Prefectural General Medical Center, Gifu; Gifu Prefectural Tajimi Hospital, Tajimi; Japanese Red Cross Nagoya First Hospital, Nagoya; Inabe General Hospital, Inabe; Hirosaki University Hospital, Reimeikyo Rehabilitation Hospital and Hirosaki Stroke Center, Hirosaki, Japan) between 2002 and 2012 due to various symptoms or for an annual health checkup. Written informed consent was obtained from all the participants and the Institutional Review Board of each participating hospital approved the study.

Type 2 DM is defined according to the criteria of the World Health Organization, as described previously (15,16). Subjects with type 2 DM had a fasting plasma glucose level of ≥ 6.93 mmol/l (126 mg/dl), a blood glycosylated hemoglobin (hemoglobin A1c) content of $\geq 6.5\%$, or were taking anti-diabetic medication. Individuals with type 1 DM, maturity onset diabetes of the young, DM associated with mitochondrial diseases or single-gene disorders, pancreatic diseases or other metabolic or endocrinological diseases were excluded from the study. Individuals on medication that may cause secondary DM were also excluded. The control individuals had a fasting plasma glucose level of < 6.05 mmol/l (110 mg/dl), a blood hemoglobin A1c content of $< 6.2\%$ and had no history of DM or of receiving anti-diabetic medication.

Selection and genotyping of polymorphisms. SNPs that were recently identified as susceptibility loci for CHD in Caucasian populations were searched for by meta-analyses of GWASs (13,14). These SNPs were examined with the dbSNP database (National Center for Biotechnology Information; <http://www.ncbi.nlm.nih.gov/SNP/>) to find SNPs with a minor allele frequency of > 0.015 in a Japanese population. Finally, 29 SNPs (data not shown) were selected and the association with type 2 DM was examined. Wild-type and variant alleles of the SNPs were determined from the original sources.

Venous blood (7 ml) was collected into tubes containing 50 mmol/l ethylenediaminetetraacetic acid (disodium salt) and genomic DNA was isolated with a kit (Genomix; Talent, Trieste, Italy). Genotypes of the 29 SNPs were determined at G&G Science (Fukushima, Japan) by a method that combines polymerase chain reaction and sequence-specific oligonucleotide probes with suspension array technology (Luminex Corporation, Austin, TX, USA). The overall call rate of genotyping of 29 SNPs was 99%. The detailed genotyping methodology was performed as described previously (17).

Statistical analysis. The χ^2 test was used to compare the categorical variables, whereas the Mann-Whitney U test was

used for analysis of the quantitative data. Allele frequencies of each SNP were compared between subjects with type 2 DM and controls by the χ^2 test. A false discovery rate (FDR) was calculated to compensate for multiple comparisons of genotypes, and $FDR \leq 0.05$ was considered to indicate a statistical significance for association. Multivariable logistic regression analysis was performed with type 2 DM as a dependent variable and age, gender (0, women; 1, men), body mass index (BMI) and the genotype of SNP as independent variables. The SNP was assessed according to dominant (the combined group of heterozygotes and variant homozygotes versus wild-type homozygotes), recessive (variant homozygotes versus the combined group of wild-type homozygotes and heterozygotes) and two additive [additive 1 (heterozygotes versus wild-type homozygotes) and additive 2 (variant homozygotes versus wild-type homozygotes)] genetic models. As fasting plasma glucose level and blood hemoglobin A1c content were not normally distributed ($P < 0.01$ by the Kolmogorov-Smirnov Lilliefors test), these parameters were compared among genotypes by the non-parametric Kruskal-Wallis test. Statistical analysis was performed with JMP version 11 and JMP Genomics version 6.0 software (SAS Institute, Cary, NC, USA).

Results

Clinical characteristics of the study subjects. The clinical characteristics of the study subjects are shown in Table I. Age, the frequency of males, BMI, the prevalence of smoking, myocardial infarction, dyslipidemia and hypertension, as well as serum concentrations of triglycerides and creatinine, were higher, whereas the serum concentrations of high-density lipoprotein (HDL) cholesterol were lower in subjects with type 2 DM compared to controls.

Associations of SNPs to type 2 DM. Allele frequencies were compared between subjects with type 2 DM and controls by the χ^2 test and five SNPs with $P < 0.05$ are shown in Table II. Among these SNPs, rs964184 (C-G) of the ZPR1 zinc finger gene (*ZPR1*) was significantly ($FDR \leq 0.05$) associated with the prevalence of type 2 DM. The genotype distributions of five SNPs were in Hardy-Weinberg equilibrium ($P > 0.05$) among subjects with type 2 DM and controls.

Multivariable logistic regression analysis with adjustment for age, gender and BMI revealed that rs964184 of *ZPR1* was significantly associated with type 2 DM in the dominant and additive 1 and 2 models, with the minor G allele representing a risk factor for this condition (Table III). As hypertriglyceridemia is an important risk factor for type 2 DM, additional multivariable logistic regression analysis was performed with adjustment for serum triglycerides concentrations or hypertriglyceridemia (serum concentration of triglycerides ≥ 1.65 mmol/l or taking anti-dyslipidemic medication) in addition to age, gender and BMI (Table III). rs964184 was also significantly associated with type 2 DM in the dominant and additive 1 models in this analysis.

Associations of rs964184 to fasting plasma glucose level and blood hemoglobin A1c content. Finally, the associations of rs964184 genotypes to fasting plasma glucose level and blood

Table I. Characteristics of the subjects with type 2 diabetes mellitus (DM) and controls.

Characteristic	Type 2 DM	Controls	P-value
No. of subjects	1444	2313	
Age, years	65.8±10.1	63.1±11.3	<0.0001
Gender, male/female (%)	67.0/33.0	57.1/42.9	<0.0001
Body mass index, kg/m ²	24.1±3.7	23.6±3.4	0.0005
Current or former smoker, %	30.3	25.5	0.0013
Myocardial infarction, %	66.3	36.5	<0.0001
Dyslipidemia, %	55.7	42.1	<0.0001
Hypertension, %	75.5	56.9	<0.0001
Serum triglycerides, mmol/l	1.79±1.39	1.43±0.87	<0.0001
Serum HDL-cholesterol, mmol/l	1.25±0.35	1.39±0.43	<0.0001
Serum LDL-cholesterol, mmol/l	3.14±1.01	3.10±0.87	0.6136
Serum creatinine, μmol/l	93.8±106.1	84.0±99.1	<0.0001
Fasting plasma glucose, mmol/l	10.46±3.91	4.96±0.64	<0.0001
Blood hemoglobin A1c, %	7.8±2.0	6.0±1.2	<0.0001

Quantitative data are mean ± standard deviation. HDL, high-density lipoprotein; LDL, low-density lipoprotein.

 Table II. Comparison of the single-nucleotide polymorphism (P<0.05) allele frequencies by the χ^2 test between subjects with type 2 diabetes mellitus (DM) and controls.

Variables	Type 2 DM (%)	Controls (%)	P-value (allele)	FDR (allele)
rs964184			0.0017	0.050
CC	728 (50.4)	1289 (55.7)		
CG	601 (41.6)	869 (37.6)		
GG	115 (8.0)	155 (6.7)		
Minor allele frequency	0.29	0.26		
Hardy-Weinberg P	0.5585	0.6024		
rs12190287			0.0101	0.119
CC	472 (32.7)	833 (36.0)		
CG	696 (48.2)	1100 (47.6)		
GG	276 (19.1)	380 (16.4)		
Minor allele frequency	0.43	0.40		
Hardy-Weinberg P	0.4960	0.5996		
rs11556924			0.0123	0.119
CC	1394 (96.5)	2191 (94.7)		
CT	49 (3.4)	121 (5.2)		
TT	1 (0.1)	1 (0)		
Minor allele frequency	0.02	0.03		
Hardy-Weinberg P	0.4044	0.6098		
rs6725887			0.0249	0.179
TT	1413 (98.2)	2280 (99.0)		
TC	26 (1.8)	22 (1.0)		
CC	0 (0)	0 (0)		
Minor allele frequency	0.009	0.005		
Hardy-Weinberg P-value	0.7295	0.8178		
rs2075650			0.0309	0.179
AA	1042 (72.2)	1576 (68.3)		
AG	359 (24.9)	666 (28.9)		
GG	42 (2.9)	65 (2.8)		
Minor allele frequency	0.15	0.17		
Hardy-Weinberg P	0.1051	0.5933		

FDR, false discovery rate.

Table III. Multivariable logistic regression analysis of rs964184 of ZPR1 zinc finger gene and type 2 diabetes mellitus with additional adjustments to age, gender and BMI.

Additional adjustments	Dominant		Recessive		Additive 1		Additive 2	
	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)
None	0.0012	1.25 (1.09-1.43)	0.1134	1.23 (0.95-1.59)	0.0036	1.23 (1.07-1.42)	0.0280	1.35 (1.03-1.75)
Serum concentrations of triglycerides	0.0209	1.18 (1.02-1.35)	0.4105	1.12 (0.86-1.45)	0.0304	1.18 (1.01-1.35)	0.1923	1.20 (0.91-1.57)
Hypertriglyceridemia	0.0101	1.19 (1.04-1.37)	0.2677	1.16 (0.89-1.50)	0.0188	1.19 (1.03-1.37)	0.1047	1.25 (0.95-1.64)

Hypertriglyceridemia was defined as a serum concentration of triglycerides ≥ 1.65 mmol/l or taking anti-dyslipidemic medication. OR, odds ratio; CI, confidence interval; BMI, body mass index.

Table IV. Association of rs964184 of ZPR1 zinc finger gene to fasting plasma glucose level and blood hemoglobin A1c content as determined by the Kruskal-Wallis test.

Parameter	Genotype of rs964184			P-value
	CC	CG	GG	
Fasting plasma glucose, mmol/l	6.9 \pm 3.3	7.1 \pm 3.4 ^a	7.3 \pm 3.8 ^a	0.0076
Blood hemoglobin A1c, %	6.8 \pm 1.8	6.9 \pm 1.7	7.3 \pm 2.2 ^a	0.0132

^aP<0.05 vs. CC.

hemoglobin A1c content were examined by the Kruskal-Wallis test (Table IV). rs964184 was significantly associated with the two parameters and the G allele was associated with the increases in fasting plasma glucose level and in blood hemoglobin A1c content.

Discussion

The associations of 29 SNPs identified as susceptibility loci for CHD by meta-analyses of GWASs to type 2 DM were examined and it was observed that rs964184 of ZPR1 was significantly associated with type 2 DM in Japanese individuals. The prevalence of type 2 DM, fasting plasma glucose level and blood hemoglobin A1c content were increased by 18.0, 6.7 and 7.4%, respectively, for individuals with the GG genotype of rs964184 compared to those with the CC genotype.

rs964184 is located in the intron region of ZPR1 at chromosome 11q23.3. ZPR1 is an essential regulatory protein for cell proliferation and signal transduction and may have multiple physiological functions (18,19). The most relevant transcription factor that binds to the promoter region of ZPR1 is peroxisome proliferator-activated receptor γ , which plays an important role in insulin sensitivity and obesity (20,21). The promoter region of ZPR1 is also bound by hepatocyte nuclear factor 4 α , which activates a variety of genes involved in glucose, fatty acid and cholesterol metabolism (22).

ZPR1 is located ~1.6 kb upstream of the APOA5-A4-C3-A1 gene complex. Previous studies have shown that several polymorphisms in or near APOA5 are significantly associated

with serum triglycerides concentrations (23-26). rs964184 of ZPR1 has been associated with serum triglycerides and this may be attributable to linkage disequilibrium with functional SNPs in APOA5, which influence metabolism of chylomicrons, very-low-density lipoprotein and HDL (27). As an increase in serum triglycerides concentration is an important risk factor for type 2 DM (28), a multivariable logistic regression analysis was performed with adjustment for serum triglycerides levels or hypertriglyceridemia in addition to age, gender and BMI. There was a significant association of rs964184 with type 2 DM in this analysis, indicating that the association was independent, at least in part, of serum triglycerides levels in the study. The previous GWASs suggested that APOA5 polymorphisms may also play an important role in the development of type 2 DM (29,30). A subgroup analysis by ethnicity of a meta-analysis revealed a significant association of the -1131T>C polymorphism of APOA5 with type 2 DM in Asian populations (31). This observation may support the hypothesis that rs964184 of ZPR1 is associated with type 2 DM through the interaction with APOA5 in Japanese individuals.

Although the contribution of rs964184 to the increased susceptibility to type 2 DM was examined in several GWASs mainly with Caucasians, the significant association was not detected (32,33). The reason for the discrepancy between the previous studies and the present results remains unclear. The variations in the minor G allele frequencies due to the ethnic differences may be, at least in part, responsible for this discrepancy. The frequencies of the CG and GG genotypes of rs964184 were 23.7 and 2.4%, respectively, in Caucasian populations (32), whereas in the present study population they were 39.1 and 7.2%, respectively. The G allele of rs964184 was therefore higher in the present population (26.8%) compared to the Caucasian population (13-14%) (23,33,34). In addition, the prevalence of type 2 DM in the present population was 38.2%, which was more than twice that reported previously (32). The higher frequency of the G allele and the higher prevalence of type 2 DM in the present population compared to those in the previous studies (33,34) may increase the statistical power to detect the association of rs964184 with type 2 DM.

In conclusion, the results indicate that rs964184 (C>G) of ZPR1 may be a susceptibility locus for type 2 DM in Japanese individuals. Validation of these findings is required in other independent subject panels or ethnic groups.

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