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SHORT REPORT

Association of serine racemase gene variants with type 2 diabetes in the Chinese Han population

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ABSTRACT

A genome-wide association study in the Chinese Han population has identified several novel genetic variants of the serine racemase (*SRR*) gene in type 2 diabetes. Our purpose was to systematically evaluate the contribution of *SRR* variants in the Chinese Han population. rs391300 and rs4523957 in *SRR* were genotyped respectively in the two independent populations. A meta-analysis was used to estimate the effects of *SRR* in 21,305 Chinese Han individuals. Associations between single-nucleotide polymorphisms and diabetes-related phenotypes were analyzed among 2,615 newly diagnosed type 2 diabetes patients and 5,029 controls. Neither rs391300 nor rs4523957 were associated with type 2 diabetes in populations. Furthermore, meta-analysis did not confirm an association between type 2 diabetes and *SRR*. In the controls, rs391300-A and rs4523957-G were associated with higher 30-min plasma glucose in an oral glucose tolerance test. The present study did not confirm that *SRR* was associated with type 2 diabetes.

INTRODUCTION

Recently, the serine racemases gene (*SRR*) was reported to have a strong association with type 2 diabetes in the first genome wide association study (GWAS) in a Chinese Han population from Taiwan[1]. This gene had not previously been associated with type 2 diabetes in either European or Asian populations, and this GWAS result has not been replicated in other ethnic populations, even in Asian population[2,3]. Furthermore, a subsequent meta-analysis of GWAS for type 2 diabetes in east Asians, which combined five different GWAS populations (including Chinese, Korean, Malay, Japanese and Filipino) also reported that rs391300 was not associated with type 2 diabetes[4]. Thus, it can be speculated that the compound genetic backgrounds of different ethnic populations might have masked the real association signal.

In the present study, we carried out a case–control study with a total of 10,204 participants to determine whether the two representative *SRR* single-nucleotide polymorphisms (SNPs) are associated with increased risk of type 2 diabetes in the Chinese Han population.

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MATERIALS AND METHODS

Two Populations From Two Independent Studies

A preliminary study was carried out in a total of 773 individuals residing in Beijing (population 1) from the cross-sectional study originally led by the Peking University People's Hospital. Type 2 diabetes cases were identified based on the 1999 World Health Organization criteria. The control individuals were recruited based on the following criteria: (i) fasting plasma glucose <6.1 mmol/L and 2-h plasma glucose <7.8 mmol/L; (ii) no family history of type 2 diabetes.

Population 2 was selected from the China National Diabetes and Metabolic Disorders Study (CNDMDS). The details of the study have been published previously[5]. The control subjects were selected based on the following criteria: (i) without family history of diabetes; (ii) ≥40 years-of-age; (iii) oral glucose tolerance test (OGTT) fasting glucose <6.1 and 2-h plasma glucose <7.8; (iv) serum triglyceride <1.7 mmol/L or high density lipoprotein <1.0 mmol/L; and (v) without history of cancer, thyroid disease and rheumatic disease. In total, 4,651 controls and 5,029 type 2 diabetes patients were enrolled. A total of 2,615 individuals newly diagnosed with type 2 diabetes by the OGTT without insulin or antidiabetic drugs therapy before carried out as part of the present study were selected for analysis.

Anthropometric and Biochemical Measurements

We collected detailed information of both study populations. Blood pressure, bodyweight, height and waist circumference were measured as previously described[6]. The glucose and insulin levels at 0, 30 and 120 min were measured by the OGTT in participants without a history of diabetes.

The homeostasis model assessment (HOMA) was used to assess β-cell function (HOMA-β) and insulin resistance (HOMA-IR)[7]. Insulin secretion (ΔI/G30) was measured as the ratio of the 30-min increment in insulin concentration to the 30-min increment in glucose concentration following oral glucose loading[8].

SNP Genotyping

In population 1, the MassARRAY iPLEX system (MassARRAY Compact Analyzer, Sequenom, San Diego, CA, USA) was used to genotype for rs391300 and rs4523957. In the CNDMD study, genotyping for SNPs was based on an Illumina GoldenGate Indexing assay.

Meta-Analysis

We searched Pubmed and the China National Knowledge Infrastructure database for relevant papers published in English or Chinese up to December 2012. Search key words included: type 2 diabetes, SRR, Chinese, GWAS. All the studies included were case–control studies of SRR-rs391300 in the Chinese Han population. We excluded studies without sufficient sample sizes of the type 2 diabetes or allele frequencies. The meta-analysis was carried out by STATA 11.0 (StateCorp LP, Lakeway Drive, College Station, TX, USA) using an additive genetic model.

Statistical Analyses

Normally distributed data were expressed as mean values ± standard deviations. Non-normally distributed variables were expressed as the median (quartile range). For each SNP, unconditional logistic regression was used to estimate the odd ratios (ORs) and 95% confidence intervals (CIs) of diabetes risk in dominant, recessive, and additive models. Adjustments were made for multiple confounding variables including sex, age and body mass index (BMI). In normal glucose tolerant and newly diagnosed diabetic populations, associations between genotype and diabetes-related phenotype (such as BMI) were examined by multiple linear regression in an additive model. A two-

Table 1 | Single-nucleotide polymorphism association with type 2 diabetes in population 1 from Beijing

SNP	Genotyping (AA/Aa/aa)		OR (95% CI)	*P**
	Case	Control		
rs391300	121/107/21	258/217/49	0.92 (0.71–1.20)	0.54
rs4523957	100/78/19	219/141/27	1.11 (0.81–1.51)	0.52

A, the major allele; a, the minor allele; SNP, single-nucleotide polymorphism. **P*-values are adjusted for age, sex and body mass index.

Table 2 | Single-nucleotide polymorphism association with type 2 diabetes in the China National Diabetes and Metabolic Disorders Study by logistic regression analysis

SNP	Case				Control				ADD		REC		DOM	
	AA	Aa	aa	MAF	AA	Aa	aa	MAF	OR (95% CI)	*P**	OR (95% CI)	*P**	OR (95% CI)	*P**
rs391300	2063	1754	347	0.29	2284	1840	354	0.29	0.98 (0.91–1.06)	0.74	1.04 (0.87–1.25)	0.63	0.96 (0.87–1.06)	0.49
rs4523957	2156	1846	338	0.29	2384	1963	373	0.29	0.99 (0.92–1.07)	0.93	1.04 (0.87–1.25)	0.62	0.98 (0.89–1.08)	0.70

A, major allele; a, minor allele; ADD, genotypic additive; CI, confidence interval; DOM, genotypic dominant; MAF, minor allele frequency; OR, odds ratio; REC, genotypic recessive. *Adjusted for age, sex and body mass index.

Table 3 | Meta-analysis of serine racemase rs391300 among Chinese Han populations

Author	Year	*n*		A allele frequency		OR	95% CI	Weight (%)	References
		Case	Control	Case	Control				
Zhang	Current study	249	524	0.30	0.30	0.998	0.791–1.260	12.09	
Zhang	Current study	4651	5029	0.29	0.29	1.014	0.953–1.080	20.02	
Li	2012	798	659	0.27	0.27	1.000	0.848–1.178	19.31	[9]
Cui	2011	698	803	0.28	0.29	0.977	0.833–1.145	15.39	[2]
Shu	2010	1019	1710	0.29	0.28	1.047	0.927–1.182	15.66	[3]
Tsai	2010	2798	2367	0.31	0.37	0.765	0.705–1.830	17.54	[1]
					Pooled OR	0.956	0.845–1.082	100	

There was an extreme heterogeneity ($P = 0.0008$, $I^2 = 85.4\%$) for meta-analysis for six studies. The odds ratio (OR) was calculated in a random model. CI, confidence interval.

Table 4 | Association between quantitative phenotypes and single-nucleotide polymorphisms in a normal glucose tolerant population

	rs4523957				*P**	rs391300				*P**
	GG	GT	TT	β		AA	AG	GG	β	
n (male/female)	v338 (96/242)	1846 (594/1252)	2156 (715/1441)			347 (101/246)	1754 (569/1185)	2063 (677/1386)		
Age	50.4 ± 8.2	50.8 ± 8.3	50.6 ± 8.5		0.64	50.6 ± 8.3	50.8 ± 8.2	50.5 ± 8.3		0.68
BMI*	22.97 ± 2.42	23.03 ± 2.42	23.06 ± 2.45	–0.04	0.49	22.97 ± 2.45	23.03 ± 2.42	23.06 ± 2.45	–0.04	0.46
FPG	5.04 ± 0.49	5.02 ± 0.53	5.00 ± 0.51	0.02	0.12	5.04 ± 0.49	5.01 ± 0.53	5.00 ± 0.51	0.02	0.13
30 m-PG	**8.33 ± 1.74**	**8.15 ± 1.74**	**8.11 ± 1.71**	**0.04**	**0.04**	**8.35 ± 1.67**	**8.15 ± 1.76**	**8.10 ± 1.71**	**0.01**	**0.02**
2hPG	5.73 ± 1.17	5.71 ± 1.10	5.72 ± 1.09	–0.01	0.69	5.78 ± 1.15	5.69 ± 1.10	5.72 ± 1.10	0.003	0.92
Fins	6.33 (4.85–8.59)	6.40 (5.00–8.54)	6.38 (5.00–8.40)	–0.003	0.78	6.33 (4.87–8.38)	6.40 (5.00–8.57)	6.46 (5.00–8.45)	–0.01	0.55
30 min-Ins	32.9 (20.82–55)	34.16 (22.61–53.11)	33.34 (21.81–52.07)	0.01	0.44	33.14 (22.02–52.79)	34.44 (23.19–53.32)	33.57 (22.02–52.79)	0.02	0.30
2 h-Ins	22.85 (13.88–34.01)	22.53 (14.2–34.78)	22.22 (14.65–34.57)	0.004	0.80	23.45 (14.28–34.69)	22.65 (14.33–35.22)	22.09 (14.52–34.47)	0.02	0.19
HOMA-B	84.78 (62.29–120.18)	86.22 (61.56–125.21)	85.55 (62.87–123.39)	–0.01	0.48	82.21 (61.81–120.37)	87.17 (62.15–124.12)	85.58 (62.88–124.62)	–0.02	0.24
HOMA-IR	1.41 (1.07–1.96)	1.44 (1.11–1.92)	1.42 (1.08–1.88)	0.001	0.95	1.41 (1.08–1.90)	1.44 (1.10–1.93)	1.44 (1.08–1.90)	–0.002	0.84
ΔI/G30†	8.89 (4.77–17.49)	9.65 (5.03–17.79)	9.46 (5.26–16.74)	–0.06	0.70	8.92 (4.98–17.49)	9.87 (5.19–17.76)	9.61 (5.17–16.97)	–0.01	0.94

*Adjusted for age, sex and body mass index (BMI), except BMI was analyzed after adjusted for sex, age. Fins, 30min-Ins, 2h-Ins, HOMA-B, HOMA-IR were natural logarithm-transformed to normal distributions before statistical analysis. †Insulin glucose ratio (ΔI30/G30) = (30 min insulin − fasting insulin)/(30 min glucose − fasting glucose). Fins, fasting serums insulin level; FPG, fasting plasma glucose; PG, plasma glucose. The bold values indicated the *p* value<0.05, which means the genotypes are associated with the 30m PG.

sided *P*-value <0.05 was considered significant. All data analyses were carried out using PLINK 1.07 (http://pngu.mgh.harvard.edu/~purcell/plink/) and SPSS Statistics for Windows, Version 17.0 (SPSS Inc. Chicago, IL, USA).

RESULTS

Association Between Genotype and Type 2 Diabetes Among Populations From Beijing and the CNDMDS

The basic characteristics of population 1 are shown in Table S1. As shown in Table 1, these two SNPs (rs391300 and rs4523957) were not associated with diabetes risk in population 1 after adjustment for age, sex and BMI in an additive model (P = 0.54/0.52, respectively).

To further study the relationship between *SRR* and type 2 diabetes, we replicated the protocol in an independent population from the CNDMDS. The basic characteristics of this population by status are shown in Table S2. In accordance with previous research, no association between these two SNPs and type 2 diabetes was observed after adjustments for age, sex, and BMI (Table 2).

Meta-Analysis of the Data From Four GWAS and the Two Current Studies

Six potentially suitable studies were identified for meta-analysis[1–4,9,10], two of which were excluded because of atypical populations[10] or the lack of detailed data[4]. The remaining four studies plus two carried out by ourselves were included in the final meta-analysis. The pooled OR was 0.956 for the Chinese Han population. However, there was an extreme heterogeneity, which was lost after exclusion of the Tsai study (P = 0.97, I^2 = 0%); the pooled OR was 1.014. This meta-analysis did not confirm an association of the A allele with type 2 diabetes (Table 3).

Association Between Quantitative Phenotypes and SNPs

We examine whether these risk alleles affected quantitative phenotypes related with type 2 diabetes in normal glucose tolerant (Table 4) and newly diagnosed diabetes populations (Table S3). We observed that in normal glucose tolerant individuals, risk allele G of rs4523957 and T of rs391300 were significantly associated with higher 30-min plasma glucose in OGTT in an additive model (P = 0.04, P = 0.02, respectively).

DISCUSSION

Overall, the results of the present study did not support those of other GWAS carried out in type 2 diabetes patients in the Chinese Han population of Taiwan. We also carried out a meta-analysis including four GWA studies and two of our own studies, the extreme heterogeneity of the analysis appeared to be as a result of the Taiwan study. In the original Taiwan GWAS, the minor allele frequency of controls in Taiwan (rs391300-A 0.37) was significantly different from that in the present study (rs391300-A 0.30). We believe this difference might lead to the heterogeneity in our meta-analysis. In the Taiwan study, the con-

trols were obtained from the Han Chinese Cell and Genome Bank, who were diagnosed according to glycated hemoglobin instead of OGTT[11]. It is possible that the patients with type 2 diabetes were mistakenly included in the control group[12]. In addition, the Taiwan study did not provide any information about comorbidities as ours did. These could have contributed to a higher minor allele frequency in controls in the Taiwan study.

In the present study, the marginal association between two SNPs and the 30-min plasma glucose levels in OGTT was observed among normal glucose tolerant individuals. However, this was not replicated in the newly diagnosed diabetic patients. In fact, the patients with newly diagnosed diabetes had different β-cell function, because the exact onset age of diabetes cannot be determined (or was unknown). The different β-cell dysfunction might have distorted relationship between SNPs and plasma glucose level. It is worth noting that the associated allele was not consistent with the risk allele for diabetes originally reported in the Taiwan study. Furthermore, a recent study that included up to 10 million individuals of European ancestry without type 2 diabetes did not find this locus associated with plasma glucose concentration in non-diabetic individuals[13]. Thus, it is possible that the association between the SNPs and 30-mins glucose level is false positive (type 1 error). Therefore, further research is required to clarify the contribution to the development of diabetes.

In conclusion, the present study shows that the genetic variants of SRR are not associated with type 2 diabetes. This hypothesis should be confirmed in a large study with sufficient statistical power.

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CONFLICTS OF INTEREST

There are no conflicts of interest.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1 | Clinical characteristics of study population 1 from Beijing.
Table S2 | Clinical characteristics of participants from the China National Diabetes and Metabolic Disorders Study.
Table S3 | Association between quantitative phenotypes and single-nucleotide polymorphisms in newly diagnosed diabetes.