SHORT COMMUNICATION

The E23K variation in the *KCNJ11* gene is associated with type 2 diabetes in Chinese and East Asian population

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The genes (*ABCC8* and *KCNJ11*) have a key role in glucose-stimulated insulin secretion and thus have always been considered as excellent susceptibility candidates for involvement in type 2 diabetes. Common polymorphisms (*KCNJ11* E23K and *ABCC8* exon16-3t/c) in these genes have been reported to be associated with type 2 diabetes in various European-descent populations. However, there were inconsistent results in previous studies in East Asian populations and no large case-control studies have been carried out in the Chinese Han population. In this study, these two variants were genotyped in about 4000 Chinese by using TaqMan technology on an ABI7900 system. A meta-analysis was also used to assess the results of association between the two variants and type 2 diabetes in East Asian populations. Our investigation confirmed the association between the *KCNJ11* E23K variant and type 2 diabetes under a recessive model (KK vs EK+EE) in the Chinese Han population (odds ratio (OR)=1.25, 95% confidence interval (95% CI) 1.04–1.50, $P=0.017$). The meta-analysis of East Asian populations also showed a strong significant association of the K allele with diabetes (OR=1.15, $P=3\times 10^{-9}$), whereas the exon16-3t/c variant (rs1799854) in *ABCC8* showed no significant association. Thus, the common E23K variant is considered as a strong candidate for type 2 diabetes susceptibility across different ethnicities.

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Type 2 diabetes is a polygenic disorder characterized by defects in insulin secretion and peripheral insulin resistance. The pancreatic β -cell adenosine triphosphate (ATP)-sensitive potassium channel (KATP), which is composed of Kir6.2 subunits (the *KCNJ11* gene) and Sur1 subunits (the *ABCC8* gene), has a central role in the regulation of glucose-induced insulin secretion by linking signals derived from glucose metabolism to cell membrane depolarization and insulin exocytosis.¹ Mutations in both genes can cause familial persistent hyperinsulinemic hypoglycemia in infancy and permanent neonatal diabetes. The *KCNJ11* and *ABCC8* genes are located at the same chromosome locus, 11p15.1, and are only 4.5 kb apart. Moreover, the strong linkage disequilibrium (LD) across the *KCNJ11* gene also extends into the *ABCC8* gene. Two representative common variants (*KCNJ11* E23K and *ABCC8* exon16-3t/c), which were in different LD blocks, have also been reported to be associated with type 2 diabetes in various ethnic populations, with fairly consistent results for the *KCNJ11* E23K variant and conflicting results for the *ABCC8* exon16-3t/c variant.^{1–4} However, a large-scale association

study of these genes has not been carried out in the Chinese Han population; and there was much inconsistency in results in East Asian studies before.^{5–9}

In this study, 1912 unrelated type 2 diabetes patients and 2041 normal controls (Supplementary methods) were recruited from Shanghai, China, by measuring their clinical characteristics (Supplementary Table 1). Both the SNPs (single nucleotide polymorphisms) mentioned above were genotyped by TaqMan SNP Genotyping Assays on an ABI7900 system (Applied Biosystems, Foster City, CA, USA). The genotyping success rates were above 98% and the concordance rates were 100% in duplicate samples. We used SHEsis¹⁰ (<http://analysis.bio-x.cn/myAnalysis.php>) to perform Hardy–Weinberg Equilibrium (HWE) tests. Logistic regression analysis and multiple linear regression analysis were performed on SPSS/Win programs (SPSS, Chicago, IL, USA) to evaluate the effects of the variants on type 2 diabetes and diabetes-related metabolic traits (Supplementary methods). In addition, a meta-analysis was used to evaluate the influence of the two variants in East Asian populations. We identified studies for the

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Table 1 Association study of rs1799854 and rs5219 variants and the risk of type 2 diabetes in the Chinese Han population

Table 1. Allelic and genotypic frequencies of SNPs in the Exon 16-3t/c (rs1799854) and E23K (rs5219) SNPs in the ABCC8 and KCNJ11 genes, respectively, in the controls and cases.											
			Genotype distribution		Risk-allele Freq.		P-value ^a				
SNPs	Genes	Risk allele		Controls n	Cases n	Controls (%)	Cases (%)	Allelic OR (95% CI)	Additive model OR (95% CI)	Dominant model OR (95% CI)	Recessive model OR (95% CI)
Exon 16-3t/c (rs1799854)	ABCC8	T	TT	704	689						
			CT	947	837	59.7	60.8	1.05 (0.95–1.15)	1.07 (0.97–1.18)	1.00 (0.84–1.21)	1.15 (1.00–1.32)
			CC	322	297			<i>P</i> =0.341	<i>P</i> _{add} =0.173	<i>P</i> _{dom} =0.961	<i>P</i> _{rec} =0.052
E23K (rs5219)	KCNJ11	K	KK	288	329						
			EK	930	863	39.4	41.2	1.08 (0.98–1.18)	1.09 (0.99–1.20)	1.05 (0.91–1.21)	1.25 (1.04–1.50)
			EE	692	656			<i>P</i> =0.127	<i>P</i> _{add} =0.088	<i>P</i> _{dom} =0.529	<i>P</i> _{rec} =0.017 PAR ^b =6.2%

Abbreviations: BMI, body mass index; CI, confidence interval; EE, homozygous for E allele; EK, heterozygous for K allele; Freq., frequency; KK, homozygous for K allele; OR, odds ratio; PAR, population attributable risk; SNP, single nucleotide polymorphism.

The association of SNPs with type 2 diabetes was assessed by logistic regression with adjustment for covariates. For regression modeling in the additive model, homozygotes for the major allele (1/1), heterozygotes (1/2), and homozygotes for the minor allele (2/2) were coded to a continuous numeric variable for genotype (0, 1, and 2). The dominant model was defined as 1/1+1/2 vs 2/2 and the recessive model as 1/1 vs 1/2+2/2.

^aAdjusted for age, gender and BMI.

^bPAR=(X-1)/X, assuming the multiplicative model where $X=(1-f)^2+2f(1-f)\gamma+f^2\gamma^2$; γ is the estimated OR and f the frequency of the risk.

meta-analysis by searching in MEDLINE (<http://www.ncbi.nlm.nih.gov/>) and conducted the meta-analysis using Comprehensive Meta Analysis software (Version 2.0, BIOSTAT, Englewood, NJ, USA) (Supplementary Methods).

The allele and genotype distributions are summarized in Table 1. Both variants were in HWE. The LD coefficients (D' and r^2) between the two variants were 0.110 and 0.005, respectively, which were consistent with those of previous studies. rs1799854 showed no association, whereas rs5219 showed a significant association with type 2 diabetes in the recessive model (KK vs EK+EE; odds ratio (OR) 1.25, 95% confidence interval (95% CI) 1.04–1.50, $P=0.017$), with age, sex and body mass index adjusted. In addition, clinical and biochemical parameters did not show any difference among the different genotypes of the rs1799854 and rs5219 variants (Supplementary Table 2). For meta-analysis, there was no significant heterogeneity (Cochran's Q -test) and publication bias (Egger's test) for rs5219 ($P=0.41$ and $P=0.15$, respectively) and rs1799854 ($P=0.92$ and $P=0.90$, respectively). For meta-analysis of the *KCNJ11* E23K variant, all eight studies^{5–9,11,12} included 7874 cases and 7629 controls and showed a strong significant association (fixed OR 1.15, 95% CI 1.10–1.21, $P=3\times 10^{-9}$, Figure 1a). For the *ABCC8* exon 16-3t/c variant, all five studies^{7,9,13,14} included 4651 cases and 4394 controls and showed no association with type 2 diabetes (fixed OR 1.05, 95% CI 0.99–1.12, $P=0.080$, Figure 1b).

In this study, two variants (rs5219 and rs1799854) that were researched fully in European-descent populations were analyzed in a large Chinese population (1912 patients with type 2 diabetes and 2041 normal controls). For a disease susceptibility allele frequency of 0.4, our sample had >99% power to detect the OR of a susceptibility gene in the range of 1.15–1.20. Furthermore, our meta-analysis study confirmed the association between the E23K variant and type 2 diabetes in the East Asian population, with an allelic OR of 1.15, and this result was significantly consistent with that in previous association studies and in several genome-wide association studies.^{2,15,16} The *KCNJ11* E23K variant tends to exert similar effects on type 2 diabetes and seems to be fairly common in East Asians (the frequency of the risk K allele was 39.4% in our Chinese Han population). Thus, this polymorphism is considered to be a strong

Figure 1 Meta-analysis of the association between the two variants and type 2 diabetes in an East Asian population. (a) *KCNJ11* E23K (rs5219) variant; (b) *ABCC8* exon 16-3t/c (rs1799854) variant. Forest plots of allelic odds ratio (OR) and overall allelic OR with 95% confidence interval (95% CI) for both variants under the fixed-effects model. Unshaded black squares indicate the allelic OR, with the size of the square inversely proportional to its variance, and horizontal lines represent 95% CIs. The pooled results are indicated by the black diamond.

candidate variant for type 2 diabetes worldwide, although its mechanism is not completely clear. The *KCNJ11* and *ABCC8* genes form the pancreatic β -cell KATP channel and have an effect on β -cell function. However, we did not investigate the traits of insulin release and insulin action in this study. Otherwise, these investigations could help us to understand the roles of the *KCNJ11* and *ABCC8* genes in the pathogenesis of type 2 diabetes.

Common polymorphisms in *KCNJ11* were detected to be in a strong LD block and the block also extended to the *ABCC8* gene in several previous studies; for instance, both *ABCC8* S1368A (rs757110) and *KCNJ11* rs5215 were in a high level of LD ($r^2 > 0.8$) with the E23K variant.^{5,7,9} In addition, rs1799854 is located in the 3'-splice site of the *ABCC8* gene and can impair normal splicing. In this study, it is not possible to exclude the *ABCC8* gene as a susceptibility gene involved in type 2 diabetes, even though no association was detected for rs1799854 in the East Asian population. To explore the effects of the *ABCC8* gene on type 2 diabetes, more tag SNPs need to be selected in further studies.

In summary, both the case-control study in the largest Chinese population so far and the meta-analysis study of East Asian populations confirmed that the *KCNJ11* E23K variant was associated with type 2 diabetes. In addition, the *KCNJ11* E23K variant is a common polymorphism worldwide and each copy of the susceptibility allele has a modest effect (OR ~ 1.15) on type 2 diabetes.

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1 Parikh, H. & Groop, L. Candidate genes for type 2 diabetes. *Rev. Endocr. Metab. Disord.* **5**, 151–176 (2004).

- 2 Prokopenko, I., McCarthy, M. I. & Lindgren, C. M. Type 2 diabetes: new genes, new understanding. *Trends Genet.* **24**, 613–621 (2008).
- 3 van Dam, R. M., Hoebee, B., Seidell, J. C., Schaap, M. M., de Bruin, T. W. & Feskens, E. J. Common variants in the ATP-sensitive K⁺ channel genes *KCNJ11* (Kir6.2) and *ABCC8* (SUR1) in relation to glucose intolerance: population-based studies and meta-analyses. *Diabet. Med.* **22**, 590–598 (2005).
- 4 Gloyn, A. L., Weedon, M. N., Owen, K. R., Turner, M. J., Knight, B. A., Hitman, G. *et al.* Large-scale association studies of variants in genes encoding the pancreatic beta-cell KATP channel subunits Kir6.2 (*KCNJ11*) and SUR1 (*ABCC8*) confirm that the *KCNJ11* E23K variant is associated with type 2 diabetes. *Diabetes* **52**, 568–572 (2003).
- 5 Koo, B. K., Cho, Y. M., Park, B. L., Cheong, H. S., Shin, H. D., Jang, H. C. *et al.* Polymorphisms of *KCNJ11* (Kir6.2 gene) are associated with Type 2 diabetes and hypertension in the Korean population. *Diabet. Med.* **24**, 178–186 (2007).
- 6 Omori, S., Tanaka, Y., Takahashi, A., Hirose, H., Kashiwagi, A., Kaku, K. *et al.* Association of CDKAL1, IGF2BP2, CDKN2A/B, HHEX, SLC30A8 and *KCNJ11* with susceptibility to type 2 diabetes in a Japanese population. *Diabetes* **57**, 791–795 (2008).
- 7 Sakamoto, Y., Inoue, H., Keshavarz, P., Miyawaki, K., Yamaguchi, Y., Moritani, M. *et al.* SNPs in the *KCNJ11*-*ABCC8* gene locus are associated with type 2 diabetes and blood pressure levels in the Japanese population. *J. Hum. Genet.* **52**, 781–793 (2007).
- 8 Tabara, Y., Osawa, H., Kawamoto, R., Onuma, H., Shimizu, I., Miki, T. *et al.* Replication study of candidate genes associated with type 2 diabetes based on genome-wide screening. *Diabetes* **58**, 493–498 (2009).
- 9 Yokoi, N., Kanamori, M., Horikawa, Y., Takeda, J., Sanke, T., Furuta, H. *et al.* Association studies of variants in the genes involved in pancreatic beta-cell function in type 2 diabetes in Japanese subjects. *Diabetes* **55**, 2379–2386 (2006).
- 10 Shi, Y. Y. & He, L. SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. *Cell Res.* **15**, 97–98 (2005).
- 11 Doi, Y., Kubo, M., Ninomiya, T., Yonemoto, K., Iwase, M., Arima, H. *et al.* Impact of Kir6.2 E23K polymorphism on the development of type 2 diabetes in a general Japanese population: The Hisayama Study. *Diabetes* **56**, 2829–2833 (2007).
- 12 Yamada, Y., Kuroe, A., Li, Q., Someya, Y., Kubota, A., Ihara, Y. *et al.* Genomic variation in pancreatic ion channel genes in Japanese type 2 diabetic patients. *Diabetes Metab. Res. Rev.* **17**, 213–216 (2001).
- 13 Ishiyama-Shigemoto, S., Yamada, K., Yuan, X., Koyama, W. & Nonaka, K. Clinical characterization of polymorphisms in the sulphonylurea receptor 1 gene in Japanese subjects with type 2 diabetes mellitus. *Diabet. Med.* **15**, 826–829 (1998).
- 14 Ohta, Y., Tanizawa, Y., Inoue, H., Hosaka, T., Ueda, K., Matsutani, A. *et al.* Identification and functional analysis of sulfonylurea receptor 1 variants in Japanese patients with NIDDM. *Diabetes* **47**, 476–481 (1998).
- 15 WTCCC. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* **447**, 661–678 (2007).
- 16 Scott, L. J., Mohlke, K. L., Bonnycastle, L. L., Willer, C. J., Li, Y., Duren, W. L. *et al.* A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. *Science (New York, NY)* **316**, 1341–1345 (2007).

Supplementary Information accompanies the paper on Journal of Human Genetics website (<http://www.nature.com/jhg>)