OMICS A Journal of Integrative Biology
Volume 19, Number 4, 2015

DOI: 10.1089/omi.2014.0162

The Uyghur Population and Genetic Susceptibility to Type 2 Diabetes: Potential Role for Variants in *CDKAL1*, *JAZF1*, and *IGF1* Genes

Manshu Song,[1,2,§] Feifei Zhao,[1,2,§] Longjin Ran,[3,§] Mamatyusupu Dolikun,[4] Lijuan Wu,[1,2] Siqi Ge,[1,2] Hao Dong,[1,2] Qing Gao,[1,2] Yanchun Zhai,[1,2] Ling Zhang,[1,2] Yuxiang Yan,[1,2] Fen Liu,[1,2] Xinghua Yang,[1,2] Xiuhua Guo,[1,2] Youxin Wang,[1,2,5] and Wei Wang,[1,2,5]

Abstract

Substantial evidence suggests that type 2 diabetes mellitus (T2DM) is a multi-factorial disease with a strong genetic component. A list of genetic susceptibility loci in populations of European and Asian ancestry has been established in the literature. Little is known on the inter-ethnic contribution of such established functional polymorphic variants. We performed a case-control study to explore the genetic susceptibility of 16 selected T2DM-related SNPs in a cohort of 102 Uyghur objects (51 cases and 51 controls). Three of the 16 SNPs showed significant association with T2DM in the Uyghur population. There were significant differences between the T2DM and control groups in frequencies of the risk allelic distributions of rs7754840 (*CDKAL1*) ($p=0.014$), rs864745 (*JAZF1*) ($p=0.032$), and rs35767 (*IGF1*) ($p=0.044$). Carriers of rs7754840-C, rs35767-A, and rs864745-C risk alleles had a 2.32-fold [OR (95% CI): 1.19–4.54], 2.06-fold [OR (95% CI): 1.02–4.17], 0.48-fold [OR (95% CI): 0.24–0.94] increased risk for T2DM, respectively. The cumulative risk allelic scores of these 16 SNPs differed significantly between the T2DM patients and the controls [17.1 ± 8.1 vs. 15.4 ± 7.3; OR (95%CI): 1.27(1.07–1.50), $p=0.007$]. This is the first study to evaluate genomic variation at 16 SNPs in respective T2DM candidate genes for the Uyghur population compared with other ethnic groups. The SNP rs7754840 in *CDKAL1*, rs864745 in *JAZF1,* and rs35767 in *IGF1* might serve as potential susceptibility loci for T2DM in Uyghurs. We suggest a broader capture and study of the world populations, including who that are hitherto understudied, are essential for a comprehensive understanding of the genetic/genomic basis of T2DM.

Introduction

Type 2 diabetes mellitus (T2DM) is a complex disease characterized by insulin resistance in peripheral tissues and dysregulated insulin secretion by pancreatic beta cells (Banerjee et al., 2014). Substantial evidence suggests that T2DM is a multi-factorial disease with a strong genetic component. High concordance rates obtained in monozygotic twins (96%) support a substantial contribution of genetic factors to T2DM (Barnett et al., 1981; Newman et al., 1987). Furthermore, 40% of first-degree relatives of T2DM patients develop diabetes as compared to 6% in the general population (KoÈbberling and Tillil, 1982). The general estimates of heritability (h^2) of T2DM is 0.49 and the relative recurrence risk for a sibling of an affected person (λ_s) to develop T2DM is 3.5 (Lander and Schork, 1994; Risch, 1990).

Multiple genetic loci have been discovered as risk factors for T2DM, most of which were detected from genome-wide association studies (GWAS) in populations of European and Asian ancestry (Ahlqvist et al., 2011; Saxena et al., 2007; Scott et al., 2007; Sladek et al., 2007; Zeggini et al., 2008), for example, loci near cyclin-dependent kinase 5 (*CDK5*), regulatory subunit-associated protein 1-like 1 (*CDKAL1*) (Saxena et al., 2007), peroxisome proliferator-activated receptor gamma

[1]School of Public Health, Capital Medical University, Beijing, China.
[2]Municipal Key Laboratory of Clinical Epidemiology, Beijing, China.
[3]College of Life Sciences, University of Chinese Academy of Sciences, Beijing, China.
[4]College of the Life Sciences and Technology, Xinjiang University, Urumqi, China.
[5]School of Medical Sciences, Edith Cowan University, Joondalup, Australia.
[§]These authors contributed equally to this work.

(*PPARG*) (Saxena et al., 2007), and juxtaposed with another zinc finger 1 (*JAZF1*) (Zeggini et al., 2008). Most of these loci are related to insulin secretion and beta-cell function, while a few are involved in insulin resistance (Ahlqvist et al., 2011; Billings and Florez, 2010). To date, more than 40 genetic loci have been reported and reproduced to be associated with T2DM or glycemic traits in Caucasian and Asian populations (Ahlqvist et al., 2011).

There are differences in the contribution of known SNPs in T2DM susceptibility genes among various ethnic populations (Klimentidis et al., 2011). SNP rs7754840 in *CDKAL1*, identified as T2DM susceptibility locus in subjects from Finland and Sweden by GWAS (Saxena et al., 2007), was replicated in Koreans (Lee et al., 2008), Han Chinese (Hu et al., 2009), Pima Indians (Rong et al., 2009), and Lebanese Arabs (Nemr et al., 2012), but showed inconsistency in risk allele frequencies and odds ratios (ORs) among these ethnic populations. SNP rs864745 in *JAZF1* was associated with T2DM in European populations (Zeggini et al., 2008), whereas it was not found to be associated with T2DM in Chinese (Hu et al., 2009). Therefore, studies in other ethnic populations could aid in determining population-specific risk variants for T2DM.

The Uyghur population, settled in Xinjiang Uyghur autonomous region, northwestern frontier area of China, accounts for 46% of the population in Xinjiang (Han Chinese accounts for 40% and Kazak accounts for 7%). It is a population presenting a typical admixture of Eastern and Western anthropometric traits (Black et al., 2006; Wang et al., 2003). Uyghurs are overwhelmingly Muslim, and have their own language, religious beliefs, and lifestyles that are very different from either Han Chinese or American/European populations. Taking dietary style as an example, the Uyghur has a high dairy intake level (over 200 g) with more flour and meat, and less bean, vegetable, and fishery products compared with Han Chinese living in the same area (Zhai et al., 2007).

The prevalence of diabetes in Uyghur was higher than other ethnic groups in Xinjiang. Tao et al. (2008) reported that the prevalence of T2DM was 8.16% in Uyghur, which was higher than 1.47% in Kazak population. Awuti et al. (2012) reported that the prevalence of T2DM for Uyghur adults was 9.5%. In addition, a cross-sectional study in Xinjiang found that 19.6% of Uyghur had diabetes, exceptionally higher than that in Han Chinese (9.1%) and Kazakh (7.3%) (Li et al., 2012). 6.23% of the Uyghur adults over 35 years old had diabetes, while 3.65% of Kazak adults had diabetes (Yang et al., 2012). For children under 17 years old, 0.77% of the Uyghur suffered from impaired fasting glucose (IFG) and diabetes, and that was 0.1% in Kazak (Zhang et al., 2012).

Up to date, there have been only two reports on association of genetics makers with T2DM in Uyghur. The G allele of adiponectin gene carriers with reduced plasma concentrations of adiponectin might be associated with insulin resistance in Uyghur (Li et al., 2007). In addition, peroxisome proliferator-activated receptor (PPAR)-gamma Pro12Ala polymorphism might affect susceptibility to diabetes in Uyghur (Li et al., 2008).

In the present case-control study, we investigated the associations between 16 SNPs susceptibility loci and T2DM, as well as the combined effects of the SNPs on the risk of T2DM in a Uyghur population.

Materials and Methods

Participants

A sample of 102 Uyghur participants was recruited from the Uyghur population at Hetian of Xinjiang, China, where the Uyghur population was less affected by the recent migration of Han Chinese. Fifty-one T2DM patients (25 men and 26 women, 54.1 ± 7.9 years old) were recruited from the local hospital as the T2DM group. The control group without diabetes was comprised of 51 health check-up participants (23 men and 28 women, 55.9 ± 9.9 years old).

Inclusion criteria for the T2DM group were: 1) ability to provide written informed consent, 2) aged more than 18 years, self-reported Uyghur ethnicity without intermarriage history with other ethnic groups within the latest three generations, 3) diagnosis of diabetes by physicians according to 1999 World Health Organization (WHO) Criteria (fasting plasma glucose greater than or equal to 7.0 mmol/L and/or 2-h plasma glucose greater than or equal to 11.1 mmol/L) (Alberti et al., 1998), and 4) diagnosis of T2DM from clinical records obtained from the patients' health care provider.

Inclusion criteria for the control group were: 1) ability to provide written informed consent, 2) aged more than 18 years, self-reported Uyghur ethnicity without intermarriage history with other ethnic groups within the latest three generations, 3) no documented clinical diagnosis of diabetes or other metabolic diseases, and 4) not taking glucose-lowering medications and had fasting and 2 h glucose values below the diagnostic thresholds for diabetes.

Anthropometric measurements, including weight, height, and waist measurements, were obtained using standardized techniques. The body mass index (BMI) was calculated by the formula weight (in kilograms)/height (in square meters). Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured with a mercury sphygmomanometer. Peripheral blood samples of patients and controls were collected in EDTA-anti-coagulated tubes after an overnight fast. Plasma glucose concentrations were measured by the glucose oxidase-peroxidase method using commercial kits. Serum total cholesterol, serum triglycerides, high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), and HbA1c were measured using standard methods (cholesterol oxidase-peroxidase-amidopyrine method, glycerol phosphate oxidase-peroxidase-amidopyrine method, enzymatic methods on a Hitachi 911 automated analyzer (Boehringer Mannheim, Mannheim, Germany), calculating by the Friedewald formula and determined by high-performance liquid chromatography, respectively).

The study was approved by the local community leaders and the ethics committee of the Capital Medical University, Beijing, China.

Selection of the candidate SNPs and genotyping

Sixteen SNPs (rs1801282 (*PPARG*), rs7754840 (*CDKAL1*), rs10811661 (*CDKN2A/B*), rs13266634 (*SLC30A8*), rs4402960 (*IGF2BP2*), rs7903146 (*TCF7L2*), rs10885122 (*ADRA2A*), rs174550 (*FADS1*), rs2191349 (*DGKB*), rs35767 (*IGF1*), rs12779790 (*CDC123/CAMK1D*), rs7961581 (*TSPAN8*), rs864745 (*JAZF1*), rs7578326 (*IRS1*), rs780094 (*GCKR*), and rs4607517 (*GCK*)) were selected for the following reasons. First, minor allele frequencies (MAF) of these selected SNPs were

more than 0.05 in both HapMap CEU data and HapMap CHB data (http://hapmap.ncbi.nlm.nih.gov/).

These SNPs were reported to be associated with T2DM in several GWAS results among European ancestry (Dupuis et al., 2010; Saxena et al., 2007; Voight et al., 2010; Zeggini et al., 2008), while 13 of them (except for rs7578326 (*IRS1*), rs780094 (*GCKR*) and rs4607517 (*GCK*)) were also evaluated in Han Chinese population (Hu et al., 2009; 2010; Liu et al., 2011; Wen et al., 2010; Zeggini et al., 2008; Zhou et al., 2010). *IRS1*, *GCKR,* and *GCK* were selected based on their roles in insulin signaling pathway (Kyoto Encyclopedia of Genes and Genomes: http://www.genome.jp/kegg/kegg2.html).

Genomic DNA was extracted from whole-blood samples using QIAamp DNA Blood Mini Kit (Qiagen, Germany) according to the manual instructions. Genomic DNA samples were subsequently diluted to 25 ng/μL. SNPs were genotyped using Mass ARRAY system (Sequenom, Inc., SanDiego, CA).

Statistical analyses

Analyses were conducted with SPSS for WINDOWS version 18.0 (SPSS, Chicago, IL, USA). Hardy-Weinberg equilibrium for genotype frequencies were tested by the Chi-square test. All continuous variables were expressed as the mean ± standard deviation (SD). Continuous variables between T2DM and control group were compared by Student's *t*-test. The differences in the frequencies of various alleles and genotypes between T2DM patients and controls were performed by Chi-square test, and Fisher's exact test was applied to the loci with a small number of alleles or genotypes (equal to or less than 5). Associations between SNPs and T2DM risks were assessed using odds ratios (ORs) with 95% confidence intervals (95% CIs) and *P* value derived from logistic regression adjusted for age and body mass index (BMI).

In addition, to evaluate the combined effects of the SNPs, cumulative scores of risk alleles were counted. Risk allele was defined as OR >1 based on the results of association analysis of candidate SNPs for T2DM. We considered an additive genetic model for each SNP, and assigned a score of 0, 1, or 2 to genotypes at the 16 loci, depending on whether subjects carried the wild-type allele or were heterozygous or homozygous for the risk allele. The count method assumed that each risk allele contributes equally and independently to the risk for T2DM. Association of cumulative risk allelic scores and T2DM were also assessed using ORs with 95% CIs and *p* value derived from logistic regression. The significant level was set at $p<0.05$, and all the analysis was two-sided.

Results

Sample characteristics

Participants' characteristics are shown in Table 1. The BMI and HbAlc were significantly higher in T2DM patients than those in control (25.0 ± 0.4 vs. 23.3 ± 0.3, $p=0.002$; 7.3 ± 2.1 vs. 5.1 ± 0.7, $p<0.001$, respectively). The SBP, DBP, total cholesterol, triacylglycerol, and glucose were also higher in T2DM patients than in the control group (135.9 ± 1.8 vs. 124.0 ± 1.5, $p<0.001$; 87.8 ± 1.4 vs. 81.8 ± 1.1, $p=0.001$; 4.9 ± 0.1 vs. 4.4 ± 0.1, $p=0.020$; 2.2 ± 0.1 vs. 1.7 ± 0.1, $p=0.006$, 9.1 ± 0.4 vs. 5.3 ± 0.1, $p<0.001$, respectively).

Table 1. Characteristics of the Study Subjects

	T2DM N = *51*	*Control* N = *51*	P *value*
Age (years)	54.1 ± 7.9	55.9 ± 9.9	0.243
Sex (Male/female)	25/26	23/28	0.843
BMI (kg/m^2)	25.0 ± 0.4	23.3 ± 0.3	**0.002**
SBP (mmHg)	135.9 ± 1.8	124.0 ± 1.5	**< 0.001**
DBP (mmHg)	87.8 ± 1.4	81.8 ± 1.1	**0.001**
Total cholesterol (mmol/L)	4.9 ± 0.1	4.4 ± 0.1	**0.020**
Triacylglycerol (mmol/L)	2.2 ± 0.1	1.7 ± 0.1	**0.006**
HDL (mmol/L)	1.1 ± 0.04	1.2 ± 0.04	0.629
LDL (mmol/L)	3.2 ± 0.1	3.0 ± 0.1	0.263
Glucose (mmol/L)	9.1 ± 0.4	5.3 ± 0.1	**< 0.001**
HbAlc (%)	7.3 ± 2.1	5.1 ± 0.73	**< 0.001**

The *p* values with statistical significance are indicated in bold numbers.

Association analysis of candidate SNPs for T2DM

Sixteen SNPs from known T2DM susceptibility loci were genotyped in 102 Uyghur participants. The genotype call rate for each SNP was >95%. There are two major causes for the missing calls. One is due to poor quality of DNA samples, which often fails to be amplified and to generate strong enough intensity of fluorescence signals over the background. The other arises when an observation (i.e., a read out of fluorescence signals) cannot be assigned unequivocally to any of the clusters of genotype, and, therefore, is subject to 'no-call' procedure. In this report, the missing calls are mainly due to the poor quality of DNA samples. Allelic frequencies of these 16 SNPs are shown in Table 2. The distributions of allelic frequencies of the 15 SNPs were all in Hardy-Weinberg equilibrium (HWE) in both cases and controls ($p>0.05$), except that of the rs4402960 ($p=0.05$) in the T2DM cases. The minor allele frequencies (MAF) of these SNPs range from 0.10 to 0.46.

Allelic frequencies of three SNPs (rs7754840 (*CDKAL1*), rs864745 (*JAZF1*), and rs35767 (*IGF1*)) were significantly different between the T2DM and control group ($p<0.05$). For rs7754840 (*CDKAL1*), frequencies of the C and G alleles were 0.24 and 0.76, respectively, in the control group. Frequencies of the C allele were significantly higher in T2DM patients than that in control group (0.46 vs. 0.24, $p=0.005$). For rs864745 (*JAZF1*), frequencies of the C and T alleles were 0.42 and 0.58 in the control group. Frequency of the T allele was significantly higher in T2DM patients than that in control group (0.71 vs. 0.58, $p=0.049$). For rs35767 (*IGF1*), frequencies of the A and G alleles were 0.21 and 0.79 in the control group. Frequency of the G allele was significantly lower in T2DM patients than that in the control group (0.67 vs. 0.79, $p=0.033$). Logistic regression analysis (adjusted for age and BMI) revealed that participants with the C allele for rs7754840 (*CDKAL1*) had a 2.32-fold [OR (95%CI): 1.19–4.54, $p=0.014$] risk of T2DM compared with the G allele. SNP rs864745 (*JAZF1*) and SNP rs35767 (*IGF1*) were also found to be significantly associated with T2DM in logistic regression analysis [OR (95% CI): 0.48 (0.24–0.94), $p=0.032$ vs. OR (95% CI): 2.06 (1.02–4.17), $p=0.044$] (Table 2). Contrary to SNP rs35767/GG, SNPs rs7754840/CC

Table 2. Association Analysis of Xandidate SNPs for T2DM in the Uyghur Participants

Locus	db SNP	Minor allele	T2DM		Control		Chi-square test	Logistic regression analysis (adjusted for age and BMI)	
			F_{minor}	P_{HWET}	F_{minor}	P_{HWET}	P *value*	*OR (95%CI)*	P *value*
CDKAL1	rs7754840	C	0.46	0.92	0.24	0.89	**0.005**	2.32 (1.19–4.54)	**0.014**
JAZF1	rs864745	C	0.29	0.48	0.42	0.09	**0.049**	0.48 (0.24–0.94)	**0.032**
IGF1	rs35767	A	0.33	0.36	0.21	0.12	**0.033**	2.06 (1.02–4.17)	**0.044**
IGF2BP2	rs4402960	T	0.26	0.05	0.30	0.64	0.182	0.99 (0.52–1.90)	0.980
CDKN2A/B	rs10811661	C	0.31	0.16	0.30	0.91	0.911	1.01 (0.51–2.01)	0.984
ADRA2A	rs10885122	T	0.10	0.44	0.13	0.29	0.443	0.67 (0.23–1.93)	0.463
CDC123/CAMK1D	rs12779790	G	0.16	0.43	0.12	0.34	0.357	1.61 (0.67–3.87)	0.299
SLC30A8	rs13266634	T	0.29	0.69	0.28	0.93	0.927	1.11 (0.57–2.15)	0.765
FADS1	rs174550	C	0.36	0.36	0.42	0.69	0.455	0.78 (0.41–1.50)	0.458
PPARG	rs1801282	G	0.14	0.27	0.17	0.15	0.542	0.62 (0.24–1.64)	0.340
DGKB	rs2191349	G	0.37	0.58	0.41	0.20	0.369	0.79 (0.42–1.49)	0.463
GCK	rs4607517	A	0.23	0.74	0.16	0.06	0.283	1.34 (0.65–2.77)	0.419
IRS1	rs7578326	G	0.32	0.39	0.23	0.74	0.200	1.51 (0.76–3.01)	0.236
GCKR	rs780094	T	0.40	0.89	0.38	0.13	0.492	0.95 (0.51–1.72)	0.873
TCF7L2	rs7903146	T	0.12	0.09	0.17	0.49	0.570	0.49 (0.12–1.22)	0.133
TSPAN8	rs7961581	C	0.35	0.41	0.29	0.34	0.621	1.70 (0.84–3.43)	0.147

P_{HWET}, p value of Hardy–Weinberg equilibrium test; F_{minor}, minor allele frequency.
ADRA2A: adrenergic α2A receptor; *CDC123/CAMK1D*: cell division cycle 123; *CDKAL1*: cyclin-dependent kinase 5 regulatory subunit associated protein 1–like 1; *CDKN2A/B*: cyclin-dependent kinase inhibitor-2A/B; *DGKB*: diacylglycerol kinase, beta 90kDa; *FADS1*: fatty acid desaturase 1; *GCK*: glucokinase; *GCKR*: glucokinase regulatory protein; *IGF1*: insulin-like growth factor 1; *IGF2BP2*: insulin-like growth factor 2 mRNA binding protein 2; *IRS1*: insulin receptor substrate 1; *JAZF1*: juxtaposed with another zinc finger 1; *PPARG*: peroxisome proliferator-activated receptor gamma; *SLC30A8*: solute carrier family 30 member 8; *TCF7L2*: transcription factor 7-like 2; *TSPAN8*: tetraspanin 8. The p values with statistical significance are indicated in bold numbers.

and rs864745/TT were more frequent in the diabetes group compared to the control group (42% vs 66.7%; 21.6% vs 5.9%; 49% vs 39.2%, respectively; Table 3).

To evaluate the combined effects of these 16 SNPs, we calculated the cumulative risk allelic scores of these 16 risk alleles that each participant had. The average of cumulative risk allelic scores of T2DM patients and control group were 17.1 ± 8.1 and 15.4 ± 7.3 ($p = 0.002$, t-test), respectively. The risk allelic scores were associated with T2DM in logistic regression analysis adjusted with age and BMI (OR: 1.27, 95% CI: 1.07–1.50, $p = 0.007$).

Discussion

Variants in more than 40 loci were identified to be associated with T2DM in more than one population by GWAS results. Some variants were associated with T2DM in one ethnic group of subjects but not in others. SNPs in *PPARG*, *CDKAL1*, *CDKN2A/B*, *SLC30A8*, *IGF2BP2*, *TCF7L2*, *ADRA2A*, *FADS1*, *DGKB*, *IGF1*, *CDC123/CAMK1D*, *TSPAN8,* and *JAZF1* were identified to be associated with T2DM in European populations by GWAS (Dupuis et al., 2010; Saxena et al.,2007; Zeggini et al., 2008). These SNPs have been confirmed by multiple studies in various populations, such as Danes, Han Chinese, and Japanese (Grarup et al., 2008; Hu et al., 2009; 2010; Liu et al., 2011; Ohshige et al., 2011; Wen et al., 2010; Zhou et al., 2010).

Study findings

In this study, the associations of SNPs in 16 respective T2DM candidate genes were investigated in Uyghurs. The Uyghur participants were recruited from Uyghur population at Hetian of Xinjiang, China, where the Uyghur population is less affected by the recent migration of Han Chinese and keeps its traditional lifestyles for generations, with its own language, religious beliefs, and marriage patterns (Black et al., 2006). Therefore, the Uyghur would be an ideal population for the study of evaluating genetic susceptibility. To our knowledge, this was the first attempt that a cohort of SNPs in *PPARG*, *CDKAL1*, *CDKN2A/B*, *SLC30A8*, *IGF2BP2*, *TCF7L2*, *ADRA2A*, *FADS1*, *DGKB*, *IGF1*, *CDC123/CAMK1D*, *TSPAN8*, *JAZF1*, *IRS1*, *GCKR,* and *GCK* were genotyped in Uyghur participants for T2DM susceptibility.

CDKAL1 is one of the most significant diabetes susceptibility genes identified to date in various populations. Intronic *CDKAL1* variant rs7754840 has been associated with T2DM, mainly due to impaired first-phase insulin release (Chistiakov et al., 2011; Stancakova et al., 2008). SNPs rs7756992, rs10946398, and rs9465871 in *CDKAL1* were also found to be associated with T2DM among European populations, Pima Indian, Han Chinese, and Korean populations in previous studies (Dehwah et al., 2010; Hu et al., 2009; Lee et al., 2008; Rong et al., 2009; Steinthorsdottir et al., 2007). Our study revealed that the C allele of rs7754840 of CDKAL1 was significantly associated with T2DM in Uyghur participants ($p = 0.014$, adjusted for age and BMI). The OR value of rs7754840 was 2.32, higher than that of Han Chinese (1.127) (Hu et al., 2009), Pima Indians (1.06) (Rong et al., 2009), and Lebanese Arabs (1.86) (Nemr et al., 2012). T2DM patients had a higher CC genotype and lower GG genotype of *CDKAL1* when compared with the control group (*Chi-*

Table 3. Frequencies of Genotypes of rs7754840 (*CDKAL1*), rs864745 (*JAZF1*), and rs35767 (*IGF1*) in T2DM Participants and Controls

						Logistic regression analysis (adjusted for age and BMI)[c]					
		T2DM	*Control*	*Chi-square test*		*Additive*		*Dominant*		*Recessive*	
SNP	*Genotype*	*No. (%)*	*No. (%)*	*Chi-square*	P	P	*OR (95%CI)*	P	*OR (95%CI)*	P	*OR (95%CI)*
rs7754840 (*CDKAL1*)	GG	15 (29.4)	30 (58.8)	17.448	**<0.001**	**0.014**	2.32 (1.19–4.54)	**0.035**	1.63 (1.04–2.57)	0.057	1.99 (0.98–3.99)
	GC	25 (49.0)	18 (35.3)								
	CC	11 (21.6)	3 (5.9)								
rs864745[a] (*JAZF1*)	TT	24 (49.0)	20 (39.2)	5.946	0.051	**0.032**	0.48 (0.24–0.94)	0.160	0.73 (0.46–1.14)	**0.025**	0.39 (0.17–0.89)
	TC	22 (44.9)	19 (37.3)								
	CC	3 (6.1)	12 (23.5)								
rs35767[b] (*IGF1*)	GG	21 (42.0)	34 (66.7)	6.853	**0.033**	**0.044**	2.06 (1.02–4.17)	**0.014**	1.75 (1.12–2.75)	0.829	1.09 (0.49–2.44)
	GA	25 (50.0)	13 (25.5)								
	AA	4 (8.0)	4 (7.8)								

[a]The number of T2DM participants is 49, due to two samples failed to call out the genotypes; [b]The number of T2DM participants is 50, due to one sample failed to call out the genotypes; [c]The logistic regression model was used to obtain the odds ratios of the minor allele with the major allele as reference group.
The *p* values with statistical significance are indicated in bold numbers.

square: 17.448, $p < 0.001$, Table 3). This result was compatible with a study among a Korean population, in which the CC genotype was significant higher in T2DM patients than that in controls (Lee et al., 2008).

Among the other loci examined in this study, SNP rs864745 in *JAZF1* also showed the most significant association with T2DM in Uyghur ($p = 0.032$, adjusted for age and BMI). SNP rs864745 resides in intron-1 of *JAZF1* gene, encoding a transcriptional repressor of the nuclear receptor subfamily-2, group C, member-2 (*NR2C2*) gene (Nakajima et al., 2004). The carriage of *JAZF1* risk variants may lead to postnatal growth restriction mainly due to affecting pancreatic β-cell mass and function (Grarup et al., 2008). The association between rs864745 and T2DM varied among populations. SNP rs864745 was strongly associated with T2DM in European participants ($p = 5.0 \times 10^{-14}$) (Zeggini et al., 2008), whereas no significant association was observed in Han Chinese ($p > 0.05$) (Hu et al., 2009) and Japanese subjects ($P > 0.05$) (Takeuchi et al., 2009). In our study, the OR value of rs864745 (0.48) was lower than that in Caucasians (1.50) (An et al., 2009), Han Chinese population (1.05–1.09) (Hu et al., 2009; Zhou et al., 2010), and Pakistani population (1.16) (Rees et al., 2011). Frequency of the CC genotype of rs864745 (*JAZF1)* was lower in T2DM patients than that in control group (Table 3, 6.1% vs. 23.5%), indicating its protective effect on T2DM in Uyghur participants.

The rs35767 polymorphism resides 1.2 kb upstream of *IGF1,* which is a biologically plausible fasting insulin-raising gene. A previous study reported that the effects of the rs35767 polymorphism near *IGF1* on fasting insulin are mediated by reduced insulin sensitivity or impaired insulin clearance; and those carriers of the GG genotype have lower insulin sensitivity as compared with subjects carrying the A allele in white Europeans (Mannino et al., 2013). The finding that the G allele of rs35767 (*IGF1*) was associated with fasting insulin and HOMA-IR in European population (Dupuis et al., 2010) as well as in Han Chinese (Hu et al., 2010) is not new.

In our study, there was a significant difference in the frequency of G allele for rs35767 (*IGF1*) between T2DM patients and controls ($p = 0.033$). T2DM patients had a lower frequency of GG genotype (42.0% vs. 66.7%) compared to that of control group. The unique life style and marriage pattern might be responsible for such an observation. Most Uyghurs live as farmers, and have different dietary habits than Han Chinese. They have high carbohydrate diets with a higher salt (more than 20 g per day), more meat, and less unsaturated fatty acids compared with Han Chinese (Zhai et al., 2007). Then, the practice of endogamy in Uyghur population might also be a reason (Mamet et al., 2005; Wang et al., 2003).

The previous studies reported that accumulative number of risk alleles may be associated with T2DM, even though these alleles were not observed to be statistically significances individually (Fontaine-Bisson et al., 2010; Yamakawa-Kobayashi et al., 2012). We calculated the cumulative risk allelic scores of these 16 risk alleles to evaluate the combined effects of these 16 SNPs, and a significant association was observed between accumulative risk allelic scores and T2DM in the Uyghur population samples ($p = 0.007$).

Study limitations

While this study provides valuable insight into the genetic difference of T2DM related loci in minority groups, it has some limitations. First, the ideal sample size should be about 300 pairs for cases and controls when we initiated this study. No multiple comparisons were attempted due to the relative small sample size that we originally designed in this pilot study. The statistical power of rs7754840 was calculated to 95% at the significance of 0.05, and those of other SNPs were less than 80% (SAS Proc Power), because the people of minority groups often refuse to participate in some investigation studies. Combined with less participant rate, the blood sampling is a very hard practice in Xinjiang, a very remote area of the northwestern frontier part of China. These are preliminary findings and further case-control studies with large samples and multiple comparisons are warranted to provide a more definite explanation about the relationships between those SNPs and T2DM based on our observation. Second, the existence of the interaction between environmental factors (e.g., life style, dietary, climates) and the T2DM susceptibility loci must be further validated in the Uyghur population. Moreover, important information on rare variants with stronger effects on T2DM could be revealed by the next-generation of whole genome sequencing when the technique routine could be available in not far future.

Conclusions and Future Outlook

This is the first study to evaluate genomic variation at 16 SNPs in respective T2DM candidate genes for the Uyghur population compared with other ethnic groups. The SNP rs7754840 in *CDKAL1*, rs864745 in *JAZF1,* and rs35767 in *IGF1* might serve as potential susceptibility loci for T2DM in Uyghurs. We suggest that a broader capture and study of the world populations, including those that are hitherto understudied or overlooked, are necessary for a comprehensive understanding of the genetic/genomic basis of T2DM. Additionally, plant OMICS research, including those with traditional plants (Sahu et al., 2014), that offer potential novel therapeutics for T2DM could be usefully is combined with studies of T2DM genetics so as to inform future personalized medicine research, global health, and society.

Acknowledgments

This study was supported by research grants from the National Natural Science Foundation of China (81273170, 30901238, and 81370083), Mason Foundation National Medical Program-ANZ-Australia (CT21946), National 12th Five-Year Major Projects of China (2012BAI37B03), ECU-Industry Collaborative Scheme 2013 (G1001368), Australian National Health and Medical Research Council (NHMRC-APP1046711), Project for Academic Human Resources Development in Institutions of Higher Learning under the Jurisdiction of Beijing Municipality (PHR201008393), Natural Science Foundation of Xinjiang Uyghur Autonomous Region (2013211A016), and Natural Science Foundation of Capital Medical University, Beijing, China (2014ZR16). Wei Wang and Manshu Song were supported by the Importation and Development of High-Calibre Talents Project of Beijing Municipal Institutions (IDHT20130213, CIT&TCD201404185), and Youxin Wang was supported by Beijing Higher Education Young Elite Teacher Project (YETP1671) and Beijing Nova Program (Z141107001814058). The authors thank the Uyghur volunteers and community leaders for their support and participation.

Author Disclosure Statement

The authors declare that no conflicting financial interests exist.

References

Ahlqvist E, Ahluwalia TS, and Groop L. (2011). Genetics of type 2 diabetes. Clin Chem 57, 241–254.

Alberti K, and Zimmet PZ. Consultation WHO. (1998). Definition, diagnosis and classification of diabetes mellitus and its complications part 1: Diagnosis and classification of diabetes mellitus—Provisional report of a WHO consultation. Diabetic Med 15, 539–553.

An P, Feitosa M, Ketkar S, et al. (2009). Epistatic interactions of *CDKN2B-TCF7L2* for risk of type 2 diabetes and of *CDKN2B-JAZF1* for triglyceride/high-density lipoprotein ratio longitudinal change: Evidence from the Framingham Heart Study. BMC Proc 3, S71.

Awuti G, Younusi K, Li LL, Upur H, and Ren J. (2012). Epidemiological survey on the prevalence of periodontitis and diabetes mellitus in Uyghur adults from rural Hotan area in Xinjiang. Exp Diabetes Res 2012, 758921.

Banerjee M, and Vats P. (2014). Reactive metabolites and antioxidant gene polymorphisms in type 2 diabetes mellitus. Indian J Hum Genet 20, 10–19.

Barnett AH, Eff C, Leslie RD, and Pyke DA. (1981). Diabetes in identical twins. A study of 200 pairs. Diabetologia 20, 87–93.

Billings LK, and Florez JC. (2010). The genetics of type 2 diabetes: What have we learned from GWAS? Ann NY Acad Sci 1212, 59–77.

Black ML, Wise CA, Wang W, and Bittles AH. (2006). Combining genetics and population history in the study of ethnic diversity in the People's Republic of China. Hum Biol 78, 277–293.

Chistiakov DA, Potapov VA, Smetanina SA, Bel'chikova LN, Suplotova LA, and Nosikov VV. (2011). The carriage of risk variants of *CDKAL1* impairs beta-cell function in both diabetic and non-diabetic patients and reduces response to non-sulfonylurea and sulfonylurea agonists of the pancreatic KATP channel. Acta Diabetol 48, 227–235.

Dehwah MA, Wang M, Huang QY. (2010). *CDKAL1* and type 2 diabetes: A global meta-analysis. Genet Mol Res 9, 1109–1120.

Dupuis J, Langenberg C, Prokopenko I, et al. (2010). New genetic loci implicated in fasting glucose homeostasis and their impact on type 2 diabetes risk. Nat Genet 42, 105–116.

Fontaine-Bisson B, Renström F, Rolandsson O, et al. (2010). Evaluating the discriminative power of multi-trait genetic risk scores for type 2 diabetes in a northern Swedish population. Diabetologia 53, 2155–2162.

Grarup N, Andersen G, Krarup NT, et al. (2008). Association testing of novel type 2 diabetes risk alleles in the *JAZF1*, *CDC123/CAMK1D*, *TSPAN8*, *THADA*, *ADAMTS9*,

and *NOTCH2* loci with insulin release, insulin sensitivity, and obesity in a population-based sample of 4,516 glucose-tolerant middle-aged Danes. Diabetes 57, 2534–2540.

Hu C, Zhang R, Wang CR, et al. (2009). *PPARG*, *KCNJ11*, *CDKAL1*, *CDKN2A*, *CDKN2B*, *IDE*, *KIF11*, *HHEX*, *IGF2BP2* and *SLC30A8* are associated with type 2 diabetes in a Chinese population. PLos One 4, e7643.

Hu C, Zhang R, Wang C, et al. (2010). Variants from *GIPR*, *TCF7L2*, *DGKB*, *MADD*, *CRY2*, *GLIS3*, *PROX1*, *SLC30A8,* and *IGF1* are associated with glucose metabolism in the Chinese. PLos One 5, e15542.

Klimentidis YC, Abrams M, Wang JL, Fernandez JR, and Allison DB. (2011). Natural selection at genomic regions associated with obesity and type-2 diabetes: East Asians and sub-Saharan Africans exhibit high levels of differentiation at type-2 diabetes regions. Hum Genet 129, 407–418.

KoEbberling J, and Tillil H. (1982). Empirical risk figures for first degree relatives of non-insulin-dependent diabetics. In: KoÈbberling J, Tattersall RB eds. *The Genetics of Diabetes Mellitus.* London: Academic Press, pp. 201–209.

Lander ES, and Schork NJ. (1994). Genetic dissection of complex traits. Science 265, 2037–2048.

Lee YH, Kang ES, Kim SH, et al. (2008). Association between polymorphisms in *SLC30A8*, *HHEX*, *CDKN2A/B*, *IGF2BP2*, *FTO*, *WFS1*, *CDKAL1*, *KCNQ1* and type 2 diabetes in the Korean population. J Hum Genet 53, 991–998.

Li LL, Kang XL, Ran XJ, et al. (2007). Associations between 45T/G polymorphism of the adiponectin gene and plasma adiponectin levels with type 2 diabetes. Clin Exp Pharmacol Physiol 34, 1287–1290.

Li LL, Ma XL, Ran JX, et al. (2008). Genetic polymorphism of peroxisome proliferator-activated receptor-gamma 2 *Pro12Ala* on ethnic susceptibility to diabetes in Uygur, Kazak and Han subjects. Clin Exp Pharmacol Physiol 35, 187–191.

Li N, Wang H, Yan Z, Yao X, Hong J, and Zhou L. (2012). Ethnic disparities in the clustering of risk factors for cardiovascular disease among the Kazakh, Uygur, Mongolian and Han populations of Xinjiang: A cross-sectional study. BMC Public Health 12, 499.

Liu C, Li H, Qi L, et al. (2011). Variants in *GLIS3* and *CRY2* are associated with type 2 diabetes and impaired fasting glucose in Chinese Hans. PLos One 6, e21464.

Mamet R, Jacobson CK, and Heaton TB. (2005). Ethnic intermarriage in Beijing and Xinjiang, China, 1990. J Comp Fam Stud 36, 187.

Mannino GC, Greco A, De Lorenzo C, et al. (2013). A fasting insulin-raising allele at *IGF1* locus is associated with circulating levels of *IGF-1* and insulin sensitivity. PLoS One 8, e85483.

Nakajima T, Fujino S, Nakanishi G, Kim YS, and Jetten AM. (2004). TIP27: A novel repressor of the nuclear orphan receptor TAK1/TR4. Nucleic Acids Res 32, 4194–4204.

Nemr R, Almawi AW, Echtay A, Sater MS, Daher HS, and Almawi WY. (2012). Replication study of common variants in *CDKAL1* and *CDKN2A/2B* genes associated with type 2 diabetes in Lebanese Arab population. Diabetes Res Clin Pract 95, e37–e40.

Newman B, Selby JV, King MC, Slemenda C, Fabsitz R, and Friedman GD. (1987). Concordance for type 2 (non-insulin-dependent) diabetes mellitus in male twins. Diabetologia 30, 763–768.

Ohshige T, Iwata M, Omori S, et al. (2011). Association of new loci identified in European genome-wide association studies with susceptibility to type 2 diabetes in the Japanese. PLos One 6, e26911.

Rees SD, Hydrie MZ, Shera AS, et al. (2011). Replication of 13 genome-wide association (GWA)-validated risk variants for type 2 diabetes in Pakistani populations. Diabetologia 54, 1368–1374.

Risch N. (1990). Linkage strategies for genetically complex traits.III. The effect of marker polymorphism on analysis of affected relative pairs. Am J Hum Genet 46, 242–253.

Rong R, Hanson RL, Ortiz D, et al. (2009). Association analysis of variation in/near *FTO*, *CDKAL1*, *SLC30A8*, *HHEX*, *EXT2*, *IGF2BP2*, *LOC387761*, and *CDKN2B* with type 2 diabetes and related quantitative traits in Pima Indians. Diabetes 58, 478–488.

Sahu J, Sen P, Choudhury MD, et al. (2014). Rediscovering medicinal plants' potential with OMICS: Microsatellite survey in expressed sequence tags of eleven traditional plants with potent antidiabetic properties. OMICS 18, 298–309.

Saxena R, Voight BF, Lyssenko V, et al. (2007). Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. Science 316, 1331–1336.

Scott LJ, Mohlke KL, Bonnycastle LL, et al. (2007). A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. Science 316, 1341–1345.

Sladek R, Rocheleau G, Rung J, et al. (2007). A genome-wide association study identifies novel risk loci for type 2 diabetes. Nature 445, 881–885.

Stancáková A, Pihlajamäki J, Kuusisto J, et al. (2008). Single-nucleotide polymorphism rs7754840 of *CDKAL1* is associated with impaired insulin secretion in nondiabetic offspring of type 2 diabetic subjects and in a large sample of men with normal glucose tolerance. J Clin Endocrinol Metab 93, 1924–1930.

Steinthorsdottir V, Thorleifsson G, Reynisdottir I, et al. (2007). A variant in *CDKAL1* influences insulin response and risk of type 2 diabetes. Nat Genet 39, 770–775.

Takeuchi F, Serizawa M, Yamamoto K, et al. (2009). Confirmation of multiple risk loci and genetic impacts by a genome-wide association study of type 2 diabetes in the Japanese population. Diabetes 58, 1690–1699.

Tao YC, Mao XM, Xie Z, et al. (2008). The prevalence of type 2 diabetes and hypertension in Uygur and Kazak populations. Cardiovasc Toxicol 8, 155–159.

Voight BF, Scott LJ, Steinthorsdottir V, et al. (2010). Twelve type 2 diabetes susceptibility loci identified through large-scale association analysis. Nat Genet 42, 579–589.

Wang W, Wise C, Baric T, Black ML, and Bittles AH. (2003). The origins and genetic structure of three co-resident Chinese Muslim populations: The Salar, Bo'an and Dongxiang. Hum Genet 113, 244–252.

Wen J, Ronn T, Olsson A, et al. (2010). Investigation of type 2 diabetes risk alleles support *CDKN2A/B*, *CDKAL1*, and *TCF7L2* as susceptibility genes in a Han Chinese cohort. PLos One 5, e9153.

Yamakawa-Kobayashi K, Natsume M, Aoki S, et al. (2012). The combined effect of the T2DM susceptibility genes is an important risk factor for T2DM in non-obese Japanese: A population based case-control study. BMC Med Genet 13, 11.

Yang YN, Xie X, Ma YT, et al. (2012). Type 2 diabetes in Xinjiang Uygur autonomous region, China. PLoS One 7, e35270.

Zeggini E, Scott LJ, Saxena R, et al. (2008). Meta-analysis of genome-wide association data and large-scale replication identifies additional susceptibility loci for type 2 diabetes. Nat Genet 40, 638–645.

Zhai F, He Y, Wang Z, and Hu Y. (2007). Status and characteristic of dietary intake of 12 minority nationalities in China. Wei Sheng Yan Jiu 36, 539–541 (in Chinese).

Zhang J, Ma YT, Xie X, et al. (2012). Prevalence and associated factors of diabetes mellitus in children of Gan, Uyghurs and Kazaks ethnicities in Xinjiang. Zhonghua Liu Xing Bing Xue Za Zhi 33, 1130–1132 (in Chinese).

Zhou DZ, Liu Y, Zhang D, et al. (2010). Variations in/nearby genes coding for *JAZF1*, *TSPAN8/LGR5* and *HHEX-IDE* and risk of type 2 diabetes in Han Chinese. J Hum Genet 55, 810–815.

Address correspondence to:
Wei Wang, MD, PhD, FFPH
Department of Global Health and Genomics
School of Medical Sciences
270 Joondalup Drive
Joondalup, Perth WA 6027
Australia

E-mail: wei.wang@ecu.edu.au

or

Youxin Wang, PhD
School of Public Health
No. 10 Xitoutiao
You An Men Wai
Fengtai District
Beijing 100069
China

E-mail: sdwangyouxin@163.com