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TITLE: The Uyghur population and genetic susceptibility to type 2 diabetes:
potential role for variants in *CDKALI*, *JAZF1* and *IGF1* genes

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Key words

Type 2 diabetes; Susceptibility loci; *CDKALI*; *JAZF1*; *IGF1*; Uyghur

Abstract

Substantial evidences suggest that type 2 diabetes (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 (*CDKALI*) ($P=0.014$), 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 *CDKALI*, 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 those 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 evidences suggest that T2DM is a multi-factorial disease with a strong genetic component. High concordance rate obtained in monozygotic twins (96%) supports 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 sib of an affected person (λ_s) to develop T2DM is 3.5 (Risch, 1990; Lander and Schork, 1994). 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 (Sladek et al., 2007; Scott et al., 2007; Zeggini et al., 2008; Ahlqvist et al., 2011; Saxena et al., 2007; Zeggini et al., 2008), e.g., loci near cyclin-dependent kinase 5 (*CDK5*), regulatory subunit-associated protein 1-like 1 (*CDKALI*) (Saxena et al., 2007), peroxisome proliferator-activated receptor gamma (*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 *CDKALI*, identified as T2DM susceptibility locus in subjects from Finland and Sweden by GWAS (Saxena et al., 2007) was replicated in Korean (Lee et al., 2008), *Han* Chinese (Hu et al., 2009), Pima Indians (Rong et al., 2009) and Lebanese Arab (Nemr et al., 2012), but showed inconsistent in risk allele frequencies and odds ratios (ORs) among these ethnic populations. SNP rs864745 in *JAZFI* was associated with T2DM in European population (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.

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 (Wang et al., 2003; Black et al., 2006). Uyghurs are overwhelmingly Muslim, and have their own language, religious beliefs and lifestyles that are very different from either *Han* Chinese population or American/European populations. Take dietary style as an example, the Uyghur has a high dairy intake level (over 200g) 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 reported that the prevalence of T2DM was 8.16% in Uyghur, which was higher than 1.47% in Kazak population (Tao et al., 2008). Awuti et al reported that the prevalence of T2DM for Uyghur adults was 9.5% (Awuti et al., 2012). 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 this 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 Uyghur population at *Hetian*

of *Xinjiang*, China, where Uyghur population was less affected by the recent migration of *Han* Chinese. Fifty one T2DM patients (25 men and 26 women, age: 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, age: 55.9 ± 9.9 years old).

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

Inclusion criteria for the control group were: Participants were included if they 1) had the ability to provide written informed consent, 2) were aged more than 18 years, self-reported Uyghur ethnicity without intermarriage history with other ethnic groups within the latest three generations, 3) did not have a documented clinical diagnosis of diabetes or other metabolic diseases, and 4) were not taking glucose-lowering medications and had fasting and 2h 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 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 (*CDKALI*), 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. Firstly, 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 (Saxena et al., 2007; Zeggini et al., 2008; Voight et al., 2010; Dupuis et al., 2010), while 13 of them (except for rs7578326 (*IRS1*), rs780094 (*GCKR*) and rs4607517 (*GCK*)) were also evaluated in *Han* Chinese population (Hu et al., 2009; Wen et al., 2010; Liu et al., 2011; Hu et al., 2010; 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, German) according to the manual instructions. Genomic DNA samples were subsequently diluted to 25ng/ μ l. SNPs were genotyped using Mass ARRAY system (Sequenom, Inc., SanDiego, CA).

Statistical analyses

Analyses were conducted in SPSS for WINDOWS version 18.0 (SPSS, Chicago, IL, USA). Hardy-Weinberg equilibrium for genotype frequencies were tested by *Chi*-square test. All continuous variables were expressed as the mean \pm 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 HbA1c 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 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).

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 were 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 were ranging from 0.10 to 0.46.

Allelic frequencies of three SNPs (rs7754840 (*CDKALI*), rs864745 (*JAZF1*) and rs35767 (*IGF1*)) were significant different between the T2DM and control group ($P<0.05$). For rs7754840 (*CDKALI*), frequencies of the C and G alleles were 0.24 and 0.76, respectively in 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 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 control group. Frequency of the G allele was significantly lower in T2DM patients than that in 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 (*CDKALI*) 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 (*JAZFI*) 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 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*, *CDKALI*, *CDKN2A/B*,

SLC30A8, *IGF2BP2*, *TCF7L2*, *ADRA2A*, *FADS1*, *DGKB*, *IGF1*, *CDC123/CAMK1D*, *TSPAN8* and *JAZF1* were identified to be associated with T2DM in European population by GWAS (Saxena et al., 2007; Zeggini et al., 2008; Dupuis et al., 2010). These SNPs have been confirmed by multiple studies in various populations, such as Danes, *Han* Chinese and Japanese (Hu et al., 2009; Wen et al., 2010; Liu et al., 2011; Hu et al., 2010; Zhou et al., 2010; Grarup et al., 2008; Ohshige et al., 2011).

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 pattern (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*, *CDKALI*, *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 study.

CDKALI is one of the most significant diabetes susceptibility genes identified to date in various populations. Intronic *CDKALI* variants rs7754840 has been found to be 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 population, Pima Indian, *Han* Chinese and Korean population in previous studies (Lee et al., 2008; Hu et al., 2009; Rong et al., 2009; Steinthorsdottir et al., 2007; Dehwah et al., 2010). Our study has 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 Arab population (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*-square: 17.448, $P < 0.001$, Table 3). This result was compatible with a study among 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 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 with *Han* Chinese. They have high carbon hydrate dietary with a higher salt (more than 20g 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 (Wang et al., 2003;

Mamet et al., 2005).

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 significance 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. Firstly, 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 than 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 usually 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. Secondly, the existence of the interaction between environmental factors (e.g., life style, dietary, climates) and the T2DM susceptibility loci is needed to be further validated in Uyghur population. Moreover, the 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 *CDKALI*, 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.

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Author Disclosure Statement

The authors declare that no conflicting financial interests exist.

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