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*Original Article*

# Association of SLC30A8 (rs13266634), CDKN2A/2B (rs10811661) and TCF7L2 (rs7903146) with the insulin resistance in type 2 diabetes in Chinese Han population in Northeast part of China

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**Abstract:** Objectives: This study was to determine the association of the polymorphism in SLC30A8 (rs13266634), CDKN2A/2B (rs10811661), HHEX (rs1111875) or TCF7L2 (rs7903146) with T2DM in Han population in Northeast part of China. Methods: Ligase detection reaction (LDR) technology was utilized to test the genotype in the four target genes in 513 cases of T2DM patients and 307 normal volunteers. Results: It was shown that there was a significant association of SNPs in CDKN2A/2B (rs10811661) or TCF7L2 (rs7903146) (P=0.0024 and 0.0007 respectively), but not in SNPs of SLC30A8 (rs13266634) and HHEX (rs1111875) with T2DM. And there was significant difference in HbA1c, FINS, HOMA-IR, and HOMA-β among the genotypes (TT, CT and CC) of CDKN2A/2B (rs10811661) (P=0.035, 0.022, 0.002 and 0.023 respectively). And the significant difference was also indicated in FPG, HbA1c, HOMA-IR, and HOMA-β among the three genotypes (TT, CT and CC) of SLC30A8 (rs13266634) (P=0.004, 0.005, 0.024 and 0.016 respectively). Conclusion: The present study revealed an association of CDKN2A/2B (rs10811661) and TCF7L2 (rs7903146) with type 2 diabetes in the Han population in the Northeast part of China, and there was also an association of SLC30A8 (rs13266634) and above-mentioned two SNPs with the insulin resistance in type 2 diabetes.

**Keywords:** CDKN2A/2B, TCF7L2, SLC30A8, T2DM, Insulin resistance

## Introduction

Type 2 diabetes is a complex metabolic disorder which is caused by both genetic and environmental factors such as food habits and life style [1]. The advent of high throughput genome-wide association (GWA) studies have facilitated the progress of discovery of genetic components for type 2 diabetes [2], and there are at least 20 loci identified today that are associated with the risk of type 2 diabetes, with the advent of GWA studies [3], such as SLC30A8 (rs13266634), CDKN2A/2B (rs10811661), HHEX (rs1111875) and TCF7L2 (rs7903146) which have been reported to be associated with type 2 diabetes in European Caucasians [2, 4-7]. And other variants, along with part of above-mentioned genetic factors have been reported to contribute to the T2DM of Chinese Han population [8-13].

Long-duration of diabetes always causes complications affecting eyes, kidneys, and the cardiovascular system, and finally morbidity and mortality among diabetic subjects [14]. And the high prevalence of diabetes with complications is significant public health problems, being responsible for a large proportion of blindness, renal replacement therapy, and cardiovascular interventions. The role of genetic factors in screening susceptibility to diabetic complications has been paid more and more attention to. Family studies have clearly indicated that susceptibility to diabetic nephropathy [15, 16]. As far as cardiovascular complications are concerned, 40-50% of which can be attributed to familial factors among individuals with diabetes [17, 18]. However, the GWA has not yet utilize widely to screen the associated genomic variants with diabetes complications.

**Table 1.** Clinical and biochemical characteristics of normal and T2DM subjects

| | Control | T2DM | *P value* |
|---|---|---|---|
| Age | 48.54 ± 9.25* | 53.39 ± 10.73 | 0.36 |
| Male/famale | 157/150 | 260/253 | 0.62 |
| BMI (kg/m$^2$) | 24.23 ± 2.97 | 25.88 ± 3.04 | <0.001 |
| FPG (mmol/l) | 5.67 ± 1.23 | 9.07 ±2.04 | <0.001 |
| HbA1c (%) | 5.64 ± 1.18 | 8.32 ± 1.65 | <0.001 |
| TC (mmol/l) | 3.88 ± 0.86 | 5.18 ± 1.16 | <0.001 |
| TG (mmol/l) | 0.99 ± 0.47 | 2.31 ± 1.03 | <0.001 |
| HDL-C (mmol/l) | 1.36 ± 0.26 | 1.14 ± 0.33 | 0.74 |
| LDL-C (mmol/l) | 3.47 ± 0.46 | 4.28 ± 0.54 | <0.01 |
| FINS (mU/L) | 9.26 ± 1.15 | 1.54 ± 0.30 | <0.001 |
| HOMA-IR | 1.15 ± 0.13 | 1.58 ± 0.19 | <0.001 |
| HOMA-β | 82.54 ± 5.93 | 71.23 ± 5.21 | <0.001 |

Data are means ± SD, median (interquartile range), or *n* (%), unless otherwise indicated. *P* represents significance of the differences between individuals from control and from T2DM groups.

In this study, to determine the association of above well-known SNPs with the T2DM in Chinese Han population in the Northeast part of China, ligase detection reaction (LDR) technology was utilized to analyze the SNP in SLC30A8 (rs13266634), CDKN2A/2B (rs10811661), HHEX (rs1111875) and TCF7L2 (rs7903146) in 513 cases of type 2 diabetes mellitus (T2DM) patients and 307 normal volunteers in Changchun, Jilin Province, and then we analyzed the association of these SNPs with the T2DM.

## Materials and methods

### *Ethical considerations*

This study was approved by the Internal Review Board (IRB) of Second Hospital of Jilin University, and each participant signed the informed consent.

### *Patients and tissue specimens*

T2DM group: 513 cases of outpatient or inpatient diagnosed with T2DM, from Feb 2012 to Dec 2015, in the Endocrinology Department, Second Hospital of Jilin University, in Changchun, Jilin Province of China. Diagnostic criteria: 1. meeting the 1999 WHO diagnosis of diabetes (fasting plasma glucose ≥7.0 mmol/L and/or 2-hour postprandial plasma glucose ≥11.1 mmol/L) and the classification criteria; 2. no ketosis within six months and other stress situations; 3. without liver and kidney dysfunction. Normal control group: 307 cases healthy persons without family history of diabetes post the health examination in the hospital within the same period. Two groups of subjects were unrelated Chinese Han population in Changchun, Jilin Province. The sodium citrate anticoagulant blood samples were collected from the cubital vein, post fasting for more than 8 hours, and were utilized to extract genomic DNA. The blood samples for the anthropometry to detect the metabolic indexes were collected simultaneously. Homeostasis model was utilized to assess the insulin resistance index (HOMA-IR) and islet β-cell function (HOMA-β).

### *DNA extraction, polymerase chain reaction and ligase detection reaction*

Blood Genomic DNA was extracted using the human genomic DNA extracting kit (Centrifugal column) (Tiangen Biochemical Technology Co., Ltd. Beijing, China).

PCR reaction: The primers were designed by the software Oligo6.0, see details in **Table 1**. The PCR reaction was performed with the specific primers and genomic DNA as template. And the reaction system was as following: template 1 μl, 10× buffer 1.5 μl, $MgCl_2$ 1.5 μl, dNTP mix 0.3 μl, primers mix 0.15 μl, Taq enzyme 0.3 ul (for total volume of 15 μl). Amplification conditions: 94°C 3 min, 94°C 15 s, 55°C 15 s, 72°C 30 s, 35 cycles, 72°C 3 min. PCR products were tested using 2% agarose gel electrophoresis.

Multiple LDR: LDR probes were designed, based on the LDR probe design principles, the LDR probe sequences were shown in **Table 2**. LDR reaction: PCR product 3 μl, 10x Taq DNA ligase buffer 1 μl, Taq DNA ligase (40 U/μl) 0.125 μl, probe (10p) 0.01 μl each, add $H_2O$ to 10 μl. LDR reaction conditions: 94°C 30 s, 56°C 3 min, 30 cycles. 1 μl extension product, adding 8 μl sample loading buffer, was denatured at 95°C for 3 min and immediately cooled in ice-water bath and sequenced.

### *Statistical analysis*

Hardy-Weinberg equilibrium method was utilized to detect the gene frequency of group representation. SPSS17.0 statistical software was utilized for all data normality test, and the data

**Table 2.** Associations of SNPs with type 2 diabetes in Chinese Han population in Northeast part of China

| Genes | SNP | Risk*/non risk allele | Risk allele frequency | | Odds ratio (95% CI) | *P* value † |
|---|---|---|---|---|---|---|
| | | | Case | Control | | |
| SLC30A8 | rs13266634 | C/T | 0.454 | 0.441 | 1.15 (0.87-1.33) | 0.2314 |
| CDKN2A/2B | rs10811661 | T/C | 0.487 | 0.432 | 1.42 (1.23-1.65) | 0.0024 |
| HHEX | rs1111875 | G/A | 0.736 | 0.724 | 1.03 (0.84-1.27) | 0.7526 |
| TCF7L2 | rs7903146 | T/C | 0.383 | 0.306 | 1.52 (1.29-1.85) | 0.0007 |

*Allele associated with higher glucose and risk of diabetes in previously published GWA studies. †Adjusted for age, gender, BMI and biochemistry laboratory. Significance was considered with a *p* value less than 0.05.

with normal distribution were expressed as mean ± S, the data without normal distribution (FINS, HOMA-IR and HOMA-β) were logarithmically transformed to normality to analyze. ANOVA was used for comparing multiple groups, $X^2$ test was used for comparing count data. P<0.05 was considered statistically significant.

## Results

### *Clinical and biochemical characteristics*

The clinical and biochemical characteristics of all subjects in the association study are summarized in **Table 1**. There was no significant difference in age, gender between the T2DM and normal control groups. However, the deregulation in plasma glucose and insulin and the high level of insulin resistance discriminated the T2DM subjects from the normal ones.

### *Analysis of genotype and allele frequencies*

We first examined the association with the risk of type 2 diabetes, of above-mentioned SNPs in SLC30A8 (rs13266634), CDKN2A/2B (rs10811661), HHEX (rs1111875) and TCF7L2 (rs7903146) (**Table 2**). It was shown that the risk frequency of SLC30A8 (rs13266634) was 0.454 in T2DM group and 0.441 in control group respectively, and there was no significant association in the SNP of zinc transporter SLC30A8 (rs13266634) with T2DM (P=0.2316 with an odds ratio (95% CI) of 1.15 (0.87-1.33)). And there was neither significant association in the SNP of HHEX (rs1111875) with T2DM (P=0.7526 with an odds ratio of 1.03 (0.84-1.27). However, the risk allele frequency was significantly different in the two SNPs of CDKN2A/2B (rs10811661) and TCF7L2 (rs7903146) between the T2DM and control groups. Both SNPs were each significantly associated with type 2 diabetes (odds ratios ranged between 1.23 and 1.65, P=0.0024 for CDKN2A/2B (rs10811661), odds ratios ranged between 1.29 and 1.85, P=0.0007 for TCF7L2 (rs7903146)).

To further confirm the association of the two SNPs with T2DM, we next examined the association between genetic variants and type 2 diabetes-related parameters (FPG, HbA1c, FINS, HOMA-IR, and HOMA-β). As shown in **Table 3**, the genotypes in CDKN2A/2B (rs10811661) and TCF7L2 (rs7903146) were significantly associated with type 2 diabetes-related phenotypes. There was significant difference in HbA1c, FINS, HOMA-IR, and HOMA-β among the genotypes (TT, CT and CC) of CDKN2A/2B (rs10811661) (P=0.035, 0.022, 0.002 and 0.023 respectively). And the significant difference was also indicated in FPG, HbA1c, HOMA-IR, and HOMA-β among the three genotypes (TT, CT and CC) of TCF7L2 (rs7903146) (P=0.004, 0.005, 0.024 and 0.016 respectively). Though the case-control analyses did not reveal a significant association of SLC30A8 (rs13266634) with T2DM, there was a significant difference in insulin resistance among the three genotypes (CC, CT and TT) of the SNP (P=0.013 for HOMA-IR, and P=0.042 for HOMA-β).

## Discussion

Approximately 2/3 of the world's population with diabetes comes from Asia, mainly from India and China. The total number of diabetes patients in China is estimated to increase to 42.3 million in 2030 [19]. And the rapid increase in prevalence of type 2 diabetes has been a major public health challenge in China. The risk factors for type 2 diabetes include obesity, physical inactivity, pregnancy, improper diet, and certain socioeconomic characteristics, most of which are associated with the lifestyle [20-26], which varies endemically and population-based prospectively [27]. Besides the important environmental contributors,

**Table 3.** Association of the identified SNP genotypes with type 2 diabetes-related parameters

| Genotypes of identified SNP | | FPG (mmol/l)* | | HbA1c (%)* | | FINS (mU/L)* | | HOMA-IR* | | HOMA-B* | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *n* | Means ± SE | *P*† | Means ± SE | *P*† | Means ± SE | *P*† | Means ± SE | *P*† | Means ± SE | *P*† |
| SLC30A8 *(rs13266634)* | 485 | | | | | | | | | | |
| CC | 162 | 9.21 ± 0.34 | | 8.68 ± 0.64 | | 1.48 ± 0.12 | | 1.47 ± 0.15 | | 68.76 ± 4.43 | |
| CT | 242 | 9.06 ± 0.28 | | 8.31 ± 0.59 | | 1.53 ± 0.15 | | 1.58 ± 0.11 | | 69.96 ± 4.62 | |
| TT | 81 | 8.82 ± 0.24 | 0.422 | 7.65 ± 0.57 | 0.137 | 1.69 ± 0.17 | 0.423 | 1.82 ± 0.18 | 0.013 | 79.98 ± 5.45 | 0.042 |
| *CDKN2A/2B* (rs10811661) | 464 | | | | | | | | | | |
| TT | 126 | 9.49 ± 0.41 | | 8.62 ± 0.51 | | 1.37 ± 0.16 | | 1.47 ± 0.19 | | 67.79 ± 4.93 | |
| CT | 246 | 8.82 ± 0.30 | | 8.42 ± 0.54 | | 1.52 ± 0.18 | | 1.51 ± 0.16 | | 69.88 ± 4.35 | |
| *CC* | 92 | 9.15 ± 0.28 | 0.173 | 7.63 ± 0.42 | 0.035 | 1.85 ± 0.16 | 0.022 | 1.92 ± 0.21 | 0.002 | 79.54 ± 5.97 | 0.023 |
| HHEX (rs1111875) | 473 | | | | | | | | | | |
| GG | 126 | 9.18 ± 0.35 | | 8.69 ± 0.57 | | 1.36 ± 0.15 | | 1.49 ± 0.11 | | 69.99 ± 5.02 | |
| *GA* | 225 | 8.90 ± 0.25 | | 8.38 ± 0.52 | | 1.59 ± 0.18 | | 1.59 ± 0.16 | | 71.38 ± 5.23 | |
| AA | 122 | 8.61 ± 0.38 | 0.386 | 7.84 ± 0.48 | 0.194 | 1.64 ± 0.25 | 0.321 | 1.65 ± 0.22 | 0.451 | 72.24 ± 5.64 | 0.341 |
| TCF7L2 (rs7903146) | 468 | | | | | | | | | | |
| TT | 121 | 9.59 ± 0.42 | | 8.85 ± 0.57 | | 1.38 ± 0.16 | | 1.31 ± 0.07 | | 67.12 ± 4.23 | |
| CT | 225 | 9.10 ± 0.32 | | 8.41 ± 0.52 | | 1.52 ± 0.13 | | 1.57 ± 0.11 | | 68.95 ± 4.51 | |
| CC | 122 | 8.21 ± 0.36 | 0.004 | 7.63 ± 0.48 | 0.005 | 1.75 ± 0.25 | 0.071 | 1.86 ± 0.23 | 0.024 | 79.52 ± 5.42 | 0.016 |

Data are *means ± SE unless otherwise indicated. †Adjusted for age, gender, and BMI. Significance was considered with a *p* value less than 0.05.

genetic determinants also play roles in type 2 diabetes susceptibility. And owing to advances in genome-wide association studies (GWASs), a few genes with common variants have been convincingly confirmed to be associated with T2DM in various populations all over world [9-13].

Recently lots of researches have focused on and found genetic variants associated with the T2DM or T2DM complications in Chinese [12, 28-30]. However, "Chinese" is a common category for all populations in China, which has a vast territory, with significantly variant climatic conditions. Actually, there are in China multiple ethnics that of various lifestyle, genetic backgrounds. Thus, it is urgent to identify the genetic variants which are associated with T2DM, in the context of populations of different areas or in the context of in China. And it has been confirmed that the prevalence of T2DM varies significantly in different areas in China [31, 32]. Besides the two metropolitan areas of Beijing and Shanghai, the Northeast part of China, including three provinces of Heilongjiang, Liaoning and Jilin, is on the top list of the highest prevalence of T2DM in China, though this area is relatively under-developed, compared to the East, South, even North part of China [32].

Various genetic variants, such as SLC30A8 (rs13266634), CDKN2A/2B (rs10811661), HHEX (rs1111875) and TCF7L2 (rs7903146) have been identified to be associated with T2DM in populations in the areas rather than Northeast part in China with the GWAS methods [8, 33, 34]. And most of them are confirmed to be also associated with the insulin resistance or insulin sensitivity. The diabetes-susceptible genes SLC30A8/ZnT8, HHEX and TCF7L2 have been shown to regulate hepatic insulin clearance or insulin metabolism, and contribute to insulin resistance in German or Taiwanese adolescents [35-37].

In present study, we evaluated the association of the above-mentioned four genetic variants with T2DM in Han population in Jilin province, in Northeast part of China. We first confirmed the association with the risk of type 2 diabetes, of CDKN2A/2B (rs10811661) and TCF7L2 (rs7903146) (**Table 2**). The risk allele frequency was significantly different in the two SNPs of CDKN2A/2B (rs10811661) and TCF7L2 (rs7903146) between the T2DM and control groups. Both SNPs were each significantly associated with type 2 diabetes. While the risk frequency of SLC30A8 (rs13266634) or HHEX (rs1111875) did not varied between the T2DM group and control group, revealing no significant association in the SNP of them with T2DM. And further association analysis confirmed the T2DM-associated CDKN2A/2B (rs10811661) and TCF7L2 (rs7903146) indicated that the two SNPs were significantly associated with type 2 diabetes–related parameters (FPG,

HbA1c, and FINS). And what's more, along with SLC30A8 (rs13266634), the two genes were associated with the insulin resistance-related phenotypes, HOMA-IR, and HOMA-β.

In summary, we firstly identified CDKN2A/2B (rs10811661) and TCF7L2 (rs7903146) are significantly associated with T2DM in Han population in Jilin province in Northeast part of China. And the two genes were also associated with the insulin resistance in these population, along with SLC30A8 (rs13266634).

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## Disclosure of conflict of interest

None.

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