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# Association of ARHGEF11 R1467H polymorphism with risk for type 2 diabetes mellitus and insulin resistance in Chinese population

**Jing Liu · Xiaohui Chen · Qian Guo · Xiaoqin Ma · Junqiang Zhang · Xiaofeng Huang · Xiaohui Zhang · Shulan Zhang**

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**Abstract** The human Rho guanine nucleotide exchange factor 11 (ARHGEF11), located on chromosome 1q21, is an activator of Rho GTPases involved in G protein signaling pathway known to regulate insulin secretion and action. The aim of our study was to evaluate the relationship between the previously reported R1467H G/A variant in ARHGEF11 and risk of type 2 diabetes mellitus (T2DM) and insulin resistance as well as metabolic traits in a Chinese population. We genotyped R1467H G/A polymorphism in 311 patients with T2DM and 328 control subjects in a Chinese population, using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) protocol and DNA sequencing methods. The genotype and allele distributions of the R1467H G/A polymorphism were significantly different between the T2DM group and the normal control group ($P = 0.024$, 0.018, respectively) and we also found that the A allele carriers (GA + AA genotype) had markedly higher risk of T2DM as compared with the wild-type GG genotype after adjustment for gender, age, and BMI (OR = 1.578, 95% CI 1.126–2.212, $P = 0.008$). Moreover, in the T2DM group the A allele carriers had higher FPG, FINS, and HOMA-IR than that of the GG homozygote ($P = 0.015$, 0.029, and 0.007, respectively). FPG and HOMA-IR levels were also significantly increased from A allele carriers to GG homozygote in the control group ($P = 0.017$, 0.012, respectively). Our investigation suggests that the R1467H polymorphism of ARHGEF11 gene may contribute to susceptibility to T2DM and insulin resistance in a Chinese population.

**Keywords** ARHGEF11 · R1467H polymorphism · Chinese · Type 2 diabetes mellitus · Insulin resistance

J. Liu (✉) · X. Chen · Q. Guo · X. Ma
Department of Endocrinology, Gansu Provincial People's Hospital, 204 West Donggang Road, Lanzhou 730000, Gansu, People's Republic of China
e-mail: liujingwelcome@126.com

J. Zhang · X. Huang · X. Zhang · S. Zhang
First Clinical Medical School of Lanzhou University, Lanzhou 730000, Gansu, People's Republic of China

## Introduction

Type 2 diabetes mellitus (T2DM) is a major public health burden and is likely to affect 366 million individuals across the globe by 2030, especially in the developing countries [1, 2]. So far genome-wide association studies have contributed significantly to search for genetic loci associated with T2DM [3], suggesting that T2DM is a complex genetic disorder influenced by interactions between multiple susceptible genetic variants and environmental factors. Likewise, insulin resistance and pancreatic beta cell dysfunction are key factors in the pathogenesis of T2DM.

The recent studies revealed that one of the strongest evidences for linkage to T2DM has similarly been identified at chromosome 1q21–q24. Within this region of linkage, there is a plausible positional candidate gene, ARHGEF11, having been analyzed among different ethnic groups [4–6]. ARHGEF11 is an activators of Rho GTPases that play a fundamental role in the regulation of G protein signaling and a number of cellular processes, including insulin secretion [7–9], insulin signaling [10, 11] and lipid metabolism [12]. Human ARHGEF11 is a member of a Rho family of guanine nucleotide exchange factors (RhoGEFs) that contain regulator of G protein signaling domains. Three closely related RhoGEFs consisting of ARHGEF1 (p115RhoGEF), ARHGEF12 (LARG) and ARHGEF11 (PDZ-RhoGEF) [13], which are involved in G protein-coupled receptor to

stimulate Rho activation [14–16]. In addition, Swiercz et al. [17] found that ARHGEF11 can be phosphorylated by tyrosine kinases and directly interact with plexin B1, a receptor for Semaphorin 4D (also known as CD100), activating Rho signaling pathways and influences axon guidance. ARHGEF11 is highly expressed in various tissues of humans, such as liver, muscle, pancreas and adipose tissue [18]. Therefore, the ARHGEF11 gene can be also considered as a good candidate for the aetiology of T2DM and insulin resistance.

The three most recent studies [4–6], in several populations, showed the association of R1467H G/A variant (dbSNP ID: rs945508) in the ARHGEF11 gene with the risk of T2DM or insulin resistance. However, contrary to the findings in the Old Order Amish [4], the minor allele A was identified as a risk variant for T2DM in Pima Indians and German Caucasians populations [5, 6]. It is well known that the inconsistency in the results of genetic variants may result from different ethnic groups and geographic regions or may be implicated in linkage disequilibrium with a true functional variant in ARHGEF11 gene. But the relationship between ARHGEF11 gene polymorphisms and the susceptibility to T2DM in Chinese has not been realized so far, as well as diabetes-related metabolic traits. Hence, we attempted to assess whether the R1467H mutation might be a risk factor for T2DM and insulin resistance in a Han population from Northwest China by PCR-RFLP.

## Materials and methods

### Subjects

A total of study subjects were comprised of 311 T2DM subjects (167 men, 144 women; age 53.10 ± 10.34 years) and 328 non-diabetic subjects (163 men, 165 women; age 52.33 ± 11.34 years). All diabetic patients were newly diagnosed T2DM inpatients and randomly recruited from Department of Endocrinology and Diabetology of Gansu Provincial People's Hospital. The T2DM patients were diagnosed according to the criteria recommended by the World Health Organization in 1999 [19]. The non-diabetic subjects (controls) with a normal 75 g oral glucose tolerance test or a fasting plasma glucose concentration <6.1 mmol/l were randomly enrolled from Medical Examination Center (MEC) of Gansu Provincial People's Hospital and were ethnically age- and gender-matched healthy subjects. All subjects, who were unrelated Northwestern Chinese Han population in Gansu Province, had no evidence of family history of diabetes. To avoid interferences with biological variables, people with previous diagnosis of type 1 diabetes, liver diseases, renal diseases and heart diseases or those, receiving treatment for hypercholesterolaemia, hypertension or T2DM, were excluded from the study. The protocol of the study was approved by the local ethics committee and written informed consent was obtained from all subjects.

### Laboratory measurements

The peripheral venous blood samples were drawn from all subjects into EDTA tubes after an overnight (>12 h) fast. Biochemical measurements (fasting plasma glucose, total cholesterol, triglyceride, high-density lipoprotein, low-density lipoprotein and fasting serum insulin) were determined by the Clinical Laboratory in Gansu Provincial People's Hospital following standardized laboratory methods. The homeostasis model assessment (HOMA) index for insulin resistance (HOMA-IR) was calculated by using the formulas: HOMA-IR = FPG (mmol/l) × Fins (mU/l)/22.5 [20].

### Genotyping of ARHGEF11 R1467H polymorphism by PCR-RFLP

Genomic DNA was extracted from EDTA anti-coagulated peripheral blood by the standard phenol–chloroform method [21]. Genotyping for R1467H G/A polymorphism of ARHGEF11 was identified by performing PCR-RFLP analysis. The forward primer 5′-ACAGGAACGGGA GGATAGC-3′ and reverse primer 5′-GGAATGGTTTT GGGGTGATG-3′ were designed using primer Premier 5.0 software (Premier, Canada). PCR reactions was carried out in a 25 μl mixed solution containing approximately 2.5 μl 10× PCR buffer, 2.0 μl dNTPs 2.5 mM, 1.0 μl each primer 20 pmol/μl, 0.2 μl *Taq* polymerase, 3 μl genomic DNA (TaKaRa, China). Amplification conditions were as follows: initial denaturation step for 5 min at 94°C followed by 35 cycles of denaturation for 30 s at 94°C, annealing for 30 s at 63°C, and extension for 50 s at 72°C, with a final extension step for 10 min at 72°C in a thermalcycler (TC-512 Thermal Cycler, techne, UK). The 377 bp PCR products were digested for 14 h at 37°C in 15 μl systems with Csp6I (MBI Fermentas) and separated by electrophoresis on 2% agarose gels stained with ethidium bromide (EB). The GG wild-type homozygote yielded two fragments of 277 and 100 bp, while the AA mutant homozygote yielded a fragment of 377 bp. The heterozygote GA produced three fragments of 377, 277 and 100 bp (Fig. 1).

### DNA sequencing

A 10% quantity of the PCR samples were confirmed the genotype by direct sequencing using dye-labeled terminators

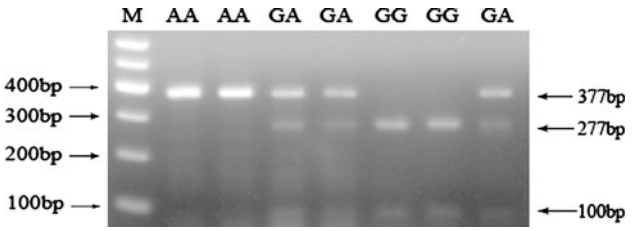


**Fig. 1** Genotypes of ARHGEF11 R1467H polymorphism by PCR-RFLP analysis. *M* represents 100 bp DNA ladder. Csp6I digestion of PCR products of the GG genotype shows two fragment (277 and 100 bp), the GA genotype shows three fragments (377, 277 and 100 bp) and the AA genotype shows one fragments (377 bp)

(BigDye terminator v3.1) on an automated ABI-PRISM 3730 Genetic Analyzer (Shanghai Sangon Co. Ltd. China) (Fig. 2a–c). And the sequencing results showed that 100% genotypes were consistent with their previous ones.

### Statistical analysis

All the statistical analysis was carried out by using the SPSS 16.0 statistical software package (SPSS Inc. Chicago, IL, USA). Normally distributed variables were expressed as mean ± SD, and those that were not distributed normally with median (25th to 75th interquartile range). Comparison between the groups was performed with the student's *t*-test or Mann–Whitney *U*-test, as appropriate. Categorical variables were compared by $\chi^2$ test. Allelic frequencies were calculated by the gene-counting method, and comparison the proportions of genotype and allele frequencies were performed using $\chi^2$ test. Hardy–Weinberg equilibrium was tested with $\chi^2$ test to compare consistency of the observed genotype frequencies with the expected genotype frequencies among the subjects. Odds ratios (OR) and 95% confidence interval (95% CI) were calculated by binary logistic regression analysis to assess the strength of the relationship between a particular allele and genotype with T2DM. Further to adjust for the possible confounding effect of gender, age and BMI, these were included as covariates in the analysis of type 2 diabetes. Power calculation was carried out using Quanto software (Version 1.0, available at http://hydra.usc.edu/gxe/), and power achieved by our sample size was 83%. A two-tailed $P < 0.05$ was set for statistical significance. The level of significance was adjusted using Bonferroni correction for testing multiple clinical characteristics of the genotypes, with a value of $P < 0.0029$ (0.05/17) was considered to be statistically significant.

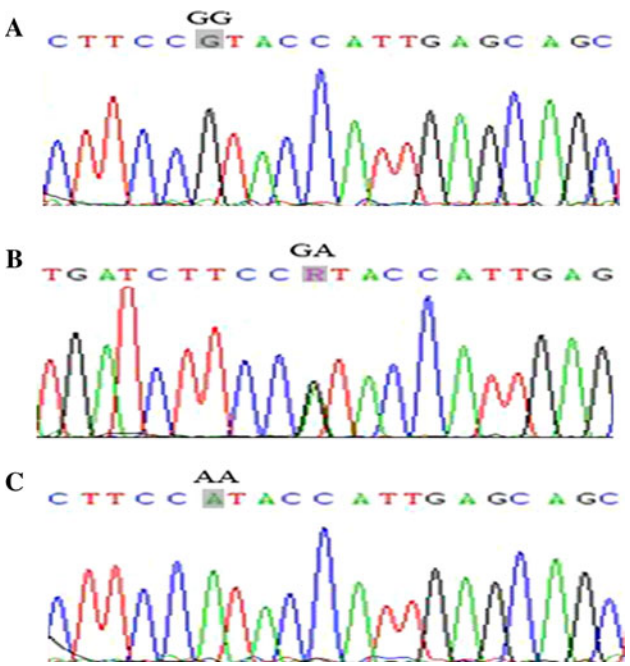


**Fig. 2** The result of DNA sequencing analysis of ARHGEF11 R1467H polymorphism. **a** Sequencing result of the GG genotype. **b** Sequencing result of GA genotype. **c** Sequencing result of AA genotype

## Results

### Characteristics of study subjects

A summary of the baseline characteristics of the study population is presented in Table 1. No statistically significant difference were identified between the two groups with regard to gender distribution, age, height, weight, BMI, and hip circumference ($P > 0.05$). However, the T2DM subjects had higher FINS ($P = 0.031$), Diastolic BP ($P = 0.003$), waist circumference, WHR, systolic BP, FPG, HOMA-IR, TC, TG, and LDL-C ($P = 0.000$). By contrast, the level of HDL-C was significantly lower in patients with T2DM compared with control subjects ($P = 0.000$).

### ARHGEF11 R1467H allele frequency and genotype distribution

The distribution of the genotypic and allelic frequency of the R1467H G/A polymorphism is shown in Table 2. The observed genotype frequencies of both the T2DM patients and the controls were not deviated from the Hardy–Weinberg equilibrium ($\chi^2 = 0.629$ $P = 0.730$; $\chi^2 = 0.358$ $P = 0.836$, respectively). The distribution of R1467H genotype was significantly different between the T2DM and control groups ($\chi^2 = 7.497$, df = 2, $P = 0.024$). The frequencies of heterozygote GA and the variant genotypes (GA + AA) were bound up with an increasing risk of T2DM as compared with the wild-type GG genotype after

**Table 1** The baseline characteristics of the study subjects

| Variables | T2DM ($n = 311$) | Controls ($n = 328$) | $P$ value |
|---|---|---|---|
| Gender (M/F) | 167/144 | 163/165 | 0.312 |
| Age (years) | 53.10 ± 10.34 | 52.33 ± 11.34 | 0.369 |
| Height (cm) | 166.17 ± 6.89 | 165.11 ± 7.53 | 0.065 |
| Weight (kg) | 68.17 ± 8.37 | 67.52 ± 9.61 | 0.151 |
| BMI (kg/m$^2$) | 24.64 ± 2.25 | 24.72 ± 2.73 | 0.726 |
| Waist circumference (cm) | 86.31 ± 7.51 | 83.15 ± 8.04 | 0.000 |
| Hip circumference (cm) | 95.03 ± 5.08 | 94.86 ± 6.89 | 0.817 |
| WHR | 0.91 ± 0.06 | 0.88 ± 0.05 | 0.000 |
| Systolic BP (mmHg) | 135.55 ± 15.96 | 126.08 ± 18.65 | 0.000 |
| Diastolic BP (mmHg) | 81.91 ± 9.68 | 79.34 ± 11.68 | 0.003 |
| FPG (mmol/l) | 11.08 ± 2.35 | 4.99 ± 0.71 | 0.000 |
| FINS (mU/l) | 8.60 (5.63–12.10) | 7.80 (5.90–10.00) | 0.031 |
| HOMA-IR | 4.02 (2.71–6.02) | 1.77 (1.27–2.19) | 0.000 |
| TC (mmol/l) | 4.79 ± 1.07 | 4.44 ± 0.96 | 0.000 |
| TG (mmol/l) | 1.94 ± 0.61 | 1.41 ± 0.46 | 0.000 |
| HDL-C (mmol/l) | 1.04 ± 0.36 | 1.21 ± 0.38 | 0.000 |
| LDL-C (mmol/l) | 2.81 ± 0.86 | 2.31 ± 0.74 | 0.000 |

Data are expressed as mean ± SD or median (25th to 75th interquartile range)

*M* male, *F* female, *BMI* body mass index, *WHR* waist-to-hip ratio, *BP* blood pressure, *FPG* fasting plasma glucose, *FINS* fasting insulin, *HOMA-IR* insulin resistance index, *TC* total cholesterol, *TG* triglyceride, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol

**Table 2** Genotypes and alleles frequencies of ARHGEF11 R1467H (G/A) between T2DM patients and controls

| Polymorphism | T2DM $n = 311$ (%) | Controls $n = 328$ (%) | $P$ value | Crude OR (95% CI) | $P$ value | Adjusted[a] OR (95% CI) |
|---|---|---|---|---|---|---|
| Genotypes | | | | | | |
| GG | 198 (63.7) | 241 (73.5) | | 1 (Ref.) | | 1 (Ref.) |
| GA | 104 (33.4) | 78 (23.8) | 0.006 | 1.623 (1.145–2.300) | 0.006 | 1.630 (1.149–2.314) |
| AA | 9 (2.9) | 9 (2.7) | 0.682 | 1.217 (0.474–3.125) | 0.719 | 1.190 (0.461–3.074) |
| GA + AA | 113 (36.3) | 87 (26.5) | 0.008 | 1.581 (1.129–2.214) | 0.008 | 1.578 (1.126–2.212) |
| Alleles | | | | | | |
| G | 500 (80.4) | 560 (85.4) | | 1 (Ref.) | | |
| A | 122 (19.6) | 96 (14.6) | 0.018 | 1.423 (1.061–1.909) | | |

*n* number of subjects, % frequencies, *Ref* reference

*P* values from $\chi^2$ test. OR (95% CI) estimated by logistic regression analysis

[a] Adjusted for gender, age, and BMI

adjustment for gender, age, and BMI (for GA: OR = 1.630, 95% CI 1.149–2.314, $P = 0.006$; for GA + AA: OR = 1.578, 95% CI 1.126–2.212, $P = 0.008$). Using the G allele as a reference, the minor A allele was significantly higher in T2DM group than that in the control group (19.6 vs. 14.6%, OR = 1.423, 95% CI 1.061–1.909, $P = 0.018$). However, no significant difference was observed in the frequency of AA genotype between the two groups after adjustment for gender, age, and BMI (OR = 1.190, 95% CI 0.461–3.074, $P = 0.719$).

## Analysis of clinical characteristics of the genotypes

Table 3 shows the relationship between the genotype of the R1467H polymorphism and clinical characteristics of the study subjects. The ARHGEF11 R1467H GA and AA genotype has been reported to be related to T2DM, insulin resistance or other metabolic parameters, so the GA heterozygote and AA homozygote variant were combined for comparison with the wild-type GG homozygote in this study. All participants' gender, age, anthropometric measurements,

**Table 3** The baseline characteristics of the study subjects according to ARHGEF11 genotype

| Characteristics | T2DM | | | Controls | | |
|---|---|---|---|---|---|---|
| | GG | GA + AA | *P* value | GG | GA + AA | *P* value |
| Gender (M/F) | 102/96 | 65/48 | 0.307 | 119/122 | 44/43 | 0.848 |
| Age (years) | 53.38 ± 10.45 | 52.61 ± 10.17 | 0.527 | 52.34 ± 11.02 | 52.30 ± 12.26 | 0.977 |
| Height (cm) | 166.08 ± 6.61 | 166.67 ± 7.15 | 0.334 | 164.77 ± 8.14 | 164.46 ± 7.27 | 0.753 |
| Weight (kg) | 67.90 ± 8.58 | 68.65 ± 8.00 | 0.447 | 66.23 ± 10.35 | 66.66 ± 9.52 | 0.732 |
| BMI (kg/m$^2$) | 24.63 ± 2.39 | 24.66 ± 2.01 | 0.889 | 24.37 ± 3.23 | 24.60 ± 2.74 | 0.550 |
| Waist circumference (cm) | 85.87 ± 7.23 | 87.07 ± 7.93 | 0.177 | 83.26 ± 8.08 | 82.86 ± 7.98 | 0.698 |
| Hip circumference (cm) | 94.23 ± 5.09 | 95.35 ± 5.31 | 0.065 | 96.09 ± 5.86 | 96.09 ± 5.26 | 0.998 |
| WHR | 0.91 ± 0.06 | 0.91 ± 0.05 | 0.826 | 0.87 ± 0.05 | 0.86 ± 0.06 | 0.537 |
| Systolic BP (mmHg) | 135.71 ± 15.88 | 135.27 ± 16.16 | 0.813 | 125.48 ± 18.74 | 127.76 ± 18.39 | 0.329 |
| Diastolic BP (mmHg) | 82.04 ± 9.63 | 81.68 ± 9.81 | 0.757 | 79.22 ± 12.01 | 79.69 ± 10.77 | 0.746 |
| FPG (mmol/l) | 10.84 ± 2.39 | 11.51 ± 2.22 | 0.015 | 4.94 ± 0.71 | 5.15 ± 0.67 | 0.017 |
| FINS (mU/l) | 7.67 (5.57–11.00) | 9.30 (6.10–13.08) | 0.029 | 7.80 (5.53–9.68) | 8.30 (6.00–9.90) | 0.178 |
| HOMA-IR | 3.73 (2.45–5.23) | 4.51 (3.10–6.50) | 0.007 | 1.57 (1.22–2.12) | 1.84 (1.39–2.39) | 0.012 |
| TC (mmol/l) | 4.75 ± 1.10 | 4.85 ± 1.02 | 0.405 | 4.43 ± 0.96 | 4.49 ± 0.94 | 0.598 |
| TG (mmol/l) | 1.95 ± 0.63 | 1.94 ± 0.59 | 0.936 | 1.40 ± 0.46 | 1.43 ± 0.48 | 0.628 |
| HDL-C (mmol/l) | 1.03 ± 0.34 | 1.07 ± 0.39 | 0.277 | 1.20 ± 0.38 | 1.22 ± 0.39 | 0.812 |
| LDL-C (mmol/l) | 2.81 ± 0.83 | 2.82 ± 0.91 | 0.876 | 2.33 ± 0.74 | 2.25 ± 0.74 | 0.419 |

Data are expressed as mean ± SD or median (25th to 75th interquartile range)

blood pressure and blood lipid profile such as TC, TG, HDL-C and LDL-C revealed no significant relationship with this polymorphism. In the analysis of Student's *t*-test and Mann–Whitney *U*-test, we found that the A allele carriers (GA + AA genotype) had significantly higher FPG, FINS, and HOMA-IR than the GG homozygote carriers in the T2DM groups ($P = 0.015$, 0.029, and 0.007, respectively). However, only two parameters, FPG and HOMA-IR in A allele carriers were higher than that in those with GG genotype in the control group and likewise were statistically significant ($P = 0.017$, 0.012, respectively). But these results were not statistically significant if the level of significance was adjusted using Bonferroni correction for testing multiple clinical characteristics of the genotypes. Although the mean FINS levels of A allele carriers were higher than those with GG homozygote in controls, the difference was not significant ($P > 0.05$).

## Discussion

In this study, we investigated the potential association between the R1467H G/A polymorphism of the ARHGEF11 gene and the risk of T2DM in a Chinese population by a case–control study. To the best of our knowledge, this is the first report demonstrating the GA genotype and A allele frequencies of R1467H were significantly different between the T2DM and control groups in a Chinese population, which suggested that R1467H variant may be useful as genetic susceptibility marker for T2DM. It is worth pointing out that our finding was consistent with results reported recently by Pima Indians [5] and German [6] investigators. And the A allele carrier (GA + AA genotype) had an approximately 1.6-fold higher risk of developing T2DM than those with the GG homozygote. The association remained significant after adjustment for gender, age, and BMI in the general population. These findings showed that the A allele at R1467H was the risk allele for T2DM and was likely to affect the progression of T2DM. However, Fu et al. [4] reported that the more common G allele was identified as a risk allele for T2DM in the Amish. Associations with opposite risk alleles in the different populations may indicate that R1467H is not the functional variant, but rather is in linkage disequilibrium with a true functional variant in ARHGEF11 gene or due to subjects from different genetic and environmental backgrounds. The nonsynonymous R1467H variant in exon 39 is a G to A substitution at nucleotide 1,467 in the encoding region of the ARHGEF11 gene, which causes a codon converting from arginine to histidine. This replacement linked to T2DM or diabetes-related traits may alter tertiary protein conformation, protein–protein interactions, or even phosphorylation events that involve the amino acid at 1,467. The nonsynonymous R1467H substitution is a good candidate to represent a causal marker within the ARHGEF11 gene although its functional significance remains to be further elucidated. It is noteworthy that the R1467H is located in the

COOH-terminal domain of ARHGEF11 protein that is responsible for homomultimerization and heteromultimerization, an event that regulates ARHGEF11 protein activity [22].

ARHGEF11 interacts with Rho subfamily G proteins, that function as molecular switches in signaling pathways that include the insulin signaling cascade [23, 24]. Therefore, ARHGEF11, whose protein product can activate Rho, was investigated as a positional candidate gene for T2DM on chromosome 1q21 and related phenotypes. Several recent studies also referred to Rho GTPases in insulin signaling via activation of the Jun NH2-terminal kinase and p38 mitogen-activated protein kinase pathways [10, 11, 25]. The Rho family small GTP-binding protein TC10 pathway functions in parallel with phosphatidylino-sitol3-kinase to stimulate fully GLUT4 translocation in response to insulin [26, 27]. Finally, a great deal of enzymes involved in lipid metabolism are regulated by GTPases including diacylglycerol kinase [12], and phospholipase D [28]. It has been acknowledged that ARHGEF11 is ubiquitously expressed and is present in various tissues of humans, including liver, muscle, pancreas and adipose tissue [18]. These findings suggest that proteins implicated in G protein signaling such as ARHGEF11 may have close relation with glucose homeostasis.

Our data showed that the FPG levels of the carriers with A allele (GA and AA genotype) were significantly higher than that of the GG wild-type homozygote in both the T2DM group and the control group. This result is similar to Bottcher et al.'s report in which the A allele carriers were closely connected with increased mean 2 h blood glucose levels in a German Caucasian cohort [6]. Rather interestingly in the current study it was noted that the FINS levels in T2DM group were of marginally statistical difference between the GG genotype and the A allele carriers ($P = 0.029$), whereas an effect that disappears in the control subjects. Furthermore, consistent with our finding on T2DM, carriers of the diabetes risk A allele tended to have a higher HOMA-IR levels in type 2 diabetic patients in a Chinese population. Similarly, Ma et al.'s report also found that the association between the R1467H polymorphism and insulin resistance during a hyperinsulinemic–euglycemic clamp in Pima Indians population [5]. These results strongly suggest additional evidence supporting the role of the ARHGEF11 variant in the pathophysiology of T2DM that may be useful in advancing clinical practice and public health genomics. The exact molecular pathogenesis of the association of this gene variant with metabolic parameters associated with diabetes remains unclear, because the nonsynonymous R1467H variant linked to T2DM or diabetes-related traits may directly change protein structure or even phosphorylation events, thus these results also suggest that the A allele carriers at R1467H in ARHGEF11 increases risk of T2DM by reducing insulin-stimulated glucose uptake, possibly as a result of increased insulin resistance.

In conclusion, we believe that R1467H G/A polymorphism of ARHGEF11 was associated with susceptibility to T2DM and insulin resistance as well as diabetes-related traits in the Chinese population, and our results suggest that A allele of this position may be used as a genetic risk marker for T2DM. Furthermore, it should be noted that larger number of samples must be examined to reach more reliable conclusions. Therefore, further studies with larger sample sizes in different ethnic populations are needed to confirm our findings and verify the molecular mechanism by which this polymorphism affects the risk of T2DM and insulin resistance.

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