Evaluation of common variants in MG53 and the risk of type 2 diabetes and insulin resistance in Han Chinese

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Abstract
Abnormally increased skeletal-muscle-specific E3 ubiquitin ligase (MG53) is associated with the inhibition of insulin signalling and insulin resistance (IR) in animal models. Four community-based studies of Han Chinese populations were included in this study to test the association of variants of MG53 and type 2 diabetes (T2D). The results showed that rs7186832 and rs12929077 in MG53 were significantly associated with T2D and impaired fasting glucose (IFG) of females in the discovery-stage case–control study and cohort study respectively of rural population but not in the replication sample of urban population. In rural population, the fasting insulin (mU/L) of the subjects with AA, AG and GG genotypes in rs12929077 were 8.70 ± 8.05, 10.71 ± 11.16 and 13.41 ± 14.26, respectively, and increased linearly in T2D cases without medication treatment (P = 0.04). This variant was significantly associated with HOMA-IR (P = 0.020) and HOMA-IS (P = 0.023). In individuals with IFG, the insulin and HOMA-IR of AG carriers were significantly higher than those of AA carriers. In urban population, after glucose loading, there were significant differences in the 30-min glucose, the area under the curve (AUC) of 30-min glucose and the AUC of 120-min glucose according to the genotypes of rs7186832 and rs12929077 in males but not females. Our findings suggest that MG53 variants might confer risk susceptibility to the development of T2D of females and IR particularly in rural population.

Keywords: MG53, Type 2 diabetes, Insulin resistance, Insulin sensitivity, Genetic association

Background
Diabetes affects approximately 10 % of the world’s adult population and is one of the leading risk factors for cardiovascular disease, renal failure and visual impairment (Shaw et al. 2010; van Dieren et al. 2010). Of all of the categories of diabetes, type 2 diabetes mellitus (T2D) accounts for approximately 90 %. In recent decades, the prevalence of T2D has increased rapidly due to ageing of the population, urbanization and lifestyle changes, making it one of the most important public health challenges in China (Xu et al. 2008; Wong and Wang 2006). Although the increase in T2D prevalence is caused by environmental factors, there is considerable evidence that T2D is highly heritable (Pyke 1979; Sandler 1984; Jirkovska 1989). Genome-wide association studies have identified a number of susceptibility loci associated with T2D (Tsai et al. 2010; Sladek et al. 2007; Scott et al. 2007). However, these loci account for only some of the genetic variants in T2D, suggesting that much remains to be discovered. Further research should reveal additional genetic factors based on the understanding of the mechanisms that are involved in the development of T2D.

Insulin resistance (IR), defined as decreased glucose uptake and disposal ability, along with defects in insulin secretion, are fundamental elements in the aetiology of T2D. In the early stage of the disease, IR is highest in skeletal muscle (Kahn 1994), which accounts for approximately two-thirds of glucose utilization after meals. Several studies have demonstrated that insulin receptor, insulin receptor substrate 1 (IRS1) and kinase activities...
are decrease in the muscles of early-T2D patients (Good-
year et al. 1995; Caro et al. 1987), suggesting that insulin
receptor and IRS1 play important roles in the insulin sig-
nalling pathway (Hepp 1980). Further studies have shown
that insulin binds insulin receptor and then activates IRS
protein tyrosine phosphorylation immediately after ini-
tiating insulin’s downstream effects, including the activa-
tion of phosphatidylinositol 3-kinase (PI3K) and the
translocation of glucose transporter 4 (Frattalli et al. 1991;
Murakami and Rosen 1991; Ma et al. 2013). In contrast
to the glucose tolerance change observed in IRS1 tissue-
specific knockout mice (Bruning et al. 1998), animals
with muscle-specific insulin receptor knockout exhib-
ted features of T2D without a change in glucose toler-
ance. Therefore, IR in skeletal muscle may be a key player
in the development of T2D, but the mechanisms that are
involved in skeletal muscle IR remain uncertain.

Recently, a report published in Nature has proposed
a new mechanism underlying IR in skeletal muscle and
metabolic syndrome, identifying a novel role of Mit-
sugumin 53 (MG53) as a muscle-specific E3 ubiquitin
ligase targeting insulin receptor and IRS1 (Song et al.
2013). The specific inhibition of the E3 ubiquitin ligase
of MG53 prevents the degradation of insulin receptor and
IRS1 and indicate that MG53, acting as an E3 ubiquitin
ligase, is a key negative regulator of insulin signal in skel-
etal muscle and that the overexpression of MG53 con-
fers a risk of metabolic disorders (Song et al. 2013). This
evidence provides important new insight into the patho-
physiology of T2D, but there is no report to date of evi-
dence for this association in a human population. Herein,
we investigated the association between MG53 polymor-
phisms and T2D and IR in four community-based studies
in Han Chinese populations.

Methods

Subjects

In the primary stage (discovery phase), our case–con-
tral study (Nantong population) consisted of 776 T2D
patients (249 men/527 women), 522 IFG subjects (180
men/342 women) and 957 NGT control subjects (331
men/626 women) who were recruited to find positive
genetic variants of MG53 for T2D. The participants
were selected from a rural population of 14,469 subjects
in two towns east of the Nantong City, Jiangsu prov-
ince in 2008. According to the diabetes diagnosis crite-
ria of the America Diabetes Association (ADA), IFG and
T2D are defined by fasting plasma glucose (FPG) levels
5.6–7.0 and ≥7.0 mmol/L, respectively, or having a self-
reported T2D history, and NGT is defined by a normal
FPG of ≤5.6 mmol/L. A questionnaire including age,
gender, nationality, education level, physical activity and
household income was given to participants to gather
demographic characteristics. The interview also included
questions associated with the diagnosis and treatment
doing diabetes, hypertension, dyslipidemia and cardiovas-
cular events. Weight, height, blood pressure and waist
circumference were measured twice by different trained
staff members. BMI was calculated as the weight (in kilo-
grams) divided by the square of the height (in metres). A
simplified version of the international physical activity
questionnaire (IPAQ) was applied to evaluate PAI by self-
reporting of 24-h physical activity. The PAI was calcu-
lated based on the hours and metabolic energy estimate
(MET) of physical activities, including sleeping (1 MET),
watching TV or sitting (1.1 MET), light activity (1.5
MET), moderate activity (4 MET), and vigorous activity
(8 MET).

The further replication stage consisted of three study
populations, also from Jiangsu province including a case–
control study of T2D in an Wuxi City urban population,
a baseline survey for cardiovascular disease cohort study
of a general rural population in Yixing City and further
average 5.18 years follow-up (from May, 2014 to Octo-
ber, 2015) was carried out. A cross-sectional study of an
IFG and impaired glucose tolerance (IGT) population
subjected to an oral glucose tolerance test (OGTT) from
the program of metabolic disease surveys in an urban
population of Gulou District, Nanjing City. Similarly to
the data acquired from the Nantong population, demo-
graphic information, disease history, family history of
diabetes, behaviour profiling and anthropometric vari-
ables, except physical activity, were acquired.

The replication case–control study of the Wuxi popula-
tion was based on a community epidemiological survey,
and unrelated ethnic Han Chinese individuals aged more
than 30 years were enrolled. A total of 1200 T2D cases
defined as above and 1200 age- and gender-matched
healthy controls were included.

HOMA indices, including HOMA-IR, HOMA-IS and
HOMA-β, which were used to assess IR, insulin sensi-
tivity and the function of islet β cells, respectively, were
estimated from serum insulin and fasting glucose in 4222
adults aged 30 years or more in the Yixing cohort study.
This population included 497 T2D cases, 875 subjects of
IFG and 2850 subjects of NGT defined as above.

During the community chronic disease survey in Gulou
District, Nanjing City, all of the subjects aged more than
40 years who were free of diabetes after fasting glucose
and 2-h postprandial glucose detection accepted an
OGTT (75 g of glucose) and venous blood samples drew
at 0, 30 and 120 min were used to access IR and pancre-
atic islet β cell function by calculating the AUCs of fast-
ing glucose and insulin. Finally, 1932 individuals were
diagnosed with prediabetes, and the numbers of IFG,
IGT (2-h blood glucose was 7.8–11.1 mmol/L after 75 g
of glucose OGTT) and both IFG and IGT were 329, 1322 and 281, respectively.

The methods were approved by the Nanjing Medical University (Nanjing) and were carried out in accordance with the approved relevant guidelines. All of the individuals provided written informed consent before participation in the study.

Sequencing analysis of MG53
MG53 (gene ID: 493829), located on human chromosome 16p11.2 and spanning 14,519 bp, is conserved in animals. To discover single nucleotide polymorphisms (SNPs) of MG53, 20 Chinese healthy control subjects and 30 extreme phenotype patients of BMI > 30 and fasting blood glucose >10 mmol/L were selected for MG53 sequencing analysis by the Sanger sequencing method. Twelve pairs of primers covering the entire region of MG53, as well as regions 2 k upstream and 1 k downstream, were designed for amplification and sequencing analysis on the basis of GenBank sequences (Ref. Seq. of MG53 NC_000016). No other variant was identified different from the International HapMap Project (HapMap Data Rel 24/phase II Nov08, on NCBI B36 assembly, dbSNP b126). Thus, two tagSNPs rs7186832 and rs12929077 were selected and further genotyped in this study (Additional file 1: Figure S1).

DNA isolation and genotyping
Blood samples were collected in K$_3$-EDTA tubes. Proteinase K digestion and phenol–chloroform extraction were used to isolate genomic DNA from whole blood. Finally, DNA was purified and diluted to 10 ng/μL. Genotyping was performed using TaqMan technology and a 7900HT Fast Real-Time PCR System (Applied BioSystems, Foster City, CA, USA). A 5-μl reaction mixture consisting of 10 ng of DNA, 2.4 μL of TaqMan universal PCR master mix, 0.1 μL of forward and reverse primers and FAM and VIC probes, and 1.2 μl of H$_2$O were prepared for PCR reaction. The TaqMan-MGB probes and primers were all ordered from Applied BioSystems. The genotype was determined automatically with the Sequence Detection System 2.1 software (95 % autocaller confidence level). Then, 5 % of the samples were repeated to determine the consistency, which was greater than 99.5 %. The genotype-calling success rates were greater than 99.9 %.

Calculations
Homeostasis model assessment (HOMA-IR, HOMA-β and HOMA-IS) was used to evaluate IR, insulin secretion and insulin sensitivity. These values were calculated using the following equations: HOMA-IR = fasting plasma glucose × fasting plasma insulin/22.5, HOMA-β = 20 × fasting plasma insulin/(fasting plasma glucose-3.5), and HOMA-IS = 1/(fasting plasma insulin × fasting plasma glucose). The unit of fasting plasma glucose was mmol/L, and the unit of fasting plasma insulin was mU/mL. The ratio of insulin increment to FPG increment 30 min after glucose loading (ΔI30/ΔG30, insulin:glucose ratio, IGR) (Seltzer et al. 1967), area under the curves (AUCs) for glucose and insulin 30 and 120 min after glucose loading, and the ratio of AUC of insulin and the AUC of glucose (insulin release index, IRI) (Stumvoll et al. 2000) were calculated to further evaluate the first-stage islet secretory responses to glycaemic stimulus.

Statistical analysis
A Kolmogorov–Smirnov test was used to determine the normality of distribution of quantitative variables. The quantitative variables of non-normal distributions were natural logarithm transformed to obtain normal distributions for statistical analysis. Levene's test was performed for homogeneity testing. Fisher's exact test was used to test for HWE in the NGT control group. The genotype and allele distribution between cases (T2D and/or IGT subjects) and NGT control individuals were compared using Chi squared (χ$^2$) tests. A comparison of quantitative variables between different genotypes was performed with ANOVA, and a general linear regression was used to adjust for covariates. An ordinal multinomial logistic regression model was performed to estimate the risk of T2D with or without adjustment for age, gender, BMI, or PAI. Bonferroni correction was used for multiple comparisons. The odds ratio (OR) and 95 % confidence intervals (CI) were used to test for association in case–control study and the hazard ratio (HR) and 95 % CI by Cox regression were used to estimate the risk of IFG and T2D in cohort study. All of the statistical analyses were performed with SPSS version 15.0 (SPSS, Inc., Chicago, USA). A two-tailed P < 0.05 was considered statistically significant.

Results
Clinical characteristics
The demographic and clinical characteristics of the four studied populations are summarized in Table 1. Age (5 years) and gender matching were performed in the Nantong, Wuxi and Yixing populations. The indices of body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), waist circumference, fasting plasma glucose (FPG), fasting plasma insulin (FINS), homeostasis model assessment (HOMA)-IR and HOMA-IS in the normal glucose tolerance (NGT), impaired fasting glucose (IFG) and T2D groups increased linearly, and the physical activity index (PAI) and HOMA-β decreased linearly (P$_{\text{trend}}$ < 0.05).
Table 1  Clinical characteristics of NGT, IFG, and T2D subjects in Nantong, Wuxi, Yixing and Gulou populations

| Characteristics | Nantong population | | | | Wuxi population | | | | | | Yixing population | | | | | | Gulou | Prediabetes (n = 1932) |
|-----------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
|                 | NGT (n = 957)     | IFG (n = 522)     | T2D (n = 776)     | NGT (n = 1200)   | IFG (n = 1200)   | T2D (n = 1200)   | NGT (n = 2850)   | IFG (n = 875)     | T2D (n = 497)     | Prediabetes (n = 1932) |
| Female (n %)    | 626 (65.41 %)     | 342 (65.52 %)     | 527 (67.91 %)     | 722 (60.17 %)    | 722 (60.17 %)    | 722 (60.17 %)    | 1685 (59.12 %)   | 535 (61.14 %)     | 287 (57.74 %)     | 1244 (64.39 %) |
| Age (years)     | 58.54 ± 9.47      | 58.05 ± 9.95      | 58.17 ± 8.56      | 56.43 ± 8.02     | 57.43 ± 9.77     | 57.43 ± 9.77     | 56.43 ± 7.01     | 57.43 ± 9.77     | 60.33 ± 10.71     | 60.86 ± 10.88 | 61.75 ± 10.30 | 58.64 ± 9.26 |
| BMI (kg/m²)     | 21.31 ± 1.50      | 25.75 ± 3.85      | 25.54 ± 3.55**    | 22.64 ± 2.86     | 24.92 ± 3.41**   | 24.92 ± 3.41**   | 22.64 ± 2.86     | 24.92 ± 3.41**   | 23.83 ± 3.31      | 24.82 ± 3.44 | 25.08 ± 3.47** | 25.07 ± 3.32 |
| Waist circumference (cm) | 75.49 ± 6.33    | 88.22 ± 10.43     | 88.96 ± 10.35**   | 81.42 ± 9.48     | 85.41 ± 9.23     | 85.41 ± 9.23     | 81.42 ± 9.48     | 85.41 ± 9.23     | 83.51 ± 9.09       | 86.37 ± 9.34 | 86.83 ± 9.36** | 86.83 ± 9.36** |
| Systolic BP (mmHg) | 113.79 ± 11.45   | 130.43 ± 19.14    | 132.72 ± 19.52**  | 118.17 ± 14.88   | 137.47 ± 21.20   | 137.47 ± 21.20   | 132.02 ± 15.65   | 135.43 ± 15.60   | 135.39 ± 16.90**  | 132.61 ± 16.77 | 135.39 ± 16.90** | 135.39 ± 16.90** |
| Diastolic BP (mmHg) | 68.28 ± 7.90     | 77.46 ± 11.10     | 78.11 ± 10.86**   | 75.83 ± 8.64     | 80.12 ± 10.25    | 80.12 ± 10.25    | 82.70 ± 8060    | 84.13 ± 8.53     | 83.64 ± 8.97**    | 78.94 ± 1047 | 83.64 ± 8.97** | 78.94 ± 1047 |
| PAI             | 63.46 ± 19.99     | 60.43 ± 20.68     | 53.81 ± 20.91**   | –                | –                | –                | –                | –                | –                | –                | –                | –                |
| FPG (mmol/L)    | 4.18 ± 0.4618     | 6.08 ± 0.37       | 8.44 ± 3.31**     | 4.51 ± 0.46      | 8.97 ± 3.52      | 4.51 ± 0.46      | 8.97 ± 3.52      | 4.51 ± 0.46      | 8.97 ± 3.52      | –                | –                | –                |
| FINS (mU/L)     | –                | –                | –                | –                | –                | –                | –                | –                | –                | –                | –                | –                |
| HOMA-β          | –                | –                | –                | –                | –                | –                | –                | –                | –                | –                | –                | –                |
| HOMA-IR         | –                | –                | –                | –                | –                | –                | –                | –                | –                | –                | –                | –                |
| HOMA-IS         | –                | –                | –                | –                | –                | –                | –                | –                | –                | –                | –                | –                |

n number of subjects, BMI body mass index, PAI physical activity index, FPG fasting plasma glucose, FINS fasting insulin, HOMA homeostasis model assessment, IR Insulin resistance, NGT normal glucose tolerance, IFG impaired fasting glucose, P probability, T2D type 2 diabetes

* Significant differences in characteristics between NGT, IGT and T2D group (* P < 0.05, ** P < 0.0001) were determined by two-tailed Student’s t-test for quantitative data (mean ± standard deviation) and two-sided Chi squared test for categorical data (%). HOMA-β and HOMA-IR are log-transformed to follow normal distribution for comparison.
Genetic association analyses of case–control study
In Nantong population, both rs7186832 and rs12929077 were confirmed to be in Hardy–Weinberg equilibrium (HWE) in the control group ($P > 0.05$). As shown in Table 2, the additive (TT vs TC vs CC) and dominant (TT vs TC + CC) models of rs7186832 were significantly associated with T2D in the Nantong population after adjusting for BMI, age, gender, and PAI. The ORs (95 % CIs) were 1.31 (1.10–1.57) and 1.21 (1.03–1.40), and the $P$ values were 0.014 and 0.003, respectively. After Bonferroni correction, the dominant model of rs7186832 remained statistically significant ($P = 0.003 \times 6$). The AG and GG genotypes (dominant) of rs12929077 carrier showed significant risk of T2D than AA genotype carrier and the OR (95 % CI) was 1.18 (1.02–1.39), $P = 0.049$, after adjustment for age, gender, BMI and PAI.

Further stratification analysis by gender showed that the dominant models of rs7186832 and rs12929077 were significantly associated with T2D in the female population, and the ORs (95 % CIs) were 1.58 (1.27–1.96) and 1.34 (1.08–1.65) with $P$ values of $3.9 \times 10^{-5}$ and 0.006, respectively (Table 3). The association strength of the two SNPs with T2D in females was even higher than that in the whole population, whereas no association was found in untreated T2D cases, and the insulin concentration observed with the variants of rs12929077 (P = 0.013) in untreated T2D cases, and the insulin concentration in the subjects with AA, AG and GG genotypes was $8.70 \pm 8.05, 10.71 \pm 11.16$ and $13.41 \pm 14.26$ mU/L, respectively. Both HOMA-IR and HOMA-IS according to the rs12929077 genotype were significantly different, with $P$ values of 0.02 and 0.023, respectively (Table 6). In the IFG group, AA genotype carriers of rs12929077 presented a relatively lower level of insulin and HOMA-IR and a higher HOMA-IS than those of the AG and GG genotypes.

No statistically significant difference in IRS1 levels was observed among the different genotypes of rs7186832 and rs12929077 in each group or in the whole population (Additional file 1: Table S2). A correlation analysis showed that IRS1 was significantly correlated with FPG (r = 0.131, $P = 0.03$) in 275 randomly selected subjects;

### Table 2 Associations of MG53 rs7186832 and rs12929077 with T2D in Nantong population

| SNP     | Group | WT/HT/MT | Additive model (WT vs HT vs MT) | Dominant model (WT vs HT + MT) |
|---------|-------|----------|---------------------------------|---------------------------------|
|         |       |          | OR (95 % CI)$^a$ OR (95 % CI)$^b$ OR (95 % CI)$^c$ | OR (95 % CI)$^a$ OR (95 % CI)$^b$ OR (95 % CI)$^c$ |
| rs7186832 | TT/TC/CC | 637/282/38 | 1.03 (0.89–1.18) 1.03 (0.89–1.18) 1.21 (1.03–1.40) | 1.06 (0.91–1.24) 1.07 (0.91–1.26) 1.31 (1.1–1.57) |
|          | NGT   |          | P = 0.717 P = 0.680 P = 0.014 | P = 0.489 P = 0.439 P = 0.003 |
|          | IFG   | 360/142/20 | P = 0.850 P = 0.070 P = 0.089 | P = 0.968 P = 0.825 P = 0.049 |
|          | T2D   | 502/247/26 | P = 0.850 P = 0.070 P = 0.089 | P = 0.968 P = 0.825 P = 0.049 |
| rs12929077 | AA/AG/GG | 570/343/41 | 0.99 (0.86–1.13) 0.99 (0.87–1.14) 1.13 (0.98–1.32) | 1.01 (0.86–1.17) 1.02 (0.87–1.19) 1.18 (1.02–1.39) |
|          | NGT   |          | P = 0.850 P = 0.070 P = 0.089 | P = 0.968 P = 0.825 P = 0.049 |
|          | IFG   | 320/182/16 | P = 0.850 P = 0.070 P = 0.089 | P = 0.968 P = 0.825 P = 0.049 |
|          | T2D   | 459/283/29 | P = 0.850 P = 0.070 P = 0.089 | P = 0.968 P = 0.825 P = 0.049 |

SNP single nuclear polymorphisms, WT wild type, HT heterozygote, MT mutant type, OR odds ratio, CI confidence interval, NGT normal glucose tolerance, IFG impaired fasting glucose, $P$ probability, T2D type 2 diabetes

$^a$ $P$ value of $\chi^2$ test for comparison of genotype between case and control groups

$^b$ Ordinal multinomial logistic regression analysis adjusted for age, gender and PAI

$^c$ Ordinal multinomial logistic regression analysis adjusted for age, gender, BMI and PAI
however, this correlation could not be replicated in the NGT or IFG subgroups or in the untreated T2D population (Additional file 1: Table S3). No statistical correlation was observed between IRS1 and FINS or HOMA-β, HOMA-IR and HOMA-IS (P > 0.05).

In the Gulou population, no significant difference in glucose, insulin or the indices of OGTT and HOMA was detected among the genotypes of MG53 SNPs (Additional file 1: Table S4) after glucose loading. Further stratification analysis showed that the 30-min glucose, the area under the curve (AUC) of 30-min glucose and the AUC of 120-min glucose increased significantly with the CC genotype (vs TT + TC) of rs7186832 and the GG genotype (vs AA + AG) of rs12929077 in males.
but not in females (Fig. 1). The results of stratification analysis by gender are listed in Additional file 1: Table S5.

Discussion
MG53, also known as tripartite motif 72 (TRIM72) in humans, is a newly identified member of the tripartite
Fig. 1 Stratification analysis showed that 30 min glucose (a1, a2), AUC of 30 min glucose (b1, b2) and AUC of 120 min glucose (c1, c2) after glucose load increase significantly in CC genotype (vs TT + TC) of rs7186832 carriers and GG genotype (vs AA + AG) of rs12929077 in males but not in females.
motif-containing (TRIM) family and is specifically expressed in the skeletal muscle and heart. The up-regulation of MG53 has been observed in high-fat diet (HFD)-induced obese mice, db/db diabetic mice, spontaneously hypertensive rats and non-human primate models of metabolic syndrome as compared to control animals. Ko et al. have confirmed that MG53 overexpression inhibits IRS1 phosphorylation and myogenesis in C2C12 myoblasts (Lee et al. 2010), and the insulin receptor and IRS1 levels notably changed when the insulin signal pathway is blocked. In this study, we observed positive association of MG53 polymorphisms and IFG and T2D in females in Nantong rural population but not in Wuxi urban populations. In Yixing rural population of cohort study, the association of MG53 polymorphisms and IFG was further replicated in females. These findings support that MG53 variants might confer risk susceptibility to the development of T2D of females in rural population.

Furthermore, in Yixing rural population, FINS linearly increased with the variation of rs12929077 in the untreated T2D population, and differential HOMA-β, HOMA-IR and HOMA-IS were observed in both the IFG group and untreated T2D population. The above results verify the population-based evidence associating MG53 with HOMA-IR, HOMA-IS, and T2D in the Han Chinese population. The findings from the present study thus confirm the role of MG53 in IR (Song et al. 2013). In addition, the genetic effects of MG53 on islet beta cell secretion and regulating blood glucose function by OGTT were evaluated in Gulou urban population and the results indicated that the 30-min glucose, AUC of 30 min glucose and AUC of 120-min glucose increased with the variation of rs7186832 and rs12929077 in males but not in females. These findings provide further evidence strengthening the impact of MG53 on the development of T2D.

We further evaluated a regional LD plot (http://www.broadinstitute.org/mpg/snap/ldplot.php) of the two positive SNPs in T2D (Additional file 1: Figure S1). The LD values \( r^2 \) were estimated for neighbouring loci and rs7186832 \( (r^2 > 0.9) \) and rs12929077 \( (r^2 > 0.8) \). We suggest that these closely linked loci need to be considered to further evaluate the genetic effect of MG53 in T2D.

Although the SNP rs7186832 in exon 3 is a synonymous variant, an online bioinformatics prediction tool (http://snpinfo.niehs.nih.gov/cgi-bin/snpinfo/snpfunc.cgi) indicated that the rs7186832 C > T variant acts in splicing regulation (Exon Splicing Silencer, ESS) for MG53, and the rs12929077 G > A variant is associated with transcription factor binding sites (TFBS) to AP2α (core match score = 0.996) and BRCA (core match score = 0.994).

The AP2α transcription factor belongs to a family of three closely related nuclear proteins that regulate genes involved in development, apoptosis, and cell cycle control (Hilger-Eversheim et al. 2000). A previous study has reported that the AP2a site acts as a positive regulator on site 5 in SLC2A10, which encodes high-affinity glucose transporter 10 (GLUT10) (Segade et al. 2005). GLUT10 is widely expressed in adult tissues, including organs that play major roles in glucose homeostasis (Rothman et al. 1995), and the haplotype of SLC2A10 is modestly associated with T2D (Lin et al. 2006). BRCA (breast cancer, early onset) encodes a nuclear phosphoprotein that plays a role in maintaining genomic stability, and a previous study has reported that after a diagnosis of, women breast cancer with a BRCA1 or BRCA2 mutation face a twofold increase in the risk of diabetes (Bordeleau et al. 2011). These data provide considerable biologic plausibility for a role of MG53 in glucose homeostasis, insulin signal regulation and T2D.

This study did not identify the IRS1 level correlating with the variants of MG53 or the HOMA index. Given the tissue specificity of MG53 and factors affecting the IRS1 level in plasma (Krutzenfeld et al. 2000; Chibalin et al. 2000), the plasma IRS1 level may only partly reflect MG53 expression. Meanwhile, this discrepancy may indicate that IRS1 might not directly interfere with the genetic effects of MG53 on pancreatic β cell function, IR or T2D, and further research on IRS1 function is warranted.

Besides the potential bias in case–control studies, there are some limitations as follows. Owing to a lack of an appropriate ELISA kit and muscle tissues, plasma MG53 expression levels could not be detected; thus, correlations between MG53 polymorphisms, MG53 expression and T2D risk could not be established in our study population. Regardless of the above limitation, this study provides updated evidence of MG53 polymorphisms, HOMA indices and T2D. In case of potential type I error, further replication study in large sample size population would be warranted.

Conclusively, our study constitutes an initial examination to investigate whether MG53 variants are associated with T2D, and the findings provide new insight into the molecular mechanism of MG53 involved in the pathogenesis of T2D through the effects on pancreatic β-cell function and IR.

**Additional file**

**Additional file 1.** Supplementary material contains supplementary tables 1 to 5 and supplementary figure.
Authors’ contributions
CS, SY and HG conceived and designed the study profile. HZ, KK, YQ, TY, YC, XZ and ZH contributed reagents/materials. HZ, KK, XC and JW performed the experiment. HZ and CS analyzed the data. HZ, CS, HG and HS wrote and revised the manuscript. All authors reviewed the manuscript. All authors read and approved the final manuscript.

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Competing interests
The authors declare that they have no competing interests.

Ethics approval and consent to participate
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