Medicine®

OBSERVATIONAL STUDY

OPEN

Type 2 Diabetes Risk Allele *UBE2E2* Is Associated With Decreased Glucose-Stimulated Insulin Release in Elderly Chinese Han Individuals

Kuanfeng Xu, PhD, Lin Jiang, MD, PhD, Mei Zhang, MD, PhD, Xuqin Zheng, MD, PhD, Yong Gu, MD, Zhixiao Wang, MD, Yun Cai, MD, Hao Dai, MD, Yun Shi, MD, Shuai Zheng, MD, Yang Chen, MD, Li Ji, MD, Xinyu Xu, PhD, Heng Chen, MD, Min Sun, MD, PhD, and Tao Yang, MD, PhD

Abstract: Recently, rs163182 in *KCNQ1*, rs7612463 in *UBE2E2*, rs7119 in *HMG20A*, and rs6815464 in *MAEA* were discovered as type 2 diabetes (T2D) loci unique to Asians, and rs13342692 in *SLC16A11* were newly reported as T2D loci in multiethnicities by genome-wide association (GWA) studies. The aim of the present study is to ascertain the potential associations between these variants and T2D risk in the Chinese population, and characterize diabetic-related quantitative traits underlying these variants.

A total of 4268 Chinese Han individuals (1754 patients with T2D and 2514 glucose-tolerant health subjects, age ≥40 years) were genotyped for these 5 variants. All the health individuals underwent an oral glucose tolerance test (OGTT), and measures of insulin release and sensitivity were estimated from insulinogenic, BIGTT, Matsuda, and disposition indices. The associations were determined by using logistic regression analysis.

After adjustment for age, sex, and BMI, rs163182 in *KCNQ1* ($P = 0.002$) and rs7612463 in *UBE2E2* ($P = 0.024$) were found to be associated with T2D risk in Chinese Han population. The risk C allele of rs7612463 in *UBE2E2* is associated with decreased IGI ($P = 0.001$), BIGTT-AIR ($P = 0.002$), CIR ($P = 0.002$), and DI ($P = 0.006$). The other 4 variants did not associate with insulin release or sensitivity.

UBE2E2 rs7612463 may mediate its diabetogenic impact on insulin response, which highly depends on the impairment of β-cell function.

(*Medicine* 95(19):e3604)

Abbreviations: AIR = acute insulin response, CI = confidence interval, CIR = corrected insulin response, DI = disposition index, GWA = genome-wide association, IGI = insulinogenic index, OGTT = oral glucose tolerance test, OR = odds ratio, T2D = type 2 diabetes.

INTRODUCTION

More than 60 genetic loci have been identified with convincing evidence of association with type 2 diabetes (T2D) risk from European large-scale genome-wide association (GWA) studies and meta-analyses, especially reported by the Diabetes Genetics Replication and Meta-analysis (DIAGRAM) Consortium.[1] The associations between these loci in European and glucose homeostasis, causal molecular mechanisms on islet function have been well characterized.[1,2] Till now, about 20 common T2D risk variants were discovered by Southeast Asian GWA studies,[3–10] and most of them were replicated in other studies for their associations with T2D risk and diabetic-related quantitative traits. However, as one of these loci in East Asians,[6] rs7612463 in *UBE2E2* was reported to be negatively associated with T2D risk in another 2 Japanese case–control studies.[11,12] And this variant was investigated for glycemic traits only based on fasting-based homeostasis model assessment of beta cell function (HOMA-β) and insulin resistance (HOMA-IR) indexes.[11] Except for rs7612463, rs163182 in *KCNQ1*,[7] rs7119 in *HMG20A*,[9] and rs6815464 in *MAEA*[10] were also reported as independent T2D tag variants, but they have not been extensively replicated in other independent studies, and the results were inconsistent. Furthermore, no glycemic traits about these variants were reported. In addition, rs13342692 in *SLC16A11*, a novel variant was newly reported in a Mexican GWA study[13] and replicated in multiethnicities by the SIGMA Type 2 Diabetes Consortium,[14] but its relationship to beta cell function and insulin resistance is still not elucidated.

Therefore, we aimed to evaluate the degree to which these 5 variants confer T2D risk in Chinese Han population. We further performed a meta-analysis on present Chinese study and other eligible Asian case–control studies to provide a quantitative assessment for these variants in Asians, which might have the possibility of reaching reliable and stable conclusions. Due to the sparse knowledge of these variants concerning the

Editor: Zhenhua He.
Received: December 8, 2015; revised: March 29, 2016; accepted: April 12, 2016.
From the Department of Endocrinology, the First Affiliated Hospital of Nanjing Medical University, Nanjing, Jiangsu, China.
Correspondence: Prof. Tao Yang, Prof. Min Sun, Department of Endocrinology, the First Affiliated Hospital of Nanjing Medical University, 300 Guangzhou Road, Nanjing, 210029, Jiangsu, China (e-mail: yangt@njmu.edu.cn, drsunm@163.com).
KX, LJ, MS, and XZ contributed equally to this work.
KX: conception and design, analysis and interpretation of data, drafting of the manuscript; ZW, MS, XZ, HD, YS, SZ, YC, and LJ: collection and selection of samples; JL, YG, YC, XX, HC, and MZ: analysis and interpretation of data; TY: conception and design, and critical revision of the manuscript; and all the co-authors gave final approval of the version to be published.
Duality of interest: The authors declare that there is no duality of interest associated with this manuscript.
This study was supported by grants from the National Natural Science Foundation of China (numbers 30971405 and 81270897), the Natural Science Foundation of Jiangsu province (number BK2012486), Jiangsu Government Scholarship for Overseas Studies (JS-2013-260), the Chinese Society of Endocrinology and National Clinical Research Center for Metabolic Diseases, and the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD).
The authors have no conflicts of interest to disclose.
Supplemental digital content is available for this article.

ISSN: 0025-7974
DOI: 10.1097/MD.0000000000003604

diabetes causing mechanisms, and improved precision of the oral glucose tolerance test (OGTT), we subsequently characterized the influence of these variants on surrogate measures of beta cell function and insulin sensitivity derived from an OGTT.

METHODS

Study Population

The present work was one part of the baseline survey from REACTION study investigating the association of diabetes and cancer, which was conducted among 259,657 adults, aged 40 years and older in 25 communities across mainland China, from 2011 to 2012.[15,16] Diabetes was diagnosed according to the criteria established by the World Health Organization. The exclusion criteria for the subjects with T2D were diabetes caused by liver dysfunction, pancreatitis, gastrointestinal diseases, malignancy, and individuals who tested positive for anti-GAD antibody. The inclusion criteria for the nondiabetic control subjects were as follows: HbA1c $\leq$6.0% (4.2 mmol/mol), fasting plasma glucose $<$ 5.6 mmol/l and 2 hours plasma glucose (postprandial glucose) $<$ 7.8 mmol/l, no family history of T2D in first-degree relatives, and no past history of a diagnosis of diabetes. Then we enrolled 2514 glucose-tolerant health individuals and 1754 patients with T2D for case–control analyses. Subsequently, 2514 health individuals were further measured for diabetic-related quantitative traits. Clinical characteristics of the study population are shown in the Electronic Supplementary Material (ESM) Table 1, http://links.lww.com/MD/A965. Written informed consent was obtained from all participants prior to investigation. The study was approved by the scientific ethics committee of the First Affiliated Hospital of Nanjing Medical University and conducted in accordance with the principles of the Helsinki Declaration II.

Derived Estimates of Insulin Release and Insulin Sensitivity From an OGTT

All participants were measured for plasma glucose and serum insulin at fasting, 30 and 120 minutes during an OGTT. The insulinogenic index (IGI), BIGTT-acute insulin response (AIR), and the corrected insulin response (CIR) were reported as indices of oral glucose-stimulated insulin release. The surrogate measures of insulin sensitivity were estimated by the Matsuda insulin sensitivity index ($ISI_{Matsuda}$) and the BIGTT-sensitivity index (SI). The BIGTT indexes integrated information on sex and BMI combined with plasma glucose and serum insulin and were calculated during an OGTT as reported. HOMA-B and HOMA-IR indexes were also calculated for comparison purposes. Beta cell function corrected for insulin sensitivity level was expressed as the disposition index (DI). For DI 1 we multiplied BIGTT-AIR with BIGTT-SI, for DI 2 we divided CIR by HOMA-IR.[17] Laboratory measurements and calculation of glycemic traits are provided in ESM Table 2, http://links.lww.com/MD/A965.

Genotyping Assay

Genomic DNA was extracted from peripheral blood (QIAamp DNA blood kit; QIAGEN, Germany). We selected 5 variants at genetic loci that had been reported to be robustly associated with T2D risk in recent GWA studies, including rs163182 in *KCNQ1*, rs7612463 in *UBE2E2*, rs7119 in *HMG20A*, rs6815464 in *MAEA*, and rs13342692 in *SLC16A11*. The genotyping of these variants was performed using Sequenom MassArray (BGI CO. LTD, China). Primer sequences and UEP primers used for MassARRAY IPLEX Genotyping are shown in ESM Table 3, http://links.lww.com/MD/A965. The genotyping success call rate was above 97% for all variants, and 215 samples measured in duplicate ($\approx$5%) were in complete concordance. The distributions of genotypes for all variants were in the Hardy–Weinberg equilibrium (all $P > 0.05$). Detailed information is shown in ESM Table 4, http://links.lww.com/MD/A965.

Statistical Analyses

Associations between the investigated 5 variants and T2D risk were tested by an additive model with adjustment for age, sex, and BMI. Correction for multiple testing was performed by the Bonferroni test. $P \leq 0.01$ was considered significant, and P value between 0.05 and 0.01 was considered nominally significant. Associations for diabetic-related quantitative traits were examined by the additive model, with adjustment for age (BIGTT indexes and DI 1) or age, sex, and BMI (all other traits). Values of serum insulin, IGI, BIGTT-AIR, CIR, HOMA-B, HOMA-IR, $ISI_{Matsuda}$, and DI were logarithmically transformed. Correction for multiple testing was performed by the Bonferroni test (correcting for 5 variants). A P value below 0.01 was considered significant, and a P value between 0.05 and 0.01 was considered nominally significant. Statistical analyses were performed using SPSS (version 18.0). Combined meta-analysis was performed using the Mantel–Haenszel procedure with a fixed effect model or the DerSimonian–Laird method with a random effect model after testing for heterogeneity. The genome-wide significance level ($P < 10^{-8}$) was considered significant for meta-analysis. The power of sample size to identify the association of these variants was calculated using the ''CaTS power calculator for genetic studies'' software (http://www.sph.umich.edu/csg/abecasis/CaTS/). Analyses of statistical power to detect quantitative trait associations were calculated using Quanto software (http://hydra.usc.edu/gxe/).

RESULTS

The genotype distribution of all the 5 investigated variants in the case and control groups did not deviate from HWE (ESM Table 4, http://links.lww.com/MD/A965). The effect directions of these variants were consistent with those reported in previous GWA studies. After adjustment for age, sex, and BMI, rs163182 in *KCNQ1* was found to be significantly associated with T2D (odds ratio [OR] 1.165, 95% confidence interval [CI] 1.055, 1.287; $P = 0.002$) and rs7612463 in *UBE2E2* showed a nominal association with T2D risk (OR 1.144, 95% CI 1.018, 1.286; $P = 0.024$) (Table 1). As for rs7119 in *HMG20A*, rs6815464 in *MAEA*, and rs13342692 in *SLC16A11*, we were unable to detect any association with T2D risk in present Chinese samples ($P = 0.076$, 0.565, and 0.515, respectively). When we combined the present data with previous reported Asian studies, the associations of rs163182 and rs7612463 with T2D were further strengthened at the genome-wide significant level ($P = 1.01 \times 10^{-16}$ and 2.01×10^{-12}, respectively). In addition, rs6815464 was also observed to reach a genome-wide significant level with T2D risk (2.45×10^{-10}), but not rs7119 or rs13342692 (ESM Table 5, http://links.lww.com/MD/A965). Moreover, to examine whether these variants also contributed to risk of obesity, the relationship between these variants and log-transformed BMI, overweight/obesity was estimated, but none of them showed any association after adjusted for age and sex, as shown in ESM Tables 6–7, http://links.lww.com/MD/A965.

TABLE 1. Association Results for the 5 Investigated Variants in the Present Population-Based East Chinese Individuals

Gene	SNP	Risk Allele	n		RAF		Additive (Unadjusted)		Additive (Adjusted)*	
			T2D	Control	T2D	Control	OR (95%CI)	*P* Value	OR (95%CI)	*P* Value
KCNQ1	rs163182	C	1737	2510	0.374	0.342	1.149 (1.050–1.257)	0.003	1.165 (1.055–1.287)	0.002
UBE2E2	rs7612463	C	1736	2495	0.799	0.777	1.141 (1.026–1.269)	0.015	1.144 (1.018–1.286)	0.024
HMG20A	rs7119	A	1740	2504	0.183	0.167	1.119 (0.999–1.253)	0.052	1.121 (0.988–1.271)	0.076
MAEA	rs6815464	C	1720	2485	0.587	0.582	1.022 (0.936–1.115)	0.63	1.029 (0.934–1.132)	0.565
SLC16A11	rs13342692	C	1741	2508	0.128	0.126	1.024 (0.899–1.165)	0.725	1.049 (0.909–1.210)	0.515

BMI = body mass index, CI = confidence interval, OR = odds ratio, RAF = risk allele frequency, SNP = single nucleotide polymorphism, T2D = type 2 diabetes.
*The results were adjusted for age, sex, and BMI.

To identify potential mediators that link these variants with T2D, we analyzed their associations with diabetic-related quantitative traits in 2514 glucose-tolerant health individuals. As shown in Table 2 and ESM Table 8, http://links.lww.com/MD/A965, after adjustment for age, sex, BMI, carriers of the risk C allele of rs7612463 in *UBE2E2* had nominally decreased 30 minutes insulin level ($P = 0.01$). For fasting insulin derived indexes (HOMA-IR and HOMA-β), none of the investigated variants exhibited significant difference. Further we estimate insulin release and insulin sensitivity derived from an OGTT. We found that carriers of the risk C allele of rs7612463 had significantly decreased IGI, BIGTT-AIR, and CIR ($P = 0.001$, $P = 0.002$, and $P = 0.002$, respectively), which are consistent with a significant decrease in the estimate of OGTT-based DI (DI2, $P = 0.006$). In addition, our results indicated that carriers of the risk C allele of rs163182 showed a tendency to be associated with decreased IGI, BIGTT-AIR, CIR, and BIGTT-SI ($P = 0.066$, 0.053, 0.071, and 0.079, respectively). But for the remaining 3 variants, our results did not show any convincing association with any diabetic-related quantitative traits (Table 2 and ESM Table 8, http://links.lww.com/MD/A965).

DISCUSSION

As the first T2D variant in East Asian, rs2237892 in *KCNQ1* was identified by 3 GWA studies.[3,4,18] Recently, rs163182 was reported as another independent T2D tag variant in *KCNQ1* by a Chinese GWA study.[7] Our case–control study and meta-analysis confirmed that rs163182 was significantly associated with T2D in Asians. Although studies indicated that other intronic T2D variants in *KCNQ1*, such as rs2237892, rs2237895, and rs2237897,[19,20] were associated with impaired β-cell function, our study did not support any association between rs163182 and diabetic-related quantitative traits. Interestingly, contradictory results were reported on the relationship between variants in *KCNQ1* and metabolic traits, including rs2237892, rs2237895, and rs2237897. A study from Liu et al[21] revealed that homozygous carriers of these 3 variants had significantly decreased BMI and waist circumferences in control individuals, another study from Yu et al showed that they were associated with lower BMI and lower incidence of overweight/obesity only in diabetic patients, but not in controls.[22] But for rs163182 in *KCNQ1*, we did not find any association with either BMI or overweight/obesity in present study. This implies that rs163182 might not be an obesity risk variant.

Following the identification of *KCNQ1*, rs7612463 in *UBE2E2* was subsequently reported as another Asian T2D risk variant in a Japanese GWA study.[6] Although 2 replicated Japanese case–control studies reported that this variant was negatively associated with T2D risk, our case–control study and further meta-analysis confirmed an association of this variant and T2D at the genome-wide significant level in Asians. Interestingly, DIAGRAM study did not detect any association between this variant and T2D in Europeans, instead they identified that rs1496653 in *UBE2E2* were the strongest causal T2D variants for Europeans.[1] This implicated that examining population-specific causal variants may provide insights into the functional biology that may differ among different ethnic groups. Actually, UBE2E2 is expressed in both human pancreas and a cultured insulin-secreting cell line, and encodes the ubiquitin-conjugating enzyme E2E2, which has been known to play a pivotal role in maintaining normal insulin biosynthesis, secretion, and signaling in pancreatic β cells.[23,24] Assessed by fasting-based homeostasis model, recently studies indicated that rs7612463 was not associated with HOMA-β or HOMA-IR,[11,25] which was confirmed by our results. However, when further applying OGTT-derived indexes, we found that rs7612463 did have a significant association with insulin release indices, including IGI, BIGTT-AIR, and CIR. As different indexes may capture different mechanisms of insulin release, our results implicated that the C risk allele of rs7612463 in *UBE2E2* might impair β-cell function by decreasing glucose-stimulated insulin response, which was confirmed by a significant decrease in 30 minutes insulin levels after applying OGTT. Furthermore, the concomitant decrease in DI suggested that, even if insulin-responsive tissues were affected by the variant, the β-cell defect may be the most profound.

As for the other 2 reported Asian T2D risk variants, rs7119 in *HMG20A* and rs6815464 in *MAEA*[9,10] did not associate with T2D in present Chinese population. Although we had sufficient power (>80% for $\alpha = 0.05$) to estimate the association reported previously, it cannot be completely ruled out that differences in study design and/or experimental procedures may underlie the discrepancy between studies. When we further combined our data with previously reported studies, we found that the association between rs6815464 and T2D risk in Asian populations reached a genome-wide significance level, but not rs7119. Furthermore, we did not find any association between rs7119 and T2D when stratified to obesity, although a study from South Asians identified that rs7178572, another variant in *HMG20A*, was significantly associated with T2D in obese cases ($P = 1.3 \times 10^{-8}$, OR = 1.11, 95%CI 1.07–1.15).[26] In addition, similar to rs7177055,[27] another variant in *HMG20A*, rs7119 associated neither insulin release nor insulin resistance in our

TABLE 2. Associations of 5 Variants With Quantitative Traits in 2514 Glucose-Tolerant East Chinese Individuals

Gene Variants	Wild Type	Heterozygote	Homozygote	β	SE	*P*	Adjusted *P*
KCNQ1 rs163182	GG	GC	CC				
HOMA-β	109.79 (84.18–157.08)	108.06 (79.37–151.46)	140.26 (79.37–150.00)	−0.008	0.007	0.17	0.245
IGI	16.45 (9.56–27.28)	14.79 (8.58–25.53)	15.06 (8.94–26.57)	−0.021	0.011	0.038	0.066
BIGTT-AIR	2086 (1550–2972)	1931 (1470–2877)	1931 (1468–2714)	−0.016	0.008	0.055	0.053
CIR	159.82 (97.47–266.20)	151.64 (88.41–240.63)	146.12 (93.66–263.51)	−0.018	0.01	0.052	0.071
HOMA-IR	2.35 (1.73–3.28)	2.31 (1.72–3.14)	2.27 (1.72–3.10)	−0.006	0.007	0.213	0.397
$ISI_{Matsuda}$	0.068 (0.041–0.110)	0.071 (0.044–0.115)	0.073 (0.047–0.120)	0.012	0.009	0.055	0.173
BIGTT-SI	7.00 ± 3.47	7.14 ± 3.40	7.41 ± 3.44	0.179	0.102	0.076	0.079
DI 1	14342 (10229–19665)	14007 (10410–18691)	14526 (10909–19494)	−0.003	0.007	0.73	0.704
DI 2	65.91 (40.05–110.05)	61.52 (38.19–107.52)	65.42 (39.92–112.22)	−0.012	0.01	0.316	0.262
UBE2E2 rs7612463	CC	CA	AA				
HOMA-β	106.28 (80.99–148.74)	111.14 (81.45–161.62)	106.94 (79.73–143.83)	0.004	0.008	0.482	0.646
IGI	14.92 (8.54–25.73)	16.98 (9.71–28.46)	15.68 (9.16–25.25)	0.042	0.013	<0.001	0.001
BIGTT-AIR	1951 (1468–2795)	2070 (1574–3224)	2019 (1465–2912)	0.03	0.009	0.002	0.002
CIR	147.66 (88.89–240.76)	168.85 (98.67–275.72)	150.93 (93.03–240.21)	0.034	0.011	0.001	0.002
HOMA-IR	2.71 (1.73–3.22)	2.38 (1.66–3.20)	2.33 (1.84–3.12)	0.001	0.008	0.692	0.88
$ISI_{Matsuda}$	0.072 (0.044–0.113)	0.066 (0.042–0.115)	0.065 (0.044–0.105)	−0.018	0.01	0.037	0.082
BIGTT-SI	7.22 ± 3.46	6.91 ± 3.41	7.05 ± 3.29	−0.209	0.116	0.074	0.071
DI 1	14001 (10167–19215)	14655 (10691–19530)	14148 (10298–18892)	0.015	0.008	0.067	0.07
DI 2	62.57 (36.89–105.65)	68.15 (44.09–119.26)	60.09 (42.78–101.57)	0.033	0.012	0.004	0.006
HMG20A rs7119	GG	GA	AA				
HOMA-β	109.43 (81.39–151.74)	105.15 (79.34–149.93)	117.19 (85.86–166.82)	0.005	0.009	0.343	0.574
IGI	15.61 (8.94–26.96)	15.38 (8.95–25.49)	14.34 (8.74–26.1)	−0.017	0.014	0.591	0.245
BIGTT-AIR	1991 (1514–2849)	1957 (1487–2935)	1976 (1512–3294)	0.007	0.11	0.476	0.511
CIR	153.70 (93.59–253.20)	153.89 (93.45–242.11)	155.29 (93.62–293.47)	−0.008	0.013	0.995	0.55
HOMA-IR	2.34 (1.75–3.17)	2.26 (1.66–3.29)	2.60 (1.69–3.57)	0.003	0.009	0.426	0.731
$ISI_{Matsuda}$	0.071 (0.044–0.115)	0.069 (0.041–0.113)	0.054 (0.036–0.102)	−0.005	0.12	0.286	0.692
BIGTT-SI	7.16 ± 3.38	7.07 ± 3.54	6.38 ± 3.87	−0.194	0.131	0.163	0.139
DI 1	14331 (10421–19331)	13910 (10351–19206)	12912 (9095–17425)	−0.015	0.01	0.149	0.113
DI 2	64.91 (38.56–111.80)	62.86 (40.49–106.54)	58.41 (39.38–95.34)	−0.01	0.013	0.625	0.451
MAEA rs6815464	CC	CG	GG				
HOMA-β	106.51 (82.06–151.87)	109.72 (79.62–154.30)	106.31 (81.90–147.89)	−0.002	0.007	0.878	0.799
IGI	14.75 (8.85–25.79)	15.89 (8.97–27.07)	15.34 (9.29–25.82)	0.002	0.011	0.796	0.847
BIGTT-AIR	2010 (1544–2840)	1965 (1468–2965)	2013 (1533–2883)	−0.003	0.008	0.893	0.853
CIR	146.83 (91.43–246.39)	157.01 (94.77–258.12)	157.45 (94.74–244.80)	−0.003	0.007	0.259	0.643
HOMA-IR	2.30 (1.69–3.18)	2.35 (1.76–3.22)	2.31 (1.71–3.17)	0.003	0.006	0.424	0.684
$ISI_{Matsuda}$	0.069 (0.043–0.115)	0.071 (0.043–0.115)	0.069 (0.043–0.107)	0.006	0.009	0.916	0.524
BIGTT-SI	7.10 ± 3.45	7.13 ± 3.44	7.05 ± 3.39	−0.019	0.099	0.868	0.845
DI 1	14220 (10448–19764)	14185 (10398–19119)	14185 (10105–19617)	0	0.007	0.918	0.957
DI 2	62.19 (39.89–107.10)	64.27 (38.56–110.43)	65.87 (39.73–112.78)	0.003	0.01	0.583	0.789
SLC16A11 rs13342692	Wild type (TT)	Heterozygote (TC)	Homozygote (CC)	β	SE	*P*	Adjusted *P*
HOMA-β	107.35 (81.22–152.12)	109.15 (80.20–154.31)	107.73 (86.92–151.08)	9.85E-05	0.01	0.875	0.992
IGI	15.60 (8.99–26.77)	15.39 (8.97–25.80)	11.40 (6.86–21.87)	−0.011	0.016	0.712	0.518
BIGTT-AIR	1990 (1506–2939)	1992 (1548–2776)	1797 (1410–2585)	−0.011	0.012	0.364	0.351
CIR	154.60 (93.64–252.37)	156.36 (95.36–245.77)	134.31 (72.81–188.40)	−0.012	0.014	0.65	0.411
HOMA-IR	2.33 (1.75–3.17)	2.29 (1.61–3.28)	2.24 (1.79–3.12)	−0.004	0.01	0.639	0.684
$ISI_{Matsuda}$	0.069 (0.043–0.114)	0.072 (0.042–0.115)	0.065 (0.039–0.092)	0.005	0.013	0.733	0.712
BIGTT-SI	7.08 ± 3.41	7.23 ± 3.55	6.79 ± 3.28	0.085	0.148	0.542	0.567
DI 1	14062 (10411–19454)	14678 (10324–19217)	12298 (9303–17243)	−0.009	0.011	0.456	0.419
DI 2	63.93 (38.67–110.07)	66.33 (43.54–107.34)	48.59 (29.92–84.92)	−0.008	0.015	0.876	0.588

The effect size corresponds to the β-coefficient (SE) per copy of T2D risk allele and was calculated using a linear regression analysis in the additive model. Except for BMI was adjusted for age and sex, all the other traits were adjusted for age, sex, and BMI. Values of insulinogenic index, BIGTT-AIR, CIR, HOMA-B, HOMA-IR, $ISI_{Matsuda}$, and DI were logarithmically transformed. BMI = body mass index, IGI = insulinogenic index, BIGTT-AIR = BIGTT-acute insulin response, BIGTT-SI = BIGTT-sensitivity index, CIR = corrected insulin response, $ISI_{Matsuda}$ = Matsuda insulin sensitivity index, DI 1 = BIGTT-AIR*BIGTT-SI, DI 2 = CIR/HOMA-IR, SE = standard error.

present study. For rs6815464 in *MAEA*, we did not find any association with the OGTT-derived traits either. Till now, the function of HMG20A and MAEA is still not clear, further functional characterization is required to elucidate their role in the pathogenesis of T2D.

No association between rs13342692 in *SLC16A11* and T2D risk or diabetic-related quantitative traits was found in our present study despite the fact that this newly discovered variant was found in Mexican and multiethnic cohorts.[14] However, as a missense variant (D127G), rs13342692 was labeled as damaging by computational prediction with SIFT[28] and thus, conclusions based on this variant alone should be interpreted with caution. Actually, SLC16A11 is expressed in liver and acts as a regulator of lipid metabolism, most notably causing an increase in intracellular triacylglycerol levels.[13] But its role in the pathogenesis of T2D is still unknown and needs further studies.

We also performed statistical power analyses for the investigated variants in different scenarios (ESM Table 9, http://links.lww.com/MD/A965), demonstrating the need for combining efforts in further meta-analyses when searching for the diabetes intermediary phenotype. Only ∼5% to 20% of heritability for most common diseases has been explained, when provided a limited glimpse into the full architecture of a given trait.[29] In this regard, further studies should be proposed to explain missing heritability, such as the identification of rare or low-frequency variants, copy number variations, gene–gene interaction, and gene–environment interaction, etc.

In conclusion, our study suggested that rs163182 in *KCNQ1*, rs7612463 in *UBE2E2*, and rs6815464 in *MAEA* are associated with T2D risk in Asians, and risk alleles of rs7612463 in *UBE2E2* might be associated with an impairment of beta cell function.

ACKNOWLEDGMENT

The authors thank the National Natural Science Foundation of China (numbers 30971405 and 81270897), the Natural Science Foundation of Jiangsu province (number BK2012486), Jiangsu Government Scholarship for Overseas Studies (JS-2013-260), the Chinese Society of Endocrinology and National Clinical Research Center for Metabolic Diseases, and the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD) for the support. The authors also thank Dr Eddie James and Dr S Alice Long at Benaroya Research Institute at Virginia Mason, Seattle, WA, US for both their revision of the manuscript.

REFERENCES

1. Morris AP, Voight BF, Teslovich TM, et al. Large-scale association analysis provides insights into the genetic architecture and pathophysiology of type 2 diabetes. *Nat Genet.* 2012;44:981–990.
2. Gaulton KJ, Ferreira T, Lee Y, et al. Genetic fine mapping and genomic annotation defines causal mechanisms at type 2 diabetes susceptibility loci. *Nat Genet.* 2015;47:1415–1425.
3. Unoki H, Takahashi A, Kawaguchi T, et al. SNPs in KCNQ1 are associated with susceptibility to type 2 diabetes in East Asian and European populations. *Nat Genet.* 2008;40:1098–1102.
4. Yasuda K, Miyake K, Horikawa Y, et al. Variants in KCNQ1 are associated with susceptibility to type 2 diabetes mellitus. *Nat Genet.* 2008;40:1092–1097.
5. Tsai FJ, Yang CF, Chen CC, et al. A genome-wide association study identifies susceptibility variants for type 2 diabetes in Han Chinese. *PLoS Genet.* 2010;6:e1000847.
6. Yamauchi T, Hara K, Maeda S, et al. A genome-wide association study in the Japanese population identifies susceptibility loci for type 2 diabetes at UBE2E2 and C2CD4A-C2CD4B. *Nat Genet.* 2010;42:864–868.
7. Cui B, Zhu X, Xu M, et al. A genome-wide association study confirms previously reported loci for type 2 diabetes in Han Chinese. *PLoS One.* 2011;6:e22353.
8. Kooner JS, Saleheen D, Sim X, et al. Genome-wide association study in individuals of South Asian ancestry identifies six new type 2 diabetes susceptibility loci. *Nat Genet.* 2011;43:984–989.
9. Sim X, Ong RT, Suo C, et al. Transferability of type 2 diabetes implicated loci in multi-ethnic cohorts from Southeast Asia. *PLoS Genet.* 2011;7:e1001363.
10. Cho YS, Chen CH, Hu C, et al. Meta-analysis of genome-wide association studies identifies eight new loci for type 2 diabetes in east Asians. *Nat Genet.* 2011;44:67–72.
11. Iwata M, Maeda S, Kamura Y, et al. Genetic risk score constructed using 14 susceptibility alleles for type 2 diabetes is associated with the early onset of diabetes and may predict the future requirement of insulin injections among Japanese individuals. *Diabetes Care.* 2012;35:1763–1770.
12. Yamakawa-Kobayashi K, Natsume M, Aoki S, et al. The combined effect of the T2DM susceptibility genes is an important risk factor for T2DM in non-obese Japanese: a population based case-control study. *BMC Med Genet.* 2012;13:11.
13. SIGMA Type 2 Diabetes ConsortiumWilliams AL, Jacobs SB, et al. . Sequence variants in SLC16A11 are a common risk factor for type 2 diabetes in Mexico. *Nature.* 2014;506:97–101.
14. SIGMA Type 2 Diabetes ConsortiumEstrada K, Aukrust I, et al. . Association of a low-frequency variant in HNF1A with type 2 diabetes in a Latino population. *JAMA.* 2014;311:2305–2314.
15. Ning G. Reaction Study Group. Risk Evaluation of cAncers in Chinese diabeTic Individuals: a lONgitudinal (REACTION) study. *J Diabetes.* 2012;4:172–173.
16. Bi Y, Lu J, Wang W, et al. Cohort profile: risk evaluation of cancers in Chinese diabetic individuals: a longitudinal (REACTION) study. *J Diabetes.* 2014;6:147–157.
17. Nielsen T, Sparsø T, Grarup N, et al. Type 2 diabetes risk allele near CENTD2 is associated with decreased glucose-stimulated insulin release. *Diabetologia.* 2011;54:1052–1056.
18. Li H, Gan W, Lu L, et al. A genome-wide association study identifies GRK5 and RASGRP1 as type 2 diabetes loci in Chinese Hans. *Diabetes.* 2013;62:291–298.
19. Qi Q, Li H, Loos RJ, et al. Common variants in KCNQ1 are associated with type 2 diabetes and impaired fasting glucose in a Chinese Han population. *Hum Mol Genet.* 2009;18:3508–3515.
20. Hu C, Wang C, Zhang R, et al. Variations in KCNQ1 are associated with type 2 diabetes and beta cell function in a Chinese population. *Diabetologia.* 2009;52:1322–1325.
21. Liu Y, Zhou DZ, Zhang D, et al. Variants in KCNQ1 are associated with susceptibility to type 2 diabetes in the population of mainland China. *Diabetologia.* 2009;52:1315–1321.
22. Yu W, Ma RC, Hu C, et al. Association between KCNQ1 genetic variants and obesity in Chinese patients with type 2 diabetes. *Diabetologia.* 2012;55:2655–2659.
23. Kawaguchi M, Minami K, Nagashima K, et al. Essential role of ubiquitin-proteasome system in normal regulation of insulin secretion. *J Biol Chem.* 2006;281:13015–13020.
24. López-Avalos MD, Duvivier-Kali VF, Xu G, et al. Evidence for a role of the ubiquitin-proteasome pathway in pancreatic islets. *Diabetes.* 2006;55:1223–1231.

25. Knowles JW, Xie W, Zhang Z, et al. Identification and validation of N-acetyltransferase 2 as an insulin sensitivity gene. *J Clin Invest.* 2015;125:1739–1751.

26. Perry JR, Voight BF, Yengo L, et al. Stratifying type 2 diabetes cases by BMI identifies genetic risk variants in LAMA1 and enrichment for risk variants in lean compared to obese cases. *PLoS Genet.* 2012;8:e1002741.

27. Harder MN, Ribel-Madsen R, Justesen JM, et al. Type 2 diabetes risk alleles near BCAR1 and in ANK1 associate with decreased β-cell function whereas risk alleles near ANKRD55 and GRB14 associate with decreased insulin sensitivity in the Danish Inter99 cohort. *J Clin Endocrinol Metab.* 2013;98:E801–E806.

28. Ng PC, Henikoff S. SIFT: predicting amino acid changes that affect protein function. *Nucleic Acids Res.* 2003;31:3812–3814.

29. Eichler EE, Flint J, Gibson G, et al. Missing heritability and strategies for finding the underlying causes of complex disease. *Nat Rev Genet.* 2010;11:446–450.