

A Genome-Wide Association Study Identifies Susceptibility Variants for Type 2 Diabetes in Han Chinese

Fuu-Jen Tsai^{1,2,3,9}, Chi-Fan Yang^{4,5,9}, Ching-Chu Chen^{6,7,9}, Lee-Ming Chuang⁸, Chieh-Hsiang Lu⁹, Chwen-Tzuei Chang^{6,7}, Tzu-Yuan Wang^{6,7}, Rong-Hsing Chen^{6,7}, Chiung-Fang Shiu⁴, Yi-Min Liu⁴, Chih-Chun Chang⁴, Pei Chen⁴, Chien-Hsiun Chen^{4,5}, Cathy S. J. Fann⁴, Yuan-Tsong Chen^{4,5,10*}, Jer-Yuarn Wu^{4,5,11*}

1 School of Post-Baccalaureate Chinese Medicine, China Medical University, Taichung, Taiwan, **2** Department of Medical Genetics, Pediatrics and Medical Research, China Medical University Hospital, Taichung, Taiwan, **3** Department of Biotechnology and Bioinformatics, Asia University, Taichung, Taiwan, **4** Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan, **5** National Genotyping Center, Academia Sinica, Taipei, Taiwan, **6** Division of Endocrinology and Metabolism, Department of Medicine, China Medical University Hospital, Taichung, Taiwan, **7** School of Chinese Medicine, College of Chinese Medicine, China Medical University, Taichung, Taiwan, **8** Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan, **9** Department of Internal Medicine, Endocrinology and Metabolism, Chia-Yi Christian Hospital, Chia-Yi, Taiwan, **10** Department of Pediatrics, Duke University Medical Center, Durham, North Carolina, United States of America, **11** Graduate Institute of Chinese Medical Science, China Medical University, Taichung, Taiwan

Abstract

To investigate the underlying mechanisms of T2D pathogenesis, we looked for diabetes susceptibility genes that increase the risk of type 2 diabetes (T2D) in a Han Chinese population. A two-stage genome-wide association (GWA) study was conducted, in which 995 patients and 894 controls were genotyped using the Illumina HumanHap550-Duo BeadChip for the first genome scan stage. This was further replicated in 1,803 patients and 1,473 controls in stage 2. We found two loci not previously associated with diabetes susceptibility in and around the genes protein tyrosine phosphatase receptor type D (*PTPRD*) ($P=8.54 \times 10^{-10}$; odds ratio [OR]=1.57; 95% confidence interval [CI]=1.36–1.82), and serine racemase (*SRR*) ($P=3.06 \times 10^{-9}$; OR=1.28; 95% CI=1.18–1.39). We also confirmed that variants in *KCNQ1* were associated with T2D risk, with the strongest signal at rs2237895 ($P=9.65 \times 10^{-10}$; OR=1.29, 95% CI=1.19–1.40). By identifying two novel genetic susceptibility loci in a Han Chinese population and confirming the involvement of *KCNQ1*, which was previously reported to be associated with T2D in Japanese and European descent populations, our results may lead to a better understanding of differences in the molecular pathogenesis of T2D among various populations.

Citation: Tsai F-J, Yang C-F, Chen C-C, Chuang L-M, Lu C-H, et al. (2010) A Genome-Wide Association Study Identifies Susceptibility Variants for Type 2 Diabetes in Han Chinese. *PLoS Genet* 6(2): e1000847. doi:10.1371/journal.pgen.1000847

Editor: Peter M. Visscher, Queensland Institute of Medical Research, Australia

Received: June 25, 2009; **Accepted:** January 18, 2010; **Published:** February 19, 2010

Copyright: © 2010 Tsai et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: This study was supported by Academia Sinica Genomic Medicine Multicenter Study; National Research Program for Genomic Medicine, National Science Council, Taiwan (National Clinical Core, NSC97-3112-B-001-014; and National Genotyping Center, NSC97-3112-B-001-015). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: chen0010@ibms.sinica.edu.tw (Y-TC); jywu@ibms.sinica.edu.tw (J-YW)

† These authors contributed equally to this work

Introduction

Type 2 diabetes (T2D) affects at least 6% of the world's population; the worldwide prevalence is expected to double by 2025 [1]. T2D is a complex disorder that is characterized by hyperglycemia, which results from impaired pancreatic β cell function, decreased insulin action at target tissues, and increased glucose output by the liver [2]. Both genetic and environmental factors contribute to the pathogenesis of T2D. The disease is considered to be a polygenic disorder in which each genetic variant confers a partial and additive effect. Only 5%–10% of T2D cases are due to single gene defects; these include maturity-onset diabetes of the young (MODY), insulin resistance syndromes, mitochondrial diabetes, and neonatal diabetes [3–5]. Inherited variations have been identified from studies of monogenic diabetes, and have provided insights into β cell physiology, insulin release, and the action of insulin on target cells [6].

Much effort has been devoted to finding common T2D genes, including genome-wide linkage, candidate-gene, and genome-wide association studies (GWAS). Whole-genome linkage scans have identified chromosomal regions linked to T2D; however, with the exception of regions 1q [7–13] and 20q, which have been repeatedly mapped, linkage results vary from study to study [14–19]. Candidate-gene studies have provided strong evidence that common variants in the peroxisome proliferator-activated receptor- α (*PPARG*) [20], potassium inwardly-rectifying channel J11 (*KCNJ11*) [21–23], transcription factor 2 isoform b (*TCF2*) [24,25], and Wolfram syndrome 1 (*WFS1*) [26] genes are associated with T2D. These genes all have strong biological links to diabetes, and rare, severe mutations cause monogenic diabetes. GWAS have accelerated the identification of T2D susceptibility genes, expanding the list from three in 2006 to over 20 genes in 2009. There are now at least 19 loci containing genes that increase risk of T2D, including *PPARG* [27], *KCNJ11* [27], *KCNQ1* [28,29],

Author Summary

Type 2 diabetes (T2D) is a complex disease that involves many genes and environmental factors. Genome-wide and candidate-gene association studies have thus far identified at least 19 regions containing genes that may confer a risk for T2D. However, most of these studies were conducted with patients of European descent. We studied Chinese patients with T2D and identified two genes, *PTPRD* and *SRR*, that were not previously known to be involved in diabetes and are involved in biological pathways different from those implicated in T2D by previous association reports. *PTPRD* is a protein tyrosine phosphatase and may affect insulin signaling on its target cells. *SRR* encodes a serine racemase that synthesizes D-serine from L-serine. Both D-serine (coagonist) and the neurotransmitter glutamate bind to NMDA receptors and trigger excitatory neurotransmission in the brain. Glutamate signaling also regulates insulin and glucagon secretion in pancreatic islets. Thus, *SRR* and D-serine, in addition to regulating insulin and glucagon secretion, may play a role in the etiology of T2D. Our study suggests that, in different patient populations, different genes may confer risks for diabetes. Our findings may lead to a better understanding of the molecular pathogenesis of T2D.

CDKAL1 [27,29–33], *CDKN2A-2B* [27,32,33], *CDC123-CAMK1D* [34], *MTNR1B* [35–37], *TCF7L2* [31,38,39], *TCF2 (HNF1B)*, *HHEX-KIF11-IDE* [27,32,33,38], *JAZF1* [34], *IGF2BP2* [27,29,32], *SLC30A8* [27,32,33,38], *THADA* [34], *ADAMTS9* [34], *WFS1* [26], *FTO* [27,31], *NOTCH2* [34], and *TSPAN8* [34]. Variants in these genes have been identified almost exclusively in

populations of European descent, except for *KCNQ1*; individually, these variants confer a modest risk (odds ratio [OR] = 1.1–1.25) of developing T2D. *KCNQ1* was identified as a T2D susceptibility gene in three GWA scans in Japanese individuals, highlighting the need to extend large-scale association efforts to different populations, such as Asian populations [28,29,40]. The association of other previously reported loci (*CDKAL1*, *CDKN2A-2B*, *IGF2BP2*, *TCF7L2*, *SLC30A8*, *HHEX*, and *KCNJ11*) with T2D were also replicated in the Japanese population [29,40,41].

To date, a GWA scan for T2D has not been conducted in the Han Chinese population, although the association of some known loci have been confirmed, including *KCNQ1* and *CDKAL1*, *CDKN2A-2B*, *MTNR1B*, *TCF7L2*, *HNF1B*, and *KCNJ11* [42–47]. Therefore, we conducted a two-stage GWA scan for T2D in a Han Chinese population residing in Taiwan. There were a total of 2,798 cases and 2,367 normal controls (995 cases and 894 controls in stage 1, 1,803 cases and 1,473 controls in stage 2). Our accomplished objective was to identify new diabetes susceptibility loci that were associated with increased risk of T2D in a Han Chinese population.

Results

Association analysis

We conducted a two-stage GWAS to identify genetic variants for T2D in the Han-Chinese residing in Taiwan. In the first stage, an exploratory genome-wide scan, we genotyped 995 T2D cases and 894 population controls using the Illumina Hap550duov3 chip (Figure 1 and Table S1). For each sample genotyped in this study, the average call rate was $99.92 \pm 0.12\%$. After applying stringent quality control criteria, high-quality genotypes for 516,737 SNPs (92.24%) were obtained, with an average call rate

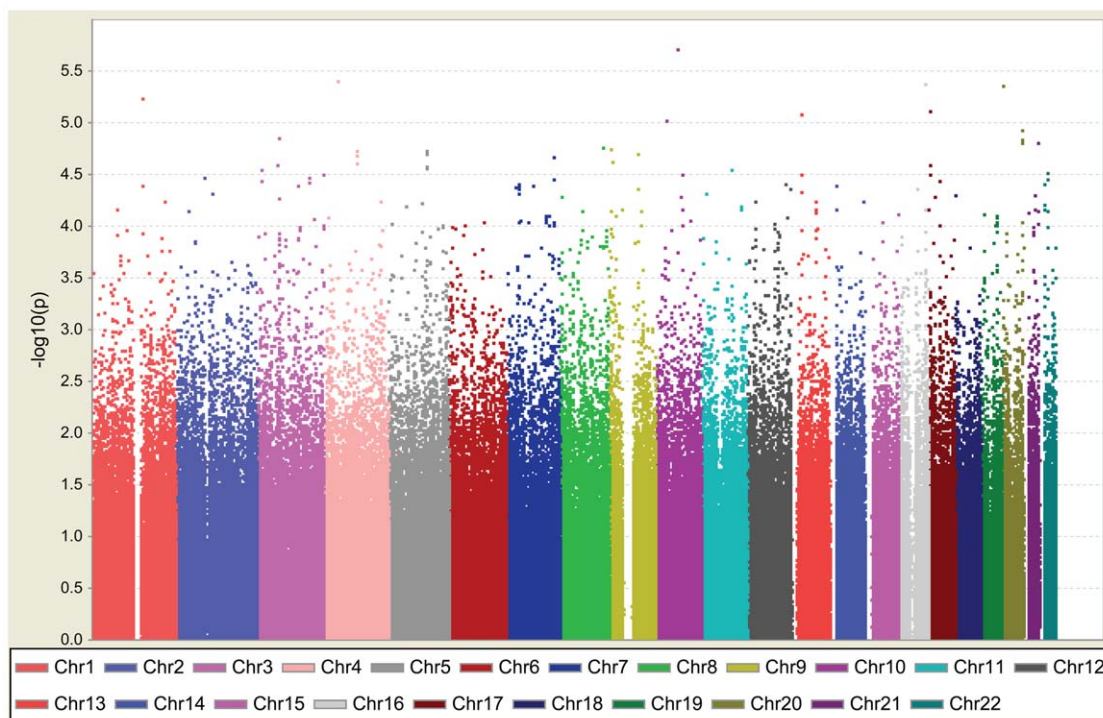


Figure 1. Graphical summary of T2D GWAS in a Han Chinese population. T2D association was determined for SNPs on the Illumina HumanHap550K-Duo chip. The y-axis represents the $-\log_{10} P$ value and the x-axis represents each of the 516,212 SNPs used in the primary scan of 995 T2D cases and 894 controls.

doi:10.1371/journal.pgen.1000847.g001

of $99.92 \pm 0.24\%$ (Table S2). The results of principal component analysis in stage 1 revealed no evidence for population stratification between T2D cases and controls ($P=0.111$, Fst statistics between populations <0.001) (Text S1; Figure S1). Multidimensional scaling analysis using PLINK [48] produced similar results (Text S1; Figure S2). Furthermore, genomic control (GC) with a variance inflation factor $\lambda = 1.078$ (trend test) did not substantially change the results of this GWAS (Table S3).

We selected eight SNPs in seven regions: rs9985652 and rs2044844 on 4p13, rs7192960 on 16q23.1, rs7361808 on 20p13, rs1751960 on 10q11.23, rs4845624 on 1q21.3, rs391300 on 17p13.3, and rs648538 on 13q12.3. These SNPs had association P values of $<10^{-5}$ at stage 1 with any of the genotype, allele, trend, dominant, and recessive models for subsequent cross-platform validation using Sequenom (Table 1; Table S3). For SNPs with weaker associations (P value between 10^{-4} and 10^{-5}), we searched

for novel susceptibility candidates for T2D as implicated by (1) gene function identified by a bioinformatics approach and (2) an animal model showing defects in glucose homeostasis caused by genes within the same subfamily. Therefore, we selected SNP rs17584499 ($P=2.4 \times 10^{-5}$ under best model) for further investigation. rs17584499 lies within protein tyrosine phosphatase receptor type D (*PTPRD*). We hypothesized that *PTPRD* might play a role in the regulation of insulin signaling, because its subfamily members leukocyte common antigen-related (*LAR*) and protein tyrosine phosphatase sigma (*PTPRS*) exhibit defects in glucose homeostasis and insulin sensitivity in knockout and/or transgenic mice [49–51].

We also evaluated the most significant SNP (rs231361) within *KCNQ1*, which was previously reported to be a diabetes susceptibility gene in a Japanese population, as well as in populations of Korean, Chinese, and European ancestry [28,29].

Table 1. Association results for Type 2 diabetes in Han Chinese.

SNP ^a	Chr.	Nearest gene(s)	Risk allele	stage	RAF (T2D)	RAF (NC)	OR (95% CI)	P value (trend)	Joint analysis of stage1+2			
									OR (95% CI)	P value (trend)	P value (perm)	P value (Fisher)
rs9985652	4	ATP8A1	A	1	0.54	0.48	1.25 (1.10–1.42)	9.30×10^{-4}				
				2	0.51	0.52	0.95 (0.86–1.05)	0.316	1.05 (0.97–1.14)	0.193	0.156	0.003
rs2044844	4	ATP8A1	C	1	0.53	0.48	1.24 (1.09–1.41)	9.30×10^{-4}				
				2	0.51	0.52	0.96 (0.87–1.06)	0.406	1.06 (0.98–1.14)	0.17	0.141	0.003
rs7192960	16	MAF	C	1	0.75	0.68	1.39 (1.20–1.61)	7.66×10^{-6}				
				2	0.73	0.70	1.12 (1.01–1.25)	0.037	1.21 (1.11–1.33)	1.33×10^{-5}	6.82×10^{-6}	4.61×10^{-6}
rs7361808	20	SIRPA	G	1	0.09	0.06	1.60 (1.24–2.05)	2.30×10^{-4}				
				2	0.06	0.06	1.07 (0.87–1.31)	0.52	1.25 (1.07–1.47)	0.005	0.005	0.001
rs1751960	10	LYZL1	G	1	0.53	0.46	1.34(1.18–1.53)	1.13×10^{-5}				
				2	0.50	0.49	1.05(0.95–1.16)	0.317	1.15(1.06–1.24)	3.78×10^{-4}	4.05×10^{-4}	4.85×10^{-5}
rs4845624	1	RORC	A	1	0.65	0.58	1.36 (1.19–1.56)	5.85×10^{-6}				
				2	0.61	0.63	0.91 (0.83–1.01)	0.07	1.05 (0.97–1.14)	0.205	0.203	6.46×10^{-6}
rs391300	17	SRR	G	1	0.69	0.63	1.31 (1.14–1.50)	9.00×10^{-5}				
				2	0.68	0.62	1.26 (1.14–1.40)	6.55×10^{-6}	1.28 (1.18–1.39)	3.06×10^{-9}	1.00×10^{-8}	5.52×10^{-8}*
rs4523957^b	17	SRR	T	1	0.71	0.65	1.30 (1.12–1.49)	3.24×10^{-4}				
				2	0.68	0.63	1.26 (1.14–1.40)	8.22×10^{-6}	1.27 (1.17–1.38)	1.44×10^{-8}	3.00×10^{-8}	1.31×10^{-8}*
rs648538	13	KATNAL1	G	1	0.67	0.61	1.28 (1.12–1.47)	2.92×10^{-4}				
				2	0.65	0.66	0.96 (0.86–1.06)	0.407	1.07(0.98–1.16)	0.116	0.125	0.001
rs17584499	9	PTPRD	T	1	0.11	0.07	1.55 (1.23–1.94)	1.41×10^{-4}				
				2	0.09	0.06	1.61 (1.33–1.95)	9.15×10^{-7}	1.57 (1.36–1.82)	8.54×10^{-10}	1.00×10^{-8}	3.07×10^{-9}*
rs231361	11	KCNQ1	T	1	0.85	0.81	1.39 (1.17–1.64)	1.49×10^{-4}				
				2	0.83	0.79	1.26 (1.11–1.44)	2.85×10^{-4}	1.30 (1.18–1.44)	2.89×10^{-7}	3.10×10^{-7}	7.64×10^{-7}*
rs231359^b	11	KCNQ1	A	1	0.85	0.81	1.36 (1.14–1.61)	4.56×10^{-4}				
				2	0.84	0.80	1.32 (1.17–1.51)	1.68×10^{-5}	1.33 (1.20–1.48)	3.43×10^{-8}	5.00×10^{-8}	1.51×10^{-7}*
rs2237895^c	11	KCNQ1	C	1	0.40	0.35	1.28 (1.12–1.46)	2.92×10^{-4}				
				2	0.39	0.33	1.30 (1.17–1.45)	6.46×10^{-7}	1.29 (1.19–1.40)	9.65×10^{-10}	1.00×10^{-8}	4.42×10^{-9}*

^aSNPs are arranged in order of decreasing P value under best statistical model in Stage 1.

^bNeighboring SNPs also significantly associated with T2D.

^cPreviously reported SNP associated with T2D [28, 29] and validated in our study.

*Empirical P value $<10^{-6}$ after 10^8 permutations.

Stage 1 (Genome scan) included 995 cases and 894 controls. Stage 2 (replication stage) included 1,803 cases and 1,473 controls. Alleles were indexed to the forward strand of NCBI Build 36. P value (trend), P value (PC), P value (permutation), and P value (meta) represent P values of trend test, principal component analysis using EIGENSTRAT, permutation, and meta-analysis using Fisher's method, respectively.

Risk allele, allele with higher frequency in cases compared to controls; RAF (T2D) and RAF (NC), risk allele frequencies in cases and controls, respectively; and OR, odds ratio for risk allele.

doi:10.1371/journal.pgen.1000847.t001

Together, these ten SNPs—the 8 SNPs with association $p < 10^{-5}$, rs17584499, and rs231361—were cross-platform validated and yielded consistent results using both Illumina and Sequenom. The concordance rate for stage 1 samples typed on the Illumina and Sequenom platforms was $99.1\% \pm 0.84\%$ (Table S4).

We took these ten SNPs and an additional 29 neighboring SNPs within the linkage disequilibrium (LD) block forward to replicate in 3,803 additional samples (stage 2; 1,803 cases and 1,473 controls). The average call rate for each sample was $96.13\% \pm 4.66\%$. After applying stringent quality control criteria, high-quality genotypes for 35 SNPs (89.7%) were obtained, with an average call rate of $98.96\% \pm 0.24\%$ (Table S2). Of the ten SNPs selected in stage 1, only three SNPs still showed a strong association in the stage 2 analysis: rs17584499 in *PTPRD* at 9p24.1-p23, rs231359 in *KCNQ1* at 11p15.5, and rs391300 in serine racemase (*SRR*) at 17p13.3 (Table 1). We were unable to replicate the association between T2D and the remaining seven SNPs in *ATP8A1/GRXCRI*, *MAF/WWOX*, *SIRPA*, *LYZL1/SVIL*, *RORC/TMEM5*, and *KATNAL1* in the stage 2 analysis (Table 1). Joint analysis of stage 1 and stage 2 data revealed consistent results with stage 2. The most significant associations were found for rs391300, rs17584499, and rs231359 (Table 1; Figure 2). These associations remained significant after calculating P values using 10^8 permutations of the disease state labels. Joint association analysis was performed with all of the 2,798 T2D cases and 2,367 controls; this could achieve a power of 0.85 to detect a disease allele with a frequency of 0.15 and an OR of 1.5, assuming a disease prevalence of 0.06, at a significant level of 0.05 (Table S5).

Identification of two novel T2D loci and confirmation of *KCNQ1* association

Two previously unknown loci were detected in our joint analysis of GWAS data. The strongest new association signal was found for rs17584499 in intron 10 of *PTPRD* ($P = 8.54 \times 10^{-10}$ [trend test]; allelic OR = 1.57, 95% confidence interval [CI] = 1.36–1.82) (Table 1; Figure 2). The second strongest signal was found with rs391300 ($P = 3.06 \times 10^{-9}$ [trend test]; OR = 1.28, 95% CI = 1.18–1.39). The nearby SNP rs4523957 also demonstrated a significant association ($P = 1.44 \times 10^{-8}$; OR = 1.27, 95% CI = 1.17–1.38). SNPs rs391300 and rs4523957 were in tight LD with one another ($r^2 = 0.942$ in HapMap HCB), and were located within the serine racemase gene (*SRR*).

SNP rs231361, located in intron 11 of *KCNQ1*, had a less significant association with T2D, and was selected in stage 1 ($P = 1.49 \times 10^{-4}$ [trend test]; OR = 1.39, 95% CI = 1.17–1.64) (Table 1). We further genotyped eight additional SNPs within the same LD block from the HapMap Asian group data: rs231359 yielded a P value of 4.56×10^{-4} with a trend test (OR = 1.36, 95% CI = 1.14–1.61) (Figure 2). rs231361 and rs231359 were in strong LD with one another ($r^2 = 1$ in HapMap HCB), and were located approximately 164 kb upstream of SNP rs2237897, which was previously reported to be significantly associated with T2D in a Japanese population [28,29]. We took rs231361, rs231359, and neighboring SNPs within the LD block forward to replicate in stage 2. Joint analysis of stage 1 and stage 2 data revealed that rs231359 had an even stronger association with T2D than did rs231361 (rs231359: $P = 3.43 \times 10^{-8}$, OR = 1.33, 95% CI = 1.2–1.48; rs231361: $P = 2.89 \times 10^{-7}$, OR = 1.3, 95% CI = 1.18–1.44).

Additional SNPs that were reported to be significantly associated with T2D in a Japanese population were further genotyped [28,29]. The average call rate for each sample was $99.12\% \pm 7.21\%$. After applying stringent quality control criteria, we obtained high-quality genotypes with an average call rate of $99.16\% \pm 0.18\%$ (Table S2). SNP rs2237895 showed the strongest association with T2D of all the

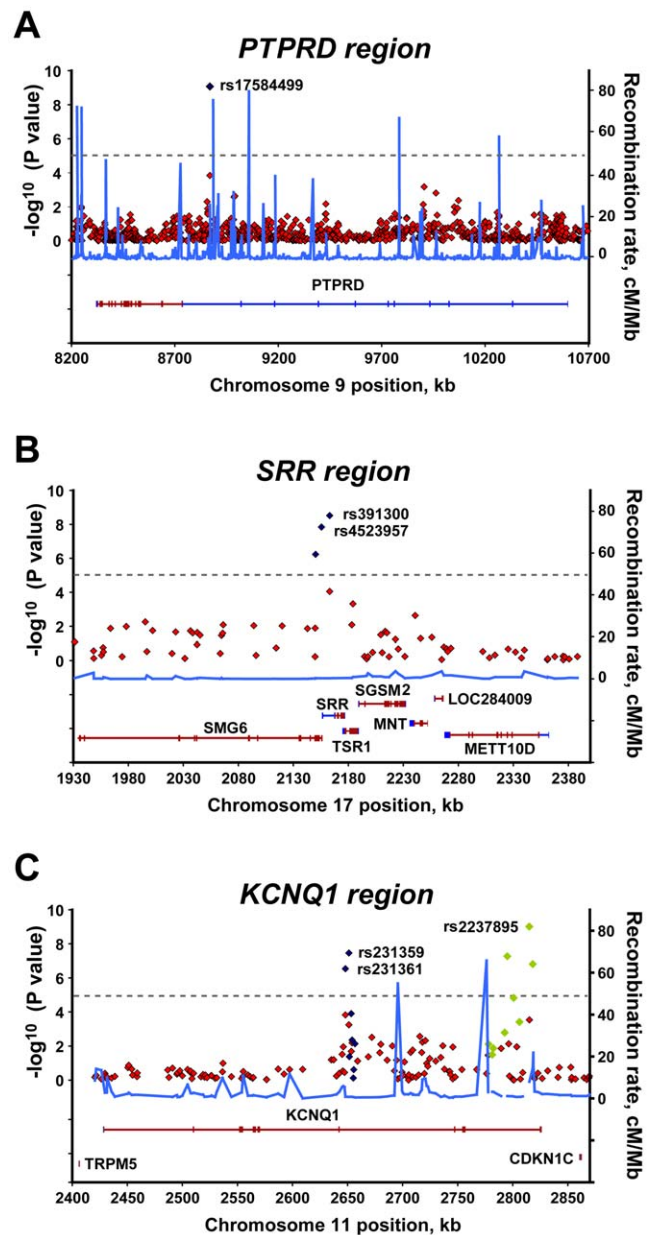


Figure 2. Regional plots of three significant associations. For each of the (A) *PTPRD*, (B) *SRR*, and (C) *KCNQ1* regions, the $-\log_{10} P$ values for the trend test from the primary scan were plotted as a function of genomic position (NCBI Build 36). The SNPs with the strongest signal and neighboring genotyped SNPs in the joint analysis are denoted by blue diamonds. Green diamonds in the *KCNQ1* region (C) represent reported T2D-associated SNPs genotyped in all samples of joint analysis. Estimated recombination rates (right y-axis) based on the Chinese HapMap population was plotted to reflect the local LD structure around the significant SNPs. Gene annotations were taken from NCBI.
doi:10.1371/journal.pgen.1000847.g002

genotyped SNPs in *KCNQ1* ($P = 9.65 \times 10^{-10}$; OR = 1.29, 95% CI = 1.19–1.40) (Figure 2 and Figure S3; Table S6). Conditioning on the rs2237895, the statistical significance of rs231361 (or rs231359) disappeared. It seems the same underlying biological effect between the 2 SNPs (Table S7).

Subsequently, we sequenced all of the exons, intron–exon boundaries, and up to 1.2 kb of the promoter region of the *KCNQ1*

gene in 50 individuals with T2D, and identified 42 polymorphic variations, including one nonsynonymous P448R polymorphism and two novel SNPs with minor allele frequency >0.03 . We then genotyped the two novel SNPs and one nonsynonymous polymorphism; however, none of these SNPs showed an association with T2D (Table S6).

Discussion

Our GWAS for T2D in a Han Chinese population found two previously unreported susceptibility genes. All of the significant variants detected in our study showed modest effects, with an OR between 1.21 and 1.57. Two loci with less-significant associations in our primary scan (stage 1), *PTPRD* and *KCNQJ*, were selected for further replication; both showed compelling evidence of association in joint analysis. The susceptibility loci we identified in this study need to be further replicated in additional populations. Of the 18 loci previously reported to be associated with T2D (with the exception of *KCNQJ*), none of the *P* values for any of the SNPs within or near the genes reached 10^{-5} using allele, genotype, trend, dominant, or recessive models (Table S8; Figure S4). Three SNPs within *CDKALI*, *JAZFI*, and *HNF1B* had the lowest *P* values, ranging from 5×10^{-4} to 10^{-5} , among the 18 known loci (Table S8). No significant associations were found within these regions in our Han Chinese population.

The strongest new signal was observed for rs17584499 in *PTPRD*. The overall *F_{st}* among 11 HapMap groups for rs17584499 was estimated to be 0.068 [52], which indicated a significant difference in allele frequencies among the populations ($P < 0.0001$, chi-square test) (Table S9). *PTPRD* is widely expressed in tissues, including skeletal muscle and pancreas, and is expressed highest in the brain. *PTPRD*-deficient mice exhibit impaired learning and memory, early growth retardation, neonatal mortality, and posture and motor defects [53]. Multiple mRNA isoforms are expressed by alternative splicing and/or alternative transcription start sites in a developmental and tissue-specific manner [54,55]. *PTPRD* belongs to the receptor type IIA (R2A) subfamily of protein tyrosine phosphatases (PTPs). The R2A PTP subfamily comprises *LAR*, *PTPRS*, and *PTPRD*. The R2A family has been implicated in neural development, cancer, and diabetes [56]. Although the complex phenotype including neurological defects seen in knockout mice could obscure the roles of these genes in glucose homeostasis, *LAR*- and *PTPRS*-deficient mice were demonstrated to have altered glucose homeostasis and insulin sensitivity [49–51]. Transgenic mice overexpressing *LAR* in skeletal muscle show whole-body insulin resistance [57]. Because R2A subfamily members are structurally very similar [54], *PTPRD* could play a role in T2D pathogenesis and should be further characterized.

The second new association locus was found for rs391300 and rs4523957 in the biologically plausible candidate gene *SRR*. *SRR* encodes a serine racemase that synthesizes D-serine from L-serine [58,59]. D-serine is a physiological co-agonist of the N-methyl D-aspartate (NMDA) class of glutamate receptors, the major excitatory neurotransmitter receptors mediating synaptic neurotransmission in the brain [60,61]. NMDA receptor activation requires binding of glutamate and D-serine, which plays a neuromodulatory role in NMDA receptor transmission, synaptic plasticity, cell migration, and neurotoxicity [62]. D-serine and *SRR* are also present in the pancreas [63]. Glutamate signaling functions in peripheral tissues, including the pancreas, and positively modulates secretion of both glucagon and insulin in pancreatic islets [64–66]. The nearby SNP rs216193 also showed significant association ($P = 2.49 \times 10^{-6}$); this SNP resides 3.8 kb

upstream from *SRR*, within Smg-6 homolog, nonsense mediated mRNA decay factor (*C. elegans*) (*SMG6*). rs216193 was in tight LD with rs391300 ($r^2 = 0.942$ in HapMap HCB). Based on their biological functions and the association results, neither *SMG6* nor any of the nearby genes *TSRI*, *SGSM2*, *MNT*, and *METT10D* were compelling candidates for association with T2D. However, *SRR* was significantly associated with T2D; thus, we suggest that dysregulation of D-serine could alter glutamate signaling and affect insulin or glucagon secretion in T2D pathogenesis.

rs7192960 also had a suggestive association with T2D ($P = 1.32 \times 10^{-5}$; OR = 1.21, 95% CI = 1.11–1.33). This SNP which lies approximately 211 kb downstream of v-maf musculoaponeurotic fibrosarcoma oncogene homolog (avian) (*MAF*) and 170 kb downstream of WW domain containing oxidoreductase (*WWOX*). *WWOX* is a tumor suppressor gene that spans the second most common human fragile site FRA16D [67,68], and is disrupted in many tumors, including pancreatic carcinoma [67,69–73]. *MAF* encodes the transcription factor c-Maf, a member of the Maf family of basic-Zip (bZip) transcription factors. c-Maf is involved in development and differentiation of the lens [74,75], kidney [76], immune system [77], adipose tissue [78], and pancreas [79]. It is expressed in α cells of the pancreatic islets [80], and is a strong transactivator of the glucagon promoter that regulates glucagon gene expression [80,81]. c-Maf is also associated with early-onset and morbid adult obesity [82].

Our GWAS revealed that *KCNQJ*, which was previously reported to be associated with T2D in several populations, was also associated with T2D in a Han Chinese population residing in Taiwan. *KCNQJ* encodes the pore-forming α subunit of a voltage-gated K^+ channel (KvLQT1), which is involved in repolarization of the action potential in cardiac muscle [83,84]. Mutations in *KCNQJ* cause long QT syndrome [85,86] and familial atrial fibrillation [87]. *KCNQJ* is widely expressed, including in the heart, brain, kidney, liver, intestine, and pancreas [88–90]. It is also expressed in pancreatic islets, and blockade of the KvLQT1 channel stimulates insulin secretion in insulin-secreting INS-1 cells [91]. *KCNQJ* knockout mice have cardiac dysfunctions [88,92] and enhanced systemic insulin sensitivity [93]. In our study, variants in the coding region did not show an association with T2D. The functional variant(s) could be located in the regulatory element of *KCNQJ*, rather than in the coding region. We did not find an association between either *CDKALI* or *IGF2BP2* and T2D, in contrast with the results described in a previous study [29], nor did we find T2D associated with various other genes identified in populations of European descent.

In conclusion, we identified two previously unknown loci that are associated with T2D in a Han Chinese population, and confirmed the reported association of *KCNQJ* with T2D. The novel T2D risk loci may involve genes that are implicated in insulin sensitivity and control of glucagon and insulin secretion: *PTPRD* may participate in the regulation of insulin action on its target cells, while *SRR* variants may alter glutamate signaling in the pancreas, thus regulating insulin and/or glucagon secretion. Our study suggests that in different patient populations, different genes may confer risks for diabetes, which may lead to a better understanding of the molecular pathogenesis of T2D.

Materials and Methods

Ethical statement

The study was approved by the institutional review board and the ethics committee of each institution. Written informed consent was obtained from each participant in accordance with institutional requirements and the Declaration of Helsinki Principles.

Subject participants

A total of 2,798 unrelated individuals with T2D, age >20 years, were recruited from China Medical University Hospital (CMUH), Taichung, Taiwan; Chia-Yi Christian Hospital (CYCH), Chia-Yi, Taiwan; and National Taiwan University Hospital (NTU), Taipei, Taiwan. All of the T2D cases were diagnosed according to medical records and fasting plasma glucose levels using American Diabetic Association Criteria. Subjects with type 1 diabetes, gestational diabetes, and maturity-onset diabetes of the young (MODY) were excluded from this study. For the two-stage GWAS, we genotyped 995 T2D cases and 894 controls in the first exploratory genome-wide scan (stage 1). In the replication stage (stage 2), we genotyped selected SNPs in additional samples from 1,803 T2D cases and 1,473 controls. The controls were randomly selected from the Taiwan Han Chinese Cell and Genome Bank [94]. The criteria for controls in the association study were (1) no past diagnostic history of T2D, (2) HbA_{1C} ranging from 3.4 to 6, and (3) BMI < 32. The two control groups were comparable with respect to BMI, gender, age at study, and level of HbA_{1C}. All of the participating T2D cases and controls were of Han Chinese origin, which is the origin of 98% of the Taiwan population. Details of demographic data are shown in Table S10.

Genotyping

Genomic DNA was extracted from peripheral blood using the Puregene DNA isolation kit (Gentra Systems, Minneapolis, MN, USA). In stage 1, whole genome genotyping using the Illumina HumanHap550-Duo BeadChip was performed by deCODE Genetics (Reykjavik, Iceland). Genotype calling was performed using the standard procedure implemented in BeadStudio (Illumina, Inc., San Diego, CA, USA), with the default parameters suggested by the platform manufacturer. Quality control of genotype data was performed by examining several summary statistics. First, the ratio of loci with heterozygous calls on the X chromosome was calculated to double-check the subject's gender. Total successful call rate and the minor allele frequency of cases and controls were also calculated for each SNP. SNPs were excluded if they: (1) were nonpolymorphic in both cases and controls, (2) had a total call rate < 95% in the cases and controls combined, (3) had a minor allele frequency < 5% and a total call rate < 99% in the cases and controls combined, and (4) had significant distortion from Hardy–Weinberg equilibrium in the controls ($P < 10^{-7}$). Genotyping validation was performed using

the Sequenom iPLEX assay (Sequenom MassARRAY system; Sequenom, San Diego, CA, USA). In the replication stage (stage 2), SNPs showing significant or suggestive associations with T2D and their neighboring SNPs within the same LD block were genotyped using the Sequenom iPLEX assay. The neighboring SNPs in the same LD were selected from the HapMap Asian (CHB + JPT) group data for fine mapping the significant signal.

Statistical analysis

T2D association analysis was carried out to compare allele frequency and genotype distribution between cases and controls using five single-point methods for each SNP: genotype, allele, trend (Cochran–Armitage test), dominant, and recessive models. The most significant test statistic obtained from the five models was chosen. SNPs with P values less than $\alpha = 2 \times 10^{-8}$, a cut-off for the multiple comparison adjusted by Bonferroni correction, were considered to be significantly associated with the traits. The joint analysis was conducted by combining the data from the stage 1 and 2 samples. We also applied Fisher's method to combine P values for joint analysis. The permutation test was carried out genome-wide for 10^6 permutations, in which the phenotypes of subjects were randomly rearranged. For better estimation of empirical P values, the top SNPs were reexamined using 10^8 permutations. Each permutation proceeded as follows: (1) the case and control labels were shuffled and redistributed to subjects, and (2) the test statistics of the corresponding association test was calculated based on the shuffled labels. The empirical P value was defined as the number of permutations that were at least as extreme as the original divided by the total number of permutations. Detection of possible population stratification that might influence association analysis was carried out using principle component analysis, multidimensional scaling analysis, and genomic control (Text S1). Quantile–quantile (Q–Q) plots were then used to examine P value distributions (Figure 3 and Figure S5).

Supporting Information

Figure S1 Principle component analysis (PCA) plot. The PCA plot shows the first two principal components, estimated by EIGENSTRAT (Price et al. Nat Genet 38: 904–909), based on genotype data from 76,673 SNPs with equal spacing across the human genome. No population stratification between the 995

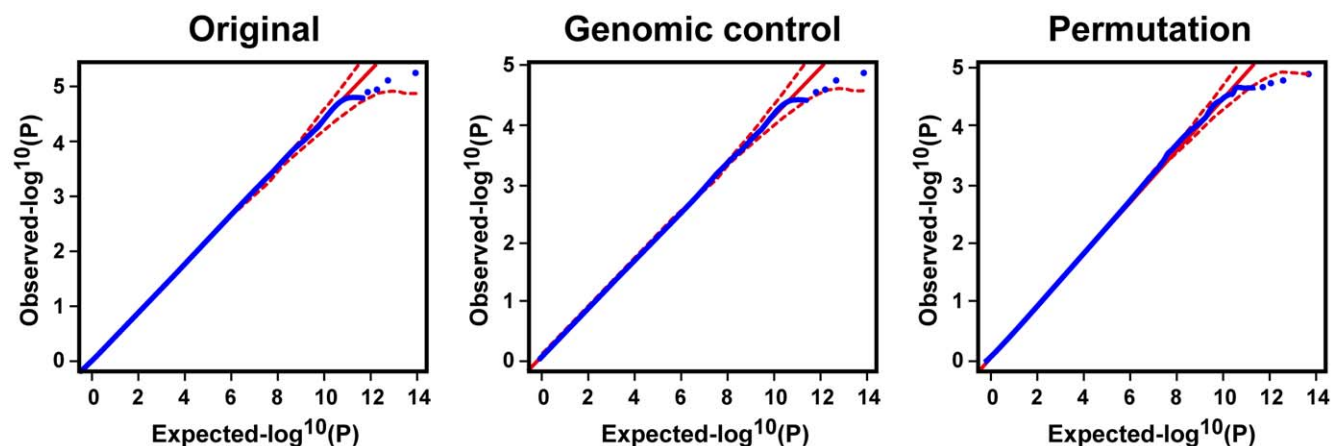


Figure 3. Q–Q plot for the trend test. Q–Q plots are shown for the trend test based on the 516,212 quality SNPs of the initial analysis of 995 cases and 894 controls. The red lines represent the upper and lower boundaries of the 95% confidence bands. doi:10.1371/journal.pgen.1000847.g003

T2D cases (green x) and 894 controls (red +) was detected ($P=0.111$, and F_{st} statistics between populations <0.001).

Found at: doi:10.1371/journal.pgen.1000847.s001 (1.18 MB TIF)

Figure S2 Multidimensional scaling analysis (MDS) plot. The MDS plot shows the first two principal components, estimated by PLINK (Zheng et al. *Am J Hum Genet* 81:559–575), based on genotype data from 516,212 SNPs. No population stratification between the 995 T2D cases (red) and 894 controls (blue) was detected (IBS group-difference empirical $P=0.192598$ for T1: case/control less similar).

Found at: doi:10.1371/journal.pgen.1000847.s002 (0.74 MB TIF)

Figure S3 LD block between rs231361 and rs223787.

Found at: doi:10.1371/journal.pgen.1000847.s003 (0.17 MB TIF)

Figure S4 Comparisons to susceptible regions reported by previous GWAS. For each of the (A) *NOTCH2*, (B) *THADA*, (C) *PPARG*, (D) *IGF2BP2*, (E) *ADAMTS9*, (F) *WFS1*, (G) *CDKALI*, (H) *JAFI*, (I) *SLC30A8*, (J) *CDKN2AB*, (K) *HHEX*, (L) *CDC123/CAMK1D*, (M) *TCF7L2*, (N) *KCNJ11*, (O) *MTNR1B*, (P) *SPAN8/LGR5*, (Q) *FTO*, and (R) *TCF (HNF1B)* regions, the $-\log_{10} P$ values from the primary scan are plotted as a function of genomic position (NCBI Build 36). The reported SNPs in previous GWAS are denoted by blue diamonds. Estimated recombination rates (right y-axis) based on the Chinese HapMap population are plotted to reflect the local LD structure around the significant SNPs. Gene annotations and numbers of transcripts were taken from NCBI.

Found at: doi:10.1371/journal.pgen.1000847.s004 (4.17 MB TIF)

Figure S5 Quantile-quantile (QQ) plots. QQ plots are shown for the four association tests, (A) allelic, (B) genotype, (C) dominant, and (D) recessive, based on the 516,212 quality SNPs of the initial analysis of 995 cases and 894 controls. The upper and lower boundaries of the 95% confidence bands are represented by the red lines.

Found at: doi:10.1371/journal.pgen.1000847.s005 (3.10 MB TIF)

Table S1 Quality control of the subject participants in stage 1.

Found at: doi:10.1371/journal.pgen.1000847.s006 (0.03 MB DOC)

Table S2 Quality control of the genotyping results.

Found at: doi:10.1371/journal.pgen.1000847.s007 (0.03 MB DOC)

Table S3 Association results in stage 1.

Found at: doi:10.1371/journal.pgen.1000847.s008 (0.05 MB DOC)

Table S4 Concordance rates for the 10 SNPs with significant associations in stage 1.

Found at: doi:10.1371/journal.pgen.1000847.s009 (0.05 MB DOC)

Table S5 Power Calculation using CaTS.

Found at: doi:10.1371/journal.pgen.1000847.s010 (0.05 MB DOC)

Table S6 Association of additional SNPs within *KCNQ1* in all T2D cases and controls in the joint analysis.

Found at: doi:10.1371/journal.pgen.1000847.s011 (0.06 MB DOC)

Table S7 Conditional analysis on rs2237895.

Found at: doi:10.1371/journal.pgen.1000847.s012 (0.03 MB DOC)

Table S8 Previously reported loci and SNPs associated with T2D.

Found at: doi:10.1371/journal.pgen.1000847.s013 (0.13 MB DOC)

Table S9 Genotype frequency and allele frequency of rs17584499 (founders only) from HapMap3.

Found at: doi:10.1371/journal.pgen.1000847.s014 (0.05 MB DOC)

Table S10 Clinical characteristics of the subjects.

Found at: doi:10.1371/journal.pgen.1000847.s015 (0.04 MB DOC)

Text S1 Supplementary methods.

Found at: doi:10.1371/journal.pgen.1000847.s016 (0.03 MB DOC)

Acknowledgments

We would like to thank all of the patients and study personnel who participated in this study. We also thank Ms. Kuan-Ching Lee for handling the samples and Mr. Yan-Hau Chen and Ms. Yi-Jung Lin for their expertise in bioinformatics.

Author Contributions

Conceived and designed the experiments: YT Chen, JY Wu. Performed the experiments: CF Yang, YM Liu, CC Chang. Analyzed the data: CF Shiu, P Chen, CH Chen, CSJ Fann. Contributed reagents/materials/analysis tools: FJ Tsai, CC Chen, LM Chuang, CH Lu, CT Chang, TY Wang, RH Chen. Wrote the paper: CF Yang, YT Chen, JY Wu.

References

- Zimmet P, Alberti KG, Shaw J (2001) Global and societal implications of the diabetes epidemic. *Nature* 414: 782–787.
- Stumvoll M, Goldstein BJ, van Haeften TW (2005) Type 2 diabetes: principles of pathogenesis and therapy. *Lancet* 365: 1333–1346.
- Ledermann HM (1995) Maturity-onset diabetes of the young (MODY) at least ten times more common in Europe than previously assumed? *Diabetologia* 38: 1482.
- Maassen JA, LM TH, Van Essen E, Heine RJ, Nijpels G, et al. (2004) Mitochondrial diabetes: molecular mechanisms and clinical presentation. *Diabetes* 53 Suppl 1: S103–109.
- Fajans SS, Bell GI, Polonsky KS (2001) Molecular mechanisms and clinical pathophysiology of maturity-onset diabetes of the young. *N Engl J Med* 345: 971–980.
- Moore AF, Florez JC (2008) Genetic susceptibility to type 2 diabetes and implications for antidiabetic therapy. *Annu Rev Med* 59: 95–111.
- Langefeld CD, Wagenknecht LE, Rotter JI, Williams AH, Hokanson JE, et al. (2004) Linkage of the metabolic syndrome to 1q23-q31 in Hispanic families: the Insulin Resistance Atherosclerosis Study Family Study. *Diabetes* 53: 1170–1174.
- Hanson RL, Ehm MG, Pettitt DJ, Prochazka M, Thompson DB, et al. (1998) An autosomal genomic scan for loci linked to type II diabetes mellitus and body-mass index in Pima Indians. *Am J Hum Genet* 63: 1130–1138.
- Elbein SC, Hoffman MD, Teng K, Leppert MF, Hasstedt SJ (1999) A genome-wide search for type 2 diabetes susceptibility genes in Utah Caucasians. *Diabetes* 48: 1175–1182.
- Vionnet N, Hani EH, Dupont S, Gallina S, Francke S, et al. (2000) Genomewide search for type 2 diabetes-susceptibility genes in French whites: evidence for a novel susceptibility locus for early-onset diabetes on chromosome 3q27-qter and independent replication of a type 2-diabetes locus on chromosome 1q21-q24. *Am J Hum Genet* 67: 1470–1480.
- An P, Hong Y, Weisnagel SJ, Rice T, Rankinen T, et al. (2003) Genomic scan of glucose and insulin metabolism phenotypes: the HERITAGE Family Study. *Metabolism* 52: 246–253.
- Wiltshire S, Hattersley AT, Hitman GA, Walker M, Levy JC, et al. (2001) A genome-wide scan for loci predisposing to type 2 diabetes in a U.K. population (the Diabetes UK Warren 2 Repository): analysis of 573 pedigrees provides independent replication of a susceptibility locus on chromosome 1q. *Am J Hum Genet* 69: 553–569.

13. Hsueh WC, St Jean PL, Mitchell BD, Pollin TI, Knowler WC, et al. (2003) Genome-wide and fine-mapping linkage studies of type 2 diabetes and glucose traits in the Old Order Amish: evidence for a new diabetes locus on chromosome 14q11 and confirmation of a locus on chromosome 1q21-q24. *Diabetes* 52: 550–557.
14. Bowden DW, Sale M, Howard TD, Qadri A, Spray BJ, et al. (1997) Linkage of genetic markers on human chromosomes 20 and 12 to NIDDM in Caucasian sib pairs with a history of diabetic nephropathy. *Diabetes* 46: 882–886.
15. Xiang K, Wang Y, Zheng T, Jia W, Li J, et al. (2004) Genome-wide search for type 2 diabetes/impaired glucose homeostasis susceptibility genes in the Chinese: significant linkage to chromosome 6q21-q23 and chromosome 1q21-q24. *Diabetes* 53: 228–234.
16. Ji L, Malecki M, Warram JH, Yang Y, Rich SS, et al. (1997) New susceptibility locus for NIDDM is localized to human chromosome 20q. *Diabetes* 46: 876–881.
17. Zouali H, Hani EH, Philipp A, Vionnet N, Beckmann JS, et al. (1997) A susceptibility locus for early-onset non-insulin dependent (type 2) diabetes mellitus maps to chromosome 20q, proximal to the phosphoenolpyruvate carboxykinase gene. *Hum Mol Genet* 6: 1401–1408.
18. Ghosh S, Watanabe RM, Hauser ER, Valle T, Magnuson VL, et al. (1999) Type 2 diabetes: evidence for linkage on chromosome 20 in 716 Finnish affected sib pairs. *Proc Natl Acad Sci U S A* 96: 2198–2203.
19. Klupa T, Malecki MT, Pezolesi M, Ji L, Curtis S, et al. (2000) Further evidence for a susceptibility locus for type 2 diabetes on chromosome 20q13.1-q13.2. *Diabetes* 49: 2212–2216.
20. Alshuler D, Hirschhorn JN, Klannemark M, Lindgren CM, Vohl MC, et al. (2000) The common PPAR γ Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. *Nat Genet* 26: 76–80.
21. Florez JC, Burt N, de Bakker PI, Almgren P, Tuomi T, et al. (2004) Haplotype structure and genotype-phenotype correlations of the sulfonylurea receptor and the islet ATP-sensitive potassium channel gene region. *Diabetes* 53: 1360–1368.
22. Gloyn AL, Weedon MN, Owen KR, Turner MJ, Knight BA, et al. (2003) Large-scale association studies of variants in genes encoding the pancreatic beta-cell KATP channel subunits Kir6.2 (KCNJ11) and SUR1 (ABCC8) confirm that the KCNJ11 E23K variant is associated with type 2 diabetes. *Diabetes* 52: 568–572.
23. Nielsen EM, Hansen L, Carstensen B, Echwald SM, Drivsholm T, et al. (2003) The E23K variant of Kir6.2 associates with impaired post-OGTT serum insulin response and increased risk of type 2 diabetes. *Diabetes* 52: 573–577.
24. Gudmundsson J, Sulem P, Steinthorsdottir V, Bergthorsson JT, Thorleifsson G, et al. (2007) Two variants on chromosome 17 confer prostate cancer risk, and the one in TCF2 protects against type 2 diabetes. *Nat Genet* 39: 977–983.
25. Winckler W, Weedon MN, Graham RR, McCarrroll SA, Purcell S, et al. (2007) Evaluation of common variants in the six known maturity-onset diabetes of the young (MODY) genes for association with type 2 diabetes. *Diabetes* 56: 685–693.
26. Sandhu MS, Weedon MN, Fawcett KA, Wasson J, Debenham SL, et al. (2007) Common variants in WFS1 confer risk of type 2 diabetes. *Nat Genet* 39: 951–953.
27. Scott LJ, Mohlke KL, Bonnycastle LL, Willer CJ, Li Y, et al. (2007) A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. *Science* 316: 1341–1345.
28. Yasuda K, Miyake K, Horikawa Y, Hara K, Osawa H, et al. (2008) Variants in KCNQ1 are associated with susceptibility to type 2 diabetes mellitus. *Nat Genet* 40: 1092–1097.
29. Unoki H, Takahashi A, Kawaguchi T, Hara K, Horikoshi M, et al. (2008) SNPs in KCNQ1 are associated with susceptibility to type 2 diabetes in East Asian and European populations. *Nat Genet* 40: 1098–1102.
30. Steinthorsdottir V, Thorleifsson G, Reynisdottir I, Benediktsson R, Jonsson T, et al. (2007) A variant in CDKAL1 influences insulin response and risk of type 2 diabetes. *Nat Genet* 39: 770–775.
31. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* 447: 661–678.
32. Saxena R, Voight BF, Lyssenko V, Burt NP, de Bakker PI, et al. (2007) Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. *Science* 316: 1331–1336.
33. Zeggini E, Weedon MN, Lindgren CM, Frayling TM, Elliott KS, et al. (2007) Replication of genome-wide association signals in UK samples reveals risk loci for type 2 diabetes. *Science* 316: 1336–1341.
34. Zeggini E, Scott LJ, Saxena R, Voight BF, Marchini JL, et al. (2008) Meta-analysis of genome-wide association data and large-scale replication identifies additional susceptibility loci for type 2 diabetes. *Nat Genet* 40: 638–645.
35. Bouatia-Naji N, Bonnefond A, Cavalcanti-Proenca C, Sparso T, Holmkvist J, et al. (2009) A variant near MTNR1B is associated with increased fasting plasma glucose levels and type 2 diabetes risk. *Nat Genet* 41: 89–94.
36. Lyssenko V, Nagorny CL, Erdos MR, Wierup N, Jonsson A, et al. (2009) Common variant in MTNR1B associated with increased risk of type 2 diabetes and impaired early insulin secretion. *Nat Genet* 41: 82–88.
37. Prokopenko I, Langenberg C, Florez JC, Saxena R, Soranzo N, et al. (2009) Variants in MTNR1B influence fasting glucose levels. *Nat Genet* 41: 77–81.
38. Sladek R, Rocheleau G, Rung J, Dina C, Shen L, et al. (2007) A genome-wide association study identifies novel risk loci for type 2 diabetes. *Nature* 445: 881–885.
39. Grant SF, Thorleifsson G, Reynisdottir I, Benediktsson R, Manolescu A, et al. (2006) Variant of transcription factor 7-like 2 (TCF7L2) gene confers risk of type 2 diabetes. *Nat Genet* 38: 320–323.
40. Takeuchi F, Serizawa M, Yamamoto K, Fujisawa T, Nakashima E, et al. (2009) Confirmation of multiple risk loci and genetic impacts by a genome-wide association study of type 2 diabetes in the Japanese population. *Diabetes* 58: 1690–1699.
41. Omori S, Tanaka Y, Takahashi A, Hirose H, Kashiwagi A, et al. (2008) Association of CDKAL1, IGF2BP2, CDKN2A/B, HHEX, SLC30A8, and KCNJ11 with susceptibility to type 2 diabetes in a Japanese population. *Diabetes* 57: 791–795.
42. Hu C, Wang C, Zhang R, Ma X, Wang J, et al. (2009) Variations in KCNQ1 are associated with type 2 diabetes and beta cell function in a Chinese population. *Diabetologia* 52: 1322–1325.
43. Wu Y, Li H, Loos RJ, Yu Z, Ye X, et al. (2008) Common variants in CDKAL1, CDKN2A/B, IGF2BP2, SLC30A8, and HHEX/IDE genes are associated with type 2 diabetes and impaired fasting glucose in a Chinese Han population. *Diabetes* 57: 2834–2842.
44. Chang YC, Chang TJ, Jiang YD, Kuo SS, Lee KC, et al. (2007) Association study of the genetic polymorphisms of the transcription factor 7-like 2 (TCF7L2) gene and type 2 diabetes in the Chinese population. *Diabetes* 56: 2631–2637.
45. Ronn T, Wen J, Yang Z, Lu B, Du Y, et al. (2009) A common variant in MTNR1B, encoding melatonin receptor 1B, is associated with type 2 diabetes and fasting plasma glucose in Han Chinese individuals. *Diabetologia* 52: 830–833.
46. Wang C, Hu C, Zhang R, Bao Y, Ma X, et al. (2009) Common variants of hepatocyte nuclear factor 1beta are associated with type 2 diabetes in a Chinese population. *Diabetes* 58: 1023–1027.
47. Zhou D, Zhang D, Liu Y, Zhao T, Chen Z, et al. (2009) The E23K variation in the KCNJ11 gene is associated with type 2 diabetes in Chinese and East Asian population. *J Hum Genet* 54: 433–435.
48. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, et al. (2007) PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 81: 559–575.
49. Ren JM, Li PM, Zhang WR, Sweet LJ, Cline G, et al. (1998) Transgenic mice deficient in the LAR protein-tyrosine phosphatase exhibit profound defects in glucose homeostasis. *Diabetes* 47: 493–497.
50. Chagnon MJ, Elchebly M, Uetani N, Dombrowski L, Cheng A, et al. (2006) Altered glucose homeostasis in mice lacking the receptor protein tyrosine phosphatase sigma. *Can J Physiol Pharmacol* 84: 755–763.
51. Batt J, Asa S, Fladd C, Rotin D (2002) Pituitary, pancreatic and gut neuroendocrine defects in protein tyrosine phosphatase-sigma-deficient mice. *Mol Endocrinol* 16: 155–169.
52. Wright S (1951) The genetical structure of populations. *Annals of Eugenics* 15: 323–354.
53. Uetani N, Kato K, Ogura H, Mizuno K, Kawano K, et al. (2000) Impaired learning with enhanced hippocampal long-term potentiation in PTPdelta-deficient mice. *Embo J* 19: 2775–2785.
54. Pulido R, Serra-Pages C, Tang M, Streuli M (1995) The LAR/PTP delta/PTP sigma subfamily of transmembrane protein-tyrosine-phosphatases: multiple human LAR, PTP delta, and PTP sigma isoforms are expressed in a tissue-specific manner and associate with the LAR-interacting protein LIP.1. *Proc Natl Acad Sci U S A* 92: 11686–11690.
55. Sato M, Takahashi K, Nagayama K, Arai Y, Ito N, et al. (2005) Identification of chromosome arm 9p as the most frequent target of homozygous deletions in lung cancer. *Genes Chromosomes Cancer* 44: 405–414.
56. Chagnon MJ, Uetani N, Tremblay ML (2004) Functional significance of the LAR receptor protein tyrosine phosphatase family in development and diseases. *Biochem Cell Biol* 82: 664–675.
57. Zabolotny JM, Kim YB, Peroni OD, Kim JK, Pani MA, et al. (2001) Overexpression of the LAR (leukocyte antigen-related) protein-tyrosine phosphatase in muscle causes insulin resistance. *Proc Natl Acad Sci U S A* 98: 5187–5192.
58. Wolosker H, Blackshaw S, Snyder SH (1999) Serine racemase: a glial enzyme synthesizing D-serine to regulate glutamate-N-methyl-D-aspartate neurotransmission. *Proc Natl Acad Sci U S A* 96: 13409–13414.
59. Wolosker H, Sheth KN, Takahashi M, Mothet JP, Brady RO Jr, et al. (1999) Purification of serine racemase: biosynthesis of the neuromodulator D-serine. *Proc Natl Acad Sci U S A* 96: 721–725.
60. De Miranda J, Panizzutti R, Foltyn VN, Wolosker H (2002) Cofactors of serine racemase that physiologically stimulate the synthesis of the N-methyl-D-aspartate (NMDA) receptor coagonist D-serine. *Proc Natl Acad Sci U S A* 99: 14542–14547.
61. Mothet JP, Parent AT, Wolosker H, Brady RO Jr, Linden DJ, et al. (2000) D-serine is an endogenous ligand for the glycine site of the N-methyl-D-aspartate receptor. *Proc Natl Acad Sci U S A* 97: 4926–4931.
62. Wolosker H, Dumain E, Balan L, Foltyn VN (2008) D-amino acids in the brain: D-serine in neurotransmission and neurodegeneration. *Febs J* 275: 3514–3526.
63. Imai K, Fukushima T, Santa T, Homma H, Huang Y, et al. (1998) Whole body autoradiographic study on the distribution of 14C-D-serine administered intravenously to rats. *Amino Acids* 15: 351–361.
64. Gono T, Mizuno N, Inagaki N, Kuromi H, Seino Y, et al. (1994) Functional neuronal ionotropic glutamate receptors are expressed in the non-neuronal cell line MIN6. *J Biol Chem* 269: 16989–16992.

65. Inagaki N, Kuromi H, Gonoi T, Okamoto Y, Ishida H, et al. (1995) Expression and role of ionotropic glutamate receptors in pancreatic islet cells. *Faseb J* 9: 686–691.
66. Bertrand G, Gross R, Puech R, Loubatieres-Mariani MM, Bockaert J (1993) Glutamate stimulates glucagon secretion via an excitatory amino acid receptor of the AMPA subtype in rat pancreas. *Eur J Pharmacol* 237: 45–50.
67. Bednarek AK, Laflin KJ, Daniel RL, Liao Q, Hawkins KA, et al. (2000) WWOX, a novel WW domain-containing protein mapping to human chromosome 16q23.3-24.1, a region frequently affected in breast cancer. *Cancer Res* 60: 2140–2145.
68. Ried K, Finnis M, Hobson L, Mangelsdorf M, Dayan S, et al. (2000) Common chromosomal fragile site FRA16D sequence: identification of the FOR gene spanning FRA16D and homozygous deletions and translocation breakpoints in cancer cells. *Hum Mol Genet* 9: 1651–1663.
69. Iliopoulos D, Guler G, Han SY, Johnston D, Druck T, et al. (2005) Fragile genes as biomarkers: epigenetic control of WWOX and FHIT in lung, breast and bladder cancer. *Oncogene* 24: 1625–1633.
70. Kuroki T, Yendamuri S, Trapasso F, Matsuyama A, Aqeilan RI, et al. (2004) The tumor suppressor gene WWOX at FRA16D is involved in pancreatic carcinogenesis. *Clin Cancer Res* 10: 2459–2465.
71. Paige AJ, Taylor KJ, Taylor C, Hillier SG, Farrington S, et al. (2001) WWOX: a candidate tumor suppressor gene involved in multiple tumor types. *Proc Natl Acad Sci U S A* 98: 11417–11422.
72. Kuroki T, Trapasso F, Shiraishi T, Alder H, Mimori K, et al. (2002) Genetic alterations of the tumor suppressor gene WWOX in esophageal squamous cell carcinoma. *Cancer Res* 62: 2258–2260.
73. Yendamuri S, Kuroki T, Trapasso F, Henry AC, Dumon KR, et al. (2003) WW domain containing oxidoreductase gene expression is altered in non-small cell lung cancer. *Cancer Res* 63: 878–881.
74. Sakai M, Imaki J, Yoshida K, Ogata A, Matsushima-Hibaya Y, et al. (1997) Rat maf related genes: specific expression in chondrocytes, lens and spinal cord. *Oncogene* 14: 745–750.
75. Kim JI, Li T, Ho IC, Grusby MJ, Glimcher LH (1999) Requirement for the c-Maf transcription factor in crystallin gene regulation and lens development. *Proc Natl Acad Sci U S A* 96: 3781–3785.
76. Imaki J, Tsuchiya K, Mishima T, Onodera H, Kim JI, et al. (2004) Developmental contribution of c-maf in the kidney: distribution and developmental study of c-maf mRNA in normal mice kidney and histological study of c-maf knockout mice kidney and liver. *Biochem Biophys Res Commun* 320: 1323–1327.
77. Agnello D, Lankford CS, Bream J, Morinobu A, Gadina M, et al. (2003) Cytokines and transcription factors that regulate T helper cell differentiation: new players and new insights. *J Clin Immunol* 23: 147–161.
78. Serria MS, Ikeda H, Omoteyama K, Hirokawa J, Nishi S, et al. (2003) Regulation and differential expression of the c-maf gene in differentiating cultured cells. *Biochem Biophys Res Commun* 310: 318–326.
79. Tsuchiya M, Taniguchi S, Yasuda K, Nitta K, Maeda A, et al. (2006) Potential roles of large maf in cell lineages and developing pancreas. *Pancreas* 32: 408–416.
80. Kataoka K, Shioda S, Ando K, Sakagami K, Handa H, et al. (2004) Differentially expressed Maf family transcription factors, c-Maf and MafA, activate glucagon and insulin gene expression in pancreatic islet alpha- and beta-cells. *J Mol Endocrinol* 32: 9–20.
81. Gosmain Y, Avril I, Mamin A, Philippe J (2007) Pax-6 and c-Maf functionally interact with the alpha-cell-specific DNA element G1 in vivo to promote glucagon gene expression. *J Biol Chem* 282: 35024–35034.
82. Meyre D, Delplanque J, Chevre JC, Lecoecur C, Lobbens S, et al. (2009) Genome-wide association study for early-onset and morbid adult obesity identifies three new risk loci in European populations. *Nat Genet* 41: 157–159.
83. Sanguinetti MC, Curran ME, Zou A, Shen J, Spector PS, et al. (1996) Coassembly of K(V)LQT1 and minK (IsK) proteins to form cardiac I(Ks) potassium channel. *Nature* 384: 80–83.
84. Barhanin J, Lesage F, Guillemare E, Fink M, Lazdunski M, et al. (1996) K(V)LQT1 and IsK (minK) proteins associate to form the I(Ks) cardiac potassium current. *Nature* 384: 78–80.
85. Neyroud N, Tesson F, Denjoy I, Leibovici M, Donger C, et al. (1997) A novel mutation in the potassium channel gene KVLQT1 causes the Jervell and Lange-Nielsen cardioauditory syndrome. *Nat Genet* 15: 186–189.
86. Wang Q, Curran ME, Splawski I, Burn TC, Millholland JM, et al. (1996) Positional cloning of a novel potassium channel gene: KVLQT1 mutations cause cardiac arrhythmias. *Nat Genet* 12: 17–23.
87. Chen YH, Xu SJ, Bendahhou S, Wang XL, Wang Y, et al. (2003) KCNQ1 gain-of-function mutation in familial atrial fibrillation. *Science* 299: 251–254.
88. Lee MP, Ravenel JD, Hu RJ, Lustig LR, Tomaselli G, et al. (2000) Targeted disruption of the Kvlqt1 gene causes deafness and gastric hyperplasia in mice. *J Clin Invest* 106: 1447–1455.
89. Demolombe S, Franco D, de Boer P, Kuperschmidt S, Roden D, et al. (2001) Differential expression of KvLQT1 and its regulator IsK in mouse epithelia. *Am J Physiol Cell Physiol* 280: C359–372.
90. Chouabe C, Neyroud N, Guicheney P, Lazdunski M, Romey G, et al. (1997) Properties of KvLQT1 K+ channel mutations in Romano-Ward and Jervell and Lange-Nielsen inherited cardiac arrhythmias. *Embo J* 16: 5472–5479.
91. Ullrich S, Su J, Ranta F, Wittekindt OH, Ris F, et al. (2005) Effects of I(Ks) channel inhibitors in insulin-secreting INS-1 cells. *Pflugers Arch* 451: 428–436.
92. Casimiro MC, Knollmann BC, Ebert SN, Vary JC Jr, Greene AE, et al. (2001) Targeted disruption of the Kcnq1 gene produces a mouse model of Jervell and Lange-Nielsen Syndrome. *Proc Natl Acad Sci U S A* 98: 2526–2531.
93. Boini KM, Graf D, Hennige AM, Koka S, Kempe DS, et al. (2009) Enhanced insulin sensitivity of gene-targeted mice lacking functional KCNQ1. *Am J Physiol Regul Integr Comp Physiol* 296: R1695–1701.
94. Pan WH, Fann CS, Wu JY, Hung YT, Ho MS, et al. (2006) Han Chinese cell and genome bank in Taiwan: purpose, design and ethical considerations. *Hum Hered* 61: 27–30.