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Genetic variation in the peroxisome proliferator-activated receptor (PPAR) and peroxisome proliferator-activated receptor gamma co-activator 1 (PGC1) gene families and type 2 diabetes

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Summary

We used a two-stage study design to evaluate whether variations in the peroxisome proliferator-activated receptors (*PPAR*) and the peroxisome proliferator-activated receptor gamma co-activator 1 (*PGC1*) gene families (*PPARA*, *PPARG*, *PPARD*, *PPARGC1A*, and *PPARGC1B*) are associated with T2D risk. Stage I used data from a genome-wide association study (GWAS) from Shanghai, China (1,019 T2D cases and 1,709 controls) and from a meta-analysis of data from the Asian Genetic Epidemiology Network for T2D (AGEN-T2D). Criteria for selection of SNPs for stage II were: 1) $P < 0.05$ in single marker analysis in Shanghai GWAS and $P < 0.05$ in the meta-analysis or 2) $P < 10^{-3}$ in the meta-analysis alone and 3) minor allele frequency > 0.10 . Nine SNPs from the *PGC1* family were assessed in stage II (an independent set of middle-aged men and women from Shanghai with 1,700 T2D cases and 1,647 controls). One SNP in *PPARGC1B*, rs251464, was replicated in stage II (OR=0.87; 95% CI: 0.77–0.99). Gene-body mass index (BMI) and gene-exercise interactions and T2D risk were evaluated in a combined dataset (Shanghai GWAS and stage II data: 2,719 cases and 3,356 controls). One SNP in *PPARGC1A*, rs12640088, had a significant interaction with BMI. No interactions between the *PPARGC1B* gene and BMI or exercise were observed.

Keywords

type 2 diabetes; PPAR; PGC1

INTRODUCTION

Type 2 diabetes (T2D) is a major cause of morbidity and mortality in the United States and many other countries worldwide. The prevalence of type 2 diabetes has doubled – from 4% to 8% – during the past 40 years in the US (Gregg et al. 2004). The increase of T2D in developing countries in Asia, including China, is similarly alarming (King et al. 1998; King and Rewers 1993).

Energy balance plays an important role in the development of T2D. High BMI (Carey et al. 1997; Chan et al. 1994; Colditz et al. 1990; Hu et al. 2001b; Ohlson et al. 1985), which reflects a positive energy balance, has been positively associated with T2D. High physical activity, another component of the energy balance equation, has been associated with lower incidence of T2D (Helmrich et al. 1991; Hu et al. 2001a; Manson et al. 1991; Sigal et al. 2004; Wei et al. 1999). Thus, it is probable that T2D is associated with genes encoding transcriptional regulators of genes affecting energy balance. The peroxisome proliferator-activated receptors (PPARs) are a family of transcription factors that regulate energy balance by promoting either energy deposition or energy dissipation (Medina-Gomez et al. 2007). The peroxisome proliferator-activated receptor gamma co-activator 1 (PGC1) family has been highlighted as an important regulator of gluconeogenesis, fatty acid oxidation, and adaptive thermogenesis (Puigserver et al. 1998).

We evaluated associations of single nucleotide polymorphisms (SNPs) in five genes in the *PPAR* and *PGC1* families (*PPARA*, *PPARG*, *PPARD*, *PPARGC1A*, and *PPARGC1B*) with T2D and their interactions with BMI and exercise, using a two-stage association study. Stage I used data from an ongoing genome-wide association study (GWAS) of T2D that included 1,019 T2D cases and 1,709 controls with additional data from a meta-analysis conducted by the Asian Genetic Epidemiology Network for T2D (AGEN-T2D). Stage II used data from an independent study of 1,700 T2D cases and 1,647 matched controls from Shanghai to test promising SNPs identified in specific stage I. Gene-exercise participation and gene-BMI interactions were evaluated in a combined dataset including Shanghai Diabetes GWAS and stage II participants (combined data from stages I and II).

METHODS

Ethics Statement

This study was approved by all relevant institutional review boards, and written informed consent was obtained from all participants prior to the study.

Stage I Study

We used data from the Shanghai Diabetes GWAS for 1,019 T2D cases and 1,709 controls and data from a meta-analysis of eight T2D GWAS (6,952 cases and 11,865 controls) of East Asian-ancestry populations (the Asian Genetic Epidemiology Network, for T2D, AGEN-T2D). The Shanghai Diabetes GWAS study is part of the AGEN-T2D.

Shanghai Diabetes GWAS—Details of the Shanghai Diabetes GWAS (SBCS/SWHS GWAS) have been described elsewhere (Shu et al. 2010). Briefly, the study included 886 incident type 2 diabetes (T2D) cases identified in the Shanghai Women's Health Study (SWHS), an ongoing population-based cohort study of approximately 75,000 women. (Zheng et al. 2005). SWHS participants were recruited between 1997 and 2000 and were aged 40 to 70 years at recruitment. In-person interviews, anthropometrics, and blood or buccal cell sample collection were carried out by trained interviewers. Study participants are being followed through biennial in-person surveys to collect information on survival status.

and occurrence of cancer, T2D, and other chronic diseases. A total of 901 women who self-reported a diagnosis of T2D since study enrollment and met the following criteria were included in the GWAS: 1) age ≥ 65 , 2) on T2D medication, 3) fasting glucose level >125 mg/dL at least twice, and 4) donated a blood sample. After quality checking, using the same method described previously for our GWAS of breast cancer (Shu, Long, Cai, Qi, Xiang, Cho, Tai, Li, Lin, Chow, Go, Seielstad, Bao, Li, Cornelis, Yu, Wen, Shi, Han, Sim, Liu, Qi, Kim, Ng, Lee, Kim, Li, Gao, Zheng, & Hu 2010), genotyping information was available for 886 participants. Included in the study were prevalent T2D cases identified from female controls of the Shanghai Breast Cancer GWAS. The latter study also contributed controls to this GWAS. Details of the Shanghai Breast Cancer GWAS, including subject recruitment, sample collection, processing, laboratory protocols, genotyping, and data cleaning procedures have been described elsewhere (Zheng et al. 2009). Of the 1,938 controls included in the breast cancer GWAS that were genotyped with Affymetrix 6.0, 17 were on T2D medication and 117 had a blood glucose level >125 (mg/dL); these participants were included as T2D cases in the current study. One of these T2D cases also participated in the SWHS. Thus, a total of 133 independent T2D cases identified from the SBCS controls were included as cases in this study. After excluding women who had a blood glucose level between 100 and 125 mg/dL and had glycated hemoglobin (HbA1C) >6.1 (n=54) or had no HbA1C data (n=28), women who were younger than age 35 at the time of diabetes diagnosis (n=4), women with a self-reported history of diabetes but who had either no information on diabetes treatment or who had a glucose level <125 mg/dL in the current study (n=8), and one participant from the SBCS control group that developed breast cancer later on, 1,709 women remained as controls for the T2D GWAS. The protocols of these studies were approved by the Institutional Review Boards of the Shanghai Cancer Institute, the Shanghai Municipal Center for Disease Control and Prevention, and Vanderbilt University, and all participants provided written informed consent. Samples included in stage I were unrelated.

Anthropometric measurements—Body weight and height were measured in the SWHS and SBCS using identical protocols. All measurements, including weight, height, and circumferences of waist and hips, were taken during in-person interviews according to a standard protocol by trained interviewers who were retired medical professionals. From these measurements, BMI was calculated as weight in kg divided by the square of height in meters (kg/m^2).

Physical Activity—Physical activity patterns were assessed during the in-person interviews. Regular exercise and sports participation were evaluated for the 10 years before the interview in the SBCS and for the 5 years before the interview in the SWHS. For SWHS participants the assessment of physical activity was obtained using a validated questionnaire (Matthews et al. 2003). In both studies participants were asked if they participated in exercise or sports activities. This was treated a dichotomous variable in all analyses. The SBCS physical activity questionnaire has not been validated, but a description of the instrument is included in a publication from our group on these subjects (Matthews et al. 2001).

Genotyping and QC—Genotyping was performed using the Affymetrix 6.0 array, which includes 906,602 SNPs. The Birdseed v2 algorithm (<http://www.broad.mit.edu/mpg/birdsuite/>) was used to call genotypes. All SBCS and SWHS samples were genotyped in Vanderbilt Microarray Shared resources. The genotyping dates overlapped. A detailed quality control (QC) procedure, which has been described previously (Shu, Long, Cai, Qi, Xiang, Cho, Tai, Li, Lin, Chow, Go, Seielstad, Bao, Li, Cornelis, Yu, Wen, Shi, Han, Sim, Liu, Qi, Kim, Ng, Lee, Kim, Li, Gao, Zheng, & Hu 2010), was used to clean the data. Briefly, QC procedures included removal of SNPs with a MAF <0.01 , Hardy-Weinberg P -

values <0.00001 , and samples with $>5\%$ missing genotypes. Three sets of SNPs genotyped on the Affymetrix SNP Array 6.0 had previously been genotyped on different platforms including: 1) 669 SNPs genotyped for 1,035 participants by using the Affymetrix Targeted Genotyping System, 2) 17 SNPs genotyped for 1,091 participants by using Taqman, and 3) 251 SNPs genotyped for 108 participants by using Sequenom. These SNP sets were used for cross-platform sample verification. The mean concordance rates were 99.5%, 98.5%, and 98.9% for the Affymetrix Targeted Genotyping, Taqman, and Sequenom, respectively, when compared with the Affymetrix SNP Array 6.0. Additionally, we included one negative control (water) and three positive QC samples (NA15510, NA10851, and NA18505) purchased from Coriell Cell Repositories (<http://ccr.coriell.org/>) in each of the 96-well plates genotyped to assess batch-to-batch validation. The average concordance rate between the QC samples was 99.8% (median: 100%). A total of 139 SNPs were directly genotyped and included in the association analyses.

Imputation—Imputation was conducted to provide complete coverage of the study genes. We imputed genotypes from the HapMap reference genotypes. The program MACH (<http://www.sph.umich.edu/csg/abecasis/MACH/>) was used for genotype imputation to determine the probability distribution of missing genotypes conditional on a set of known haplotypes, while simultaneously estimating the fine-scale recombination map. Imputation was based on 570,441 autosomal SNPs genotyped in stage I that passed the QC procedure, with the phased Asian data from HapMap Phase II (release 22) as the reference. Hapmap Phase II data were used as the reference, since these data contain a greater selection of SNPs. Only data with high imputation quality ($RSQR >0.3$ for MACH) were included in the current analysis. We excluded all SNPs with a $MAF <0.05$ from the imputed SNPs for analysis. A total of 319 SNPs in our candidate genes met these criteria and were included in our analyses.

Asian Genetic Epidemiology Network, for T2D (AGEN-T2D)—This consortium was organized for genetic studies of diverse complex traits in 2010. Eight studies included 6,952 T2D cases and 11,865 controls from the Korea Association Resource Study (KARE), the Singapore Diabetes Cohort Study (SDCS), the Singapore Prospective Study Program (SP2), the Singapore Malay Eye Study (SiMES), the Japan Cardiometabolic Genome Epidemiology Network (CAGE), the Shanghai Diabetes Genetics Study, (SBCS/SWHS GWAS or SDGS), the Taiwan T2D Study (TDS), and the Cebu Longitudinal Health and Nutritional Survey (CLHNS). The study design and T2D diagnosis criteria of each study have been described previously (Shu, Long, Cai, Qi, Xiang, Cho, Tai, Li, Lin, Chow, Go, Seielstad, Bao, Li, Cornelis, Yu, Wen, Shi, Han, Sim, Liu, Qi, Kim, Ng, Lee, Kim, Li, Gao, Zheng, & Hu 2010). Each study obtained approval from the appropriate institutional review board and written informed consent from all participants. Participants were genotyped with high-density SNP genotyping platforms covering the entire human genome. In most studies, only unrelated samples with missing genotype call-rates $<5\%$ were included for subsequent genome-wide association analyses. IMPUTE, MACH, or BEAGLE were used with haplotype reference panels from the JPT and CHB founders (JPT+CHB+CEU and/or YRI in some studies) on the basis of HapMap build 36 (release 21, 22, 23a or 24). Only imputed SNPs with high genotype information content (proper_info >0.5 for IMPUTE and $Rsq >0.3$ for MACH and BEAGLE) were used for the association analysis.

Stage II Study

The stage II study included 967 incident T2D cases and 913 controls from the SWHS and 733 male incident T2D cases and 734 controls from the Shanghai Men's Health Study (SMHS), an ongoing, population-based cohort study of 61,491 men aged 40 to 74 years at study enrollment (Cai et al. 2007), for a total of 1,700 T2D cases and 1,647 controls.

Genotyping was performed on the iPLEX™ Sequenom MassARRAY® platform. Polymerase chain reaction (PCR) and extension primers were designed by using the MassARRAY Assay Design 3.0 software (Sequenom, Inc). PCR and extension reactions were performed according to the manufacturer's instructions, and extension product sizes were determined by mass spectrometry using the Sequenom iPLEX system. On each 96-well plate, two negative controls (water), two blinded duplicates, and two samples from the HapMap project were included. We also included 65 participants who had been genotyped by using the Affymetrix 6.0 array on the Sequenom genotyping platform.

Statistical analyses

Quantitative demographic and lifestyle parameters were compared between cases and controls by using the Mann-Whitney rank sum tests or ANOVA, where appropriate. Chi-squared statistics were used to evaluate differences between cases and controls for categorical variables.

Stage I Study—Single-marker association analyses were carried out to evaluate associations with T2D risk. Odds ratios (ORs) and 95% confidence intervals (CI) were estimated using logistic regression models with adjustment for BMI. Meta-analysis was performed by an inverse-variance method assuming fixed effects with Cochran's Q test to assess between-study heterogeneity. METAL software (<http://www.sph.umich.edu/csg/abecasis/Metal>) was used for the meta-analysis.

Selection criteria for the top SNPs to take forward to stage II were: 1) a $P < 0.05$ (BMI-adjusted) in the single-marker analysis from the Shanghai Diabetes GWAS and $P < 0.05$ in the BMI-adjusted meta-analysis or 2) $P < 10^{-3}$ in the meta-analysis alone and 3) MAF $\geq 10\%$ and $r^2 \geq 0.80$, if the SNP was imputed. If SNPs that met either of these criteria were in linkage disequilibrium with each other, only one tagSNP was chosen for further evaluation ($r^2 > 0.80$). Nine SNPs met at least one of these criteria (all SNPs in the *PGC1* family and in the *PPARGC1A* and *PPARGC1B* genes) and were independent of each other (r^2 cut point = 0.80) and were moved to the stage II study. Age was available in SBCS/SWHS GWAS, but not in all the studies in AGEN-T2D. Therefore, in combining results from SBCS/SWHS with the results from the meta-analysis we only adjusted for BMI.

Stage II Study—Single-marker association analyses were carried out to evaluate associations with T2D risk. Odds ratios (ORs) and 95% confidence intervals (CI) were estimated using logistic regression models with adjustment for age, sex, and BMI. The association between genotype and T2D risk was evaluated based on an additive genetic model. We also performed analyses with a combined dataset, using the SBCS/SWHS GWAS population from stage 1 and stage 2 data (2,719 cases and 3,356 controls).

Interaction analyses—We conducted interaction analyses using combined data from the stage I and II studies (2,719 cases and 3,356 controls). Stratified analyses were performed to investigate interactions between SNPs and exercise participation and BMI categories. Tests for interaction were performed by including interaction terms in the analysis. All analyses were performed using SAS (version 9.1). All P values presented are based on two-tailed tests. P values presented in this paper were not corrected for multiple testing.

RESULTS

Characteristics of the study participants included in stage I (SBCS/SWHS GWAS) and Stage II are presented in Table 1. In Stage I, cases were older, had a higher BMI and WHR, and were more likely to exercise than controls. The reason why controls were younger in

Stage I is most likely because controls were drawn from a breast cancer case-control study whose participants were younger. In Stage II, cases had higher BMI and WHR, while no differences in exercise participation were observed (Table 1).

A total of nine SNPs were selected for stage II, from the *PPARGC* family. Only 2 SNPs out of the 9 SNPs had a P value <0.05 in the meta-analysis (see Table 1 in appendix). No SNP from the *PPAR* family met the criteria for validation in Stage II. Results from the single SNP analysis and from the meta-analysis are shown in Table 2. Three SNPs were in the *PPARGC1A* gene (rs12640088, rs12503529, and rs3796407) and six in *PPARGC1B*. Only one SNP (rs251464) in *PPARGC1B* was replicated in stage II (Table 2). The OR for this SNP in stage II under the additive model was 0.87 (95%CI: 0.77–0.99); $P=0.03$). In combined data from stages I and II, two SNPs were associated with T2D ($P<0.05$), rs251464 and rs1549188, both in the *PPARGC1B* gene. Four SNPs were associated with T2D in the same direction in stage I and in stage II.

To explore possible gender specific effects, we repeated the analysis stratified by gender. In men one SNP (rs741580) in *PPARGC1B* was replicated in stage II, while none of the SNPs were replicated in women in stage 2 (see Appendix Table 2). In combined data from stages I SBCS/SWHS GWAS and stage II, six SNPs were associated with T2D in women ($P<0.05$), three in the *PPARGC1A* and three in the *PPARGC1B* gene (see Appendix Table 3).

Associations between genotype and exercise participation are presented in Table 3. We found that rs1549188 was associated with higher risk of T2D in the non-exercise group only, while another SNP, rs251464, was associated with lower risk of T2D in the non-exercise group only. No significant interactions between genetic variation and exercise participation were observed.

In analyses stratified by BMI categories (BMI ≤ 25 and BMI >25) we found that rs251464 was associated with lower risk of T2D for the low BMI category only, while rs1549188 was associated with higher risk of T2D in the low BMI group (Table 4). In the high BMI group, rs12640088 was associated with lower risk of T2D. The P value for multiplicative interaction with BMI was significant only for rs12640088.

DISCUSSION

Using a comprehensive study approach, we investigated associations between polymorphisms in two related families of genes involved in energy balance and glucose and lipid metabolism, *PPAR* and *PGC1*, with T2D. In addition, we investigated interactions between validated SNPs with BMI and exercise participation and T2D.

We chose two related families of genes for their likely impact on energy balance and the biological plausibility of a role in the development of insulin resistance. No SNPs in the *PPAR* gene family were associated with T2D in stage I. Some studies have linked *PPARA* to components of the metabolic syndrome and T2D in Caucasian populations (Evans et al. 2001; Robitaille et al. 2004; Tai et al. 2002; Tai et al. 2005; Uthurralt et al. 2007). One cross-sectional study reported an association of a haplotype of *PPARA* with age of T2D diagnosis among European subjects (Flavell et al. 2005). Genetic variation in the *PPARD* gene has been associated with higher fasting plasma glucose concentrations (Hu et al. 2006) and with the conversion from impaired glucose tolerance to T2D in the STOP-NIDDM trial (Andrulionyte et al. 2006). No association between this gene and T2D was found in a Korean population or in our population (Shin et al. 2004; Villegas et al. 2011). However in a study conducted among Han Chinese, one SNP in *PPARD* rs6902123 was significantly associated with T2D and combined T2D and impaired fasting glucose (Lu et al. 2012). The minor allele frequency for this SNP in our population (SBCS/SWHS GWAS) was 0.036,

thus we not had limited power to detect association. The *PPARG* gene has been associated with higher susceptibility to obesity and insulin sensitivity (Tonjes and Stumvoll 2007) and the Ala allele of the P12A polymorphism in *PPARG* was associated with T2D in a GWAS (Scott et al. 2007) and has been replicated in many studies, but our study found no association between this polymorphism and T2D. In the SBCS/SWHS GWAS study the minor allele frequency for this SNP was 0.05 and thus, it is possible that we did not have enough power to detect an association. With 1019 cases and 1079 controls in the SBCS/SWHS GWAS, we had enough power to detect only moderate sized associations (with MAF =0.05 we had only 80% power to detect an effect size of 1.4 or greater). However, in the meta-analysis, with 6,952 T2D cases and 11,865 controls we had enough power to detect only moderate sized associations (with MAF =0.07 we had ~ 87% power to detect an effect size of 1.2 or greater with a $P<0.001$). Similar to our study, other study in Han Chinese found no association between the Ala allele of the P12A polymorphism in *PPARG* and T2D (Wang et al. 2009) and in a meta-analysis of 22 studies no association between this SNP with the susceptibility for T2D diabetes in Chinese Han population (Guo et al. 2011).

Nine SNPs in the *PPARGC* family of genes were associated with T2D in stage I. However, only one SNP, in *PPARGC1B*, rs251464, was replicated in stage II. In a study conducted in a Han Chinese population, no association between 6 SNPs from *PPARGC1A* and T2D were found while a common haplotype of *PPARGC1A* was associated with higher T2D risk (Zhu et al. 2009). Two common SNPs in the *PPARGB1A* gene (Gly482Ser and Thr394Thr) have been associated with T2D previously but not in this study. In our population, these two SNPs were not in linkage disequilibrium with the SNPs that were evaluated in our study for replication. The Gly482Ser SNP in *PPARGC1A* has been associated with insulin resistance and susceptibility to T2D in some (Ambye et al. 2005; Kunej et al. 2004; Sun et al. 2006; Zhang et al. 2007), but not all, studies (Nelson et al. 2007; Stumvoll et al. 2004; Wang et al. 2005). A meta-analysis of 8 studies, including 5 European-ancestry populations, one Pima Indian population, and one Japanese population, suggested that the Gly482Ser *PPARGC1A* variant was associated with T2D risk (Barroso et al. 2006). The other SNP of *PPARGC1A*, Thr394Thr, has been associated with body fat and T2D in South Asians from India (Vimaleswaran et al. 2005; Vimaleswaran et al. 2006). The lack of association between these common variants and T2D in our study might not be related to lack of power. We had enough power in the meta-analysis to detect moderate sized associations. The SNPs may not be in LD with the causal variants originally reported (if other than the GWAS variant), or context with other variables. The lack of association may be due to the absence of the causal allele in Asian populations

The expression of both *PPARGC1A* and *PGC1B* co-activators was lower in both diabetic subjects and family history-positive, non-diabetic Mexican Americans, further supporting a possible role for these genes in the etiology of T2D (Patti et al. 2003). Genetic associations of *PPARGC1B* with T2D were examined in a Korean population of 775 T2D patients and 316 controls (Park et al. 2006), but results were not significant after correction for multiple testing.

The *PPARGC* transcriptional co-activators play a critical role in the maintenance of glucose, lipid, and energy homeostasis and are likely involved in the pathogenic conditions such as obesity and diabetes (Lin et al. 2005). Expression of these genes may influence insulin sensitivity, as well as energy expenditure, thereby contributing to the development of human obesity (Esterbauer et al. 1999). The *PPARGC* transcriptional co-activators are major regulators of crucial aspects of energy metabolism (Spiegelman 2007) and energy balance plays an important role in the development of T2D. *PGC1A* has been implicated in increasing oxidation of fatty acids via increasing mitochondrial capacity and function, making this co-factor a key candidate for the treatment of lipotoxicity (Medina-Gomez,

Gray, & Vidal-Puig 2007). Ectopic expression of *PGC1A* in white fat cells conferred on them some of the properties of brown fat, including induction of uncoupling protein (UCP), a mitochondrial uncoupler and dissipator of heat. Since then, it has become clear that *PGC1A* can coactivate a large repertoire of transcription factors, including most members of the nuclear receptor family (Lin, Handschin, & Spiegelman 2005). It has been reported that variation of *PGC1B* may contribute to the pathogenesis of obesity (Andersen et al. 2005). In this study we investigated possible interactions between SNPs in these 2 genes and BMI and exercise participation, the main components of energy balance. Expression of the *PPARGC1A* gene may influence insulin sensitivity, as well as energy expenditure, thereby contributing to the development of human obesity (Esterbauer, Oberkofler, Krempler, & Patsch 1999).

Strengths of the current study include its relatively large size, and the two-stage design, which permitted an independent evaluation of the results of stage I. Furthermore, we had detailed information about exercise participation and BMI measurements, which allowed us to pursue investigations of gene-exercise interactions and gene-BMI-interactions. A limitation of our study is that we performed a relatively large number of tests, which may have increased the number of type I errors. We did not correct for multiple testing and our association results would not survive multiple testing correction. However, the study is hypothesis driven and is based on results from other studies and therefore does not suffer from limitations often associated with GWAS discovery analyses. Age was only available in SBCS/SWHS GWAS, but not in all the studies in AGEN-T2D and we were comparing results from this study with the results from the meta-analysis, thus we only adjusted for BMI in the SBCS/SWHS GWAS. We might have had limited power to evaluate interactions between SNPs with low a MAF and BMI and exercise participation. We only had enough power to detect moderate sized interactions (for an effect size of 1.4 or greater we had at least 80% power for a MAF=0.10).

In summary, variation in the *PPARGC1B* gene may be associated with T2D among middle-aged Chinese men and women. Our data does not suggest interactions between this gene and BMI or exercise participation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Characteristics of the study populations from stages I and II.

	STAGE I SBCS/SWHS GWAS				STAGE II		
	All	Controls N=1709	Cases N=1019	<i>P</i> value ^I	All	Controls N=1647	Controls N=1700
Age (years) Median	50.7	47.9	55.5	<0.01	61.66	61.69	61.56
BMI (kg/m²) Mean	24.0	22.8	26.3	<0.01	25	24.20	26.34
WHR Mean	0.82	0.80	0.84	<0.01	0.87	0.85	0.88
Ever smoker (%)	2.8	2.8	2.8	0.93	32.06	31.57	32.53
Regular exercise (%)	31.1	29.4	33.8	0.02	42.72	43.78	41.53
Education (%)							
None	7.8	8.4	6.9	<0.01	16.31	14.51	18.06
Elementary	21.0	24.4	15.2		16.01	15.60	16.41
High school	61.1	57.0	68.1		51.84	51.85	51.82
Third level	10.1	10.2	9.8		15.36	17.42	13.36
Men (%)	N/A	N/A	N/A		43.83	44.57	56.88

^I *P*-values were calculated by t test for all continuous variables with the exception of age because was not it was normally distributed and a Wilcoxon two-sample test was used; for comparison of categorical variables a χ^2 -square test was used.

Table 2

Associations between SNPs and T2D in stage I¹, stage II, and combined data(stages I and II combined)

SNP number	Gene	STAGE I				STAGE II		COMBINED DATA ⁵	
		SBCS/SWHS GWAS			AGEN-T2D Meta analysis	OR ⁴ (95% CI)	P value	OR ⁴ (95% CI)	P value
		MAF	Risk Allele /other	OR ³ (95% CI)	P value				
rs12640088 ²	PPARGC1A	0.14	A/C	0.80 (0.67–0.96)	0.01	0.81 (0.73–0.89)	0.03	0.93 (0.84–1.04)	0.24
rs12503529 ²	PPARGC1A	0.12	T/C	0.72 (0.58–0.88)	0.001	0.78 (0.68–0.88)	0.04	0.94 (0.83–1.06)	0.33
rs3796407	PPARGC1A	0.13	A/G	1.37 (1.13–1.66)	0.001	1.27 (1.18–1.36)	0.03	1.06 (0.94–1.20)	0.32
rs1422429 ²	PPARGC1B	0.30	T/C	1.00 (0.88–1.14)	0.97	1.20 (1.16–1.24)	0.002	0.98 (0.90–1.06)	0.58
rs251464 ²	PPARGC1B	0.17	C/G	0.86 (0.74–1.01)	0.06	0.82 (0.76–0.88)	0.004	0.88 (0.80–0.96)	0.007
rs741580	PPARGC1B	0.16	A/G	1.06 (0.90–1.25)	0.46	0.83 (0.77–0.89)	0.009	0.97 (0.87–1.08)	0.99
rs1549188	PPARGC1B	0.16	A/G	1.27 (1.07–1.50)	0.007	1.21 (1.15–1.27)	0.02	1.13 (1.02–1.26)	0.02
rs4705385 ²	PPARGC1B	0.11	T/C	0.80 (0.66–0.98)	0.03	0.78 (0.70–0.86)	0.01	0.91 (0.81–1.03)	0.16
rs109075 ²	PPARGC1B	0.37	T/C	1.14 (1.00–1.29)	0.048	1.22 (1.17–1.27)	0.03	1.07 (0.99–1.16)	0.08

¹ Criteria for selection for stage II: 1) P value <0.05 in single marker analysis Shanghai GWAS and P<0.05 in the meta-analysis or 2) P<10⁻³ in the meta-analysis alone and 3) MAF 10% and if imputed, MACH_RSQ>0.80.

² Imputed SNPs, MACH_RSQ=0.99;

³ Adjusted for BM

⁴ Adjusted for age, BMI, and sex. The odds ratio reported is for the risk allele determined in the meta-analysis.

⁵ Combined data: Stage II and SBCS/SWHS GWAS.

Table 3
Associations of SNPs with T2D stratified by exercise participation categories (combined datasets)^I

SNP number	No exercise		Exercise	
	OR (95% CI)	P value	OR (95% CI)	P value
rs12640088	0.95(0.82–1.10)	0.51	0.91(0.76–1.08)	0.27
rs12503529	0.99(0.85–1.17)	0.97	0.73(0.71–1.05)	0.14
rs3796407	0.98(0.83–1.15)	0.81	1.20(0.99–1.46)	0.06
rs1422429	1.02(0.92–1.14)	0.68	0.91(0.79–1.04)	0.15
rs251464	0.82(0.72–0.93)	0.002	0.97(0.84–1.13)	0.70
rs741580	0.92(0.80–1.06)	0.24	1.05(0.89–1.24)	0.56
rs1549188	1.19(1.03–1.36)	0.02	1.04(0.88–1.24)	0.58
rs4705385	0.85(0.72–0.99)	0.04	1.04(0.86–1.27)	0.68
rs109075	1.09(0.98–1.21)	0.10	1.05(0.92–1.19)	0.49

^I Combined data: Stage II and SBCS/SWHS GWAS; Adjusted for age, BMI, and sex.

Table 4
Associations of SNPs with T2D stratified by BMI categories (combined datasets)¹

SNP number	BMI 25		BMI>25	
	OR (95% CI)	P value	OR (95% CI)	P value
rs12640088	1.06(0.91–1.25)	0.42	0.83(0.71–0.98)	0.02
rs12503529	1.04(0.87–1.23)	0.67	0.94(0.72–1.02)	0.08
rs3796407	1.02(0.86–1.21)	0.83	1.08(0.97–1.21)	0.19
rs1422429	0.99(0.88–1.11)	0.84	1.12(0.94–1.34)	0.44
rs251464	0.83(0.72–0.95)	0.008	0.95(0.85–1.07)	0.16
rs741580	0.92(0.79–1.06)	0.24	0.91(0.80–1.04)	0.71
rs1549188	1.22(1.05–1.43)	0.009	1.03(0.89–1.19)	0.30
rs4705385	0.92(0.77–1.09)	0.36	1.08(0.93–1.25)	0.34
rs109075	1.08(0.96–1.20)	0.20	0.92(0.78–1.09)	0.16

¹ Combined data: Stage II and SBCS/SWHS GWAS; Adjusted for age and sex.