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Genetic variation and Insulin Resistance in middle age Chinese men

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Abstract

Insulin resistance (IR), a necessary condition to T2D development is potentially reversible. Understanding factors associated with IR, including genetic susceptibility, is important for T2D prevention.

We investigated the effect of variants in the first three genes in the insulin signaling pathway and genes identified from genome wide association studies (GWAS) of T2D quantitative traits with IR (fasting insulin and the homeostasis model assessment of IR, HOMA-IR) and evaluated gene-environment interactions with IR traits among 1879 non-diabetic middle-aged men from a population-based prospective study conducted in Shanghai, China.

One candidate gene, *IGF1*, was associated with fasting insulin and HOMA-IR. We observed 5 BMI-gene interactions ($p < 0.05$) with HOMA-IR (*INRS* rs7254060, *INRS* rs7254358, *GLU4* rs12054720, *GLU4* rs2113050 and *GLU4* rs7713127) and 7 BMI-gene interactions with fasting insulin (*INRS* rs7254060, *INRS* rs7254358, *INRS* rs1017205, *INRS* rs1799817, *GLU4* rs12054720, *GLU4* rs2113050 and *GLU4* rs7713127). There were 4 WHR-gene interactions with HOMA-IR (*INRS* rs10417205, *INRS* rs12971499, *INRS* rs7254060, *INRS* rs7254358), 5 WHR-gene interactions with fasting insulin (*INRS* rs10417205, *INRS* rs7254060, *INRS* rs7254358, *GLU4* rs2113050 and *GLU4* rs7713127), 8 physical activity-gene interactions with HOMA-IR (*INRS* rs10411676, *INRS* rs11671297, *INRS* rs2229431, *INRS* rs12461909, *INRS* rs610950, *INRS* rs10420382, *IRS2* rs913949 and *IRS2* rs2241745) and 5 physical activity-gene interactions with fasting insulin (*INRS* rs2229431, *INRS* rs12461909, *INRS* rs10420382, *IRS2* rs913949 and *IRS2* rs2241745).

Our results suggest that BMI, WHR and physical activity may modify IR-associated variants.

INTRODUCTION

Diabetes is a growing public health problem. In the United States, the disease afflicts more than 20 million people with most cases having Type 2 diabetes (T2D). T2D is characterized by insulin resistance (IR) and impaired insulin secretion. Of these two features, insulin resistance is potentially reversible. Thus, understanding factors associated with the development of IR is of critical importance for the prevention of T2D as those most at risk can start preventive measures early. IR results from the interaction of environmental factors with a predisposing genetic background (1). Thus, understanding the gene-environment context in which IR develops is critical to preventing insulin resistance and reducing T2D incidence. However epidemiological data investigating interactions of genes and environmental factors and IR is limited.

The precise mechanisms mediating IR are unknown. The metabolic action of insulin is regulated by a cascade of molecular events starting with insulin binding to its receptor (2). Activation of the insulin signaling pathway induces translocation of glucose transporter 4 (*GLUT4*) to the cell surface, allowing glucose import into the cell. Thus, genes in the insulin signaling pathway, such as the insulin receptor (*INSR*) and the insulin receptor substrate 1 and 2 (*IRS1* and *IRS2*), and *GLUT4* represent excellent candidates for IR. A single nucleotide polymorphism (SNP) near *IRS1* has been associated with IR in a genome wide association study (GWAS) of T2D related quantitative traits (3). Other loci identified in GWAS of T2D related quantitative traits associated with IR include, insulin-like growth factor 1 (*IGF1*), and glucokinase (hexokinase 4) regulatory protein (*GCKR*) (4).

IR is associated with several environmental factors, including those that contribute to obesity. Visceral fat in obese patients is a stronger correlate of insulin resistance than subcutaneous fat (5). High physical activity levels are also associated with lower incidence of insulin resistance (6). Exercise, therefore, is an important intervention strategy for the prevention and treatment of muscle insulin resistance and T2D (7).

In this project, we investigated associations of genes related to insulin resistance with fasting insulin levels and the homeostasis model assessment of insulin resistance (HOMA-IR) in 1879 middle-aged men free of T2D at baseline. In this study, we used the first three genes in the insulin signaling pathway (*INSR*, *IRS1* and *IRS2*), *GLUT4* and two genes discovered in GWAS of insulin resistance (*IGF1* and *GCKR*). We also investigated whether any associations were modified by body mass index (BMI), waist to hip ratio (WHR), and physical activity. All of which are thought to play an important role in the pathogenesis of insulin resistance, and thereby, provide a more complete picture of the factors associated with insulin resistance.

Methods

Study Population: The Shanghai Men's Health Study

The Shanghai Men's Health Study (SMHS) is a population-based cohort study of men (aged 40–74 years) living in urban Shanghai, China. Recruitment for the SMHS was conducted between 2002 and 2006. A total of 83 058 eligible male residents of eight communities in

urban Shanghai were invited to participate and 61480 men who had no prior history of cancer were enrolled in the study (response rate: 74.0%). Information was collected on demographic characteristics, disease history and lifestyle factors by trained interviewers. Participants were asked to provide biological samples, including a blood or cheek cell sample and a spot urine sample. The study protocols were approved by the Institutional Review Boards of Vanderbilt University, Nashville, TN and the Shanghai Cancer Institute, Shanghai, China. All participants provided written informed consent.

At the time of the in-person interview, a 10 ml blood sample was drawn into an EDTA vacutainer tube. The samples were kept in a portable Styrofoam box with ice packs (0–4 °C) and were processed within 6 hours. In a sub-cohort of 3978 participants who had no history of diabetes at baseline and who provided a fasting blood sample, we measured levels of disease-related biomarkers, including glucose and insulin. Blood glucose was measured by the Vanderbilt Clinical Nutrition Center using an ACE clinical chemistry system. Insulin was measured at Vanderbilt's Hormone Assay & Analytical Services Core laboratory by radioimmunoassay (RAI) using a double antibody procedure. We studied random samples of 1879 participants with fasting glucose values < 7 mmol/L. The HOMA-IR scores were calculated according to the model developed by Matthews et al., which derives an estimate of insulin sensitivity from the mathematical modeling of fasting plasma glucose and insulin concentrations (8).

Physical Activity

Assessment of physical activity was obtained using a validated physical activity questionnaire (PAQ) (9). The PAQ contained separate sections to collect information on physical activity related to exercise/sports, daily living activities, and commuting to/from work. For exercise/sports physical activity, participants were asked if they had engaged in regular exercise/sports during the preceding five years. Exercisers were asked to report details for up to three types of exercises/sports (i.e., type, hours/week, and years of participation). For daily living physical activity, participants were asked about walking, stair climbing, bicycling, and household chores. For commuting-related physical activity, participants were asked whether they walked, bicycled, drove a vehicle, or rode a bus to work. Summary energy expenditure values (metabolic equivalent task [MET]-hr/day) for individual activities were estimated using a compendium of physical activity values (10). Summary energy expenditure values for exercise/sports participation were estimated as MET-hours/day/year by using the weighted average of energy expended in all exercise/sports-related activities reported over the preceding 5 years. Summary energy expenditure values were also calculated for activities related to daily living and commuting. Finally, we calculated totals for physical activity related to exercise/sports participation, daily living, commuting, and an overall physical activity total (total METs), by combining all types of physical activities.

Anthropometrics

Anthropometric measurements of weight, height, and waist and hip circumferences were taken twice according to a standard protocol. If the difference between the first two measurements was larger than 1 cm for circumferences or 1 kg for weight, a third

measurement was taken. The average of the two closest measurements was applied in the present study. From these measurements, the following variables were created: body mass index (BMI), weight in kg divided by the square of height in meters; and waist-hip ratio (WHR), waist circumference divided by hip circumference.

Genotyping

We genotyped haplotype tagging SNPs in the 6 study genes (*INSR*, *IRS1*, *IRS2*, *GLUT4*, *IGF1*, *GCKR*), using pairwise tagging, with Tagger software (11), to define tag SNPs (12;13) with a linkage disequilibrium (LD) threshold of $r^2 \geq 0.80$, minor allele frequency (MAF) greater than or equal to 0.10, and the most updated HapMap release with the Han Chinese (CHB) as reference genotypes. Tagging did not include any upstream or downstream regions of the study genes. Genotyping assays were performed on the iPLEX™ Sequenom MassARRAY® platform (Sequenom, Inc., San Diego, CA). On each 96-well plate, two negative controls (water), two blinded duplicates, and two samples from the HapMap project were included.

Genotyping assays were performed on the iPLEX™ Sequenom MassARRAY® platform (Sequenom, Inc., San Diego, CA). Polymerase chain reaction (PCR) and extension primers were designed with the MassARRAY Assay Design 4.0 software, and alleles of each SNP were detected through matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry on the MassARRAY Analyzer 4 system (Sequenom, San Diego, CA, USA). In each 96-well plate, two negative control (water) and two samples from the 1000 Genomes project were used in the Sequenom assay. Genotype concordance rate for 44 positive QC samples from the 1000 Genomes project was between 90.9% and 100% with a mean of 99.5%. Genotype calling rates ranged from 97.3% to 99.8% with a mean rate of 98.9%. All blanks and duplicate samples showed consistent results (i.e., no genotype or identical genotypes). All SNPs passed checks of the Hardy-Weinberg equilibrium with P values greater than 0.05.

Data analyses

Effects of SNPs on quantitative traits were analyzed by a linear regression model, under an additive model, indexing exposure to the minor allele of each SNP. We performed an adjustment for age and BMI. Values of HOMA-IR and insulin were logarithmically transformed as they did not have a normal distribution. For interactions with environmental factors we also used linear regression and included interaction terms in the analyses. All analyses were performed using SAS (version 9.1, SAS Institute, Cary, North Carolina). All P values presented are based on two-tailed tests. P values presented in this paper were not corrected for multiple testing.

RESULTS

The general characteristics of the participating study population by quintiles of HOMA-IR are presented in Table 1. Participants in the upper quintile of HOMA-IR had a higher BMI and WHR, and had lower METs of physical activity compared to those in the lower quintile.

A total of 83 SNPs were included in the analysis (Supplementary table). We found that 3 SNPs in the *IGF1* gene were associated with fasting insulin and 2 of them were also associated with HOMA-IR (Table 2). The P value after correction for multiple testing (Bonferroni correction) was 0.0006 and none of the P values passed the significance threshold.

We observed seven significant BMI-gene interactions ($p < 0.05$) with fasting insulin (*INRS* rs7254060, *INRS* rs7254358, *INRS* rs1017205, *INRS* rs1799817, *GLU4* rs12054720, *GLU4* rs2113050 and *GLU4* rs7713127), while only 5 of those BMI-gene interactions were significant with HOMA-IR (*INRS* rs7254060, *INRS* rs7254358, *GLU4* rs12054720, *GLU4* rs2113050 and *GLU4* rs7713127) (Table 3).

Table 4 shows associations between SNPs and WHR with HOMA-IR and fasting insulin. We observed four WHR-gene interactions with HOMA-IR, all in the *INRS* gene (*INRS* rs10417205, *INRS* rs12971499, *INRS* rs7254060, *INRS* rs7254358) and 5 WHR-gene interactions with fasting insulin (*INRS* rs10417205, *INRS* rs7254060, *INRS* rs7254358, *GLU4* rs2113050 and *GLU4* rs7713127) (Table 4). When we looked at interactions between these SNPs and total METs of physical activity (Table 5), we found that 8 physical activity-gene interactions had a P value for interaction less than 0.05 with HOMA-IR (*INRS* rs10411676, *INRS* rs11671297, *INRS* rs2229431, *INRS* rs12461909, *INRS* rs610950, *INRS* rs10420382, *IRS2* rs913949 and *IRS2* rs2241745), but only 4 of those physical activity-gene interactions had a $P < 0.05$ with fasting insulin (*INRS* rs2229431, *INRS* rs12461909, *INRS* rs10420382, *IRS2* rs913949 and *IRS2* rs2241745). The two other SNPs had a P value of marginal significance ($0.07 > p = 0.05$). The P value after correction for multiple testing (Bonferroni correction) was 0.0005 and none of the P values for the gene-environment interactions passed the significance threshold.

DISCUSSION

In this study, we evaluated common genetic variants in the *INSR*, *IRS1*, *IRS2*, *GLUT4*, *IGF1* and *GCKR* genes in participants of the Shanghai Men's Health study for their association with insulin resistance quantitative traits. The genes in this project were selected based on their physiological roles in insulin signaling and glucose uptake as well as genes identified in GWAS of insulin resistance related quantitative traits. We also investigated the interaction between the genetic variants with three modifiable environmental risk factors of insulin resistance (BMI, WHR, and total METs of physical activity) on HOMA-IR and fasting insulin.

Only one of the six genes in this study, *IGF1*, showed significant association with both fasting insulin and HOMA-IR. The other five genes investigated showed no evidence of association with either of these two traits, though several interesting interactions were observed. Interactions between the insulin receptor gene (*INSR*) with BMI, WHR, and total METs of physical activity with HOMA-IR and fasting insulin were found. SNPs in the *GLUT4* gene showed interactions with BMI and fasting insulin and with WHR with HOMA-IR and fasting insulin. We also found that 2 SNPs from the *IRS2* gene showed interactions with total METs of physical activity with HOMA-IR and fasting insulin.

Given the strong correlation between fasting insulin and HOMA-IR ($R^2=0.96$), it is unsurprising that genetic variation in the *IGF1* gene was significant for associations with both. *IGF1* encodes the insulin-like growth factor which has structural homology with insulin. The *IGF1* receptor activates many of the same signaling pathways as insulin (14). *IGF1* SNPs have also been associated with insulin sensitivity in a Chinese population (15) and with body composition in another study (16). Genetic variation in the *IGF1* gene has been associated with HOMA-IR (4) and height in GWAS in a Korean population (17) and has shown an interaction with BMI (18), though one was not observed in the present study. The most significant SNP in *IGF1* was rs5742714, which resides in the 3' UTR of this gene. According to HaploReg, this SNP alters a number of regulatory motifs and may thus alter transcriptional regulation (19). This SNP was associated with interactions with current and past BMI as a risk factor for cancer in a Japanese population (20). The SNP does not have any significant LD with the SNPs identified by prior GWAS. While the P values would not remain significant after Bonferroni correction, when evaluated on a per-gene basis, *IGF1* may deserve further study or may show differential effects on insulin and HOMA-IR depending on unknown factors, though it appears interactions with BMI, WHR, and MET do not explain this association.

While no association between the other genes and fasting insulin or HOMA-IR was found, some genes showed evidence of an interaction with measures of body fat including BMI and WHR. *INSR* and *GLUT4* showed evidence of interaction with BMI impacting association with HOMA-IR and insulin resistance. Six of the seven SNPs showing interaction with BMI were among the six SNPs showing interaction with WHR. This is somewhat unsurprising given the correlation between BMI and WHR ($R^2=0.35$).

INSR, *IRS1*, and *IRS2* encode the first components of the insulin signaling pathway with tyrosine phosphorylation of these proteins occurring after the binding of insulin to its receptor (2). Several rare mutations have been identified in *INSR* that confer moderate to severe insulin resistance (21;22), but identification of more common variants that confer insulin resistance has not been successful (23;24). *IRS2* knockout mice developed insulin resistance (25), but studies conducted in humans found no such associations with insulin resistance (26–28), while haplotypes of the *IRS2* gene appeared to act as a modulator of insulin resistance in a Japanese population (29). Being overweight appears to modify the effect of the G1057D (rs1805097) polymorphism in *IRS2* toward a higher risk of T2D (30). Among obese individuals, the D1057 allele and the CA haplotype in the *IRS2* gene could be useful genetic markers for individuals who are particularly susceptible to insulin resistance (29). These SNPs were not genotyped in the present study nor were its proxies significantly associated with insulin resistance either by interaction measures or single SNP analysis. The other gene analyzed in our study, *GLUT4*, encodes an insulin-sensitive transporter with a critical role in glucose homeostasis and diabetes pathogenesis (31). *GLUT4* disruption in mice caused severe insulin resistance (32). However, genetic studies on humans have had varied results (33–37).

There were several reasons why we chose to study associations between these candidate genes with insulin resistance and interactions with BMI and WHR. Given the strong correlation between obesity and insulin resistance, the extent and distribution of adiposity is

likely to influence the effect of genes that are associated with insulin resistance (38). Obesity is associated with decreased expression of insulin receptors at the cell surface and lower tyrosine kinase activity (39). In addition, impaired insulin-stimulated glucose uptake in skeletal muscle from severely obese patients is accompanied by a deficiency in insulin receptor signaling (40).

Finally, our analysis of interaction effects between SNPs and physical activity suggests a role for SNPs in *INSR* and *IRS2* in regulation of insulin or HOMA-IR. Exercise may affect skeletal insulin action by influencing specific events in the insulin signaling pathway, in particular, at the level of the insulin receptor substrate proteins (41;42). Animal studies show that *GLUT4* translocation is the major mechanism by which exercise increases glucose uptake in skeletal muscle (43). No interactions between *GLUT4* and physical activity were observed in this study; however, we found physical activity-gene interactions with the *INRS* and *IRS2* genes with HOMA-IR and fasting insulin. None of the SNPs in *INSR* or *IRS2* that showed interaction with physical activity showed either single-marker association with HOMA-IR or fasting insulin, nor were they the same SNPs associated in other interaction analyses of WHR or BMI. Given the relatively large number of SNPs required to tag the 181.7kb *INSR* gene, findings of interactions or other associations in this gene may be less remarkable than those found for other loci, such as *GLUT4* and its interaction with BMI and WHR. When we corrected for multiple testing using the Bonferroni correction, none of the P values for the gene-environment interactions passed the threshold for significance. Although the Bonferroni method is very conservative, and easy to interpret, correcting for multiple testing in gene-environment interaction studies is inherently more complicated than in standard single-SNP association studies.

Genes not implicated in the present study include *IRS1* and *GCKR*. SNPs in *GCKR* have been associated with lower HOMA-IR in a Japanese population (44), and in a Chinese population, loci in this gene have been associated with obesity, T2D, and non-alcoholic liver disease (45–47), both highly correlated to insulin resistance. However, no association between *GCKR* and fasting insulin in a Chinese population was found (48).

Strengths of the current study include that it was a relatively large study, and had good coverage of the genetic variation of these genes. Furthermore, we had detailed information about total physical activity of the participants, which allowed us to pursue investigations of gene-physical activity interactions. A limitation of our study is that we performed a relatively large number of tests which may have increased the risk for Type 1 error and no replication has been done. However, the study is hypothesis driven and the candidate genes selected are based on results from other studies.

In summary, in our study of insulin resistance related quantitative traits among middle-aged Chinese men we found one of the six candidate genes (*IGF1*) had evidence for association. There was modest evidence of interaction with BMI (*INSR* and *GLUT4*), and WHR (*INSR*, *GLUT4*, and *IRS2*); and two genes had modest evidence of interaction with physical activity (*INSR* and *IRS2*). Future studies may further investigate the role of these loci in the regulation of insulin resistance and related traits.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Characteristics of participants by quintiles of HOMA-IR in the Shanghai Men's Health Study

Table 1

	Q1	Q2	Q3	Q4	Q5
Age (Median and interquartile range)	48 (45–53)	48 (45–53)	48 (45–55)	49 (45–55)	49 (45–59)
BMI* (Mean ± SD)	21.29 ± 2.52	22.25 ± 2.44	23.27 ± 2.59	24.33 ± 2.63	25.29 ± 3.06
WHR** (Mean ± SD)	0.86 ± 0.05	0.88 ± 0.06	0.89 ± 0.05	0.91 ± 0.06	0.91 ± 0.05
Total METs*** (Mean ± SD)	8.36 ± 5.05	7.79 ± 4.46	7.32 ± 4.86	7.60 ± 4.81	7.19 ± 4.34

* Body Mass Index

** Waist to Hip Ratio

*** Metabolic equivalent task (MET)-hr/day

Table 2
Associations between HOMA-IR and fasting insulin and SNPs in the *IGF1* gene in the Shanghai Men's Health Study*

SNP ID	Gene	MAF	Minor allele	HOMA-IR			Fasting insulin			r ² with index SNP
				Beta	SE	P value	Beta	SE	P value	
rs5742714	<i>IGF1</i>	0.18	G	-0.07	0.028	0.01	-0.07	0.026	0.007	index
rs17796225	<i>IGF1</i>	0.16	C	-0.07	0.029	0.02	-0.07	0.027	0.01	0.834
rs17032623	<i>IGF1</i>	0.27	C	0.03	0.02	0.24	0.04	0.02	0.04	0.072

* adjusted for age, BMI

Table 3
Interactions between SNPs and BMI and HOMA-IR and fasting insulin in the Shanghai Men's Health Study*

SNP ID	Gene	MAF	HOMA-IR			Fasting insulin			r ² with index SNP
			Beta	SE	P value	Beta	SE	P value	
rs10417205	<i>INRS</i>	0.41	0.01	0.007	0.06	0.01	0.007	0.03	0.006
rs1799817	<i>INRS</i>	0.28	0.01	0.007	0.12	0.01	0.007	0.048	0.002
rs7254060	<i>INRS</i>	0.11	0.02	0.009	0.008	0.02	0.009	0.008	index
rs7254358	<i>INRS</i>	0.47	-0.02	0.007	0.01	-0.01	0.007	0.02	0.002
rs12054720	<i>GLUT4</i>	0.23	0.01	0.007	0.06	0.01	0.06	0.04	0.587
rs2113050	<i>GLUT4</i>	0.13	-0.01	0.007	0.04	-0.01	0.007	0.03	0.996
rs7713127	<i>GLUT4</i>	0.23	-0.01	0.007	0.04	-0.01	0.007	0.03	index

* adjusted by BMI, age, SNP

Table 4
Interactions between SNPs and WHR with HOMA-IR and fasting insulin in the Shanghai Men's Health Study*

SNP ID	Gene	MAF	HOMA-IR			Insulin			r ² with index SNP
			Beta	SE	P value	Beta	SE	P value	
rs10417205	<i>INRS</i>	0.41	0.96	0.42	0.02	0.98	0.39	0.01	0.003
rs12971499	<i>INRS</i>	0.19	0.79	0.38	0.03	0.57	0.36	0.11	0.426
rs7254060	<i>INRS</i>	0.11	1.25	0.47	0.01	1.18	0.44	0.01	0.002
rs7254358	<i>INRS</i>	0.47	-1.05	0.38	0.006	-0.95	0.36	0.01	index
rs2113050	<i>GLUT4</i>	0.13	-0.77	0.39	0.05	-0.75	0.37	0.04	index
rs7713127	<i>GLUT4</i>	0.23	-0.75	0.39	0.06	-0.73	0.37	0.04	0.996

* adjusted for age, BMI, WHR and SNP

Table 5

Interactions between SNPs and total METs of physical activity and HOMA-IR and fasting insulin in the Shanghai Men's Health Study*

SNP ID	Gene	MAF	HOMA-IR			Insulin			r ² with index SNP
			Beta	SE	P value	beta	SE	P value	
rs10411676	<i>INRS</i>	0.08	0.01	0.006	0.04	0.01	0.006	0.05	0.000
rs11671297	<i>INRS</i>	0.32	0.009	0.004	0.04	0.008	0.004	0.07	0.000
rs2229431	<i>INRS</i>	0.33	0.02	0.008	0.01	0.02	0.008	0.009	0.085
rs12461909	<i>INRS</i>	0.18	0.01	0.004	0.003	0.01	0.004	0.01	index
rs6510950	<i>INRS</i>	0.17	0.01	0.005	0.02	0.008	0.004	0.06	0.524
rs10420382	<i>INRS</i>	0.39	0.01	0.004	0.009	0.01	0.004	0.02	0.778
rs913949	<i>IRS2</i>	0.32	0.01	0.005	0.005	0.01	0.005	0.003	index
rs2241745	<i>IRS2</i>	0.26	0.01	0.005	0.02	0.01	0.005	0.01	0.723

* adjusted for age, BMI, exercise participation and SNP