Clinical and Population Studies

Obesity Genotype Score and Cardiovascular Risk in Women With Type 2 Diabetes Mellitus

Meian He; Marilyn C. Cornelis; Paul W. Franks; Cuilin Zhang; Frank B. Hu; Lu Qi

Objective—To investigate the associations between obesity-predisposing genetic variants, cardiovascular biomarkers, and cardiovascular disease (CVD) risk in women with preexisting type 2 diabetes mellitus.

Methods and Results—We genotyped polymorphisms at nine established obesity loci in 1,395 women with diabetes from the Nurses’ Health Study: 449 women developed CVD, and 946 women did not develop CVD. A genetic risk score (GRS) was derived by summing risk alleles for each individual. Four polymorphisms (rs9939609 [FTO], rs11084753 [KCTD15], rs10838738 [MTCH2], and rs10938397 [GNPDA2]) showed nominally significant associations with CVD. The GRS combining all obesity loci was linearly related to CVD risk ($P = 0.013$ for trend). The odds ratio was 1.08 per risk allele (95% confidence interval, 1.02–1.15; $P = 0.01$) after adjustment for body mass index and other conventional risk factors. Women with the highest quartile of GRS had 53% (95% confidence interval, 6%–122%) increased CVD risk, compared with those in the lowest quartile of GRS ($P = 0.024$). In addition, a higher GRS was associated with lower adiponectin levels ($P = 0.02$). Further adjustment for body mass index and other covariates did not change the association ($P = 0.006$). A higher GRS was also correlated with lower levels of high-density lipoprotein ($P = 0.01$).

Conclusion—Obesity-predisposing variants may jointly affect CVD risk among women with diabetes. (Arterioscler Thromb Vasc Biol. 2010;30:327-332.)

Key Words: Cardiovascular disease ■ type 2 diabetes ■ obesitygene ■ polymorphism

Obesity is a major risk factor for the development of type 2 diabetes mellitus and cardiovascular disease (CVD).1 The underlying mechanisms involve insulin resistance, endothelial damage, inflammation, and dyslipidemia,2 resulting largely from overactivation of adipose depots. Because of a strong link between obesity and the risk of diabetes and CVD, obesity-predisposing genetic variants may adversely affect CVD risk in people with diabetes. The identification of genetic risk markers might help elucidate the mechanisms underlying the relationship between diabetes and CVD.

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Recently, genomewide association studies (GWAS) have identified multiple risk variants that are associated with risk of obesity. These variants localize to the FTO, MC4R, TMEM18, SH2B1, KCTD15, MTCH2, NEGR1, and GNPDA2 genes.3–6 To our knowledge, no study has examined associations between these variants and CVD risk among people with diabetes. We aimed to address this issue in women from the Nurses’ Health Study. We also examined the associations between these polymorphisms and levels of adipocytokines, lipids, and inflammatory and endothelial biomarkers.

Methods

Study Population

The Nurses’ Health Study began in 1976 with the recruitment of 121,700 female registered nurses (aged 30–55 years). In 1989 and 1990, a total of 32,826 women provided blood samples. Medical history, lifestyle information, and disease diagnoses were updated every 2 years using a validated questionnaire.7 Women with type 2 diabetes were identified by self-report methods that were confirmed with a validated supplementary questionnaire. For cases diagnosed before the 1998 cycle, we used the National Diabetes Data Group criteria8 to define diabetes. The validity of this method has been established.9 We used the 1997 American Diabetes Association diagnostic criteria for diabetes diagnoses from 1998 onward.10 The Human Research Committee at Brigham and Women’s Hospital in Boston, Massachusetts, approved this study. All participants gave written informed consent.

CVD Ascertainment

Cardiovascular disease cases were defined as those in whom fatal coronary heart disease (CHD), nonfatal myocardial infarction (MI),...
coronary artery bypass grafting or percutaneous transluminal coronary angioplasty, fatal stroke, or nonfatal stroke had occurred between baseline and follow-up through 2004. Fatal CHD was confirmed if there was fatal MI confirmed by hospital records or on autopsy with the next of kin’s permission. Nonfatal MI was confirmed according to the criteria of the World Health Organization by reviewing medical records for documentation of symptoms plus either typical electrocardiographic changes or elevated cardiac enzymes levels. The methods used to ascertain CVD events were described in detail elsewhere. Only CVD events that occurred after the onset of diabetes are included in the present report. In total, 1,395 white women with European ancestry were included. Among them, 449 CVD events occurred (including 347 CHD and 102 fatal and nonfatal stroke events); 946 women remained event free during follow-up.

Assessment of Covariates and Plasma Levels of Biochemical Markers

Blood samples were collected in 1989 and 1990 and were analyzed by previously reported methods. To minimize bias and interassay variation, study samples were selected from randomly ordered case-control pairs. The methods for measuring lipids and other biomarkers are described in detail elsewhere. The coefficients for individuals with complete data, assuming that the missing values for individuals with missing genotypes were not associated with disease status. In sensitivity analyses, results were similar when subjects with missing genotypes were excluded.

Statistical Analyses

Hardy-Weinberg equilibrium was assessed by a $\chi^2$ test. We used unconditional logistic regression to estimate odds ratios (ORs) for CVD and CHD risk, adjusting for age (in years), BMI, physical activity (<1.5, 1.5–5.9, 6.0–11.9, 12.0–20.9, or ≥21.0 metabolic equivalent hours per week), smoking status (never, past, or current [1–14.9, 15.0–24.9, or ≥25.0 pack-years]), alcohol intake (non-drinker or drinker [0.1–4.9, 5.0–9.9, 10.0–14.9, or ≥15.0 g/d]), duration of diabetes, menopausal status (pre or postmenopausal [never, past, or current hormone use]), and HbA1c levels (in quintiles). To normalize the distributions, plasma C-reactive protein, tumor necrosis factor receptor 2, E-selectin, adiponectin, intracellular cell adhesion molecule 1, and leptin were logarithmically transformed. Generalized linear models were used to compare geometric mean values of quantitative traits across quintiles of the GRS after adjustment for covariates. In addition, we used a restricted cubic spline regression model to examine the associations between GRS (as continuous variables) and risk of CVD among women with diabetes. A statistical package was used for the analyses (SAS, version 9.0 for UNIX; SAS Institute Inc, Cary, North Carolina). All probability values are 2 sided.

Results

Baseline Characteristics of Diabetic CVD Cases and Controls

Table 1 presents the baseline characteristics of persons who did and did not develop CVD during follow-up. Women who developed CVD were older, more likely to be postmenopausal, had higher HbA1c levels, and had diabetes for a longer duration than those who remained free of CVD. There were no significant differences in BMI, alcohol intake, physical activity, or smoking between those with CVD and those without CVD.

Obesity Gene Variants and CVD Risk

Of the nine SNPs examined, rs9939609 (FTO), rs7498685 (SH2B1), and rs10838738 (MTCH2) showed positive associations with BMI, whereas the associations between other SNPs (rs17700633 [MC4R], rs6548238 [TMEM18], rs11084753 [KCTD15], rs2815752 [NEGRI], and rs10938397 [GNPDA2]) and BMI were not statistically significant; however, the direction of effect of these SNPs on BMI was consistent with the published data. In sensitivity analysis, we obtained similar results when we used BMI in 1990 (data not shown). Obesity risk variants of rs9939609 (FTO), rs11084753 (KCTD15), rs10838738 (MTCH2), and rs10938397 (GNPDA2) showed nominally significant associations with increased CVD risk even after adjustment for BMI and other potential covariates. Similar results were observed for rs9939609 (FTO) and rs10938397 (GNPDA2) with CHD risk. (P < .05 for all; supplementary Table 1).

GRS and CVD Risk

The GRS was positively associated with baseline (in 1976) BMI ($P = .04$ for trend) but was not associated with other factors, including age, blood pressure, smoking status, alcohol consumption, and physical activity (data not shown). Women who developed CVD had a significantly higher GRS (mean, 6.40; SE, 0.10) than those who were free of CVD.
The positive association between the GRS and CVD risk was dose dependent \((P = 0.013\) for trend) after adjustment for age, BMI, smoking status, alcohol consumption, physical activity, postmenopausal status, hypertension, HbA1c level, and duration of diabetes. The OR (95% confidence interval [CI]) for CVD associated with each point scored, corresponding to one risk allele, was 1.08 (1.02–1.15) \((P = 0.003)\) (Table 2). Women in the highest GRS quartile \((GRS > 8.0)\) were at 53% greater risk of developing CVD \((OR, 1.53; 95\% CI, 1.06–2.22; \(P = 0.024\)) than those in the lowest GRS quartile \((GRS < 5.0)\) \((P = 0.003)\) (Table 2). In the sensitivity analysis, we removed the SNP that deviated from the Hardy-Weinberg equilibrium \((rs7498665)\) and obtained similar results. After adjustment for conventional risk factors, the OR (95% CI) for CVD associated with each point scored was 1.10 (95% CI, 1.04–1.18; \(P = 0.003)\). Results were similar when the outcome was restricted to CHD. In addition, we used a restricted cubic spline regression model to investigate the associations continuously. The regression splines demonstrated a linear relationship between GRS and the risk of CVD among women with diabetes (Figure).

### Associations of GRS With Plasma Biomarkers

To elucidate the potential mechanisms underlying the observed associations, we further examined the associations between the GRS and biochemical risk factors for CVD, including adipocytokines, lipids, and markers of inflammation and endothelial dysfunction (Table 3). In the single SNP association analysis, the SNPs rs9939609 in \(FTO\) and rs11084753 in \(KCTD15\) were significantly associated with adiponectin levels; SNP rs17700633 in \(MC4R\) was associated with high-density lipoprotein (HDL) levels after adjustment for other conventional risk factors. The GRS (in quartiles) was significantly associated with lower plasma adiponectin levels. Adjustment for age, smoking status, alcohol consumption, physical activity, postmenopausal status, HbA1c level, and duration of diabetes did not change results materially, nor did further adjustment for BMI. In addition, the GRS was significantly associated with lower HDL levels. Adjustment for covariates, except BMI, did not change the association; further adjustment for BMI attenuated the association. No significant associations were observed with other biomarkers. However, in the models further adjusting for adiponectin and HDL, the associations between GRS and CVD/CHD remained significant with modest attenuation (data not shown).

### Discussion

In this study, we examined the joint effects of previously reported obesity-predisposing loci derived from GWASs on the risk of CVD in diabetic women. Our data indicate that the accumulation of obesity risk alleles significantly increases CVD risk independently of BMI and other conventional CVD risk factors.

People with diabetes are more obese and have a 2- to 3-fold higher risk of CVD than the general population.\(^6\) Our data indicate that the obesity-predisposing genetic variants may increase CVD risk among people with diabetes. Because gene variants generally uncorrelated with environmental factors,
the observed associations are unlikely to be the result of confounding factors. The associations are independent of BMI, suggesting that the genetic effects may be largely mediated by the changes in fat accumulation that is not fully captured by BMI or that the gene variants act pleiotropically, influencing obesity and CVD risk through distinct pathways.

Because the individual genetic effects on CVD are moderate, we combined the variants by computing GRS, which congregates information from multiple genetic variants. We found that persons in the highest quartile of the GRS had a 53% greater risk of developing CVD than those in the lowest quartile of the GRS. We chose this approach because it accounts to some extent for an individual’s genetic background and provides a broader characterization of the individual’s risk profile. Although the GRS we computed did not explicitly account for the individual effect sizes for each SNP, previous studies that have compared weighted with unweighted GRSs reported similar effects for both models. This may be because the effects for each allele tend to be normally distributed in most populations studied to date; thus, alleles with larger effects are counterbalanced by those with smaller effects. When summing these effects, the weighted mean approximates that of the unweighted mean.

Obesity may influence CVD risk through systemic inflammation, insulin resistance, endothelial dysfunction, hypertension, and dyslipidemia. In the present study, the GRS was inversely associated with the plasma adiponectin levels. Adiponectin is secreted by adipose tissue, can improve insulin action and glucose and lipid metabolism, and has been related to lower CVD risk in epidemiology studies. The association between the GRS and adiponectin was not mediated by BMI in the present study, which is consistent with our observations for CVD risk. We also observed an inverse and significant association of GRS with HDL. High-density lipoprotein is an established protective factor for CVD, by preventing cholesterol accumulation in the artery wall and blocking inflammation, antioxi-

Figure. Odds ratio of cardiovascular disease among women with diabetes mellitus according to genetic risk score. Dashed lines are 95% confidence interval. Relative risks were estimated using unconditional logistic regression, controlled for age, body mass index, smoking status, alcohol consumption, physical activity, postmenopausal status, and duration of diabetes.
to be confounded by population stratification. The magnitude of the associations of the individual gene variants and CVD risk is generally modest. We acknowledged that our study may be underpowered to detect such modest effects, which may explain the lack of statistical association for some of these variants. In addition, our study includes only women and it remains to be determined whether the effects observed herein are similar in men with diabetes and in nonwhites.

In summary, we found that a GRS, composed of nine obesity-predisposing variants, is significantly associated with an increased risk of CVD in women with type 2 diabetes. The genetic effects are not fully mediated by the changes in biochemical risk factors, such as adiponectin and HDL level. Future studies are needed to investigate the potential mechanisms underlying the links among obesity genes, diabetes, and CVD risk.

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Disclosure

None.

References


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Supplemental Material
Supplementary table 1 Association between nine gene variants and the risks of CVD and CHD in diabetic women (continued)

<table>
<thead>
<tr>
<th>SNPs</th>
<th>Genotypes</th>
<th>CVD n (%)</th>
<th>CHD n (%)</th>
<th>Controls n (%)</th>
<th>CVD; OR (95% CI)</th>
<th>CHD; OR (95% CI)</th>
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<tbody>
<tr>
<td>rs9939609</td>
<td>TT</td>
<td>120(28.2)</td>
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<td>FTO</td>
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<td>219(51.5)</td>
<td>172(52.3)</td>
<td>426(47.3)</td>
<td>1.32(1.01-1.72)</td>
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<td>AA</td>
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<td>184(43.2)</td>
<td>150(45.1)</td>
<td>373(40.5)</td>
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<td>437(48.5)</td>
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<td>153(47.1)</td>
<td>378(41.9)</td>
<td>1.17(0.92-1.50)</td>
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<td></td>
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<td>TMEM18</td>
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<td>1.01(0.78-1.30)</td>
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<td>11(2.5)</td>
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<td>rs7498665</td>
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<td>SH2B1</td>
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<td>201(45.0)</td>
<td>156(45.2)</td>
<td>403(43.6)</td>
<td>1.01(0.79-1.30)</td>
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<td>180(19.5)</td>
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</tbody>
</table>
Supplementary table 1 Association between nine gene variants and the risks of CVD and CHD in diabetic women (continued)

<table>
<thead>
<tr>
<th>SNPs</th>
<th>Genotypes</th>
<th>CVD n (%)</th>
<th>CHD n (%)</th>
<th>Controls n (%)</th>
<th>CVD; OR (95% CI)</th>
<th>CHD; OR (95% CI)</th>
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<td>MTCH2</td>
<td>AG</td>
<td>205(46.3)</td>
<td>163(47.7)</td>
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<td>354(37.4)</td>
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<td>104(30.1)</td>
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<td>155(44.8)</td>
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<td>1.50(1.09-2.06)</td>
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</table>

Data are n (%). ORs were estimated adjusting for age, BMI, smoking, alcohol consumption, physical activity, postmenopausal status, HbA1c and duration of diabetes.