

SORCS1: A Novel Human Type 2 Diabetes Susceptibility Gene Suggested by the Mouse

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OBJECTIVE—A small number of susceptibility genes for human type 2 diabetes have been identified by candidate gene analysis or positional cloning. Genes found to influence diabetes or related traits in mice are likely to be susceptibility genes in humans. *SorCS1* is the gene identified as responsible for the mouse chromosome 19 *T2dm2* quantitative trait locus for fasting insulin levels, acting via impaired insulin secretion and increased islet disruption in obese females. Genes that impair compensatory insulin secretion in response to obesity-induced insulin resistance may be particularly relevant to human diabetes. Thus, we sought to determine whether variation in the human SORCS1 gene was associated with diabetes-related traits.

RESEARCH DESIGN AND METHODS—We assessed the contribution of variation in SORCS1 to human insulin-related traits in two distinct Mexican-American cohorts. One cohort (the Mexican-American Coronary Artery Disease [MACAD] cohort) consisted of nondiabetic individuals, allowing assessment of genetic association with subclinical intermediate insulin-related traits; the second cohort (the San Antonio Family Diabetes Study [SAFADS]) contained individuals with diabetes, allowing association analyses with overt disease.

RESULTS—We first found association of SORCS1 single nucleotide polymorphisms and haplotypes with fasting insulin levels and insulin secretion in the MACAD cohort. Similar to our results in the mice, the genetic association was strongest in overweight women. We then observed association with diabetes risk and age at diagnosis in women of the SAFADS cohort.

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CAD, coronary artery disease; LD, linkage disequilibrium; MACAD, Mexican-American Coronary Artery Disease; QTL, quantitative trait locus; SAFADS, San Antonio Family Diabetes Study; SNP, single nucleotide polymorphism.

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CONCLUSIONS—Identification of SORCS1 as a novel gene affecting insulin secretion and diabetes risk is likely to provide important insight into the biology of obesity-induced type 2 diabetes. *Diabetes* 56:1922–1929, 2007

Obesity and type 2 diabetes have reached epidemic proportions in Western societies. Numerous twin and familial aggregation studies have established an inherited basis for type 2 diabetes susceptibility (1). Variants in a small number of genes have been identified that contribute to diabetes risk. A number of these were examined based on their known function (the candidate gene approach). Examples of such candidate genes include peroxisome proliferator-activated receptor γ (*PPARG*) and the pancreatic β -cell inwardly rectifying potassium channel Kir6.2 (*KCNJ11*) (2–5). Another approach to diabetes gene discovery is the whole-genome approach, using linkage or association to interrogate the entire genome. A benefit of this approach is that it does not require knowledge of the underlying pathophysiology of the disease. This approach led to the discovery of calpain-10 (*CAPN10*) and *TCF7L2* as genes that contribute to diabetes risk (6,7). Each of the validated diabetes genes contributes modestly to overall risk of developing diabetes, consistent with the concept that diabetes is a complex genetic condition wherein multiple genes contribute moderately to overall risk (8).

A relatively unexploited additional approach to diabetes gene discovery is to translate findings from murine genetics to humans. Genes that contribute to diabetes risk in mice may also contribute to this disease in humans. Genetic heterogeneity underlying the phenotype, the segregation of other unknown modifier loci, and a highly variable environment makes the study of diabetes genetics difficult in humans. Genetic analysis in model organisms enables the study of genetically homogeneous strains in a controlled environment. Inbred mouse strains replicate much of the genetic diversity of the human population, and it is likely that variation at many of the same gene loci are responsible for diabetes susceptibility in both organisms (9). The genetic component of complex traits can then be dissected through the creation of congenic strains wherein a chromosomal segment containing a single modifier locus is fixed within a defined genetic background, increasing the sensitivity to detect relatively small independent effects of these loci (10).

We have focused on obesity-induced type 2 diabetes in the mouse as a model of human type 2 diabetes. Obesity leads to insulin resistance; however, most obese individu-

als do not develop diabetes. Diabetes is thought to develop in obese insulin-resistant individuals when the β -cells become unable to secrete adequate insulin to overcome the insulin resistance (11,12). Thus, we anticipate that many genes conferring susceptibility to diabetes will turn out to affect the ability of the pancreas to compensate for insulin resistance with an augmented insulin response.

In an F2 intercross between two strains differing in susceptibility to obesity-induced diabetes, we mapped *T2dm2*, a quantitative trait locus (QTL) on mouse chromosome 19 affecting fasting insulin levels (13). Using interval-specific congenic mouse strains, we recently identified *SorCS1* as the gene underlying *T2dm2* (14). Genetic variation in *SorCS1* had its most dramatic effect in obese female mice, where it led to decreased fasting insulin levels via reduced insulin secretion following a glucose challenge and pancreatic islet disruption; insulin sensitivity was not affected.

The region of mouse chromosome 19 harboring the *SorCS1* gene is syntenic with human chromosome 10q. We previously reported linkage between type 2 diabetes and a region on chromosome 10q (15). Linkage and association of type 2 diabetes and diabetes-related traits to this region were subsequently reported in a number of studies (16–28). Indeed, our prior genome-wide scan in Mexican Americans detected a susceptibility locus for type 2 diabetes and age of diabetes onset to a broad region on chromosome 10q, which includes the human *SORCS1* gene (15). In the current study, we examined *SORCS1* as a candidate gene for human type 2 diabetes. We utilized two Mexican-American family cohorts: one free of diabetes but extensively phenotyped for insulin-related traits and a second cohort with numerous diabetic individuals. This allowed us to assess whether *SORCS1* was associated with not only fasting insulin (the trait used to identify this gene in mice), but also with insulin sensitivity or insulin secretion, two processes that contribute to plasma insulin levels and, when deranged, lead to diabetes. Thus, this study had the advantage of the ability to assess association with intermediate traits as well as with risk and age of onset of overt disease.

Similar to our findings in mice, *SORCS1* variants were associated with fasting insulin and insulin secretion in humans. *SORCS1* variants were also associated with diabetes risk and age of onset of diabetes. This is the first genetic association study that translated a diabetes susceptibility gene identified in mice to humans. This study identifies a novel potential gene for human diabetes and also suggests that the underlying mechanism is altered insulin secretion into the bloodstream, highlighting the importance of factors affecting β -cell compensatory responses to the development of type 2 diabetes.

RESEARCH DESIGN AND METHODS

Associations with fasting insulin, insulin resistance, and insulin secretion were assessed in participants in the Los Angeles Mexican-American Coronary Artery Disease (MACAD) Study, a study of coronary artery disease (CAD) and insulin resistance in Mexican Americans (29,30). In the present report, 102 two-generation Mexican-American families were included. In total, 548 subjects (adult offspring of probands with CAD and the spouses of those offspring) were extensively phenotyped, and 808 subjects (adult offspring along with their spouses and parents) were genotyped. The phenotyped offspring were healthy and free of clinically overt diabetes and CAD; thus, the observed associations represent preclinical manifestations that may predispose to the onset of diabetes.

The hyperinsulinemic-euglycemic clamp (31) was performed as previously described with an insulin infusion rate of 60 mU/m² per min (29). The glucose

infusion rate (M , given in mg · kg⁻¹ · min⁻¹) over the last 30 min of steady-state insulin and glucose concentrations reflects glucose uptake by all tissues of the body (primarily insulin-mediated glucose uptake in muscle) and is therefore a direct physiologic measurement of tissue insulin sensitivity (31).

The oral glucose tolerance test consisted of baseline glucose and insulin measurements, followed by administration of 75 g oral glucose with blood draws at 30, 60, 90, 120, and 180 min. Insulin secretion indexes were obtained from glucose and insulin measurements at the oral glucose tolerance test 30-min time point to estimate early insulin secretion (30). We used the insulin-to-glucose ratio (IGR30, $\mu\text{IU} \cdot \text{dl}^{-1} \cdot \text{mg}^{-1} \cdot \text{ml}^{-1}$), the 30-min insulin value divided by the 30-min glucose value. Such indexes have correlations of 0.31–0.51 with acute insulin secretion directly measured by frequently sampled intravenous glucose tolerance testing (32).

Associations with diabetes risk were assessed in subjects from the San Antonio Family Diabetes Study (SAFADS) (15). The SAFADS cohort comprises 574 individuals from 32 extended Mexican-American families, ascertained from a proband with type 2 diabetes. The prevalence of diabetes in SAFADS is 20.6% (19.6% in female and 22.1% in male subjects) with a mean age of onset of 48.2 years (48.7 years in female and 47.7 years in male subjects). Female subjects comprise 58.9% of the cohort, and their average BMI was 30.6 ± 0.39 kg/m². These families ranged in size from 2 to 53 members, with a median size of 16 individuals per family. Diabetes was defined as a fasting plasma glucose level ≥7.0 mmol/l (126 mg/dl) or a self-report of physician-diagnosed diabetes under treatment with either oral antidiabetes agents or insulin.

The institutional review boards of the University of California Los Angeles, Cedars-Sinai Medical Center, and the University of Texas Health Science Center at San Antonio approved all procedures, and all subjects gave informed consent.

In both studies, peripheral blood lymphocytes were transformed and immortalized for all participants to establish a perpetual source of genomic DNA.

Genotyping. In the initial screen, seven single nucleotide polymorphisms (SNPs) within *SORCS1* were selected using information from the HapMap Project (33), such that each SNP would 1) have a high frequency of the minor allele in the Caucasian population, 2) represent one region of high linkage disequilibrium (LD) (haplotype block) as measured by D' (<http://www.hapmap.org>), and thus 3) contribute to testing the hypothesis that the human genomic region syntenic to the *T2dm2* linkage peak was associated with fasting insulin (Table 1 and Fig. 1). Three SNPs flanking this region (two in the neighboring *SORCS3* gene and one ~330 kb telomeric to *SORCS1*) were also genotyped in the initial screen (Table 1).

Next, 13 additional SNPs were selected to refine the haplotype structure of the syntenic region (Table 1). Genotyping was performed using TaqMan MGB technology as previously described (29,34). PCR primers and TaqMan MGB probes for the SNPs genotyped are listed in online appendix Tables 1 and 2 (available at <http://dx.doi.org/10.2337/db06-1677>).

Statistical analysis. Methods of analyses in the two human cohorts were selected based on the characteristics of the different populations. The MACAD cohort is composed of small families and marrying-in spouses; therefore, the generalized estimating equation (35) approach was selected to utilize data from all phenotyped subjects. We evaluated association using this robust variance estimation approach to test hypothesized associations between phenotypes and genotypes while accounting for familial correlations present in the data. The PROC GENMOD procedure in SAS (version 9.0; SAS Institute, Cary, NC) was used for the association analysis in which a sandwich estimator was used, assuming exchangeable correlation. Family was taken as the cluster factor, i.e., members from the same family were assumed to be correlated. Both fasting insulin and IGR30 were log transformed to better approximate conditional normality and homogeneity of variance. A dominant genetic model was assumed in all the association analyses. Analyses were conducted using age and sex as covariates, unless otherwise specified. To confirm the associations detected with the original set of 10 screening SNPs (7 in *SORCS1* and 3 flanking), the entire sample was permuted by shuffling the fasting insulin phenotype between individuals, and subsequent association analyses were computed 1,000 times to obtain the distribution of the test statistic under the null hypothesis of no association. The empirical P values were obtained as the proportion of the 1,000 replicates that had P values less than or equal to the nominal one that was obtained from the actual (unshuffled) data. The resulting P values were essentially unchanged. As subsequent analyses were confirmatory of the hypotheses generated from these original SNPs, further permutation testing was not performed.

Haploview 3 was used to determine haplotype frequencies and to delineate haplotype blocks (36). Haploview constructs haplotypes by using an accelerated expectation-maximization algorithm. Haploview was used to calculate LD (the D' statistic) between each pairwise combination of all 20 SNPs used in haplotype block determination. To determine haplotype blocks, Haploview

TABLE 1
Association of individual SNPs with fasting insulin in MACAD

SNP position	Location in SORCS1	Major/minor allele	Minor allele frequency	Fasting insulin (pmol/l)			
				1/1	1/2 and 2/2	P	
SORCS3							
*rs813756	106,761,882	~1.56 Mb cen	T/G	30.2	109.92 ± 3.80 (265)	112.07 ± 3.66 (282)	0.39
*rs1670008	106,792,219	~1.53 Mb cen	G/C	31.1	109.85 ± 3.80 (262)	112.15 ± 2.80 (285)	0.31
SORCS1							
Haplotype block 1							
rs2788677	108,458,473	Intron 7	T/C	46.0	108.27 ± 5.45 (159)	114.44 ± 3.30 (386)	0.14
rs10748924	108,477,170	Intron 6	C/T	35.5	110.50 ± 4.45 (225)	114.08 ± 3.66 (319)	0.33
Haplotype block 2							
rs7067660	108,492,751	Intron 5	T/C	7.1	113.94 ± 3.09 (469)	103.97 ± 7.10 (76)	0.22
*rs10736189	108,502,070	Intron 5	C/G	41.9	104.47 ± 4.38 (180)	114.73 ± 1.22 (352)	0.032
rs10509818	108,512,646	Intron 4	G/A	24.4	107.19 ± 3.59 (316)	120.18 ± 4.45 (229)	0.0056
*rs2249022	108,532,319	Intron 3	A/G	40.4	103.32 ± 4.09 (193)	115.30 ± 3.52 (341)	0.017
rs821994	108,547,162	Intron 3	C/T	37.4	104.61 ± 3.95 (211)	117.53 ± 3.87 (334)	0.041
Haplotype block 3							
rs822000	108,550,027	Intron 3	A/G	41.6	101.45 ± 3.87 (192)	118.75 ± 3.80 (353)	0.0038
*rs2243454	108,567,901	Intron 3	C/T	47.5	116.45 ± 5.60 (150)	108.99 ± 3.01 (387)	0.49
rs1538417	108,573,589	Intron 3	G/A	21.3	108.56 ± 3.37 (329)	118.67 ± 4.88 (213)	0.050
*rs4390282	108,579,740	Intron 2	T/G	30.1	105.40 ± 3.52 (251)	116.16 ± 4.02 (285)	0.032
*rs1537919	108,681,751	Intron 2	G/A	34.0	112.58 ± 4.45 (232)	109.99 ± 3.37 (300)	0.59
Haplotype block 4							
rs10748932	108,805,465	Intron 1	A/G	9.5	114.08 ± 3.16 (447)	105.33 ± 6.24 (98)	0.68
rs11193188	108,805,896	Intron 1	G/A	17.5	113.94 ± 3.529 (374)	109.20 ± 4.52 (170)	0.65
*rs11193190	108,808,277	Intron 1	T/C	39.9	106.69 ± 3.66 (223)	114.87 ± 3.80 (310)	0.43
rs7086426	108,837,197	Intron 1	G/A	20.2	112.15 ± 3.59 (346)	113.37 ± 4.66 (199)	0.87
Haplotype block 5							
rs607437	108,870,445	Intron 1	T/C	40.7	116.52 ± 5.09 (178)	110.78 ± 3.44 (463)	0.51
rs685316	108,883,709	Intron 1	G/A	34.3	117.74 ± 4.88 (226)	108.99 ± 3.37 (317)	0.36
*rs7897974	108,886,554	Intron 1	G/A	25.5	108.13 ± 3.23 (289)	114.73 ± 4.52 (243)	0.18
rs1251753	108,888,293	Intron 1	A/G	47.6	116.16 ± 5.96 (146)	111.50 ± 3.16 (396)	0.43
Flanking SNP							
*rs1530248	109,250,514	~330 kb tel	T/C	44.7	112.86 ± 4.81 (174)	110.28 ± 3.23 (364)	0.88

Data are means ± SE (n). P values were obtained from generalized estimating equation models. 1/1 are homozygotes for the major allele at each SNP, 1/2 are heterozygotes, and 2/2 are homozygotes of the rare allele. SNP locations are the basepair positions in the University of California Santa Cruz Genome Browser hg17 assembly, which is based on the National Center for Biotechnology Information build 35. Significant associations are indicated in bold. *The 10 SNPs used in the initial screen. cen, centromeric to SORCS1; tel, telomeric to SORCS1.

searches for regions of strong LD ($D' > 0.8$) running from one marker to another, wherein the first and last markers in a block are in strong LD with all intermediate markers (solid spine of LD algorithm). Based on the pedigree structures and genotype data of all individuals in each pedigree, haplotypes were constructed as the most likely set (determined by the maximum likelihood method) of fully determined parental haplotypes of the marker loci for each individual in the pedigree, using the simulated annealing algorithm implemented in the program Simwalk2 (37).

To assess the association between the SNPs and the traits diabetes and age of diabetes onset in the SAFADS cohort, which is composed of large pedigrees, a measured genotype approach was used in the framework of variance component-based pedigree analysis, as implemented in SOLAR (38). Random effects accounting for residual polygenic effects were included in the model to account for the relatedness among family members. The model was adjusted for the effects of age, age², and BMI. The minor allele counts at the SNP (0, or 1, or 2) were included as a covariate in the analysis. The likelihood of the model where the effect of this covariate was estimated by likelihood maximization was compared with the likelihood of the null model where the effect of the genotypes was fixed at zero. Two times the difference between the natural logarithm of the likelihoods of the two models is distributed asymptotically as a χ^2 statistic with 1 d.f. Since these findings represent confirmation of a prior hypothesis, they were not adjusted for multiple comparisons.

RESULTS

Subjects in the MACAD Study were selected to exclude those with diabetes; however, based on their ethnic background, these individuals bear an enrichment of alleles

predisposing to type 2 diabetes and have a high frequency of physiologic abnormalities predisposing to type 2 diabetes. This cohort has been extensively phenotyped for both insulin resistance and insulin secretion, two factors contributing to plasma insulin levels. Thus, this is an ideal population to examine the effects of genetic variation in SORCS1 on these intermediate phenotypes that are deranged in type 2 diabetes.

SORCS1 is a very large gene, encompassing >500 kb of human chromosome 10. Our studies in the mice localized the causative variant(s) to a region of 242 kb spanning part of the promoter and first exons of the gene (14). To first test the hypothesis that variation in the human SORCS1 gene contributes to fasting insulin levels in the MACAD population, we chose to focus on this same region of the gene. We performed an initial screen with seven SNPs within the region syntenic to the maximal extent of the linkage peak underlying *T2dm2* in the mouse (14) and three flanking SNPs. We analyzed these SNPs for association with fasting insulin (the trait used to localize the gene in mice) in the MACAD cohort. This screen identified three SNPs within SORCS1 that showed evidence of association with fasting insulin (rs10736189, rs2249022, and rs4390282) (Table 1). To confirm these findings, we used a permutation test to derive empirical P values for the

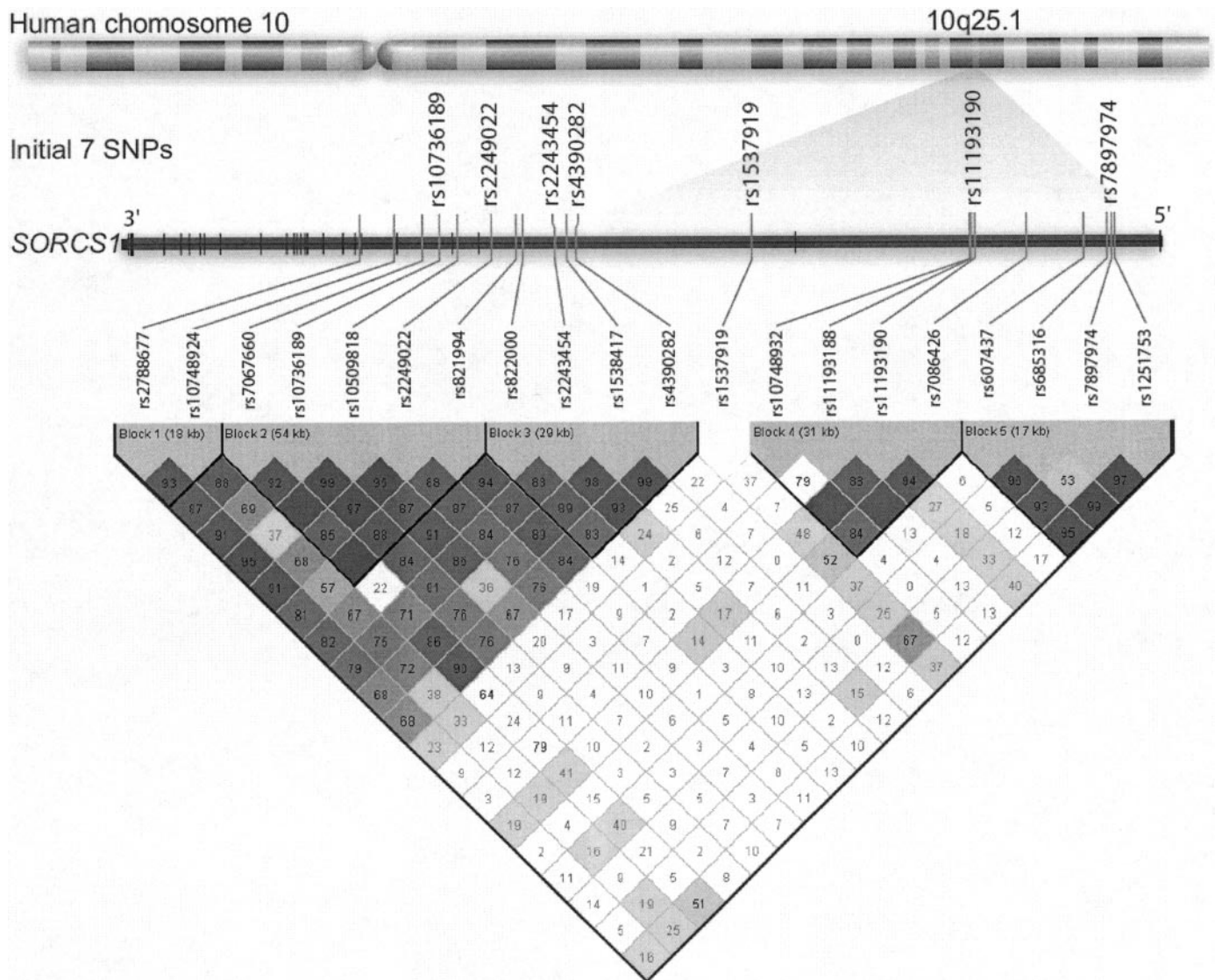


FIG. 1. Location of SORCS1 SNPs and haplotype structure in the MACAD cohort. The upper portion of the figure shows the human SORCS1 gene structure (exons = black lines) on the minus strand of human chromosome 10 and the corresponding location of the SNPs studied. We performed an initial screen of the region syntenic to the *T2dm2* QTL in the MACAD population using seven SNPs representing each major haplotype block. This identified three SNPs (rs10736189, rs2249022, and rs11193190) that were associated with fasting insulin levels. We subsequently screened an additional 13 SNPs in this region to refine the haplotype structure within this population. The LD plot at the bottom of the figure displays D' values (%) for each pair of SNPs in the box at the intersection of the diagonals from each SNP. Dark solid blocks indicate $D' = 100\%$ for the corresponding pair of variants. Five haplotype blocks were identified.

associations of each of the SNPs with fasting insulin. These P values were essentially unchanged from those obtained by our association tests. None of the three flanking SNPs showed evidence of association with fasting insulin (Table 1), suggesting that the causal variation lies within SORCS1 and is not due to long-range LD to neighboring genes.

To confirm the hypothesis that variation in SORCS1 is associated with fasting insulin, we then selected and genotyped 13 additional SNPs in this region to determine the haplotype structure within this population. We identified five haplotype blocks in the MACAD cohort (Fig. 1). Only SNPs located in blocks 2 and 3 displayed association with fasting insulin (Table 1). Thus, we analyzed haplotypes from only these two blocks for association with fasting insulin and other insulin-related traits. Fasting insulin can be a reflection of insulin sensitivity or insulin secretion. To examine the mechanism behind the alter-

ations in fasting insulin of the associated block 2 and 3 SNPs, we also tested the haplotypes for association with indexes of insulin secretion (IGR30) and insulin sensitivity (M value). Three haplotypes within blocks 2 and 3 were significantly associated with fasting insulin levels or IGR30 (Table 2). For each associated haplotype, the direction of effect was consistent between fasting insulin and IGR30. No haplotypes were associated with the M value, suggesting that, as observed in the mice, variation in SORCS1 affects fasting insulin primarily by influencing insulin secretion.

Because the effects of *T2dm2* were most pronounced in obese female mice (14), we stratified the cohort by weight and sex and then examined the associations of fasting insulin and IGR30 with the three associated haplotypes in blocks 2 and 3. Despite reduced sample size, the effects of haplotype 1-2-2-2 within block 2 were still observed in overweight females (Table 2), whereas no significant as-

TABLE 2
Association of haplotypes in SORCS1 with fasting insulin and insulin secretion in MACAD

Block	Associated haplotype	Haplotype frequency (%)	Fasting insulin (pmol/l)			IGR30		
			Noncarrier	Carrier	P	Noncarrier	Carrier	P
Whole cohort								
Block 2	1-2-2-2	21.0	106.19 ± 3.52 (290)	121.90 ± 5.24 (163)	0.0032	0.66 ± 0.03 (282)	0.71 ± 0.04 (159)	0.20
Block 2	1-2-1-2-1	3.5	111.64 ± 3.01 (421)	114.80 ± 13.78 (32)	0.86	0.67 ± 0.02 (411)	0.86 ± 0.09 (30)	0.049
Block 3	2-1-2-2	17.7	106.05 ± 3.09 (327)	120.11 ± 5.38 (156)	0.010	0.66 ± 0.02 (316)	0.71 ± 0.04 (152)	0.086
Female (BMI ≥25 kg/m ²)								
Block 2	1-2-2-2-2	21.9	115.88 ± 5.67 (124)	134.24 ± 7.32 (75)	0.0098	0.73 ± 0.04 (121)	0.83 ± 0.06 (74)	0.085
Block 2	1-2-1-2-1	2.3	120.90 ± 4.52 (190)	162.66 ± 30.71 (9)	0.12	0.75 ± 0.03 (188)	1.13 ± 0.15 (7)	0.059
Block 3	2-1-2-2	17.9	116.74 ± 4.66 (147)	132.74 ± 8.32 (68)	0.11	0.72 ± 0.04 (143)	0.80 ± 0.06 (68)	0.30

Data are means ± SE (n). SNPs contained within these blocks are shown in Fig. 1. Allele 1 represents the common allele; allele 2 is the rare allele. IGR30 is given in $\mu\text{IU} \cdot \text{dl} \cdot \text{mg}^{-1} \cdot \text{ml}^{-1}$.

sociations of these haplotypes with insulin were observed in either males or lean individuals (data not shown).

The MACAD Study design focused on intermediate insulin-related traits and thus specifically excluded individuals with type 2 diabetes. To ask whether variation in the SORCS1 haplotype blocks are also related to diabetes susceptibility, we then genotyped SNPs from block 2 in the SAFADS cohort, which includes individuals with type 2 diabetes. This block was chosen because it contained the haplotype associated with fasting insulin and IGR30 in overweight women.

We detected associations with diabetes risk and age at diagnosis for most block 2 SNPs in this cohort (Table 3) as well as haplotypes based on these SNPs (Table 4). Consistent with our findings in mice and in the MACAD cohort, these associations were only observed in females. Within block 2, haplotype 1-2-2-2-2 (comprised of SNPs rs7067660, rs10736189, rs10509818, rs2249022, and rs821994) was associated with decreased diabetes risk and a later age of diabetes diagnosis in SAFADS. This same haplotype was associated with increased fasting insulin and IGR30 in MACAD. These data suggest that SORCS1 variation present on this haplotype acts to increase insulin secretion and plasma insulin levels, resulting in protection from developing type 2 diabetes and a later onset of type 2 diabetes in those who do develop it. While some but not all of the five SNPs in block 2 displayed significant associations (Tables 1 and 3), the mean trait levels for each of the five SNP alleles that define haplotype 1-2-2-2-2 were in the same direction as the haplotype itself.

DISCUSSION

We previously positionally cloned *SorCS1* as the gene underlying a QTL affecting plasma insulin levels in obese

mice (14). The primary mechanism for this reduction was reduced secretion of insulin into the bloodstream, accompanying an increased disruption of islet morphology. These studies localized the causative variant to the first few exons of *SorCS1*. Here, we have sought to address the question of whether genetic variation within the same region of human SORCS1 is associated with plasma insulin levels and insulin secretion and to determine whether these variants are associated with altered risk of developing type 2 diabetes.

We have detected associations of SNPs within two haplotype blocks with diabetes or diabetes-related traits (fasting insulin and insulin secretion) in two different Mexican-American populations. These SNPs are distinct from those in mice yet were associated with similar phenotypes as those observed in mice (14), suggesting that the effect of SORCS1 on insulin secretion is directly relevant to the development of type 2 diabetes in humans. The risk ratios for type 2 diabetes observed for these SORCS1 variants are similar to those reported for SNPs in other type 2 diabetes susceptibility genes (8,39).

A strength of our study was the use of two distinct but complementary Mexican-American cohorts: one that specifically excluded subjects with type 2 diabetes but underwent detailed phenotyping of intermediate traits for diabetes (MACAD) and another containing diabetic subjects (SAFADS), enabling us to test for association with diabetes and age of diabetes onset. Since diabetic subjects may have either higher or lower fasting insulin levels, depending on the stage of their disease and medication history, assessment of relationships with fasting insulin must be confined to nondiabetic subjects. Thus, we did not test for association of SORCS1 variants with fasting insulin in SAFADS. That the effect is sex specific imposes a

TABLE 3
Association of block 2 SNPs with diabetes risk in SAFADS female subjects

SNP	Major/minor allele	Minor allele frequency (%)	Relative risk for diabetes		Age at diagnosis	
			2/2*	P	Effect on age at diagnosis	P
rs7067660	T/C	5.2	1.09	0.55	Decrease	0.43
rs10736189	C/G	40.7	0.86	0.041	Increase	0.17
rs10509818	G/A	23.7	0.80	0.0018	Increase	0.0055
rs2249022	A/G	42.3	0.86	0.040	Increase	0.16

*Relative risk of 2/2 compared with 1/1. Allele 1 represents the common allele; allele 2 is the rare allele. Associations were observed in female subjects only and have been adjusted for BMI.

TABLE 4
Association of block 2 haplotypes in SORCS1 with diabetes risk in SAFADS female subjects

Block	Associated haplotype*	Minor allele frequency (%)	Relative risk for diabetes		Age at diagnosis	
			Risk†	P	Effect on age at diagnosis	P
Block 2	1-1-1-1-1	51.7	1.08	0.067	NA	0.23
Block 2	1-2-2-2-2	22.3	0.78	0.0020	Increased	0.010

*Allele at fifth position inferred from MACAD haplotypes. †Relative risk of bearing 2 copies of haplotype vs. no copies of haplotype. Allele 1 represents the common allele; allele 2 is the rare allele. Associations were observed in female subjects only and have been adjusted for BMI.

further reduction in the number of subjects available for analysis; hence, we lacked power to assess the relationship between SORCS1 variants and fasting insulin in the SAFADS cohort.

The human SORCS1 gene is located on chromosome 10q25. In the SAFADS cohort, we had previously observed linkage in this population between type 2 diabetes and age of type 2 diabetes onset with a broad region of human chromosome 10 encompassing SORCS1 (15). Other rodent (9) and human (16–28) linkages to type 2 diabetes-related traits have been mapped to regions containing SORCS1, raising the possibility that this gene may have broad relevance to the development of type 2 diabetes.

Recently, variants within *TCF7L2* have been identified as predictors of type 2 diabetes (7). This gene resides 5.8 Mb telomeric to SORCS1. Thus, it was possible that the effects of SORCS1 variants on insulin levels and diabetes could have been a consequence of variants of the *TCF7L2* gene. We tested this possibility by determining whether known SNPs within the two genes are in LD; they were not (data not shown). In addition, results from the SNPs flanking SORCS1 suggest that our results are due solely to variation within the SORCS1 gene itself.

SORCS1 belongs to a family of five proteins (sortilin, SORLA [LR11], and SORCS-1, -2, and -3) that are composed of a vacuolar protein sorting-10 domain, a single transmembrane domain and a short cytoplasmic tail (40). A leucine rich-repeat domain between the vacuolar protein sorting-10 and transmembrane segments characterizes the SORCS family. SORCS1 is most highly expressed in the brain, heart, kidney, pancreatic islets, and β -cell lines (14,41). Our congenic mice have reduced insulin levels due to impaired insulin secretion in vivo, but not in isolated islets in vitro, suggesting impaired delivery of insulin to the bloodstream. SORCS1 is known to bind platelet-derived growth factor-BB (42), which plays a role in blood vessel development. Our working hypothesis is that SORCS1 plays a role in the maintaining or expanding islet vasculature during islet growth and compensation for insulin resistance. Impaired vascular structure may directly affect insulin secretion to the blood and may also lead to disrupted islet architecture in areas of insufficient vascular flow (43,44).

The insulin deficiency and the pancreatic islet phenotypes associated with *T2dm2* were only evident in obese mice, not in their lean littermates, suggesting the mouse *SorCS1* variants are insufficient to affect islet morphology in the absence of a challenge to increase β -cell mass and/or function (14). Also, we only observed the genotype effect on plasma insulin levels in female mice. Thus, it is striking that in humans we also observed a genotype effect primarily in overweight female subjects. This may be coincidental or may be reflective of a sexual dimorphism

resulting from a complex interaction among SORCS1 genotype, other genes, and/or the hormonal milieu present in women but not men. Future studies will be required to resolve this issue. It is possible that other variants of SORCS1 may influence islet biology in a wider array of individuals.

Block 2 haplotype 1-2-2-2-2 was associated with increased fasting insulin and IGR30 in MACAD and with decreased diabetes risk in SAFADS; no association with insulin sensitivity was observed. This may be interpreted as inherited preservation in insulin secretion leading to protection against developing diabetes for individuals bearing this particular haplotype. Until further genotyping and/or sequencing is conducted, we cannot determine which SNP(s) on this haplotype are responsible for the observed associations. As this study represents our initial exploration of this very large (>500 kb) gene, we cannot rule out the possibility that other regions of the gene will also harbor functional variants or that additional SNPs showing associations (either protective or predisposing to diabetes) will be detected within this region. Any such additional associations will become evident as more complete genotyping and analysis are carried out. The goal of this study is to present the successful translation of a murine diabetes gene to humans, to stimulate further investigation of SORCS1 in other human cohorts.

Using a mouse model that mimics common obesity-associated diabetes, we identified *SorCS1* as the gene underlying the *T2dm2* locus (14). This locus affects in vivo insulin secretion, an important process in compensating for the insulin resistance of obesity. Factors affecting the ability of the pancreatic islets to maintain sufficient compensation for insulin resistance are likely important determinants of diabetes risk. Here, we demonstrate that distinct allelic variants of the SORCS1 gene are associated with alterations in fasting insulin levels, insulin secretion (intermediate traits), and type 2 diabetes risk (overt disease) in Mexican Americans. These findings represent the first example of variants in a gene identified through positional cloning in a model organism affecting type 2 diabetes in humans. Combined, these data strongly suggest that alterations in SORCS1 directly affect these phenotypes and provide validation that SORCS1 is a novel type 2 diabetes susceptibility gene.

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