Association of \textit{SSTR2} Polymorphisms and Glucose Homeostasis Phenotypes
The Insulin Resistance Atherosclerosis Family Study

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OBJECTIVE—This study evaluated the influence of somatostatin receptor type 2 (\textit{SSTR2}) polymorphisms on measures of glucose homeostasis in the Insulin Resistance Atherosclerosis Family Study (IRASFS). \textit{SSTR2} is a G-protein–coupled receptor that, in response to somatostatin, mediates inhibition of insulin, glucagon, and growth hormone release and thus may affect glucose homeostasis.

RESEARCH DESIGN AND METHODS—Ten single nucleotide polymorphisms (SNPs) spanning the gene were chosen using a SNP density selection algorithm and genotyped on 1,425 Hispanic-American individuals from 90 families in the IRASFS. These families comprised two samples (set 1 and set 2), which were analyzed individually and as a combined set. Single SNP tests of association were performed for four glucose homeostasis measures—insulin sensitivity (SI), acute insulin response (AIR), disposition index (DI), and fasting blood glucose (FBG)—using generalized estimating equations.

RESULTS—The \textit{SSTR2} locus was encompassed by a single linkage disequilibrium (LD) block (D' = 0.91–1.00; \(r^2 = 0.09–0.97\)) that contained four of the ten SNPs evaluated. Within the \textit{SSTR2}-containing LD block, evidence of association was observed in each of the two sets and in a combined analysis with decreased SI (\(P_{\text{homozygous}} = 0.16; P_{\text{meta-analysis}} = 0.0024–0.0030\)), decreased DI (\(P_{\text{homozygous}} = 0.35 to -5.16; P_{\text{meta-analysis}} = 0.0075–0.027\)), and increased FBG (\(P_{\text{homozygous}} = 2.30; r_{\text{meta-analysis}} = 0.045\)). SNPs outside the \textit{SSTR2}-containing LD block were not associated with measures of glucose homeostasis.

CONCLUSIONS—We observed evidence for association of \textit{SSTR2} polymorphisms with measures of glucose homeostasis. Thus, variants in \textit{SSTR2} may influence pathways of SI to modulate glucose homeostasis. \textit{Diabetes} \textbf{58}:1457–1462, 2009

\textbf{S}omatostatin (SRIF) is a hormone found throughout the body that inhibits the action or expression of several hormones including insulin, glucagon, and growth hormone (1). SRIF is an important regulator of the endocrine system, exerting its effects through interactions with pituitary growth hormone, thyroid-stimulating hormone, and several hormones of the gastrointestinal tract. Five G-protein–coupled receptors (SRIF receptor type [\textit{SSTR}] 1–5) that bind SRIF exist, each exerting differential effects based on the receptor bound and localization. \textit{SSTR2} is found primarily in the pancreas, pituitary, and stomach (2). Several studies have evaluated the effects of SRIF on the respective receptors. Rat studies have found that \textit{SSTR2} ligands mediate inhibition of glucagon release, whereas \textit{SSTR5} mediates inhibition of insulin release (3). In addition, a study of pancreatic islets from \textit{SSTR2} knockout mice supported the finding in rats, concluding that SRIF inhibition of glucagon release is primarily regulated through interaction with \textit{SSTR2}, whereas insulin secretion is primarily regulated by \textit{SSTR5} (4). Human studies of the pancreas revealed slightly different results, suggesting that SRIF can inhibit insulin release through the \textit{SSTR2} receptor (5). In contrast, Cheng et al. (6) observed that \textit{SSTR2} mediates the SRIF-induced increase in insulin secretion, although the increase was detected only in the presence of arginine vasopressin. Based on these studies, it is apparent that whereas \textit{SSTR2} does appear to mediate the regulation of insulin and glucagon, the exact mechanism remains obscure.

To date, no studies have assessed the effects of \textit{SSTR2} polymorphisms on glucose homeostasis traits in population-based studies. The Insulin Resistance Atherosclerosis Family Study (IRASFS) is a multicenter study investigating the genetic contributions to glucose homeostasis and adiposity and includes a population suitable for studying such effects. Previous studies in the IRASFS of \textit{SSTR2} as a positional candidate gene for alterations in the amount of visceral adiposity and includes a population suitable for studying such effects. Previous studies in the IRASFS of \textit{SSTR2} as a positional candidate gene for alterations in the amount of visceral adiposity.
the goal of defining the biological pathways through which this receptor exerts its effects.

**RESEARCH DESIGN AND METHODS**

The IRASFS was designed to investigate the genetic determinants of insulin resistance and adiposity that are important risk factors in the development of type 2 diabetes and atherosclerosis. Using well-developed technical methods to accurately assess quantitative measures of glucose homeostasis and adiposity, the IRASFS examines the familial aggregation and genetic contributions for those phenotypes. Hispanic Americans from the IRASFS were recruited from two centers: San Antonio, Texas, and San Luis Valley, Colorado. The study design, recruitment, and phenotyping have previously been described in detail (9). Briefly, 90 multigenerational Hispanic-American families (1,425 individuals) were recruited in two phases denoted set 1 (45 families: 33 from San Antonio, Texas, and 12 from San Luis Valley, Colorado; 827 individuals) and set 2 (45 families: 27 from San Antonio, Texas, and 18 families: 33 from San Antonio, Texas, and 12 from San Luis Valley, Colorado; 592 individuals). Recruitment was independent of type 2 diabetes or cardiovascular disease status, although 14.2% of individuals were diagnosed with type 2 diabetes. Table 1 summarizes the study design, recruitment, and phenotyping data.

Measures of glucose homeostasis were assessed by frequently sampled intravenous glucose tolerance tests (10) with MINMOD analyses (11). $S_r$ was calculated using MINMOD software. AIR was calculated as the mean insulin increment in plasma insulin concentration above basal in the first 8 min after glucose administration. DI was calculated as DI = $S_r$ × AIR. FBG values were obtained using standard methods. Individuals with type 2 diabetes were excluded from analysis.

Single nucleotide polymorphisms (SNPs) were selected using the SNPbrowser 3.0 (Applied Biosystems) SNP density selection algorithm. The genomic interval assessed included the SSTR2 gene ±15 kb, which extended coverage to two adjacent linkage disequilibrium (LD) blocks, as assessed by evaluation of the HapMap CEU population. Density was selected at 2 kb with a minor allele frequency (MAF) >5%. Supplementary Table A1, available in an online appendix at http://diabetes.diabetesjournals.org/cgi/content/full/db08-0189/DC1, contains the characteristics of SNPs selected for genotyping. Primers for PCR amplification and extension were designed using SpectroDESIGNER software, and genotyping was performed on the Sequenom MassARRAY system (12).

SNPs were examined for Mendelian inconsistencies in their genotypes using PedCheck (13). Inconsistent genotypes (n = 43) were converted to missing (0.002% error rate). Maximum likelihood estimates of allele frequencies were computed using the largest set of unrelated individuals and genotypes tested for departures from Hardy-Weinberg equilibrium proportions. To validate SNP coverage, the aggressive tagging option of Tagger (14) implemented in the program Haploviev (15) was used to assess coverage in the HapMap CEU population.

**TABLE 1**

Demographic summary of IRASFS Hispanic-American participants

<table>
<thead>
<tr>
<th></th>
<th>Set 1</th>
<th>Set 2</th>
<th>Combined sets 1 and 2</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>42.2 ± 14.5 (40.9)</td>
<td>43.5 ± 14.7 (41.6)</td>
<td>42.8 ± 14.6 (41.3)</td>
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<tr>
<td>Female sex (%)</td>
<td>57</td>
<td>60</td>
<td>58</td>
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<tr>
<td>Glucose homeostasis</td>
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<tr>
<td>$S_r$ (×10^{-m} min^{-1}/[pmol/l])</td>
<td>2.0 ± 2.0 (1.5)</td>
<td>2.1 ± 1.8 (1.7)</td>
<td>2.0 ± 1.9 (1.5)</td>
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<tr>
<td>AIR (pmol/l)</td>
<td>685 ± 631 (537)</td>
<td>709 ± 687 (529)</td>
<td>695 ± 655 (534)</td>
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<tr>
<td>DI (10^{-m} min^{-1})</td>
<td>1,193 ± 1,266 (887)</td>
<td>1,222 ± 1,201 (933)</td>
<td>1,205 ± 1,238 (919)</td>
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<tr>
<td>FBG (mg/dl)</td>
<td>105.3 ± 33.6 (93.5)</td>
<td>102.7 ± 36.5 (93.5)</td>
<td>103.2 ± 34.8 (93.5)</td>
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Data are means ± SD (median) unless otherwise indicated.
### RESULTS

A total of 10 SNPs at the SSTR2 locus were genotyped on 1,425 Hispanic individuals. The majority of SNPs in this study had an MAF >0.15. Using the Haploview Tagger program, a subset of the genotyped SNPs (six SNPs genotyped in the HapMap Build 36 dataset) captured common variation (HapMap CEU, MAF >0.05, aggressive tagging algorithm) with a mean $r^2 > 0.70$. D' values were high between SNPs within the SSTR2 gene (rs11077670, rs1880486, rs714925, rs998571, and rs7220818; D' ≥0.8) and declined 5' and 3' to these markers (D' <0.6; Fig. 1).
In Table 2, the majority of the SNPs in the SSTR2-containing LD block ($D' > 0.91; r^2 > 0.10$) were associated (or trending toward association) with $S_1$ ($P < 0.011$), $DI$ ($P < 0.036$), and $FBG$ ($P < 0.019$). Association with $AIR$ was not detected. Similar association was detected with individuals in set 2, although many of the SNPs showed a decrease in the magnitude of association. $S_1$ and $FBG$ were the most consistently associated glucose homeostasis traits, with three of five SNPs in high LD ($D' > 0.77; r^2 > 0.08$) showing association or trending toward association ($P < 0.039$ and $P < 0.030$, respectively; Table 3). In the combined analysis (Table 4), the magnitude of association increased within the LD block ($D' > 0.85; r^2 > 0.09$; Fig. 1) from that seen in either set independently. $S_1$ was the most consistently associated trait within the LD block ($P_{meta-analysis} < 0.0030$). $DI$ was also associated, with four of the five SNPs in high LD showing association ($P_{meta-analysis} < 0.027$). Association with $FBG$ decreased in the combined analysis, and only one SNP remained significant (rs714925, $P_{meta-analysis} = 0.045$).

**DISCUSSION**

This study of SSTR2 gene polymorphisms represents the first detailed population genetic analysis of this gene in relation to glucose homeostasis phenotypes. Whereas the current study design is limited by the SNP selection method, i.e., density versus tagging algorithms, the high LD in the region facilitated sufficient coverage of genetic variation. Strengths of this study include the use of a large Hispanic-American cohort and analysis of quantitative measures of glucose homeostasis. Moreover, since the Hispanic-American subjects were divided into two independent sets with identical recruitment criteria and similar demographic data, results can be compared between the...
The magnitude of association increased when the two sets of Hispanic Americans were combined. Tests of between-population heterogeneity were nonsignificant for the most significantly associated SNPs and traits (Table A2 in the online appendix). Within the LD block, associations were observed between SSTR2 SNPs and $S_1$ ($P_{\text{meta-analysis}} = 0.0024–0.0030$) and DI ($P_{\text{meta-analysis}} = 0.0075–0.027$). Examination of the genotypic means revealed a consistent pattern of decreased $S_1$ (β_{homozygous} = −0.16) and DI (β_{homozygous} = −0.35 to −5.16) associated with the minor allele (Table 4; online appendix Table A2). In addition, evidence of association was observed with FBG ($P_{\text{meta-analysis}} = 0.045$), with increased values (β_{homozygous} = 2.30) associated with the minor allele (Table 4; online appendix Table A2). Taken together, these findings are consistent with genetic variants at the SSTR2 locus contributing to insulin resistance, which

### TABLE 4
Genotypic means for quantitative measures of glucose homeostasis calculated in the combined IRASFS Hispanic-American population

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>SNP</th>
<th>Alleles†</th>
<th>MAF</th>
<th>Genotypic means ± SD (n)*</th>
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**Boldface data indicate P values <0.05.** *Genotypic means ± SD (n) for homozygotes of the major allele (1/1) as well as heterozygotes (1/2) and homozygotes of the minor allele (2/2). †Major/minor alleles. ‡Test for association among each SNP-trait combination using a series of generalized estimating equations. §Meta-analysis P value computed by sequential oligogenic linkage analysis with the modeling of set 1 or set 2 affiliation as a random effect in a variance component–measured genotype model.**

two populations, resulting in an ideal replication population, and can be combined to give maximal power for detecting association.

In total, 10 SNPs located at the SSTR2 locus were genotyped on 1,425 Hispanic Americans. The most consistent region of association fell within the LD block encompassing the SSTR2 gene, with three of the five SNPs highly correlated ($r^2 > 0.78$; Fig. 1) and driving the association. IRASFS Hispanic Americans were separated into two distinct groups for analysis, and results were compared between groups. Although set 1 individuals exhibited stronger association between polymorphisms in SSTR2 and $S_1$ as well as DI and FBG, similar evidence of association or trends was detected in set 2, albeit of lesser magnitude. Thus, association was detected in two independent sets of Hispanic Americans.
results in higher circulating blood glucose levels. Evaluation of \textit{SSTR2} SNPs for association with type 2 diabetes resulted in an overall lack of association, with only a single SNP showing nominal association (\( P = 0.040 \); data not shown). Lack of association should be viewed cautiously, as the result could be attributed to modest sample size for type 2 diabetes (\( n = 181 \)).

In addition, we evaluated \textit{SSTR2} in a collection of 605 African Americans from the IRASFS who were recruited and phenotyped in a similar manner. No evidence of association was observed (data not shown). This absence of association could reflect observable differences in LD patterns in the Hispanic- and African-American populations as reflected in the HapMap reference populations (CEU and YRI, respectively; online appendix Figs. A1 and A2), which results in less efficient tagging of variation in African populations (\( r^2 = 0.47 \); MAF > 0.05, aggressive tagging algorithm). In addition, lack of association could be attributed to reduced power due to smaller sample size or ethnicity-specific genetic differences.

\textit{SSTR2} polymorphisms appear to play a role in the modulation of glucose homeostasis through mechanisms consistent with the known biological function of the protein. SRIF inhibits growth hormone and glucagon secretion in a subtype-selective process through \textit{SSTR2} (1). Both growth hormone and glucagon exhibit potent antagonistic effects on insulin action by increasing hepatic gluconeogenesis and glycogenolysis. In addition, growth hormone decreases peripheral glucose utilization (20). Decreased utilization of peripheral glucose would directly affect \( S_1 \) as measured herein. Evidence of association with DI is likely to be attributed to associations with \( S_1 \) due to the mathematical relationship (DI = \( S_1 \times \text{AIR} \)). The physiological effect of decreased glucose utilization coupled with increased glucose production would result in elevated blood glucose levels, as observed through the association with FBG. Lack of association with AIR could be attributed to the subtype-selective actions of SRIF to inhibit insulin secretion, predominantly through \textit{SSTR5} (1). Based on these findings, additional research is warranted to identify a true functional variant(s), as it is unlikely the causal variant was genotyped on these individuals; i.e., SNPs were chosen based on position. Although evidence of association was detected between SNPs in the \textit{SSTR2} coding region and glucose homeostasis phenotypes, magnitude of the association was not completely consistent throughout all analyses, suggesting that the association is due to LD with the true functional variant. Additionally, a more thorough evaluation of the proximal 5’ genomic region may provide insight into a causal mechanism, possibly through interaction with the proposed promoter(s) (21–23). In conclusion, \textit{SSTR2} polymorphisms are associated with glucose homeostasis and appear to be involved through modulation of \( S_1 \).

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**REFERENCES**