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POLYMORPHISMS NEAR SOCS3 ARE ASSOCIATED WITH OBESITY AND GLUCOSE HOMEOSTASIS TRAITS IN HISPANIC AMERICANS FROM THE INSULIN RESISTANCE ATHEROSCLEROSIS FAMILY STUDY

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Abstract

The SOCS3 gene product participates in the feedback inhibition of a range of cytokine signals. Most notably, SOCS3 inhibits the functioning of leptin and downstream steps in insulin signaling after being expressed by terminal transcription factors, such as STAT3 and c-fos. The SOCS3 gene is located in the chromosome region 17q24–17q25, previously linked to body mass index (BMI), visceral adipose tissue (VAT), and waist circumference (WAIST) in Hispanic families in the Insulin Resistance Atherosclerosis Family Study (IRASFS). A high density map of 1536 single nucleotide polymorphisms (SNPs) was constructed to cover a portion of the 17q linkage interval in DNA samples from 1425 Hispanic subjects from 90 extended families in IRASFS. Analysis of this dense SNP map data revealed evidence of association of rs9914220 (located 10 kb 5' of the SOCS3 gene) with BMI, VAT, and WAIST (P-value ranging from 0.003 to 0.017). Using a tagging SNP approach, rs9914220 and 22 additional SOCS3 SNPs were genotyped for genetic association analysis with measures of adiposity and glucose homeostasis. The adiposity phenotypes utilized in association analyses included BMI, WAIST, waist to hip ratio (WHR), subcutaneous adipose tissue (SAT), VAT, and visceral to subcutaneous ratio (VSR). Linkage disequilibrium (LD) calculations revealed three haplotype blocks near SOCS3. Haplotype Block 1 (5' of SOCS3) contained SNPs consistently associated with BMI, WAIST, WHR, and VAT (P-values ranging from 2.00×10^{-4} to .036).

Haplotype Block 3 contained single-SNPs that were associated with most adiposity traits except for VSR (P-values ranging from 0.002 to 0.047). When trait associated SNPs were included in linkage analyses as covariates, a reduction of VAT LOD score from 1.26 to .76 above the SOCS3 locus (110 cM) was observed. Multi-SNP haplotype testing using the quantitative pedigree disequilibrium test (QPDT) was broadly consistent with the single-SNP associations. In conclusion, these results support a role for SOCS3 genetic variants in human obesity.

Keywords

Suppressor of Cytokine Signalling 3; Genetic Association; Single Nucleotide Polymorphisms; Obesity/Glucose Homeostasis Traits

Introduction

The Insulin Resistance Atherosclerosis Family Study (IRASFS) is a multi-center, family-based study with the goal of identifying genetic components underlying quantitative measures of adiposity and glucose homeostasis. An initial linkage scan and subsequent fine mapping revealed suggestive evidence of linkage of Body Mass Index (BMI; LOD=2.81), Visceral Adipose Tissue (VAT; LOD=3.11), and waist circumference (WAIST; LOD=2.50) to a ~40cM interval (74–114 cM) on chromosome 17q24–17q25 in the Hispanic cohort (1,2). Though VAT was consistently the trait with the greatest evidence of linkage on chromosome 17q, VAT and WAIST showed greatly diminished LOD scores following adjustment for BMI (1,2), consistent with the linked locus/loci contributing to overall adiposity rather than VAT specifically. In an effort to identify the genes contributing to this evidence of linkage, a high-density tagging single nucleotide polymorphism (SNP) map was constructed that surveyed a proximal portion of the linkage region (from 96 to 112 cM). Among genes near or containing adiposity-associated SNPs in this densely mapped region is SOCS3 (rs9914220, 10 kb 5' of SOCS3, associated with BMI, VAT, and WAIST with p-values ranging from .003 to .017). SOCS3 is a gene implicated in obesity as a feedback inhibitor of the leptin signal, and in diabetes due to its similar inhibition of insulin-signaling components (3–6). We have used a haplotype-tagging SNP approach to test whether variants within or near SOCS3 affect quantitative measures of obesity and/or diabetes in the IRASFS Hispanic cohort. We hypothesize that more extensive investigation of genetic variants within or near SOCS3 will reveal SNPs associated with quantitative adiposity or glucose homeostasis phenotypes. Subsequently, we hypothesize that by adjusting the adiposity linkage intervals by adiposity-associated SOCS3 SNPs, we will observe a supportive decrease in the LOD scores.

Materials and Methods

Subjects

The study design and recruitment of the IRASFS Hispanic and African American cohorts are described in detail (11). This report summarizes studies on the 90 multigenerational Hispanic families (1425 individuals), which consisted of an urban sample from San Antonio, TX and a rural sample from San Luis Valley, CO. Individuals were recruited over a 2.5 year period at the clinical centers on the basis of large family size: proband having at least four living siblings and five living offspring among the four siblings. All subjects have provided informed consent. Though the subjects were recruited on the basis of family size and not disease status, approximately 13.7% have been diagnosed with type 2 diabetes. Table 1 summarizes the primary phenotypes measured on study participants.

Phenotypes

The IRASFS subjects have been extensively phenotyped for quantitative measures of adiposity and glucose homeostasis, the process of which was described in detail elsewhere (1,2,11). Adiposity traits collected for IRASFS Hispanic participants included the standard anthropometric measures: Body Mass Index (BMI, kg/m^2), waist circumference (WAIST, cm), and waist-to-hip ratio (WHR). In addition to standard anthropometric measures of adiposity, measures of adiposity at specific abdominal depots were obtained from computed tomography (CT) scanning. The collected CT-derived measures of adiposity include visceral adipose tissue (VAT, cm^2), subcutaneous adipose tissue (SAT, cm^2), and visceral to subcutaneous ratio (VSR).

Measures of glucose homeostasis were assessed using the frequently sampled intravenous glucose tolerance test (FSIGT), and minimal model analyses (MINMOD software program), in order to calculate insulin sensitivity (S_I) and glucose effectiveness (S_G) (12,13). Other FSIGT-derived measures of glucose homeostasis included acute insulin response (AIR) and the disposition index (DI). Plasma glucose (GFAST) and insulin (FINS) levels (or concentrations) were also obtained. Diabetes-affected individuals were excluded from SNP association analysis with quantitative measures of glucose homeostasis.

DNA Preparation

Genomic DNA was purified from whole blood PUREGENE DNA isolation kits (Gentra Inc., Minneapolis, MN, USA). Quantification of the purified DNA was performed by fluorometric assay (Hoefer DyNA Quant 200 fluorometer; Hoefer Pharmacia Biotech Inc., San Francisco, CA, USA). of 5 ng/ μl .

SNP Genotyping and Selection

Initial genotyping across the 17q region was performed by the Center for Inherited Disease Research with the Illumina Goldengate© 1536 SNP Genotyping Assay and BeadLab (Illumina, Inc., San Diego, CA). The 1536 SNPs cover a 6.85 Mb interval at a SNP density of 1 per 4.46 kb. HapMap coverage of the mapped region in CEU & YRI populations for SNPs of $\text{MAF} \geq 10\%$ at an r^2 of 0.65 is greater than 80% and 60%, respectively.

SOCS3 SNP genotyping was performed using the iPLEX MassARRAY SNP genotyping system (Sequenom Inc., San Diego, CA, USA), which utilizes mass tagging to differentiate between alleles (14). SNP selection for candidate gene analysis was performed in a ~13 kb genomic region 5' to SOCS3, the ~3 kb SOCS3 genic region, and an ~31 kb region 3' to SOCS3. CEU HapMap tagging SNPs with an r^2 threshold of 0.8 were initially selected, and supplemented with HapMap (www.hapmap.org) genotyped SNPs, as well as those of dbSNP. According to the tagging function of HapMap, the 15 tagging SNPs and the 13 SNPs of dbSNP capture over 80–90% of the genomic variation of the region in CEU, YRI, and CHB ancestral populations. No SNPs selected for genotyping had a listed minor allele frequency (MAF) of less than 5%. Each pedigree has previously been examined for consistency of stated family structure and is described in detail elsewhere (15). Maximum likelihood estimates of allele frequencies were computed using the largest set of unrelated individuals ($n=228$) and tested for departures from Hardy-Weinberg equilibrium proportions (HWE) using a chi squared goodness of fit test. The largest set of unrelated individuals was also used to calculate the D' and r^2 inter-SNP linkage disequilibrium (LD) statistics. Each of the SNPs evaluated in this work were examined for Mendelian inconsistencies in their genotypes using the program PEDCHECK (16). Any genotypes inconsistent with Mendelian inheritance that could not be resolved by examination of the genotyping data were converted to missing.

Statistical Analysis

Single-SNP analysis was performed using the variance components method as implemented in the software package, SOLAR (<http://www.sfbr.org/solar/>). Briefly, analysis consisted of the two degree of freedom overall test of genotypic association and the three individual models defined by the *a priori* genetic models (i.e., dominant, additive, recessive). To minimize type 1 error, we considered the individual genetic models only when the genotypic association test suggested an association, or after adjusting the individual genetic model P-values by a Bonferroni correction. Tests were computed by adjusting measures of adiposity and glucose homeostasis for age, gender and recruitment center (San Antonio, TX and San Luis Valley, CO) and, in parallel, adjusting for age, gender, BMI, and recruitment center. When necessary, quantitative traits were transformed to best approximate the distributional assumptions of the test (i.e., conditional normality and homogeneity of variance). The adiposity phenotypes were transformed by taking the square root of VAT and SAT, and the natural logarithm of BMI, WHR, and VSR after the addition of one. The glucose homeostasis phenotypes were transformed by taking the natural logarithm of FINS and SI after the addition of one and by taking the sine of the square root of AIR and DI.

The data were also analyzed using the quantitative pedigree disequilibrium tests (QPDT), using two, three, and four marker moving windows, which assess haplotype association. The QPDT uses a moving window analysis method, which forms multi-SNP haplotypes out of progressively adjacent SNPs (by physical position). In other words, it includes SNP 1, then SNP 1 and adjacent SNP 2 (2 marker), then SNPs 1–2 and SNP 3 (3 marker), and finally SNPs 1–4 (4 marker). The process then repeats, but it begins to form the haplotypes starting with SNP 2. All of the different individual combinations of alleles comprising these small haplotypes are tested for over- or under-transmission to offspring in nuclear families whose trait variance departs from the expected. A global p-value is generated by the QPDT, which represents the overall significance based on the association tests of all the individual allele combinations of that haplotype, and these are what are reported (in Supplementary Figure 2 and Supplementary Figure 3). In addition, we report the most associated and sufficiently common (frequency $\geq 5\%$) individual haplotypes underlying the global p-values (Supplementary Figure 4). The QPDT uses the expectation-maximization algorithm to estimate the haplotype frequencies of individuals whom have an ambiguous phase, and is generally robust to potential population stratification.

To test whether a particular subset of SNPs contributed either directly, or through linkage disequilibrium, to the evidence for linkage to the QTL, a subset of trait-associated SNPs was entered into the QTL linkage analysis and the change in the magnitude of the LOD score calculated. If the polymorphism directly or indirectly contributes to the evidence for linkage, the initial LOD score will be reduced in a model that includes the polymorphisms as a covariate.

Results

Twenty-eight SNPs have been genotyped within or near SOCS3 in 1425 Hispanic IRASFS subjects and tested for association with quantitative adiposity and glucose homeostasis traits. Eight SNPs are located in the 5' upstream/promoter region, 3 SNPs in the 3'-UTR, and 17 SNPs in the 3' region that is distal to the UTR (see Figure 1). The small SOCS3 genic region (~3 kb) has no genotyped SNPs due to the small number and low informativeness of tagging SNPs in the HapMap database (there are 2 tagging SNPs that were in the 3'-UTR, and were genotyped here). Prior mutation scanning approaches have revealed no common genic SOCS3 SNPs linked to obesity or diabetes traits (7–10). The genotyped SNPs did not depart from Hardy-Weinberg Equilibrium (Supplementary Table 1). The Gabriel et al. method of haplotype block definition, as implemented by Haploview, shows the presence of a 10 kb haplotype block 5' of SOCS3, an 11 kb block 3' of SOCS3, and a <1 kb block further downstream in the 3'

direction (17,18, Figure 1, Supplementary Figure 1). The 5' Gabriel-defined haplotype block is designated as Haplotype Block 3, the 11 kb 3' block is designated as Haplotype Block 2, and the <1 kb block further downstream is designated as Haplotype Block 1.

Analysis of the single SNP data reveals evidence for association of multiple SOCS3 SNPs with adiposity measures, the most prominent of which are 4 highly correlated SNPs that are ~10 kb 5' of the gene and within 2 kb of one another (Table 2). rs9914196, rs9914220, rs8070204, and rs8074003 were associated with BMI, WAIST, WHR, and VAT (P-values ranging from 2×10^{-4} to .036; MAF=12–14%). rs8070204 shows additional association with VSR (P-value of .002). SOCS3 SNPs of the 3'-UTR/downstream region also show evidence of association with adiposity measures, the most prominent of which was rs7221341 association with BMI, WAIST, WHR, VAT, and SAT (P-values range from .002 to .036; MAF=31%). Notable also is rs4969168's association with WAIST, WHR, and SAT (P-values range from .004 to .044; MAF=26%). rs2280148 is associated with multiple measures of adiposity, but is poorly polymorphic (MAF=1%). Finally, the 3' SNPs rs8076673 and rs6501199 show nominal evidence of association with VAT (P-values range from .034 to .047; MAF=16%–30%).

A second analysis was performed adjusting for BMI in addition to age, gender, and center, to evaluate evidence of association after adjustment for overall body size (Table 2). In the promoter region, the highly correlated SNPs rs9914196, rs9914220, and rs8070204 retain some evidence of association with WAIST and WHR (P-values range from .003 to .046). Other 5' SNPs, rs8070204 (VAT; P-value of .060) and rs8074003 (WHR; P-value of .097), have reduced evidence of association. SNPs of the 3' region, e.g. rs7221341, also have reduced evidence for association, but evidence of association for rs4969168 with WAIST and WHR remains (P-values range from .009 to .015). Similarly, evidence for association of rs16971055 with WAIST and WHR remains following BMI adjustment (P-values range from .013 to .018; MAF=7%).

The 4 highly correlated and closely spaced SNPs in the promoter region have nominal evidence of association with glucose homeostasis measures when adjusting for age, gender, and center. rs9914196, rs9914220, rs8070204, and rs8074003 are associated with S_I and SG (P-values range from .002 to .050). rs8074003 is additionally associated with FINS and DI (P-values range from .020 to .040). There is no evidence for association between glucose homeostasis measures and SNPs 3' to the coding region (Data not shown). To evaluate the influence of body size on evidence of association with glucose homeostasis measures, the association analysis was repeated adjusting for BMI, in addition to age, gender, and center. Adjustment for BMI results in limited evidence for association of SOCS3 SNPs with glucose homeostasis traits (Data not shown; P-values range from .007 to .048). Only a single SNP of the 3'-UTR, rs8069976, is associated (with FINS) by a p-value of < .01 after BMI adjustment.

The phenotypic effects of common (MAF>5%) adiposity trait-associated SNPs under SOLAR are presented as genotypic means in Table 3. A single SNP that is associated with a trait under the 2df model is tested for association with the dominant, additive, and recessive *a priori* genetic models. Table 3 illustrates that most SOCS3 SNPs associated under the 2df model operated by a recessive model, in which two copies of the minor allele are protective from obesity. Inheriting two minor alleles of SNP rs8070204, which was one of the correlated SNPs in the promoter/5' region of SOCS3, was associated with a 38.9 cm² decrease in VAT and a 3.5 kg/m² decrease in BMI in IRASFS Hispanics.

The QPDT was performed on the genotyped SNPs in order to determine if SOCS3 variants are part of multi-SNP haplotypes that show association with adiposity traits. The QPDT shows results that are partially supportive of the single-SNP results as calculated by SOLAR (Supplementary Figures 2 and 3). However, in contrast to the SOLAR results, there are no SOCS3 SNPs in the 5' region that were associated with adiposity measures. In the 3' region,

there are associations of multi-marker SNP haplotypes with BMI, WAIST, WHR, SAT, and VSR (P-values range from .001 to .045). Additional adjustment of the QPDT adiposity measure association results by BMI causes changes in the genetic association profile of 3' SOCS3 SNPs (Supplementary Figure 3). There is no longer association of multi-marker haplotypes with WAIST. However, association of multiple SNP haplotypes is observed with WHR (association seems to shift further in the 3' direction; P-values range from .022 to .036), VAT (P-value of .031), and VSR (most association persists; P-values range from .001 to .043). The SNPs rs8076673 and rs7221341 are prominently associated with adiposity measures after BMI adjustment due to their inclusion in nearly all WHR, VAT, and VSR-associated haplotypes. When the genotyped SOCS3 variants are tested for association with glucose homeostasis measures via QPDT, while adjusted for age, gender, and center, there is again limited evidence of association (Data Not Shown). The SNPs of the 5' region comprised haplotypes that were nominally associated with S_G and S_I (P-values range from .017 to .049). Elsewhere in the genotyped SOCS3 interval, there is no association with glucose homeostasis measures before or after BMI adjustment (Data Not Shown).

The IRASFS previously found linkage of BMI, VAT, and WAIST (only before adjustment for BMI) to a ~40 cM region (74–114 cM) of chromosome 17q24–17q25 (1,2). Similarly, the present results reflect an association of SOCS3 SNPs with whole-body adiposity, but preferential deposit of increased adiposity to the abdomen and viscera. Therefore, we have adjusted the 17q adiposity linkage intervals by adiposity-associated SOCS3 SNP genotypes, which theoretically capture meaningful haplotypic variation. Subsequently, this adjustment will enable us to conclude if polymorphism in SOCS3 accounts for IRASFS linkage results. After adjusting the chromosome 17q BMI, WAIST, and VAT linkage intervals by adiposity-associated SOCS3 SNP genotype, there is a corroborative decrease in the LOD score for VAT at a minor peak just over the mapped position of SOCS3. The SNPs selected with which to adjust the linkage intervals included rs7221341, rs7222391, rs4969168, rs9914220, rs8070204, rs9914196, rs8074003, rs6501199, rs8076673, and rs16971055. The minor linkage peak for VAT at the approximate location of the SOCS3 gene (maps to 110 cM) was most affected by adjustment for SOCS3 SNP genotypes, decreasing from a LOD of 1.26 to .76 when adjusted for age, gender, and associated SOCS3 SNPs (Figure 2). There is a more modest decrease from a LOD of 2.83 to 2.57 at 94 cM under the largest VAT linkage peak. Linkage evidence for VAT or LOD reduction after SNP adjustment was not apparent when BMI was also used as a covariate (Figure 2). The linkage interval for BMI on 17q shows a largely unaffected LOD score after adjustment for SOCS3 SNP genotype (Supplementary Figure 5). The linkage interval for WAIST on 17q modestly decreases from a LOD of 1.84 to 1.62 at 95 cM, the site of the greatest evidence for linkage. As with VAT, linkage evidence for WAIST and LOD reduction after SNP adjustment was not apparent when BMI was also used as a covariate (Supplementary Figure 6).

Discussion

In this study we have evaluated a region on 17q24–q25 for association with measures of adiposity in Hispanic subjects from the IRASFS. Initial analysis of the 1536 SNP data from this linkage-defined interval revealed evidence for association of SNP rs9914220 with BMI, VAT, and WAIST with P-values ranging from 0.003 to .017. This SNP is approximately 10 kb upstream of the SOCS3 gene. SOCS3 plays a central role in feedback inhibition of the leptin signaling pathway. Expression of SOCS3 is induced by STAT3 transcription factors, which are an endpoint of the leptin receptor's JAK-STAT signaling pathway. Once expressed, SOCS3 modulates the leptin signal through direct binding of signal transduction components that utilize phosphorylated tyrosines (including the leptin and insulin receptors, JAK proteins; 20–22). Abnormally functioning or expressed SOCS3 may be a result of inherited genetic variation within or near the gene, and may be partly responsible for the “leptin resistance” observed in

obesity (23,24). Furthermore, SOCS3's ability to inhibit shared downstream leptin and insulin signaling components (e.g. IRS proteins), and the finding that SOCS3 is downstream of major inflammatory mediators (such as TLR-4 and TNF- α), suggests that SOCS3 may be a direct genetic determinant of obesity and diabetes (6,25,26).

To further investigate the influence of SOCS3 in obesity and diabetes, we used a tagging SNP approach to comprehensively evaluate this gene for association with quantitative adiposity and glucose homeostasis measures. The 3.3 kb SOCS3 gene is within the ~47 kb genotyped region composed of 3 major LD blocks (Gabriel method, Figure 1). We found evidence for association between SNPs both 5' and 3' of the gene and adiposity measures.

Strongest evidence of association was with adiposity measures in the Promoter region, 3'-UTR, and 3'-downstream region of the gene (Haplotype Block 3, Haplotype Block 1; Figure 1; Table 2). Adiposity association in the promoter region was most prominent between BMI, WAIST, WHR, VAT and the highly correlated SNPs rs9914196, rs9914220, rs8070204, and rs8074003 (P-values ranged from 2.0×10^{-4} to .036). Meanwhile, adiposity association in the 3'-UTR was most prominent between BMI, WAIST, WHR, SAT and the SNPs rs4969168 and rs2280148 (P-values ranged from .004 to .044). Finally, adiposity association in the 3'-downstream region was most prominent between all tested adiposity traits except VSR and the SNP rs7221341 (P-values ranged from .002 to .036). Adjustment for BMI caused SNP association with WAIST and WHR to be emphasized, which implies a primary influence of SOCS3 SNPs on whole-body adiposity with preferential deposition of abdominal fat (P-values ranged from .003 to .046; Table 2). Analyses with measures of glucose homeostasis suggest that polymorphism in SOCS3 has a primary effect upon adiposity, and that any genetic association with glucose homeostasis measures is mediated through adiposity. There is no evidence of association with glucose homeostasis traits in BMI-adjusted analysis of the 5'/promoter region. Moreover, there is only limited evidence for association in the 3' region between SNPs in Haplotype Blocks 1 and 2 and glucose homeostasis traits. The only strong glucose homeostasis trait association following BMI adjustment was rs8069976's with FINS in the 3'UTR (P-value is .007, Data not Shown).

When the adiposity-associated tagging SNPs rs7221341, rs7222391, rs4969168, rs9914220, rs8070204, rs9914196, rs8074003, rs6501199, rs8076673, and rs16971055 were used to adjust the BMI, VAT, and WAIST linkage intervals on chromosome 17q, (Supplementary Figures 2–4) it was clear that the most significant effect was observed at a minor LOD peak within the VAT linkage interval (Figure 2). The adiposity-associated tagging SNPs were utilized as a unit to adjust the linkage peak because, in theory, these SNPs capture meaningful genomic information. The LOD score decreases at the minor VAT peak from 1.26 to .76, which is the approximate mapped position of the SOCS3 gene (110 cM). Subsequently, the LOD reduction in BMI-unadjusted VAT suggested that the positional evidence of linkage with VAT (when considered as a component of overall adiposity) at this minor peak is partly explained by these SOCS3 SNPs. Because BMI-unadjusted VAT was the only linkage interval with a modest linkage peak directly above the mapped position of SOCS3, this was unsurprising. These results lend additional significance to single-SNP associations with VAT prior to BMI adjustment, which includes the previously noted adiposity-associated SNPs of Blocks 1 and 3, and further suggests a mechanism for SOCS3 SNPs in increased overall adiposity with preferential deposition of abdominal fat. Though the LOD reduction is supportive of our single-SNP association results, we note that the major linkage peaks for VAT, BMI, and WAIST are largely unaffected by adjustment for SOCS3 SNPs. Thus, we do not conclude that SOCS3 is the major contributor to our evidence of linkage in the region overall.

These results have not been adjusted for type I error as presented, and should be considered with some caution, especially in regard to the glucose homeostasis results. One perspective on

accounting for type I error would utilize a standard Bonferroni correction for all SNP and trait comparisons. Such a correction may be too stringent since there is a strong prior hypothesis that this gene possesses obesity-associated variants, which is based on a body of physiologic evidence. Additionally, there is a non-trivial amount of inter-trait and inter-SNP correlation present in this data set, which makes the number of conducted tests difficult to determine. For instance, the estimated genetic correlation between BMI and WAIST, WHR, SAT, or VAT ranges from an r^2 of .61 to .94, and the mean inter-SNP D' is .56. The correlations between adiposity phenotypes and SNPs present a scenario for which a consistent multiple corrections strategy has not been proposed. Subsequently, a finite threshold of significance following a multiple comparisons adjustment could potentially mischaracterize the results.

To date, there have been no reports of functional analyses that directly link SOCS3 gene expression to the region of most prominent association. However, Haplotype Block 1 (Figure 1 and Supplementary Figure 1) is 10 Kb in size, and it contains associated SNPs in high LD (e.g. rs9914196, rs9914220, rs8070204, rs8074003). Since SOCS3 is downstream of many critical biological pathways, such as the leptin receptor, insulin receptor, and toll-like receptors, the SOCS3 promoter should contain many response elements. Most prior studies of the SOCS3 promoter have focused on the “functional” region ~1 to 3 Kb upstream of the gene.

We have evaluated the 10 kb Haplotype Block 1 using the bioinformatics tool, MatInspector. A twenty-one base pair sequence for each variant (10 bps on either side of each SNP) was scanned using MatInspector, restricting the results to humans and requesting a sequence alignment (28). As is common with this approach, several SNPs appear to be in putative transcription factor binding sites. However, rs9914220 and rs8074003 are within putative response elements that are congruent with SOCS3 biology and expression. The SNP rs9914220 was adjacent to a putative growth factor independent 1 (GFI1) response element (Matrix Similarity=.919). GFI1 is a transcriptional repressor that regulates cell differentiation in hematopoietic stem cells, T cells, neutrophils, and dendritic cells. In performing its function, some evidence suggests that GFI1 inhibits SOCS3 expression (29). The SNP rs8074003 was within the core sequence for putative X-box-binding protein 1 (XBP-1; Matrix Similarity=.891) and hypoxia-inducible factor response elements (Matrix Similarity=.946). XBP-1 is a transcription factor that is critical in responding to endoplasmic reticulum stress, which can be caused by obesity through accumulation of misfolded proteins, lipids, glucose deprivation, and excessive demand for secretory proteins. XBP-1 knock-outs show hyper-activation of JNK, reduced insulin receptor signalling (partly through inhibition of IRS-1), and systemic insulin resistance (30). Additionally, mechanically stressed adipocytes that are distant from the circulation may become hypoxic. Expression of the hypoxia-inducible factor-1 alpha is elevated in mouse models of obesity, and can promote increased levels of leptin (31). The preceding suggests that SNP interference in the promoter binding of GFI1, XBP-1, or hypoxia-inducible factor-1 alpha may lead to abnormal expression of SOCS3, which would then have implications in obesity and diabetes. Assuming that any of these putative response elements are functionally meaningful, and the association of SOCS3 SNPs is confirmed, this could provide a starting point for further study.

Contrary to the negative results of prior genetic analyses of SOCS3 and the immediate promoter region (7–10), we report that multiple SNPs farther 5' and 3' of the genic region are associated with adiposity, and nominally so with glucose homeostasis measures. Furthermore, these SNPs provide an explanation for the modest VAT linkage peak just over the mapped position of SOCS3, previously observed in the IRASFS Hispanics (1,2). Three distinctive haplotype blocks were identified upon the determination of the LD structure, and all three contained associated genetic variants. The chief mechanism by which these SNPs affect obesity appears to be through overall body mass with preferential deposit of adipose tissue in the abdominal and visceral area. The implications of these SNPs in diabetes are more limited, and much of

the observed effect on glucose homeostasis traits is mediated through increased adiposity. Interestingly, a majority of the minor allele effect of common associated genetic variants was protective. Clearly, these SNPs and SNP haplotypes should be tested for association with obesity and diabetes traits in other cohorts for validation. Consistently associated, sufficiently common, genetic variants with even a modest prevalence or effect on SOCS3 could be highly significant in the pathogenesis of obesity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

A schematic representing the genomic locations and number of genotyped SOCS3 SNPs. SNP locations are not to scale; however, efforts were made in placing them so they approximated the inter-variant distance. The demarcated haplotype blocks are estimated using the Gabriel method and inter-SNP D' and r^2 calculations, see Supplementary Figure 1.

Figure 2.

The chromosome 17q VAT linkage interval with and without adjustment for BMI and/or SOCS3 SNP genotype. Microsatellite appear at the top of the diagram, the maximum log of the odds of linkage (LOD score) is on the Y axis, and genetic distance in centimorgans the X axis. The LOD plot is adjusted for age and gender. The black line represents the linkage scan without adjustment for SOCS3 SNPs, the line represents the linkage scan with adjustment for SOCS3 SNPs, the lower black line represents the linkage scan with adjustment for BMI, lower dashed line represents the linkage scan with adjustment for SOCS3 SNPs and BMI. SOCS3 maps to 110 cM on this plot.

Visceral Adipose Tissue; Chromosome 17; Covariates: Age, Sex

Table 1

Demographics of the IRASFS total Hispanic cohort.

	Hispanic individuals in IRASFS	
Phenotype	Mean (SD)	Median
<i>Demographic</i>		
Age (yrs)	42.8 (14.6)	41.3
Female gender (%)	58	
<i>Adiposity</i>		
Body Mass Index (kg/m ²) (BMI)	28.9 (6.1)	28.1
Waist Circumference (cm)	89.8 (14.3)	89.1
Weight (kg)	77.6 (18.2)	75.5
Waist/Hip Ratio (WHR)	0.86 (0.08)	0.85
Subcutaneous Fat Area L4/L5 (cm ²) (SAT)	338.7 (154.7)	313.7
Visceral Fat Area L4/L5 (cm ²) (VAT)	113.8 (61.2)	105.9
Visceral: Subcutaneous Ratio (VSR)	0.38 (0.21)	0.33
<i>Glucose Homeostasis</i>		
Insulin Sensitivity (S _I ; X10 ⁻⁵ min ⁻¹ /[pmol/L])	2.0 (1.9)	1.5
Acute Insulin Response (AIR, pmol/L)	695 (655)	534
Disposition index (DI; 10 ⁻⁵ min ⁻¹)	1205.3 (1238.2)	918.7
glucose effectiveness (S _G ; min ⁻¹)	0.02 (.009)	0.02
fasting insulin (FINS)	16.1 (12.9)	13
fasting glucose (FGLU; mg/dL)	103.2 (34.8)	93.5

Table 2

Single-SNP Genotypic Association (2df test) Results in the IRASFS Hispanics. The physical location, minor allele frequency (MAF), and SNP designations are in the far left columns. Results adjusted by standard parameters (age, gender, center) and those adjusted by standard parameters and BMI are presented. SAT is not included in the BMI adjusted results due to high correlation with BMI ($r^2 \geq .90$). A dot (“.”) represents no association. Note that only phenotypes with at least one associated SNP are displayed below, and no glucose homeostasis results are presented.

ADJUSTED FOR AGE, CENTER, GENDER													ADJUSTED FOR BMI		
SNP	MAF	Alleles	BMI	WAIST	WHR	VAT	SAT	VSR	WAIST	WHR					
3'	rs12449451	20%	C/T	.	.	.	.	.	.	.	.	.			
	rs4789575	13%	A/T	.	.	.	.	.	.	.	.	.			
	rs11651398	6%	C/T	.	.	.	.	.	.	.	.	.			
	rs4436839	41%	A/C	.	.	.	.	.	.	.	.	.			
	rs16971055	7%	A/G	.	.	.	.	.	0.013	0.018					
	rs7222391	6%	A/G	.	.	.	.	.	.	.	.	.			
	rs12936911	2%	C/T	.	.	.	.	.	.	.	.	.			
	rs17642091	10%	A/G	.	.	.	.	.	.	.	.	.			
	rs8076673	16%	A/G	.	.	0.047	.	.	.	.	.	.			
	rs7221341	31%	C/T	0.021	0.035	0.007	0.036	0.002	.	.	0.025				
	rs6501199	30%	C/G	.	.	.	0.034	.	.	.	.	.			
	rs4447485	12%	C/T	.	.	.	.	.	.	.	.	.			
	rs11077359	22%	C/T	.	.	.	.	.	.	.	.	.			
	rs7216115	5%	G/T	.	.	.	.	.	.	.	.	.			
	rs12944581	29%	C/G	.	.	.	.	.	.	.	.	.			
5'	rs8069976	12%	A/C	.	.	.	.	.	.	.	.	.			
	rs8071356	12%	G/T	.	.	.	.	.	.	.	.	.			
	rs4969168	26%	A/G	.	0.011	0.004	.	0.044	0.009	0.015					
	rs4969169	14%	C/T	.	.	.	.	.	.	.	.	.			
	rs2280148	1%	A/C	0.003	0.005	0.011	.	.	0.023	.	.	.			
	rs11868378	24%	A/G	.	.	.	.	.	.	.	.	.			
	rs4969170	46%	A/G	.	.	.	.	.	.	.	.	.			
	rs9914196	13%	A/C	0.018	0.006	0.001	0.012	.	.	.	0.012				
	GENE														

	ADJUSTED FOR AGE, CENTER, GENDER											ADJUSTED FOR BMI		
	SNP	MAF	Alleles	BMI	WAIST	WHR	VAT	SAT	VSR	WAIST	WHR			
	rs9914220	14%	C/T	0.019	0.006	0.001	0.012	.	.	.	0.011			
	rs8070204	13%	A/G	0.017	0.003	2.00E-04	0.003	.	0.002	0.046	0.003			
	rs8074003	12%	C/T	0.036	0.035	0.011	0.022	.	.	.	.			
	rs4994934	13%	C/T	.	.	.	.	.	.	.	.			
	rs4969172	46%	C/T	.	.	.	.	.	.	.	.			

Table 3

The genotypic means of associated SNPs under the 2df test of SOLAR, which have MAF >5% with standard deviations in parentheses. Beginning from the far left, the columns list: the SNP designations, the physical location of the SNP, the traits with which the SNP was associated, the genetic model with which the SNP was associated, and the MAF of the SNP. The far right column contains the genotypic means for each associated trait and each SNP: 1/1 represents individuals homozygous for the major allele, 1/2 represents heterozygous individuals, and 2/2 represents minor allele homozygotes. The SNP marked with *** is the most associated of the four highly-correlated promoter SNPs (rs9914220, rs8074003, rs8070204, and rs9914196). Units: Body Mass Index (BMI) kg/m², Waist circumference (WAIST) cm, Waist to Hip Ratio (WHR) Visceral Adipose Tissue (VAT) cm², Subcutaneous Adipose Tissue (SAT) cm², fasting insulin (FINS) mg/dL

SNP	Region	Trait	Best Model	MAF	Genotypic Means (SD)		
					1,1	1,2	2,2
rs7221341	3' of gene	BMI	RECESSIVE	31%	28.9 (6.28)	29.2 (5.97)	27.4 (5.40)
		WAIST			89.8 (14.3)	90.3 (14.3)	86.7 (13.6)
		WHR			.856 (.084)	.859 (.085)	.842 (.092)
		VAT			111 (57.2)	120 (66.2)	104 (62.5)
		SAT			339 (156)	345 (154)	304 (143)
rs8069976	3' of gene	FINS	RECESSIVE	12%	15 (12)	15 (9)	10 (7)
rs4969168	3'-UTR	WAIST	RECESSIVE	26%	90 (15)	90 (14)	87 (14)
		SAT			337 (155)	347 (158)	307 (124)
rs8070204***	Promoter	BMI	RECESSIVE	13%	29.0 (6.23)	28.8 (5.94)	25.5 (5.07)
		WAIST			89.9 (14.4)	90.0 (13.8)	80.2 (11.5)
		WHR			.855 (.085)	.860 (.084)	.805 (.085)
		VAT			115 (62.2)	114 (59.1)	76.1 (45.6)
		SAT			341 (153)	337 (158)	280 (144)