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Genome-wide study of subcutaneous and visceral adipose tissue reveals novel sex-specific adiposity loci in Mexican Americans: The Insulin Resistance Atherosclerosis Family Study

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Ethical statement: Participants included in this study were recruited from clinical centers in San Antonio, TX and San Luis Valley, CO. The Institutional Review Board of each clinical (UT Health Science Center San Antonio Review Board and Colorado Multiple Institutional Review Board, respectively) and analysis (Wake Forest School of Medicine) site approved the study protocol and all participants provided written informed consent.

Conflict of Interest: The authors have no conflict of interest to disclose.

Abstract

Objective—This study aimed to explore genetic mechanisms of regional fat deposition, which is a strong risk factor for metabolic diseases beyond total adiposity.

Methods—A genome-wide association study of 7,757,139 SNPs in 983 Mexican Americans (N_{male}=403, N_{female}=580) from the Insulin Resistance Atherosclerosis Family Study (IRASFS) was performed. Association analyses were performed with and without sex stratification for subcutaneous adipose tissue (SAT), visceral adipose tissue (VAT), and visceral-subcutaneous ratio (VSR) obtained from computed tomography (CT).

Results—The strongest signal identified was SNP rs2185405 (MAF=40%, $P_{VAT}=1.98\times10^{-8}$) with VAT. It is an intronic variant of the GLIS family zinc finger 3 gene (*GLIS3*). In addition, SNP rs12657394 (MAF=19%) was associated with VAT in males ($P_{male}=2.39\times10^{-8}$; $P_{female}=2.5\times10^{-3}$). It is located intronically in the serum response factor binding protein 1 gene (*SRFBP1*). On average, male carriers of the variant had 24.6cm^2 increased VAT compared to non-carriers. Subsequently, genome-wide SNP-sex interaction analysis was performed. SNP rs10913233 (MAF=14%, $P_{int}=3.07\times10^{-8}$) in *PAPPA2* and rs10923724 (MAF=38%, $P_{int}=2.89\times10^{-8}$) upstream of *TBX15* were strongly associated with the interaction effect for VSR.

Conclusions—Six loci were identified with genome-wide significant associations with fat deposition and interactive effects. These results provided genetic evidence for a differential basis of fat deposition between genders.

Keywords

Genetics; Hispanics; Family Studies; Computed Tomography; Obesity

Introduction

Obesity is a global health epidemic affecting more than 500 million individuals worldwide and responsible for nearly three million deaths each year (1). Previous studies have confirmed obesity as a strong risk factor for many metabolic diseases including cardiovascular disease (CVD), type 2 diabetes (T2D), metabolic syndrome, certain types of cancer, stroke, and hypertension (2, 3). However, the exact mechanisms underlying these associations have been poorly elucidated.

Recent research has suggested obesity is not a homogeneous condition and regional fat distribution affects glucose and lipid metabolism beyond total body adiposity (4). For example, visceral adipose tissue (VAT) has been shown to be responsible for the increased mortality and risk for metabolic disorders while subcutaneous adipose tissue (SAT) is thought to be benign (5). Genetic studies have been successful in identifying genetic loci responsible for regional fat distribution using measures including waist circumference (WAIST) and waist-hip ratio (WHR). However, anthropometric measures can be impacted by skeletal structure and aging (6) and cannot differentiate between regional fat depots, e.g. visceral and subcutaneous adipose tissue, and therefore bias studies. Currently, computed tomography (CT) is considered as the gold standard for the measurement of adipose tissue deposition (7, 8). However, due to cost and accessibility reasons, only one genome-wide

association study (GWAS) has been published focusing on directly measured SAT and VAT with genome-wide significant signals (9).

Numerous evidence has suggested strong sex specificity for regional adipose tissue distributions with females having a higher proportion of gluteal-femoral body fat whereas males have more in the abdominal (visceral) region (8, 10). This observation suggests a potentially different mechanism for fat deposition in different genders. In 2015, the Genetic Investigation of Anthropometric Traits (GIANT) consortium published a genetic study of adipose tissue deposition using WHR and identified 49 (33 new) signals associated with 20 loci demonstrating sex-specific effects (11). Until now, no formal genome-wide SNP-sex interaction analysis has been performed in Mexican Americans.

Here we report a genetic study of sex-specific adipose tissue deposition using CT measures including SAT and VAT in the Insulin Resistance Atherosclerosis Family Study (IRASFS). Genome-wide and exome chip association studies were combined to provide a more comprehensive scan of both common and rare variants. As adiposity deposition differs between genders, sex-stratified analyses as well as genome-wide SNP-sex interaction analyses were performed.

Materials and Methods

Insulin Resistance Atherosclerosis Family Study (IRASFS)

The study design, recruitment, and phenotyping for the IRASFS have been previously described (12, 13). In brief, the IRASFS is a family-based study designed to investigate the genetic and environmental basis of insulin resistance and adiposity. Individuals included in this cohort (N=1,417 individuals, 90 pedigrees) were Mexican Americans recruited from in San Antonio, TX and San Luis Valley, CO. Since a diagnosis of diabetes was not required for participation, about 12.7% of individuals had diabetes. The study protocol was approved by the Institutional Review Board of each participating clinical and analysis site and all participants provided written informed consent.

Phenotypes

Measures of adiposity were obtained using a standardized protocol. BMI was calculated as weight in kilograms divided by height in meters squared. Computed tomography (CT) scans were performed to estimate visceral and subcutaneous fat area (VAT and SAT, respectively; cm^2). This procedure consisted of a single scout of the abdomen followed by a 10-mm thick axial image. Axial images were obtained at L4-L5 disc space using a standard protocol. CT images were sent to a centralized reading center at the University of Colorado Health Sciences Center. VAT and SAT were computed from these data as previously described (7). Visceral-subcutaneous ratio (VSR) was computed as the ratio of VAT and SAT. In addition, glucose homeostasis traits were also obtained in IRASFS, e.g. acute insulin response (AIR), metabolic clearance rate of insulin (MCRI), fasting plasma glucose (GFAST) and insulin (FINS), and homeostatic model assessment of beta-cell function and insulin resistance ($HOMA_B$ and $HOMA_{IR}$). Phenotype acquisition and variable calculations have been previously described (12, 14).

Genotyping and Quality Control

Genotyping

GWAS genotyping was supported through the Genetics Underlying Diabetes in Hispanics (GUARDIAN) Consortium (15) using the Illumina OmniExpress and 1S arrays (Illumina Inc.; San Diego, CA, USA) and exome chip genotyping was carried out on the Illumina HumanExome Array. A detailed description of genotyping platforms and quality controls has been published (13).

Imputation

Imputation was performed using IMPUTE2 (16) and the 1000G phase I V3 integrated reference panel. All IRASFS samples genotyped on the OmniExpress and 1S arrays were imputed together. Imputed variants were filtered with a confidence score >0.90 and an information score >0.50. Imputation quality was evaluated using 10,000 SNPs with both exome chip and imputation coverage randomly selected from 32,729 overlapping SNPs.

Statistical Analysis

GWAS and Exome Chip

Phenotypes were transformed to approximate the distributional assumptions of normality and homogeneity. Specifically, VSR was natural log transformed and SAT and VAT were square-root transformed. Admixture estimates were calculated using maximum likelihood estimation of individual ancestries as implemented in ADMIXTURE (17). For SNPs available in both GWAS imputation and exome chip, exome chip genotypes were always used for analysis. Imputation quality was evaluated with 10,000 overlapping SNPs between GWAS imputation and exome chip, which were selected based on minor allele frequencies (MAF). Concordance analysis was performed between the two platforms and an r-square value was computed for each variant.

Tests of association between individual variants and quantitative traits were computed using the Wald test from the variance component model implemented in Sequential Oligogenic Linkage Analysis Routines (SOLAR) (18). Rare variants (both genotyped and imputed) with MAF <1% were removed, resulting a total of 7,708,309 variants. Genetic associations were calculated adjusting for age, recruitment center, admixture estimates, and sex (for sex-combined analysis only). The primary inference was the additive model. A lack of fit to the additive model was tested using the orthogonal contrast. If the lack-of-fit test was significant ($P<0.05$), the model with the “best” p-value as the minimum of the dominant, additive, and recessive genetic models was selected. For robust estimation purposes, the dominant and recessive genetic models were not computed if there were less than 10 and 20 individuals homozygous for the minor allele, respectively (this threshold is not applicable for SNPs with the non-significant lack-of-fit test). Interaction analysis was performed to test the beta-coefficient of the interaction variable using the same genetic model as the main effect. Genome-wide significance was defined as a P value less than 5×10^{-8} and suggestive significance was defined as a P value less than 5×10^{-7}. A genetic locus is defined as a

genetic region (<1MB) with a cluster of correlated variants (r^2>0.4). A novel signal is defined as one that is more than 500kb away from a known CT phenotype-associated locus.

Replication

Replication of loci that attained genome-wide significance in IRASFS was undertaken among Mexican American participants from the Multi-ethnic Study of Atherosclerosis (MESA; n=485). MESA is a multiethnic cohort of participants that were free of clinical cardiovascular disease at enrollment.(19) Protocols were approved by the Institutional Review Board at each participating institution. All participants provided written informed consent. Assessment of adiposity by CT has been previously described.(20) Genotypes from MESA were obtained from the Affymetrix Genome-Wide Human SNP Array 6.0 with imputation to the 1000G phase I V3 integrated reference panel. The statistical analysis in MESA followed the same protocol as described for IRASFS.

Results

A demographic summary of the study samples is shown in Table S1. Overall, individuals were overweight with an average BMI greater than 28.3kg/m^2. In total, 983 and 1,205 individuals were analyzed for GWAS imputed and exome chip SNPs, respectively. Compared to GWAS, an additional 222 samples were included in the exome chip data, of which 150 were individuals with T2D. This resulted in modestly increased age and adiposity traits (P<0.001). In addition, mean trait values of adiposity phenotypes were significantly different between females and males, i.e. females had significantly larger amounts of SAT (P<0.0001) while males had larger amounts of VAT (P<0.0001). The ratio between VAT and SAT (VSR) was two times greater in males than females (P<0.0001), suggesting males have the predisposition to store fat viscerally. Overall, 7,708,309 SNPs with MAF≥1% were analyzed with SAT, VAT, VSR, and VAT_BMI (VAT with additional adjustment of BMI). A complete list of quantile-quantile (q-q) plots can be found in Figure S1.

Imputation quality

The majority of the SNPs analyzed were derived from statistical imputation as opposed to direct genotyping. To evaluate imputation quality, concordance analysis for 10,000 SNPs overlapping between exome chip and imputation was performed. Overall, SNP genotypes were well-matched between the two platforms with an r^2>0.95 (Figure S2). However, for rare variants with MAF<1%, the imputation quality varied and therefore these were excluded from analysis.

Association Results

Association analyses were computed for SAT, VAT, VAT_BMI, and VSR adjusting for age, sex, recruitment center, and admixture estimates. The association results are summarized in Figure S3, Table 1. The strongest signal identified was SNP rs2185405 ($P_{rec}=1.98\times10^{-8}$, MAF=40%) with VAT. It is an intronic SNP located in the GLIS family zinc finger 3 gene (*GLIS3*). Association of this variant in MESA was nonsignificant (P=0.63, Table S3). In addition, suggestive evidence of association was observed with SAT (rs2223471 and

rs4746598), VAT (rs2131949 and rs78596136), VAT_BMI (rs4243443 and rs12657394), and VSR (rs1504143) (Table S2).

Sex-stratified Association Analysis

Results of the sex-stratified association analysis are summarized in Figure S4 and Table 1. Overall, five SNPs from two loci reached genome-wide significance ($P<5\times10^{-8}$). Two directly genotyped intronic SNPs (rs12657394, MAF=18.9%, $P_{male}=2.39\times10^{-8}$" $P_{female}=4.10\times10^{-3}$; rs2914610, MAF=19.2%, $P_{male}=4.55\times10^{-8}$, $P_{female}=5.58\times10^{-3}$) within the serum response factor binding protein 1 gene (*SRFBP1*) on chromosome 5 were strongly associated with VAT_BMI in males ($r^2=0.98$). Three imputed SNPs (rs1002945, rs13247968, and rs1830005, $r^2>0.91$) located downstream of the sorting nexin 13 gene (*SNX13*) were significantly associated with VAT_BMI in males but not females (rs13247968, MAF=42.9%, $P_{male}=7.63\times10^{-9}$, $P_{female}=0.36$). Analysis of significant results in MESA failed to provide replication (P>0.35, Table S3) although a consistent direction of effect was observed for the two variants in *SRFBP1* (rs12657394 and rs2914610). Signals of suggestive significance ($P<5\times10^{-7}$) are summarized in Table S2.

SNP-sex Interaction Analysis

Results of the SNP-sex interaction are summarized in Figure S5 and Table 1. SNP rs9289345 was strongly associated with the interaction variable for VAT_BMI ($P_{int}=3.73\times10^{-8}$, MAF=1.3%). It is an intronic SNP located within transmembrane and coiled-coil domain family 1 gene (*TMCC1*). An intronic SNP within pappalysin 2 gene (*PAPPA2*) (rs10913233, $P_{int}=3.07\times10^{-8}$, MAF=13.8%) was associated with the interaction variable for VSR. SNP rs10923724, located upstream of T-box 15 gene (*TBX15*), was strongly associated with the interaction variable for VSR ($P_{int}=2.89\times10^{-8}$, MAF=38.1%). Association of these variants in MESA was non-significant (P>0.15, Table S3).

Assessment of Previously Identified Signals

Nine previously identified CT loci (9, 21, 22) were evaluated herein (Table S4). Consistent with previous findings, the fat mass and obesity-associated gene (*FTO*) signal was modestly associated with SAT in IRASFS (rs9922619, $P_{SAT}=4.01\times10^{-3}$, $P_{male}=5.98\times10^{-2}$, $P_{female}=6.20\times10^{-2}$) yet no interactive effect ($P_{SAT\_INT}=0.41$) was detected. In addition, SNP rs2123685 on chromosome 7 was modestly associated with SAT and VAT in males ($P_{SAT}=4.15\times10^{-2}$, $P_{VAT}=3.55\times10^{-3}$) and rs7374732 on chromosome 3 was modestly associated with VSR in males ($P_{VSR}=3.21\times10^{-2}$). Furthermore, 49 adipose deposition signals identified by GIANT using WHR were evaluated for association with all four phenotypes (Table S4). Overall, 21 signals were significantly associated (P<0.05) with at least one of the four CT phenotypes and 18 of the 21 signals exhibited gender-specific effects (P<0.05 for gender stratified or interaction analysis). The strongest signal observed was SNP rs2645294 ($P_{VSR\_INT}=1.20\times10^{-5}$). It is located in the 3′-UTR region of the tryptophanyl TRNA synthetase 2 mitochondrial gene (*WARS2*) and has been previously reported to be associated with WHR (11). Interestingly, SNP rs10923724, which is about 500kb downstream of rs2645294, was identified to have a significant sex-interactive effect ($P_{INT}=2.89\times10^{-08}$) with VSR. Further analysis revealed that the two SNPs were in modest linkage disequilibrium (LD) in IRASFS ($r^2=0.59$) and strong LD in Europeans ($r^2=0.93$ in

1000G CEU). However, the previous study revealed no sex specificity for SNP rs2645294 (11), suggesting a potentially different biology represented by WHR and VSR.

Discussion

Here we present a study combining genome-wide and exome chip arrays to investigate the genetic determinants of adipose tissue deposition in Mexican Americans from the IRASFS. The differentiation between SAT and VAT using CT provided more refined adiposity phenotypes compared to anthropometric measures. In addition, as SAT and VAT distributions vary by sex, sex-stratified as well as SNP-sex interaction analyses were performed. Signals with significant sex-specific effects were identified.

SNP rs2185405, an intronic SNP within GLIS family zinc finger 3 gene (*GLIS3*), was found to be genome-wide significant for the main effect with VAT without sex stratification ($P_{dom}=1.98\times10^{-8}$, MAF=40%)(Figure 1). On average, the carriers of the minor allele T have 19.91 cm^2 less VAT compared to non-carriers. *GLIS3* is a member of the GLI-similar zinc finger protein family and encodes a nuclear protein with five C2H2-type zinc finger domains. It functions as both a repressor and activator of transcription and is specifically involved in the development of pancreatic beta-cells, the thyroid, eye, liver and kidney (23). Previous studies have shown this gene is associated with diabetes in multiple ethnicities (24, 25). *In vitro* experiments suggested *GLIS3* modulates pancreatic beta-cell apoptosis via the regulation of a splice variant of the BH3-only protein Bim (26). Since VAT has been shown to be a risk factor for metabolic disorders (4, 5), it is possible that VAT is involved in the modulation of beta-cell function through *GLIS3*. Further evaluation of the SNP using glucose homeostasis traits in IRASFS revealed a significant association with insulin clearance (P=0.03) and a suggestive association with fasting insulin (P=0.09). However, acute insulin response (AIR) was not significant (P=0.64).

For sex-stratified analyses, five SNPs from two loci (*SRFBP1*, 7p21.1) reached genome-wide significance. *SRFBP1*, also named as *p49/STRAP*, was associated with VAT_BMI in males. Regional plots indicate long-range LD covering a 200kb region (Figure 2). The strongest signal in the region was an intronic SNP rs12657394 under a dominant model ($P_{male}=2.39\times10^{-8}$; $P_{female}=0.0041$; MAF=18.9%). In males, the minor allele carriers had a 24.6cm^2 increase in VAT compared to common allele homozygous individuals. Conditional analysis using rs12647394 as a covariate abolished the association signal, suggesting only one independent signal exists in *SRFBP1*. Previous studies have shown that the protein encoded by *SRFBP1* specifically interacts with an acidic amino acid motif in the N-terminus of GLUT4 in adipose cells, suggesting a possible role in biosynthesis and/or processing of GLUT4 in adipocytes (27). However, no biological evidence has been identified to explain sex specificity. Further evaluation of glucose homeostasis traits in IRASFS with and without sex stratification revealed modest association signals for rs12657394 with fasting insulin ($P_{add}=7.92\times10^{-3}$), fasting glucose ($P_{rec}=0.025$), $HOMA_{IR}$ ($P_{add}=0.03$) and $HOMA_B$ ($P_{dom}=0.03$). However, no sex-specific patterns were observed. Extended examination of this region in the GIANT consortium with WAIST, BMI, and WHR stratified by sex failed to replicate this signal. This could be due to ethnic heterogeneity as well as the limitations of anthropometric measures compared to CT-derived fat deposition.

At 7p21.1, SNP rs13247968 (MAF=42.5%, $P_{male}=7.63\times10^{-9}$, $P_{female}=0.36$) as well as two other highly correlated SNPs ($r^2\geq0.9$) were strongly associated with VAT after adjusting for BMI under the dominant model in males. On average, male carriers of the rs13247968 minor allele (G) had a 16.4 cm^2 increase of VAT compared to homozygous major allele carriers. The regional plot (Figure 3) reveals a tight cluster of signals located downstream of *SNX13* (Sorting nexin 13 gene, also known as *RGS-PX1*). The RGS (Regulator of G protein) domain of the encoded protein can function as a GTPase-activating protein for G alpha subunits of heterotrimeric G proteins while the PX (Phox) domain works as a sorting nexin protein involved in intracellular trafficking (28). Studies have shown that the protein can target lysosomes and delay lysosomal degradation of the EGFR (Epidermal growth factor receptor) (28). Further examination of this region in the GIANT consortium identified rs1990467, 100kb proximal to rs13247968, was associated with WC_BMI in males ($P=2.6\times10^{-4}$)(Figure S6) (29, 30). However, the correlation between rs13247968 and rs1990467 was poor ($r^2=0.00$ in IRASFS, $r^2<0.08$ in 1000G). Previous genetic studies have suggested *SNX13* is strongly associated with HDL cholesterol in European ancestry individuals (31). Evaluation of SNP rs13247968 with circulating cholesterol levels and BMI in IRASFS failed to detect significant associations ($P>0.05$). Interestingly, rs13247968 is 300kb upstream of histone deacetylase 9 gene (*HDAC9*), which encodes an important histone deacetylase that regulates transcriptional regulation, cell cycle progression, and developmental events. Genetic studies have suggested *HDAC9* is associated with multiple phenotypes including coronary artery disease (CAD), BMI, and vigorous physical activity in European and Hispanic populations (rs2107595, rs2853552, rs12666612, respectively) (32, 33). However, no strong LD was detected between rs13247968 and these previously identified *HDAC9* signals ($r^2<0.01$).

The pappalysin 2 or pregnancy-associated plasma protein A2 gene (*PAPPA2*) located on chromosome 1 (rs10913233, $P_{Add}=3.07\times10^{-8}$, MAF=13.8%) was associated with the SNP-sex interaction effect for VSR (Figure 4). The protein encoded by *PAPPA2* has been proposed as a biomarker for preeclamptic placentae in pregnant women. It works as a protease specifically cleaving insulin-like growth factor binding protein 5 (IGFBP5) and thus plays an important role in regulating IGFBP5 levels (34). Diseases related to this gene include HELLP-syndrome and developmental dysplasia of hip (35, 36). Human population genetic studies have suggested this gene is associated with height (37). Previous mice studies have identified this gene to be associated with body size and weight, bone size and shape, postnatal growth retardation with more pronounced phenotypes in female mice compared to male mice (38). Further examination of this region in the GIANT Consortium identified modest signals for WHR and WHR_BMI in females (rs10913190, $P_{WHR}=5.6\times10^{-4}$, rs10913282 $P_{WHR\_BMI}=8.6\times10^{-4}$; $r^2<0.01$ with rs12090061 in IRASFS) (Figure S7).

SNP rs10923724 was significantly associated with the SNP-sex interaction variable with VSR ($P_{add}=2.89\times10^{-8}$, MAF=38%) (Figure 5). Sex-stratified analysis revealed rs10923724 was nominally associated in males and females with opposite directions of effect ($P_{male}=3.84\times10^{-4}$, $\beta_{male}=-0.97$; $P_{female}=2.31\times10^{-3}$, $\beta_{female}=0.10$). It is an intergenic SNP located at 1p12, 14kb upstream of *TBX15* and 27kb downstream of the tryptophanyl tRNA synthetase 2 gene (*WARS2*). *WARS2* is one of the two isoforms of mitochondrial

aminoacyl-tRNA synthetases that catalyzes the aminoacylation of tRNA (23). The T-box 15 gene (*TBX15*) is a member of the T-box family. The family encodes phylogenetically conserved transcription factors that regulate developmental processes (23). Diseases related to the gene product include Cousin Syndrome and congenital heart malformations (39, 40). Interestingly, this locus has been identified as one of the top WHR genetic signals in multiple ethnicities i.e. rs2645294 ($P=1.7\times10^{-19}$) and rs984222 ($P=8.69\times10^{-25}$) were associated with WHR without sex-specific patterns (11, 41). In IRASFS, the two SNPs were highly correlated ($r^2=0.87$) with strong associations with the interaction variable of VSR (rs2645294, $P_{VSR\_int}=1.2\times10^{-5}$; rs984222, $P_{VSR\_int}=2.63\times10^{-7}$). However, they were not associated with non-interactive effects of VSR and WHR ($P>0.10$). SNP rs10923724 has nominal correlations with the previous two SNPs ($r^2<0.58$) and was not associated with WHR ($P=0.60$). Analysis of rs10923724 conditioned by rs2645294 and rs984222 revealed nominal association ($P=0.040$) in males and no association in females ($P=0.98$). These results suggest there are multiple signals in the region with and without sex-specific heterogeneity. Interestingly, GTEx Portal suggested a strong eQTL signal for rs10923724 with *WARS2* expression in multiple tissues including skeletal muscle and adipose tissue ($P=3.3\times10^{-27}$) (42). Taken together, 1p12 is an interesting locus with complicated signals combining sex-specific and non-sex-specific mechanisms.

Although significant signals have been identified, study limitations do exist. First, the majority of the SNPs analyzed were from statistical imputation as opposed to direct genotyping. Although SNP genotypes were highly concordant between platforms, the imputation quality was reduced for rare variants with MAF<1% (Figure S2). Therefore, only SNPs with MAF≥1% were included in analysis. More specifically, the most significant results with the exception of one were common variants. Another limitation was the small number of available individuals which largely limited the study power, i.e. interaction and sex-stratified analyses. Replication efforts focused on Mexican American participants from MESA; however, a modest sample size (n=485) likely impacted the lack of replication observed. In addition, limited biological evidence was found to support sex-specific signals identified by genetic studies. This is likely attributed to the small number of biological studies that have been focused on sex-specific mechanisms of adipose deposition.

Increasing evidence has supported disease susceptibility heterogeneity related to adipose tissue depots: subcutaneous adipose tissue is benign while visceral adipose is correlated with metabolic risks (5). The use of CT scans has enabled a more direct estimate of regional adiposity, i.e. differentiate between subcutaneous and visceral adipose tissue. In addition, CT scans are less prone to user bias as all scans were conducted under the same protocol and results were sent to a centralized reading center. In contrast, anthropometric measures can often be biased by age, sex, clinical site, etc. (6). CT measures can include bias as well. For example, females predominantly store body fat in the gluteal-femoral region while males store fat in abdominal region (8). However, CT measures in this study were obtained by axial slices at the L4-L5 disc space and therefore no gluteal adipose tissue was measured. This may explain why fewer female signals were observed.

In summary, we computed a combined study of genome-wide and exome chip arrays in the IRASFS Mexican American cohort. Adiposity phenotypes included were SAT, VAT, VSR,

and VAT_BMI. Sex stratification and formal SNP-sex interaction analyses were conducted to search for signals with sex-specific effects. These findings support a genetic basis to the differential mechanism of adipose tissue distribution by sex. Moreover, these results highlighted the importance of using more refined measures of adiposity (CT scans) as well as the added utility of research in minority populations where an increased prevalence of adiposity-related diseases may be associated with a different genetic architecture.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Study questions

What is already known about this project

- Genetic variation contributes to body fat deposition.
- Males and female have distinct fat deposition patterns.
- Regional fat deposition is a strong risk factor for metabolic disease.

What does your study add

- Novel genetic signals associated with body fat deposition were identified.
- Novel genetic signals with sex-specific fat deposition effects were identified.
- Previously identified fat deposition genetic signals were replicated.

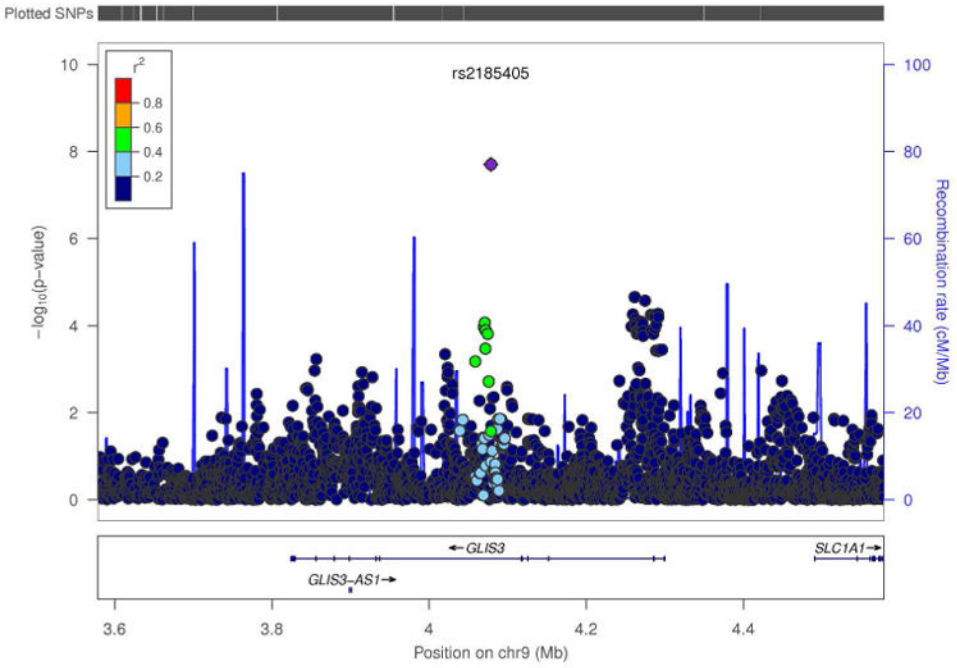
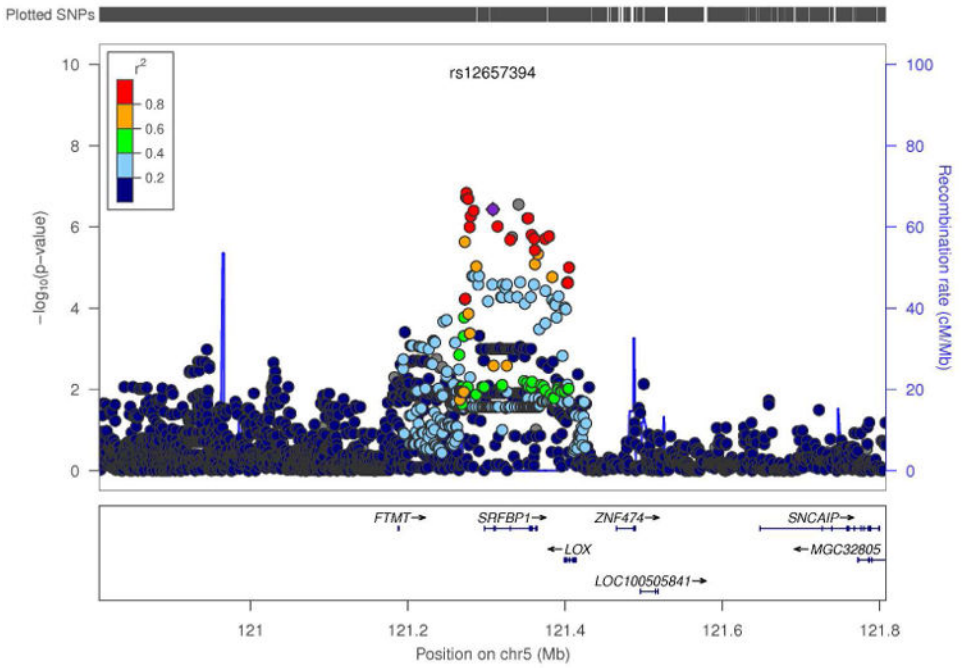
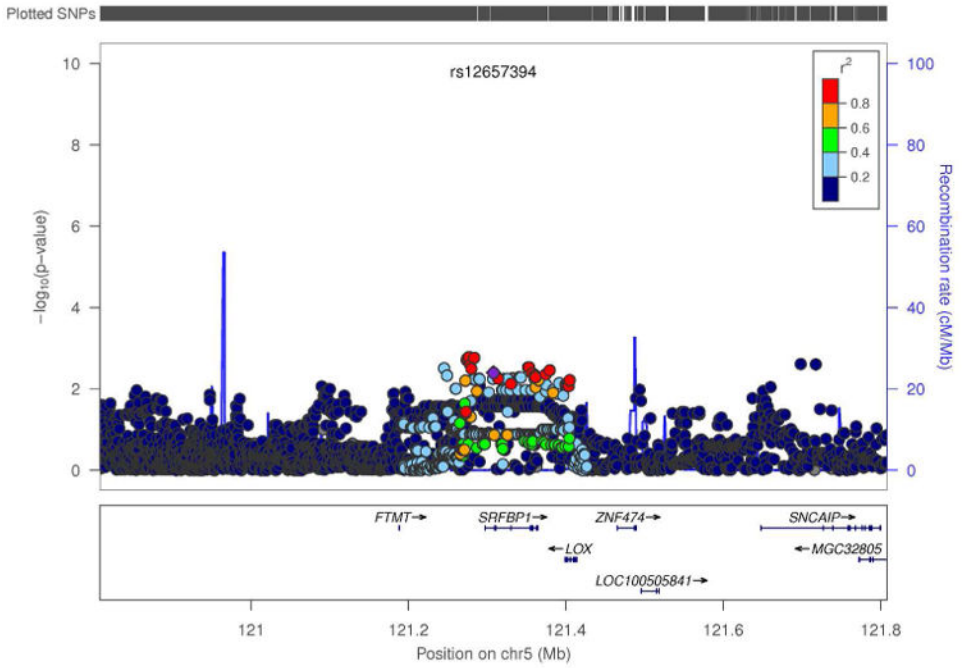


Figure 1.

Regional plots of *GLIS3* for association with visceral adipose tissue (VAT) in IRASFS Mexican Americans combining genome-wide and exome chip datasets.



a



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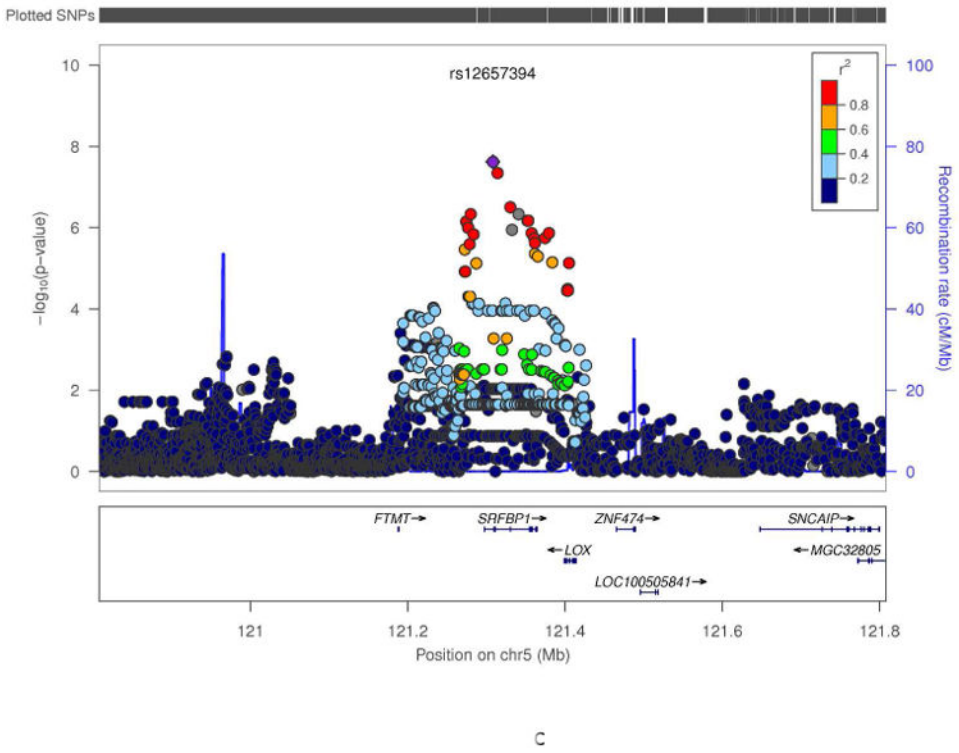
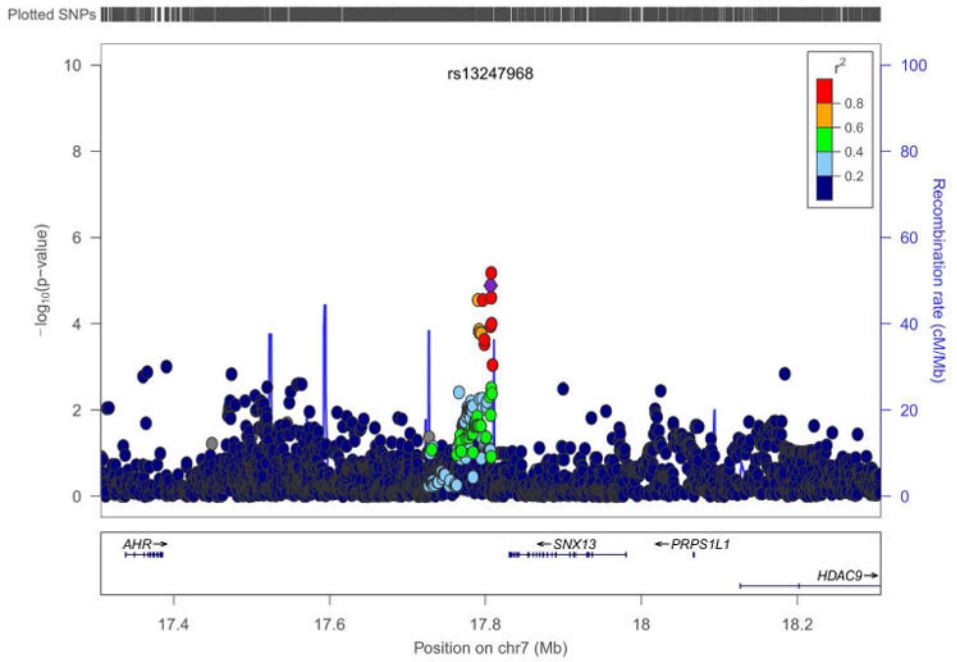


Figure 2.

Regional plots of *SRFBP1* for association with visceral adipose tissue adjusted by BMI in IRASFS Mexican Americans combining genome-wide and exome chip datasets. **A.** without sex stratification, **B.** females only, C. males only.



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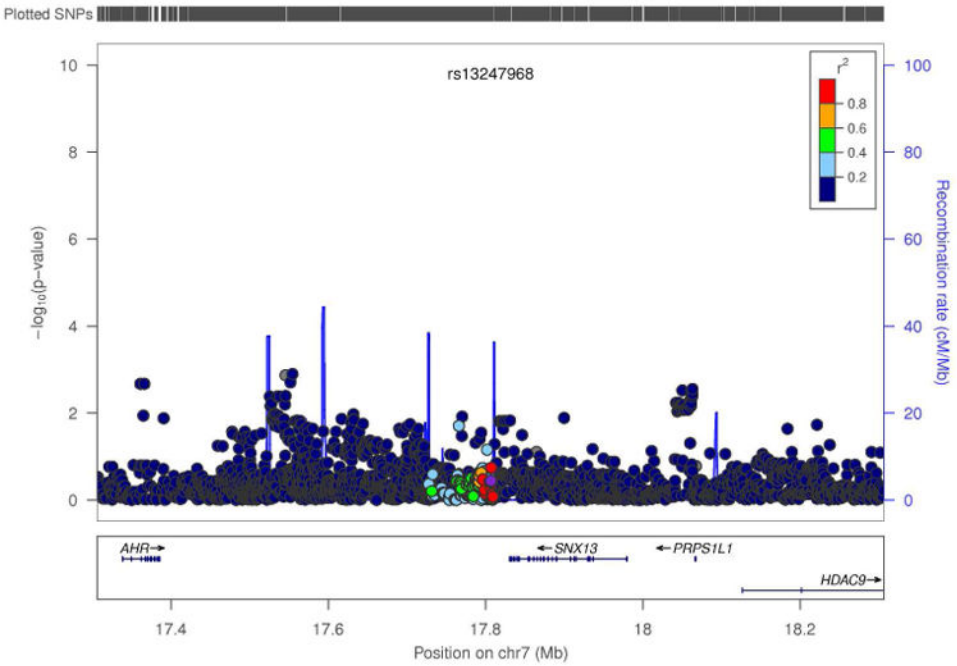

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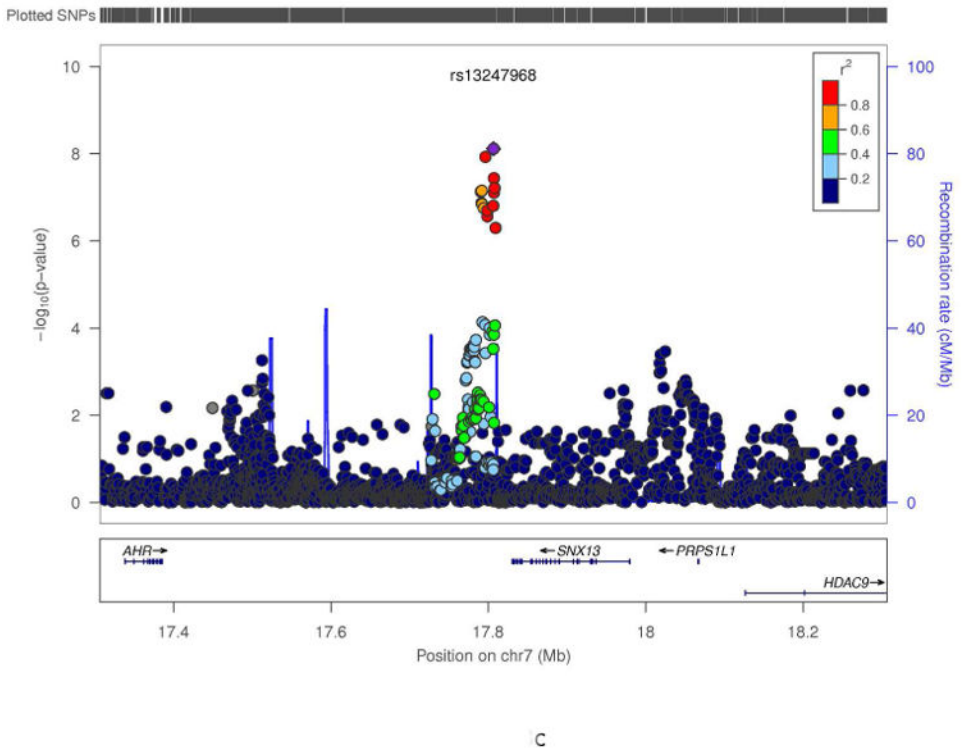

Figure 3.

Regional plots of the rs13247968 locus for association with visceral adipose tissue adjusted by BMI in IRASFS Mexican Americans combining genome-wide and exome chip datasets. **A.** without sex stratification, **B.** females only, **C.** males only.

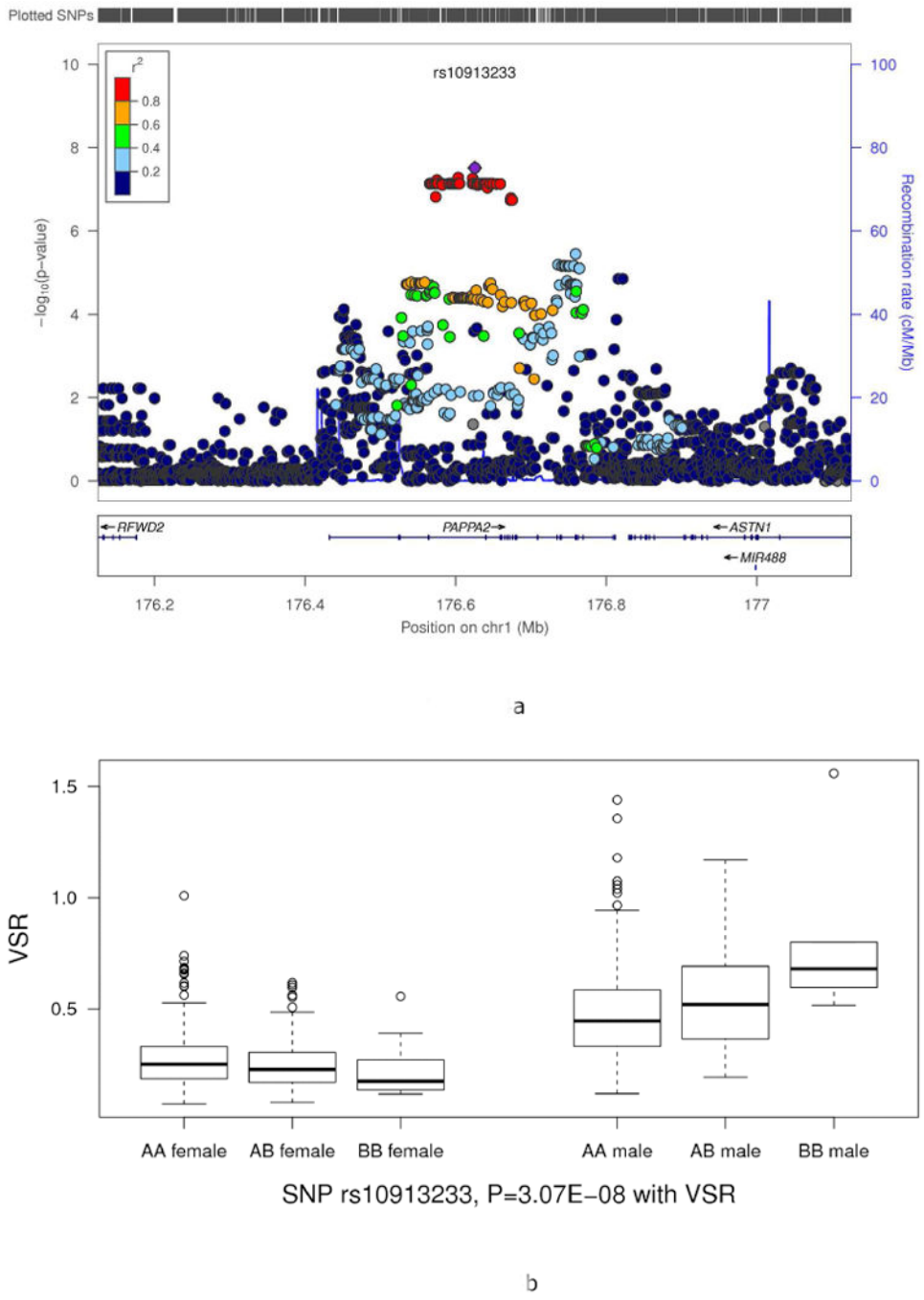

Figure 4.

SNP rs10913233 was associated with the SNP-sex interaction variable for VSR. **A.** Regional plots of the rs10913233 locus for interaction analysis, **B.** genotypic means of rs10913233 SNP-sex interaction analysis results stratified by sex.

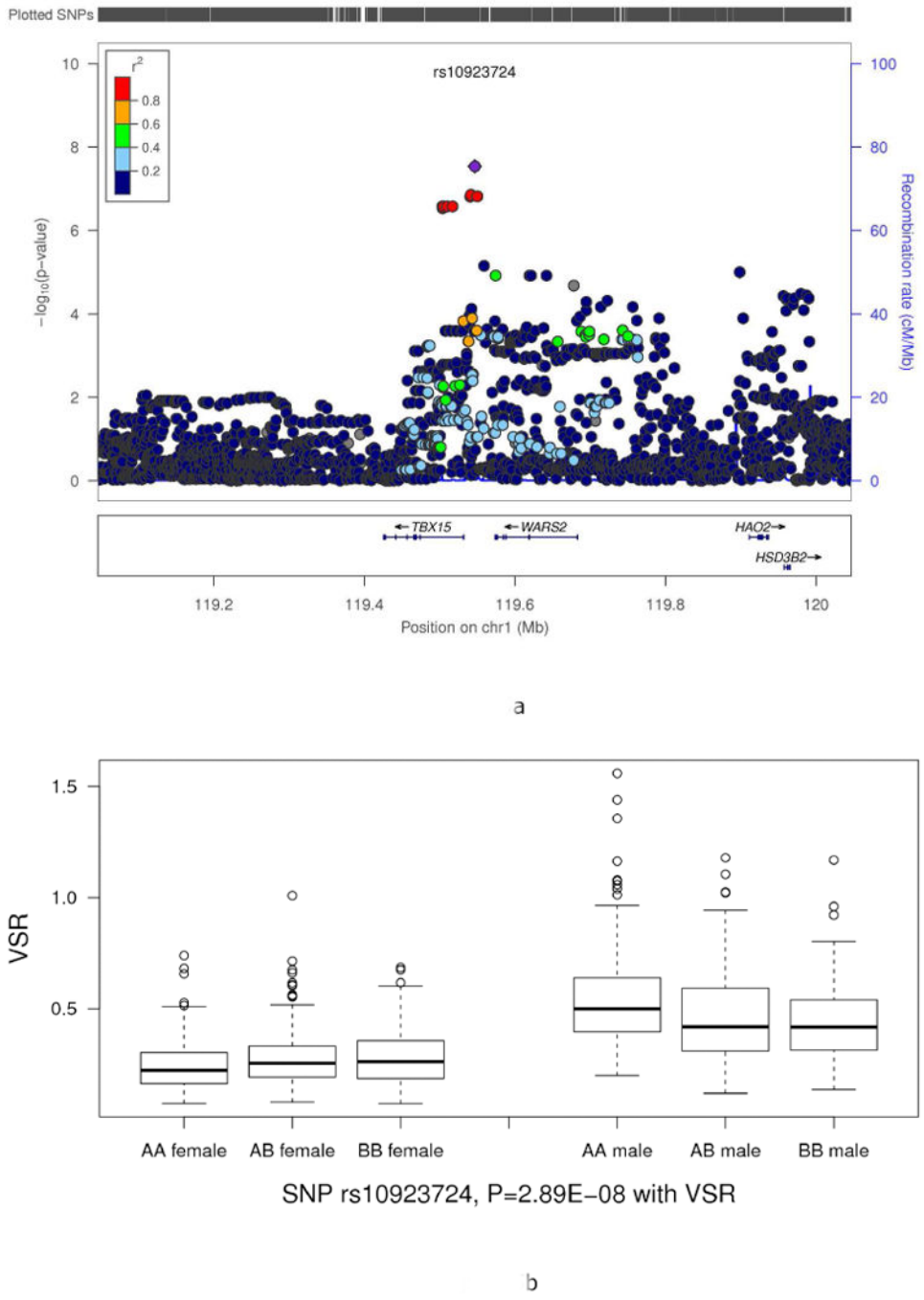

Figure 5.

SNP rs10923724 was associated with the SNP-sex interaction variable for VSR. **A.** Regional plots of the rs10923724 locus for interaction analysis, **B.** genotypic means of rs10923724 SNP-sex interaction analysis results stratified by sex.

Table 1

Genome-wide significant signals from SNP association analyses

SNP[a]	Chr:Pos	Gene	Alleles[e]	RAF[f]	Beta	P_overall (N=983)	Beta	P_female (N=580)	Beta	P_male (N=403)	P_interaction (N=983)
Visceral Adipose Tissue (VAT)											
rs2185405	9:4078851	*GLIS3*	T/C	0.40	-0.90	**1.98E-08**[d]	-0.76	1.36E-04[d]	1.11	1.10E-05[d]	0.69[d]
Visceral Adipose Tissue adjusted by BMI (VAT_BMI)											
rs12657394	5:121308199	*SRFBP1*	A/G	0.19	0.62	3.68E-07[c]	0.35	4.10E-03[b]	1.09	**2.39E-08**[c]	1.10E-03[c]
rs2914610	5:121314168	*SRFBP1*	A/G	0.19	0.59	9.78E-07[c]	0.34	5.58E-03[b]	1.07	**4.55E-08**[c]	1.53E-03[c]
rs1002945	7:17796659	*AHR-SNX13*	A/T	0.43	0.50	2.80E-05[c]	0.10	0.33[b]	1.09	**1.17E-08**[c]	1.37E-04[c]
rs13247968	7:17806817	*AHR-SNX13*	G/T	0.42	0.53	1.30E-05[c]	0.10	0.36[b]	1.11	**7.63E-09**[c]	1.59E-04[c]
rs1830005	7:17807563	*AHR-SNX13*	C/T	0.43	0.55	6.72E-06[c]	0.15	0.18[b]	1.07	**3.67E-08**[c]	8.70E-04[c]
rs9289345	3:129579506	*TMCC1*	G/A	0.01	0.35	0.51[b]	1.40	0.017[c]	-4.59	2.90E-04[c]	**3.73E-08**[b]
Visceral Subcutaneous Adipose Ratio (VSR)											
rs10913233	1:176625427	*PAPPA2*	T/A	0.14	-0.011	0.68[b]	-0.087	4.94E-03[b]	0.13	4.66E-03[c]	**3.07E-08**[b]
rs10923724	1:119546842	*TBX15/WARS2*	C/T	0.38	-0.0078	0.68[b]	0.099	2.30E-03[c]	-0.098	3.84E-04[b]	**2.89E-08**[b]

[a] SNP in build GRCh37/hg19;

[b] Additive model;

[c] Dominant model;

[d] Recessive model;

[e] Minor/Major allele;

[f] Reference allele based on minor allele.

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