Body mass index is a mediumiator of the genetic association between STK39 and blood pressure in Mexican women

Alicia Huerta-Chagoya
National Autonomous University of Mexico
Hortensia Moreno-Macías
Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán
María Luisa Ordoñez-Sánchez
Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán
Clicerio González-Villalpando
Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán
Carlos Alberto Aguilar-Salinas
Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán
Gerardo Gamba
National Autonomous University of Mexico
G.-Behn-Eschenburg Sebastián
National Autonomous University of Mexico
Montserrat Marroquín-Rodríguez
Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán
Teresa Tusié-Luna
National Autonomous University of Mexico
María Chávez-Canales (maria@iibiomedicas.unam.mx)
Unidad de Investigación UNAM-INC, Instituto Nacional de Cardiología Ignacio Chávez

Research Article

Keywords: body mass index, hypertension, Mexican, rs6749447, STK39, SPAK, mediation, adipose tissue

Posted Date: March 16th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1328727/v1

Abstract

Background

Although obesity causally relates to hypertension, the underlying genetic mechanisms are not completely understood. *STK39* gene, encoding the SPAK kinase, associates with both hypertension and body mass index (BMI). In a murine model, we previously showed that the inactivation of SPAK resulted in a hypotensive and obesity-resistant phenotype.

Methods

We analyzed the mediator effect of BMI on the association between *STK39* and systolic blood pressure (SBP) in a sample of 2,853 Mexican adults. We also assessed the *STK39* expression patterns in human adipose tissue, a relevant tissue determining BMI.

Results

We found that a *STK39 locus*, tagged by rs6749447 genetic variant, has a positive and significant direct effect on blood pressure in women (B(SE) = 0.073(0.028), P = 0.010), as well as an indirect effect through the BMI (B(SE) = 0.010(0.004), P = 0.024), therefore showing that BMI is a mediator of *STK39* and SBP. None of the effects were significant in men (direct effect: B(SE) = 0.030(0.031), P = 0.329; indirect effect: B(SE) = 0.124(0.255), P = 0.626). Additionally, we found that *STK39* is expressed in subcutaneous adipose tissue with similar steady-state levels to *PPARG* and *LIPE* genes, and its expression is higher in women than in men (P = 0.0008).

Conclusions

Our results shed light on the genetic basis of obesity-induced hypertension and outline a specific *locus* within *STK39* as an important modulator of this process.

Introduction

The prevalence of obesity and its associated comorbidities have reached pandemic proportions. Nearly two billion obese and over 650 million overweight adults were reported by the World Health Organization in 2016.[1] By the year 2025, obesity prevalence is expected to increase to 18% in men and 21% in women.[2]

The epidemiological relationship between the excess of adiposity and the increase in blood pressure has been extensively documented. The mechanisms involved in the pathogenesis of obesity-related hypertension includes the activation of the sympathetic nervous and renin-angiotensin-aldosterone

systems, adipocyte dysfunction, insulin resistance, as well as structural and functional renal changes.[4] In addition, Mendelian randomization analyses have supported that BMI causally relates to hypertension and, at clinic trials, modest reductions in weight have resulted in a decrease in blood pressure (BP) in hypertensive patients.[5]

BMI is influenced by a number of variables, including sex. Males tend to accumulate more visceral fat, leading to the android body shape, which has been highly correlated to increased cardiovascular risk. In contrast, females accrue more fat in the subcutaneous depot prior to menopause. Although this may confer protection against the negative effects of the metabolic syndrome, with aging fat deposition may shift to favor the visceral depot.[6] Accordingly, the prevalence of both hypertension and high BMI increase more in women than in men during aging.[7] Some explanations involve sex hormones given their ability to affect vascular tone, smooth muscle cell growth, the sympathetic nervous system and, importantly, adiposity distribution. The genetic studies of BMI have identified more than 100 loci associated with sex-driven adipose distribution.[8] Similarly, a recent study showed evidence of heterogeneity of the genetic effects of hypertension-associated variants between males and females and, that the variants leading sex-specific genetic susceptibility may relate to genes involved in steroid hormone receptor biosynthesis pathway.[9]

Alongside studies in human populations, rodent models have been a valuable resource for the better understanding of hypertension and its relation to adiposity. In a recently published study, a hypotensive $SPAK^{T243A/T243A}$ mice also exhibited an obesity-resistant phenotype. SPAK or Ste20-related proline/alanine rich kinase is a member of the GCK superfamily of serine/threonine kinases[10] that has a key role in the development of hypertension. Prevention of SPAK activity in mice, by generating the knockin mice colony $SPAK^{T243A/T243A}$ that no longer can be phosphorylated and thus activated by WNK upstream proteins, resulted in decreased expression and phosphorylation of the renal thiazide-sensitive Na^{+}-Cl^{-} cotransporter NCC, with the consequent salt remediable arterial hypotension phenotype[11].

Remarkably, $SPAK^{T243A/T243A}$ mice fed with a high-fat diet developed a significantly lower increase in body weight than their respective controls, despite no significant differences in food intake. The resistance to obesity was associated with increased UCP1-mediated thermogenesis in the brown adipose tissue and higher skeletal muscle mitochondrial content, resulting in increased whole-body oxygen consumption and thermogenesis. Thus, the inactivation of SPAK resulted in an obesity-resistant phenotype[13], which brings powerful evidence of the interplay between hypertension and BMI.

In humans, several research groups have associated *STK39* gene, encoding SPAK kinase, with hypertension across diverse populations, though a few have failed to replicate such association.[14–16] In a study using an integrated approach of comparative genomics between humans and pigs, Kim *et al.* reported syntenic chromosomal regions associated with back-fat thickness in pigs and subscapular skinfold thickness in humans.[17] Among those common genes for obesity and blood pressure was *STK39*, suggesting that it could be both a hypertension and obesity susceptibility gene.[18] Akiyama *et al.* also supported this by identifying an association between a variant within *STK39* and BMI in the

Japanese population.[19] Regarding the hypertension sex-differentiated effect, there is evidence that both SPAK expression and activity increase in the kidney of females compared to males in both rats and mice and that ovariectomy ablated these differences.[20] McCarthy *et al.* reported an additional example of sex hormones modulating *STK39* by showing an estradiol-induced increase in gene expression of *STK39* and its paralog *OSR1* in the immature hypothalamus of rats.[21]

Given the experimental and genetic evidence implicating the SPAK kinase in regulating both body mass and blood pressure, the aim of this study is to disentangle the direct and putative body-mass-mediated effect of *STK39* genetic variants on blood pressure in Mexican adults. Because, particularly in women, blood pressure increases with age accompanied by weight gain, we also aim to study sex-stratified SPAK effects.

Methods

Study subjects

Study sample comprised 2,853 Mexican individuals (with parents and grandparents born in Mexico). Participants were recruited from *i*) the Outpatient Diabetes Clinic of the Department of Endocrinology and Metabolism of the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán (INCMNSZ) (N = 1953) and *ii*) the "Mexico City Diabetes Study" [13], a population-based cohort of the Instituto Nacional de Salud Pública (INSP) (N = 900).

Men or women aged 25 years or older, with BMI greater than 20 but lower than 40 kg/m^2 were included. Pregnant women, individuals with drug addictions and under the use of systemic corticosteroids in pharmacologic doses were excluded. Additional exclusion criteria included active liver disease (defined as AST (SGOT) or ALT (SGPT) > 2.0x upper limit of the normal range, alkaline phosphatase (ALK-P) > 1.5x upper limit of the normal range, or total bilirubin > 1.5x upper limit of the normal range) and significant renal dysfunction (defined as serum creatinine > 1.7 upper limit of the normal range or nephrotic syndrome).

Measurements

All assessments were performed at morning, after a 9-12hr fasting period. The evaluation comprised a clinical examination using standardized questionnaires, anthropometric measurements, and a blood draw. Demographic information and a medical history, including personal and family history of common chronic diseases was obtained.

All serum samples were kept frozen until processed in a central laboratory certified by the External Comparative Evaluation of Laboratories Program of the College of American Pathologists (Departamento de Endocrinología y Metabolismo, INCMNSZ, Mexico City). Clinical chemistry parameters and the lipid profile were measured using commercially available reagents (Synchron CX5 delta, Beckman Coulter).

Immunonephelometric methods were applied for the measurement of C-reactive protein (BN ProSpec, Siemens). Insulin concentrations were measured using an ELISA method (AxSYM, Abbott).

Resting BP was measured under standard procedures and recommendations. All observers were standardized by a physician. After 5 minutes of rest, BP was measured by auscultation using a standard clinical sphygmomanometer. Measurements were made on the right arm in a sitting position with the elbow at the level of the right atrium using an appropriately sized cuff.

Arterial hypertension was diagnosed according to the AHA guidelines. Diagnosis of type 2 diabetes was done following the American Diabetes Association criteria, *i.e.*, fasting plasma glucose values ≥ 126 mg/dl, current treatment with a hypoglycemic agent or casual glucose values ≥ 200 mg/dl.

DNA purification, SNP genotyping and quality control

Genomic DNA was isolated from whole blood using the QIAmp 96 DNA Blood Kit. Purity and concentration were obtained with a NanoDrop ND 1000.

Genotype data were obtained from the Illumina OMNI 2.5 array. SNP imputation was performed by pre-phasing with HAPI-UR34 version 1.01 and imputation with IMPUTE version 2.2.0 with the 1000 Genomes Phase I integrated variant set32 (build 37 and haplotype release date in August 2012) serving as our reference panel. The analysis included all imputed variants with minor allele frequency (MAF) ≥ 1% and INFO score ≥ 0.6. We focused on *STK39* genetic region (GRCh37/hg19: 168,810,530 – 169,104,651 bp) and conducted genotype data quality control using PLINK.[22] SNPs with 5% or more missing data within the full dataset were removed. In addition, samples with 5% or more missing data and SNPs with < 5% MAF within the full dataset were also removed. All analyzed SNPs were tested for Hardy Weinberg equilibrium (HWE) (P value < $1x10^{-6}$). SNPs that failed to pass HWE test were excluded from further analyses.

In order to control for population stratification, a principal components analysis was performed on ~ 300,000 random genetic variants with MAF > 0.05, using EIGENSTRAT.[23] The top 2 principal components (PC) were used as covariates for correcting for population stratification, as they accounted for most of the total variance.

Blocks definition

Given the differences in linkage disequilibrium across human populations, instead of selecting candidate variants based on literature, we chose a selection method through which we defined blocks of correlated SNPs within *STK39* genomic region based on high linkage disequilibrium (LD) using HAPLOVIEW.[24] Blocks were defined by the 4-gamete rule algorithm. Although the software predicted 26 blocks, they were grouped into 5 larger and visually clear blocks–containing 10 or more SNPs–. For each of these 5 blocks, we randomly chose one representative SNP at the middle positions. The selected SNPs showed a high correlation with the remaining variants within each block. For block 3, we specifically chose rs6749447,

as it was previously associated with BP in Europeans.[14] Thus, we analyzed 5 representative SNPs within the *STK39* genomic region.

Association and mediation analyses

Before performing mediation analysis, we assessed the genetic association of the 5 representative SNPs with the levels of systolic blood pressure (SBP) using separate linear regression models adjusted for sex, age, squared age, status of diabetes, blood pressure medication as a binary covariate, recruitment site, and the top 2 PC. Since BMI was in the causal pathway between the selected SNPs and SBP, BMI was not included as covariate. We first assumed additive models and noticed that the effects were only seen among risk homozygous carriers. Thus, we tested the existence of non-additive effects as previously reported. [25] Briefly, we first fitted dominance deviation models and for variants showing evidence of non-additivity ($p < 0.05$ for the dominance deviation regression coefficient), we classified them as potentially-showing genotypic effects consistent with complete dominance or recessive action. Then, we fitted a second model setting the heterozygous genotype class as the reference category and the homozygous classes as different categories. Because the effect of the associated variants was only seen in risk homozygous *vs.* heterozygous ($P < 0.05$), we used recessive models in further analyses.

For those SNPs showing significant association with the SBP, we also evaluated their association with BMI. We performed the analyses via linear regression models adjusted for sex, age, squared age, status of diabetes, recruitment site and the top 2 PC.

As discussed before, given the already documented sex dimorphism of adipose tissue depots and its consequent underlying sex-related morbidities, including hypertension, we also assessed the effect of *STK39* variants on both BMI and hypertension through stratified models by sex. We used R software[26] for statistical analyses. Bonferroni correction was used to control for multiple testing.

When a SNP showed significant association with both SBP and BMI, we evaluated the role of BMI as a mediating variable between the SNP and SBP. We assessed the mediation effect of BMI through path analysis and estimated the standard errors by the bootstrap method. Comparative Fit Index (CFI), Tucker-Lewis Index (TLI), and Root Mean Squared Error of Approximation (RMSEA) were used for assessing the goodness of fit of the models. We used Mplus 8.4 software for this part of the analysis.

Functional association analyses

Although the role of SPAK in kidney function is well understood, it is not the case for adipose tissue, a relevant tissue determining BMI. Given the above, we used available Affymetrix Human Genome U133 Plus 2.0 array data to perform gene expression analyses. This dataset was independent of the discovery sample and comprised 74 unrelated Mexican case/control individuals recruited as part of a Familiar Combined Hyperlipidemia study. Fat biopsies from umbilical subcutaneous adipose tissue were collected. Supplementary table 6 summarizes the clinical characteristics of the subjects. We normalized CEL files using the RMA function, which applies background subtraction and quantile normalization. We

calculated expression values for genes with multiple probe sets by taking the median value of all probe sets for that gene.

We first assessed the expression levels of *STK39* and 1Mb surrounding genes, as well as the expression levels of two known highly expressed genes in adipose tissue (*PPARG* and *LIPE*). We also analyzed the expression levels of a set of genes which are part of the signaling cascade of *STK39*: *WNK1/4*, *OSR1*, *SLC12A1-3* and *SLC12A5*. Then, we assessed the differential expression of *STK39* gene based on several phenotypes and on rs6749447 genotype: i) by regressing the expression levels on either hypertensive and overweight status adjusted for sex and ii) by regressing the expression levels on the genotype counts of a recessive model adjusted for sex and BMI.

Finally, to better understand the functional implications of rs6749447 locus, we used the Common Metabolic Diseases Knowledge Portal (2021 Dec 15; https://md.hugeamp.org), which aggregates genetic, epigenomic and computational results from multiple diseases and traits. We also used GTEx Portal to assess rs6749447 effect on the expression of *STK39* and nearby genes.[27]

Results

Our sample included 2,853 Mexican individuals, of which 59.3% were women. On average, participants belonged to the sixth decade of life and showed borderline values indicative of hyperglycemia, hypertriglyceridemia or hypercholesterolemia. A large proportion of individuals had obesity, type 2 diabetes or high blood pressure (34.4, 38.6, 29.5%, respectively). Although a greater proportion of women were obese, men showed higher prevalence of hypertension, as well as a worse metabolic profile, as demonstrated by their higher levels of triglycerides, GGT, uric acid and creatinine (Table 1).

Table 1
Sample description.

	All	Women	Men	
	Mean ± sd (%)	**Mean ± sd (%)**	**Mean ± sd (%)**	**P**
N	2853	1693	1160	-
Sex (% women)	59.34	-	-	-
Age (years)	58.02 ± 10.4	58.28 ± 10.33	57.63 ± 10.5	0.1066
Obesity (%)	34.41	39.05	27.61	**< 0.001**
BMI (kg/m^2)	28.66 ± 4.52	29.06 ± 4.74	28.07 ± 4.09	**< 0.001**
Waist-hip ratio	0.94 ± 0.08	0.91 ± 0.08	0.97 ± 0.06	**< 0.001**
HTA (%)	29.50	27.95	31.83	**0.0437**
SBP (mmHg)	126.55 ± 18.99	126.2 ± 19.49	127.08 ± 18.21	0.2564
DBP (mmHg)	78.77 ± 10.29	78.04 ± 10.41	79.87 ± 10.01	**< 0.001**
BP medication (%)	34.23	38.70	27.68	**< 0.001**
Type 2 diabetes (%)	38.63	38.33	39.05	0.7283
Fasting glucose (mmol/l)	6.65 ± 3.52	6.58 ± 3.44	6.75 ± 3.64	0.2179
Fasting insulin (µIU/ml)	77.81 ± 55.01	76.4 ± 47.68	79.74 ± 63.65	0.3159
Hba1c (%)	7.49 ± 2.33	7.54 ± 2.33	7.42 ± 2.33	0.4487
Triglycerides (mmol/l)	2.26 ± 1.98	2.07 ± 1.27	2.53 ± 2.68	**< 0.001**
Total cholesterol (mmol/l)	5.29 ± 1.14	5.37 ± 1.09	5.17 ± 1.22	**< 0.001**
HDL cholesterol (mmol/l)	1.12 ± 0.33	1.21 ± 0.33	1 ± 0.29	**< 0.001**
GGT (IU/l)	25.49 ± 37.97	23.08 ± 28.11	29.12 ± 49.06	**0.0348**
ALT (IU/l)	28.27 ± 19.69	27.62 ± 18.74	29.24 ± 21.02	0.2381
HS-CRP (mg/dl)	3.11 ± 5.04	3.43 ± 4.96	2.67 ± 5.14	0.1547
Uric acid (mg/dl)	5.72 ± 5.79	5.04 ± 1.45	6.68 ± 8.74	**< 0.001**
Creatinine (mg/dl)	1.32 ± 3.3	1.11 ± 1.89	1.62 ± 4.61	**0.0257**

HTA is defined as SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg. Obesity is defined as BMI ≥ 30 kg/m^2. Women *vs.* men comparisons. P value from t-student test or chi square test.

We started the analyses with 3,912 SNPs within the *STK39* genetic region. After quality control, our dataset included 744 SNPs. All of them were grouped into five LD blocks and the following SNPs:

rs2044680, rs4668016, rs6749447, rs56088988 and rs10930316 (Supplementary Fig. 1) represented each one. As expected, a pairwise $r^2 < 0.2$ was found between pairs of variants (Supplementary Table 1). As previously mentioned, this unbiased approach was chosen over the evaluation of candidate SNPs from other studies because of differences in patterns of linkage disequilibrium across populations.

After multiple comparison correction, two SNPs, representative of the second and third blocks, showed a statistically significant association with SBP (rs4668016: B(SE)=-3.74(1.22) mmHg, P = 0.0021 and rs6749447: B(SE) = 3.46(0.96) mmHg, P = 0.0003) (Fig. 1 and Supplementary Table 2). We considered a significant P value of 0.003 (0.05/15 comparisons [5 SNPs assessed in the whole sample, in women-only and in men-only]). The effect of both SNPs was mainly seen in women-only (rs4668016: Women B(SE)=-5.31(1.55) mmHg, P = 0.0006 *vs.* Men B(SE) = 1.045(0.565) mmHg, P = 0.0645 and rs6749447: Women B(SE) = 4.652(1.255) mmHg, P = 0.0002 *vs.* Men B(SE) = 1.681(1.480) mmHg), P = 0.2563). Although not reaching statistical significance, a fixed-effects meta-analysis showed that 65.4% and 57.3% of the variability in blood pressure effects estimates was due to sex (rs4668016: I^2 = 65.4%, P = 0.089 and rs6749447: I^2 = 57.3%, P = 0.126). ((Fig. 1 and Supplementary Table 2).

We then continued the analysis only with the SNPs that showed significant association with SBP. When assessing the effect of the two SBP-associated SNPs with BMI levels, we found a nominal association of rs6749447 in the whole sample without stratifying by sex (B(SE) = 0.54(0.22) kg/m^2, P = 0.0127) (Fig. 1 and Supplementary Table 3). Risk allele G of rs6749447 variant showed a frequency of 0.423 in the sample (Supplementary Table 2).

As seen with SBP, the effect of rs6749447 over BMI was also stronger in women compared with men (Women: B(SE) = 0.81(0.3) kg/m^2, P = 0.0067; Men: B(SE) = 0.19(0.31) kg/m^2, P = 0.540), although the heterogeneity test did not reach statistical significance (I^2 = 51.6%, P = 0.151). After Bonferroni correction, the effect in women was statistically significant. We considered a significant P value of 0.008 (0.05/6 comparisons [2 SNPs assessed in the whole sample, in women-only and in men-only]) (Fig. 1 and Supplementary Table 3).

The effect of rs6749447 on SBP was stronger in postmenopausal women (age ≥ 55 years) (B = 7.216 mmHg, P = 3.78×10^{-05}) than in the whole female sample, premenopausal (age < 50 years) or menopausal women aged between 50 and 55 (B = 4.351 mmHg, P = 5.55×10^{-04}; B=-1.74 mmHg, P = 0.487 and B = 3.046 mmHg, P = 0.1806, respectively) (Supplementary Table 4).

The prevalence of the risk homozygote individuals (GG genotype) was 18.3%. No difference was found between the prevalence of the risk homozygote women and men (Women: GG *frequency* = 18.28%, Men: GG *frequency* = 18.31%, chi square P value = 1) (Supplementary Table 5).

In order to assess between a direct or a BMI-mediated effect of rs6749447 variant on systolic blood pressure and given the observed sex-related differences in the effect estimates, we performed a path analysis in both men and women separately. We stratified these analyses based on our previous findings

showing genotype effects mainly in women but not in men. After checking the model information in women (CFI = 1.0; TLI = 1.0 and RMSEA 90% CI (0,0.07), we found that besides having a direct effect on both SBP and BMI in women-only, rs6749447 showed an indirect BMI-dependent effect on SBP (B(SE) = 0.010 mmHg (0.004), P = 0.024). That is, 12% of the effect of rs6749447 on SBP is mediated by BMI in women. Interestingly, we found that rs6749447 also showed an indirect BMI-dependent effect on T2D (B(SE) = 0.007(0.004), P = 0.047). Regarding to the model considering men only, the model fit parameters were CFI = 0.99, TLI = 0.95 and RMSEA 90% CI (0,0.08); The rs6749447 variant did not show any direct effect on either SBP or in BMI (SBP: B(SE) = 0.030 mmHg (0.031), P = 0.329; BMI: B(SE) = 0.016 kg/m^2 (0.033), P = 0.624) (Figs. 1 and 2).

To further highlight the pleiotropic effects of SPAK in humans, we extracted the association P values of rs6749447 and surrounding ± 50 kb variants from the Common Metabolic Diseases Knowledge Portal. We found that besides renal traits, this genetic locus shows an enrichment of associations with anthropometric as well as sleep-wake patterns, such as excessive daytime sleepiness, short sleep duration or frequent insomnia symptoms ($P < 5x10^{-04}$) (Supplementary table 6).

When examining the expression level of *STK39* in subcutaneous adipose tissue, we found it was comparable with that of *PPARG* and *LIPE* genes, which are known to be highly expressed genes in adipose tissue. By examining the expression of the 7 genes surrounding 1Mb of *STK39 locus*, only *NOSTRIN* was found to be expressed at comparable levels. In contrast, *CERS6*, *G6PC2* and *DHRS9* showed low expression levels. Regarding the genes taking part in the *STK39* signaling cascade, *WNK1*, *OSR1* and *SLC12A2* genes also showed expression in subcutaneous adipose tissue. Interestingly, *STK39* gene expression was higher in women than in men (P = 0.0008). We did not observe the same sex dimorphic gene expression above in either *WNK1*, *OSR1* or *SLC12A12* genes (Fig. 3, Supplementary Tables 7 and 8).

Finally, we found an increase in *STK39* gene expression in adipose tissue of hypertensive individuals (B = 0.279, P = 0.0488). No statistically significant association was found between the *STK39* expression and overweight/obesity status, nor between non-risk and risk rs6749447 carriers, assuming a recessive model (Supplementary Table 8).

Discussion

There is robust evidence implicating the SPAK kinase as an important regulator of blood pressure. However, its involvement in the development of obesity was recently suggested. [13] In line with the above, our results showed that the rs6749447 variant, tagging a *locus* of 76.8 kb span within the *STK39* gene, increases both SBP and BMI levels in Mexican people. The rs6749447 variant was previously associated with the risk of hypertension in Europeans and Middle-Easterns[14], but not in Asian adult population.[28] In addition, in the Chinese population, rs3754777 (in LD with rs6749447 [r^2 in Mexicans = 0.40] and also within *STK39* LD block 3, ~ 25 Kb apart from rs6749447) was associated with diastolic blood pressure in overweight children.[29]

Interestingly, we found that the effect of rs6749447 over both SBP and BMI was stronger in women. Accordingly, in two Swedish cohorts, variant rs35929607 (in LD with rs6749447 [r^2 in Mexicans = 0.42] and also within *STK39* LD block 3, ~ 6 Kb apart from rs6749447) was found to be significantly associated with hypertension risk in women, but not in men.[30] Considering that no difference between women and men was found in the prevalence of the risk allele in our cohort, these findings suggest the influence of additional factors on the sex-dependent effect of rs6749447. In line with McCarthy *et al.* findings in hypothalamus of rats[21], here, we report that in subcutaneous adipose tissue, *STK39* also showed higher expression in women than in men.

We also found that the effect size of the rs6749447 variant over SBP was stronger among postmenopausal women, suggesting that its effect may depend on the body fat distribution and hormonal changes. Although very large sample sizes are required to statistically confirm sex-genotype interactions, sex differences have been previously reported in terms of the relationship between metabolic risk and fat distribution[6], potentially driven by hormonal influences.[31]

Here, we found a statistically significant association between *STK39* and body mass index. Consistently, in the Japanese population, rs2390669 variant within *STK39* (within LD block 3 and ~ 50 kb upstream from rs6749447, pairwise LD r^2 in Mexicans = 0.24) achieved a genome-wide significant association with BMI.[19] In addition, we previously reported that SPAK inactivation in mice results in an obesity-resistant phenotype characterized by increased thermogenesis in brown adipose tissue, reduced insulin resistance and elevated mitochondrial activity in skeletal muscle.[13]

Previous studies had implicated two obesity-related genes, *LEP* and *PCSK1*, with increased blood pressure levels.[32, 33] Some mechanisms of obesity-related hypertension include insulin resistance, sodium retention, activation of the renin-angiotensin-aldosterone axis and altered vascular function.[4] Our results showed that rs6749447 has direct effects on both BMI and SBP. The last one was BMI-independent, meaning rs6749447 influence SBP even in lean individuals. Besides this independent and direct effect of rs6749447 on BMI and blood pressure, we found a mediated effect of BMI between the association of the rs6749447 and blood pressure, where 12% of the effect of rs6749447 over SBP is mediated by BMI. To our knowledge, this is the first report implicating a BMI-mediated effect of *STK39* on blood pressure. This mediation by BMI on SBP is mainly seen in women. No direct significant associations were found between rs6749447 and glycemic traits or insulin resistance indexes. However, we found an indirect effect of rs6749447 over T2D mediated by BMI in women. Further research is needed to understand the mechanisms by which the rs6749447 effect over BMI mediates the SBP increase and potentially other metabolic traits, and it raises the question of whether carrier women will benefit more from an adiposity-reduction program to control and prevent hypertension.

Given its clear relationship with BMI, we only analyzed the *STK39* expression patterns in adipose tissue. *STK39* showed a significantly higher expression in women than in men. Also, a higher *STK39* expression in adipose tissue was found in individuals with hypertension as compared with individuals with normotension. We found *STK39* is expressed at comparable levels to *PPARG* or *LIPE* genes in

subcutaneous adipose tissue. *STK39* is part of a signaling cascade that regulates sodium and potassium transport across the plasma membrane, which includes *WNK1,4/OSR1/STK39/SLC12A1,2,3,5* genes.[34] Our results showed that *WNK1*, *OSR1* and *SLC12A2* and *SLC12A3* genes were also expressed in subcutaneous adipose tissue. However, *WNK4*, *SLC12A1* and *SLC12A5* gene expression were null or very low. The above supports the relevant role of WNK/SPAK pathway in obesity development. Even though the function of *STK39* surrounding genes is also of metabolic interest (*i.e. NOSTRIN*, *CERS6*, *G6PC2*, *DHRS9, ABCB11* and *LRP2)*, according with Genotype-Tissue Expression (GTEx) Project, rs6749447 ± 50 kb variants seem to regulate expression of *STK39* gene only.

Even though little is known about the role of *STK39* in adipose tissue, evidence has started to arise. However, the study of additional key biological tissues and pathways is required to understand the relationship among *STK39* gene expression, BMI, and blood pressure. For instance, variants near rs6749447 have been found associated with sleep-wake patterns. Given that there is evidence pointing out that rs6749447 and other linked variants are eQTLs of *STK39* expression in brain basal ganglia (data from GTEx Project), previously reported as a regulator of sleep-wake behavior, the study of the role of STK39 in other relevant tissues should also be taken into consideration for future studies.

Conclusions

In summary, we replicated a previous association of *STK39* gene with blood pressure and found a novel partial mediation of this effect by body mass index, specifically in Mexican women. Interestingly, we found that *STK39* expression in subcutaneous adipose tissue is higher in women than in men and that its relative expression level was comparable with that of highly transcribed adipocyte genes, such as *PPARG* and *LIPE*. Therefore, our results point out a potential role of *STK39* gene as an important regulator of obesity-induced hypertension.

Declarations

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was conducted with the approval of the Ethics and Research Committees of all participating Institutions. All participants gave written informed consent before we included them in the study.

CONSENT FOR PUBLICATION

All authors have approved the publication of the manuscript.

AVAILABILITY OF DATA

The genotype dataset supporting the conclusions of this article is available upon request. The microarray data can be accessed in MIAME compliant format from the NCBI Gene Expression Omnibus (GEO)

database (GSE17170).

COMPETING INTERESTS

The authors declare that they have no competing interests.

FUNDING

Partial funding was obtained from CONACyT grant to A H-C and M C-C CF-19-87794 and 262077 to T T-L and from “Fronteras de la Ciencia” grant No. 23 to GG. M C-C was funded by PAPIIT IN203620 and IA28522 from PAPIIT UNAM. C A-S was supported by a grant from the “Consejo Nacional de Ciencia y Tecnología (CONACyT)” (S0008-2009-1-115250) and a research grant by Sanofi. The MCDS (Mexico City Diabetes Study) has been supported by the following grants: RO1HL 24799 from the National Heart, Lung, and Blood Institute; Consejo Nacional de Ciencia y Tecnología 2092, M9303, F677-M9407, 251M, 2005-C01-14502, and SALUD 2010-2151165; and Consejo Nacional de Ciencia y Tecnología (CONACyT) [Fondo de Cooperación Internacional en Ciencia y Tecnología (FONCICYT)] C0012-2014-01-247974.

AUTHORS’ CONTRIBUTIONS

A.H.-C., C.A.A., T.T.-L. M.C.-C. conceived, designed, and oversaw the study. C.G.-V and the Study Group on Metabolic Diseases provided patient samples and genetic data. M.L.-O.-S., performed the genotyping. A.H.-C., H.M.-M. and -S, S.G.B.-E. performed quality control of the data and the statistical analyses. A.H.-C. and H.M.-M., analyzed and interpreted the data. A.H.-C. and H.M.-M performed and analyzed the expression assays in human samples. A.H.-C., H.M.-M., T.T.-L. and M.C.-C. drafted the manuscript, with input from C.A.A.-S., G.G., S.G.B and M.M.-R. All authors reviewed and approved the manuscript.

ACKNOWLEDGMENTS

We thank Saúl Cano-Colín, Norma Vázquez and María Elena González for their technical help.

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Figures

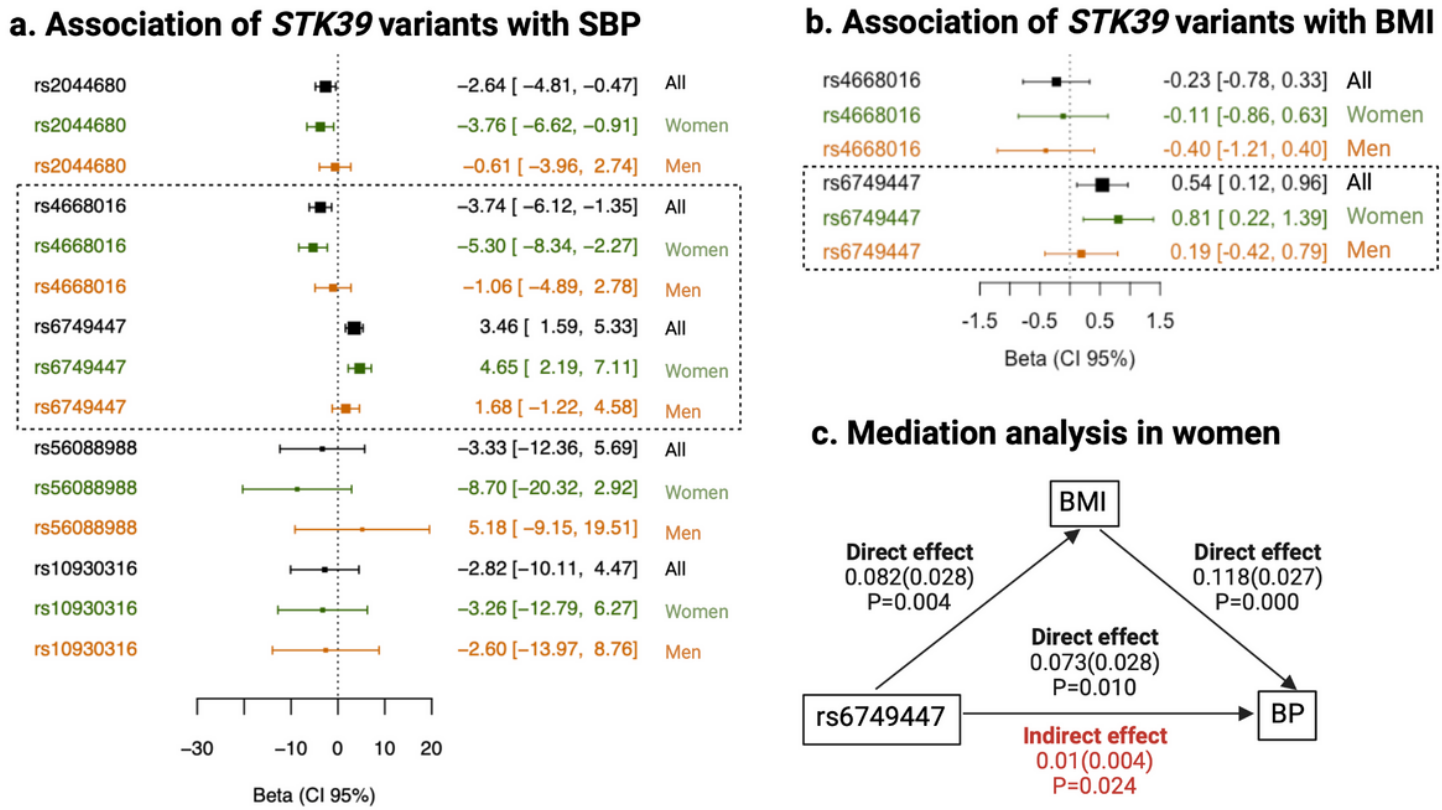

Figure 1

BMI mediated effect on the relationship of rs6749447 and blood pressure. a Association analysis of five representative SNPs within STK39 gene with SNP in the whole sample. **b** Association analysis of two blood pressure related SNPs with BMI in the whole sample, in women-only and in men-only. **c** Mediation analysis of rs6749447. Because no direct effects of rs6749447 on any of the traits were seen in men, the indirect effects of the SNP were only analyzed in women-only. The standardized path coefficients are shown. BP: blood pressure.

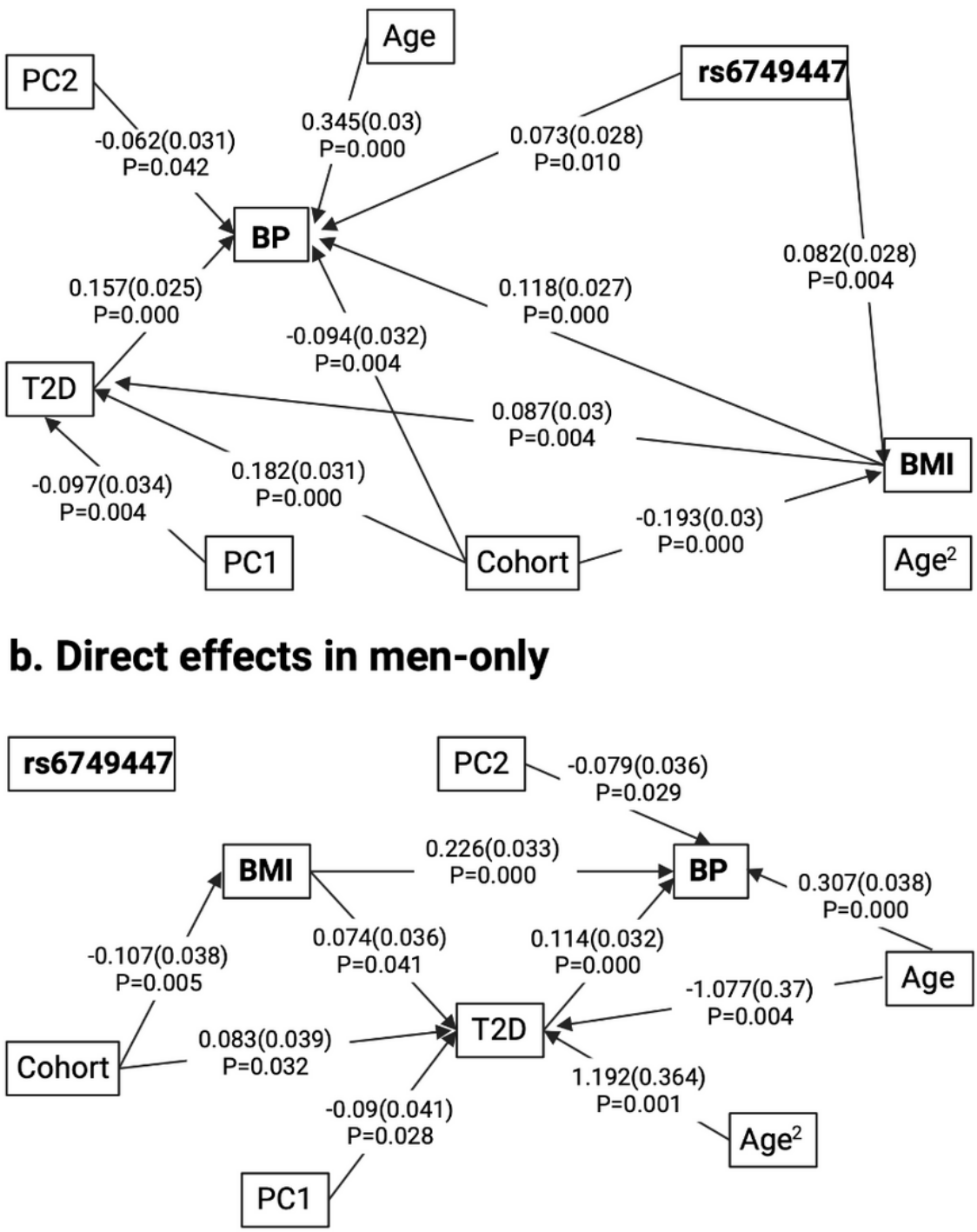

Figure 2

Standardized path coefficients. Direct effects between considered traits in women-only (**a**) and in men-only (**b**). BP: blood pressure, T2D: type 2 diabetes, PC1: ancestry top first principal component, PC2: ancestry top second principal component.

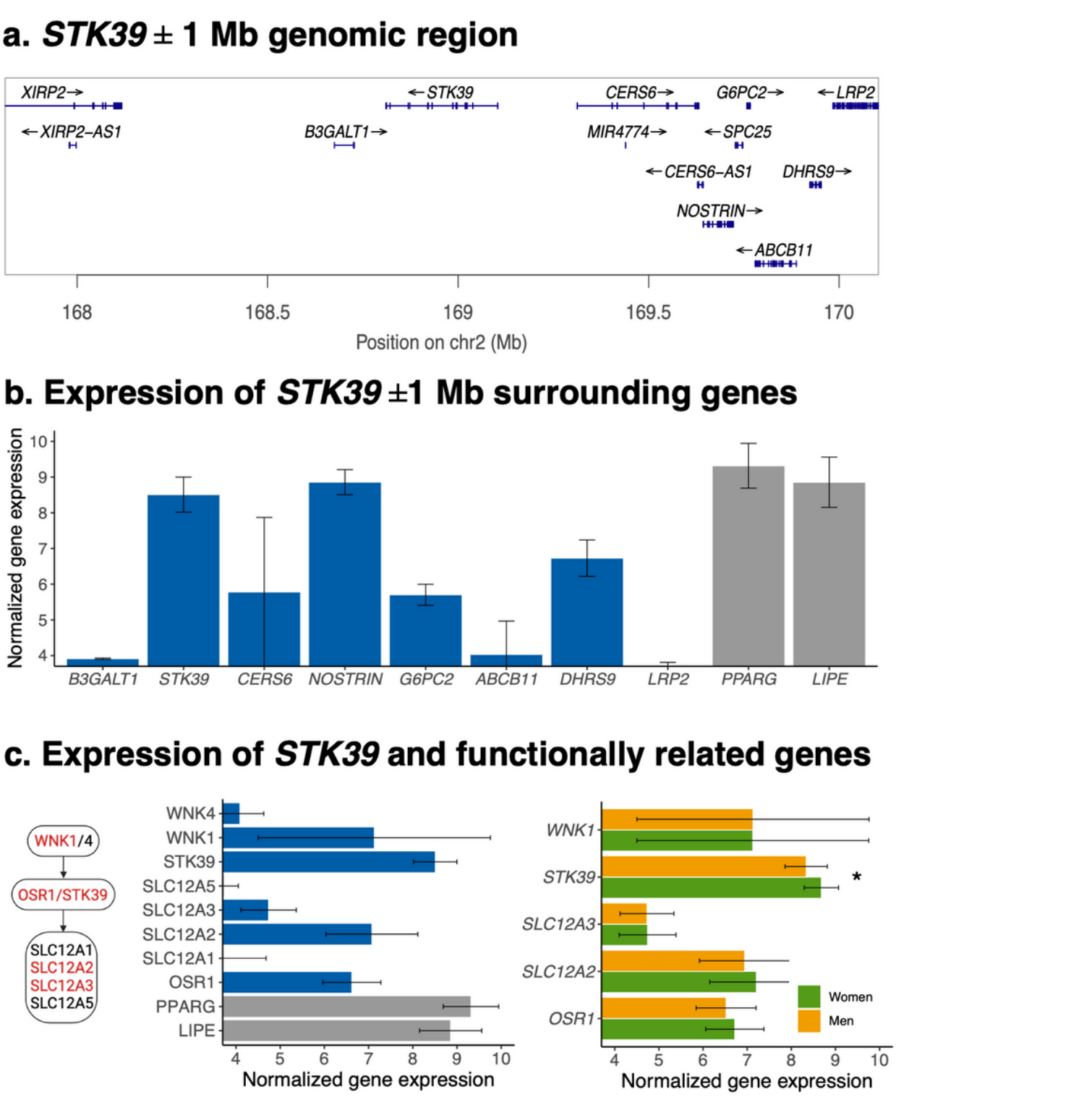

Figure 3

Analysis of gene expression for *STK39* and surrounding genes in subcutaneous adipose tissue. a. Representation of *STK39*±1Mb genomic region. **b.** Normalized gene expression of *STK39* and 1 Mb surrounding genes (blue bars) in adipose tissue, as well as two known highly expressed genes in adipose tissue (grey bars). **c.** Normalized gene expression of *STK39* and functionally related genes taking part in the same signaling cascade. Left diagram shows the genes upstream and downstream *STK39*, as

reported in literature. Only *WNK1*, *OSR1*, *STK39*, *SLC12A2* and *SLC12A3* genes showed to be expressed in adipose tissue (center plot and red letters in the left diagram). From those, *STK39* gene showed significant differences in the expression levels by sex in adipose tissue (P<0.05) (right plot). Normalized expression of 4 is considered as baseline. Data from Affymetrix U133 plus 2.0 microarrays of 75 human adipose tissue biopsies.

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