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Association between MCU Gene Polymorphisms with Obesity: findings from the *All of Us* Research Program

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Abstract: Obesity is a public health crisis, and its prevalence disproportionately affects African Americans in the United States. Dysregulation of organelle calcium homeostasis is associated with obesity. The mitochondrial calcium uniporter (MCU) complex is primarily responsible for mitochondrial calcium homeostasis. Obesity is a multifactorial disease in which genetic underpinnings such as single-nucleotide polymorphisms (SNPs) may contribute to disease progression. The objective of this study was to identify genetic variations of MCU with anthropometric measurements and obesity in the All of Us Research Program. Methods: We used an additive genetic model to assess the association between obesity traits (body mass index (BMI), waist and hip circumference) and selected MCU SNPs in 19,325 participants (3,221 normal weight and 16,104 obese). Eleven common MCU SNPs with a minor allele frequency ≥ 5% were used for analysis. Results: We observed three MCU SNPs in self-reported Black/African American (B/AA) men, and six MCU SNPs in B/AA women associated with increased risk of obesity. However, none of the SNPs in the White study participants were associated with obesity (P≤0.05). Conclusions: This study found associations of MCU SNPs with obesity providing evidence of a potential predictor of obesity susceptibility in B/AA adults.

Keywords: SNP; MCU; obesity; All of Us

1. Introduction

According to 2017-2018 data from the National Health and Nutrition Examination Survey, more than 76 million American adults which represents 42.8% of the population are obese [1]. If present trends continue, by 2030 78% of American adults are projected to be overweight or obese [2]. Obesity is defined as the accumulation of excessive fat resulting from energy imbalance. Furthermore, obesity perpetrates a financial burden of $150 - $210 billion annually in associated medical costs with the medical cost for people who have obesity being $1,429 higher than those of normal weight [5, 6]. According to the Centers for Disease Control (CDC), there are disparities in obesity burden across racial and ethnic groups, with the highest prevalence of obesity in non-Hispanic Black adults (38.4%) overall, followed by Hispanic adults (32.6%) and non-Hispanic White adults (28.6%) [3, 4]. Furthermore, obesity is a highly polygenic disease in which genetic variations such as single-nucleotide polymorphisms (SNPs) may contribute to an individual's development of this disease. Previous studies have established a significant association between several genes such as fat mass and obesity-associated (FTO) gene, melanocortin-4 receptor (MC4R) gene, and leptin receptor (LEPR) gene with obesity [7-10]. The public health, financial burden, and health inequity associated with obesity underpins the importance of elucidating the genetic basis of this polygenic disease.

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The All of Us (AoU) Research Program, funded by the National Institutes of Health (NIH), is a robust research tool that enables the assessment of biological, clinical, social, and environmental determinants of health and disease while simultaneously reflecting diversity in the United States [5]. The primary aim of the AoU Research Program is to understand the association between biomarkers and genes with disease risk [6]. Earlier studies implicated a role for mitochondrial dysfunction in the pathogenesis of obesity [11]. Mitochondria are dynamic organelles responsible for energy production, calcium homeostasis, and cell death. The mitochondrial calcium uniporter (MCU) is a multimeric complex that regulates calcium influx into the mitochondrial matrix [12]. Calcium is a vital internal messenger that regulates energy metabolism [13]. Several studies previously demonstrated that obesity leads to cytosolic and/or organelle calcium flux dysregulation [14]. A critical gap exists in our understanding of the potential role of MCU genetic variants in obesity susceptibility. Studies of MCU allelic variants and their association with obesity are not fully understood. Findings from this study contribute to understanding the link between the MCU gene and obesity susceptibility. Given this significant gap in the literature, we aimed to elucidate the genetic etiology of eleven MCU SNPs with anthropometric traits and obesity in self-reported White and B/AA subsamples of the AoU Research Program. Moreover, associations found in this study could hold promise for finding novel therapeutic targets. This is of particular importance in the current era of increased use of weight loss management medications [15].

2. Materials and Methods

2.1. AoU Participant Cohort Construction

This study was performed on cross-sectional and genomic data generated from AoU participant data collected as previously described [16] using the AoU Researcher Workbench, a cloud computing platform. This cloud-based platform allows authorized researchers to annotate groups of participants data based on specific inclusion and/or exclusion criteria (Cohort Builder), create datasets for analysis using the health information of the cohorts (Dataset Builder), and Jupyter Notebooks to save datasets using high-powered queries Python 3 and R programming languages. Our cohort and dataset construction and code/R commands used for analysis are in the Research Workbench workspace 'MCU Genetic Variation, Obesity, and Vascular Disease' and are available to authorized United States researchers at the AoU research workbench (https://www.researchallofus.org/data-tools/workbench/) or from the corresponding author upon reasonable request.

We utilized the Dataset Builder to create participant datasets categorized into two groups: those with normal weight (body mass index (BMI): 20-24.9) and those classified as obese (BMI: 30+), determined through observation Systemized Nomenclature of Medicine –Clinical Terminology (SNOMED-CT) codes [17]. Our inclusion criteria encompassed self-reported Black/African American (B/AA) and White participants, aged 18-80, with a recorded sex at birth of either female or male, and identified as not Hispanic or Latino ethnicity. Also, participants were not pregnant.

2.2. Measurement of Biochemical and Anthropometric Traits

Each participants' clinic visit consisted of biospecimen collection (blood, urine, saliva), physical measurements including physiologic (blood pressure and heart rate), and anthropometric (height, weight, waist circumference, and hip circumference) [5]. BMI uses weight (kilograms)/height (meters squared) and is a widely accepted indicator of adiposity. Anthropometric measurements, waist and hip circumference, were obtained using standardized protocols from the phenotypes and exposures (PhenX) toolkit [27].

These measurements are also indicators of abdominal obesity. Biochemical traits were measured in the biospecimen samples collected for: total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides, and hemoglobin A1c (HbA1c) levels.

2.3. SNP Selection and Genotyping

Participants who consented to participate in the AoU research program donated fresh whole blood as a primary source of DNA [5]. Subsequently, this DNA was sent to genome centers for genomic analysis, including whole genome sequencing (WGS) and genotyping. Details on biospecimen collection and processing, genome center sample receipt, WGS library construction, sequencing, array genotyping, and genomic data curation are previously described [5]. The candidate gene approach was used to select genetic variants in the MCU gene located on chromosome 10q22.1 and GRCh38/hg38 position base-pair coordinates chr10:72696113-72851477. Employing dbSNP, we curated SNPs based on their minor allele frequency (MAF) within African American (AA) populations. Specifically, we focused on SNPs showing an intermediate frequency, falling between the MAFs observed in the native ancestral populations of European and African descent. For this study, 11 SNPs were selected with an MAF ≥ 5% for further analyses. The MAFs for the analyzed SNPs ranged from 5.2% to 52.9%.

2.4. Statistical Analysis (Descriptive statistics and regression analysis)

To summarize the study variables, mean ± standard deviation (SD) was used to present the descriptive statistics of the study population. For this study, we examined the first physiologic, physical, and anthropometric measurement logged for each participant. Outliers and missing values were removed using distribution-based outlier detection. The Tukey statistical test was used to analyze differences between multiple groups in GraphPad Prism 9.4 [20]. Association analyses between MCU SNPs and normal continuous outcomes (BMI, waist circumference, hip circumference) and categorical outcomes (obesity), we used multivariate linear and logistic regression under an *a priori* additive model using age, and HbA1c levels as covariates for model adjustments. We adjusted for HbA1c as studies have widely established that obesity and type II diabetes are comorbidities in American adults [21]. The beta (β) values were used to determine the direction of effect for each MCU SNP.

3. Results

3.1. Clinical Characteristics of the Study Population

The clinical characteristics of the study population stratified by self-reported race, BMI, and sex at birth are summarized in Table 1. A total of 19,325 participants (3,221 normal weight and 16,104 obese) were included in this study from the AoU cohort, of whom 77.9% (12,546/16,104) were female and 22.1% (3,558/16,104) were male Anthropometric measurements and biochemical characteristics were compared by weight status across sex at birth in White participants (Table 1) and in B/AA participants (Table 1). Additionally, clinical characteristics were compared by weight status across self-reported race stratified by sex at birth (Table 1). The anthropometric measurements (weight, waist circumference, hip circumference, BMI) and HDL levels were significantly higher in subjects with obesity than subjects with normal weight independent of self-reported race and sex at birth (Table 1).

Sex-specific differences in weight, waist circumference, hip circumference, BMI, total cholesterol, LDL, HDL, triglycerides, HbA1c levels, and systolic blood pressure

(SBP) of White study participants were observed with White obese women having higher values of hip circumference, total cholesterol, LDL, and HDL levels (Table 1). White men with obesity had higher weight, triglycerides, waist circumference, and SBP levels relative to White women (Table 1). Sex-specific differences in weight, waist circumference, hip circumference, BMI, total cholesterol, LDL, HDL, triglycerides, HbA1c levels, diastolic, and systolic blood pressure of B/AA study participants were observed with B/AA obese women having higher values of hip circumference, total cholesterol, LDL, and HDL levels (Table 1). Obese B/AA men had higher weight, waist circumference, triglycerides, SBP, and HbA1c levels relative to B/AA women (Table 1).

Table 1: Clinical Characteristics of the Study Population

Variables	**Normal Weight (BMI 20-24.9)**				**Obese (BMI 30+)**			
	White Men	**White Women**	**B/AA Men**	**B/AA Women**	**White Men**	**White Women**	**B/AA Men**	**B/AA Women**
	(N=744)	(N=1369)	(N=493)	(N=615)	(N=4113)	(N=6532)	(N=1429)	(N=4030)
Age (years)	62.87±13.74^{A}	60.41±13.77	59.29±11.78^{E}	56.86±13.46	64.28±11.52^{B}	60.42±12.39	59.39±10.5^{F}	57.77±12.08
Weight (kg)	76.85±10.65^{A,C} (n=702)	63.81±9.98^{D} (n=1337)	73.7±13.28^{E,G} (n=469)	66±13.12^{H} (n=590)	106.02±15.42^{B} (n=3944)	91.51±15.98 (n=6355)	102.84±17.79^{F} (n=1356)	92.84±17.69 (n=3868)
BMI (kg/m^2)	24.39±2.9^{A,C} (n=677)	23.48±2.86^{D} (n=1271)	23.78±3.02^{G} (n=469)	24.04±3.38^{H} (n=540)	33.8±4.16^{B} (n=3878)	34.09±4.84 (n=6078)	33.4±4.6^{F} (n=1376)	34.33±5.15 (n=3782)
WC (cm)	91.9±8.9^{A,C} (n=614)	81.4±9.77^{D} (n=1246)	87.53±9.08^{E,G} (n=459)	84.85±10.48^{H} (n=568)	115.13±11.3^{B} (n=3400)	105.11±12.52 (n=5747)	111.98±12.56^{F} (n=1310)	105.87±12.7 (n=3828)
.HC (cm)	99.28±6.35^{C} (n=618)	98.89±6.91^{D} (n=1230)	97.75±6.91^{G} (n=443)	98.82±7.64^{H} (n=542)	115.23±9.35^{B} (n=3417)	119.73±10.88 (n=5694)	115.39±10.16^{F} (n=1318)	119.79±11.34 (n=3763)
LDL (mg/dL)	96.64±34.53^{A,C} (n=384)	106.37±32.44^{D} (n=716)	95.3±33.2^{G} (n=257)	101.84±33.26^{H} (n=303)	104.23±35.4^{B} (n=2448)	111.02±32.58 (n=3495)	104.88±34.8 (n=771)	108.29±32.86 (n=2069)
HDL (mg/dL)	47.23±15.43^{A,C} (n=539)	60.29±16.68^{D} (n=992)	51.87±17.05^{E,G} (n=384)	57.12±16.61^{H} (n=473)	41.45±10.71^{B} (n=3382)	50.73±13.05 (n=4812)	44.43±12.99^{F} (n=1129)	50.96±13.19 (n=3121)
TG (mg/dL)	112.74±54.91^{A,C} (n=524)	101±47.96^{D} (n=987)	104.14±51.39^{G} (n=379)	102.02±49.89 (n=469)	147.47±64.01^{B} (n=3117)	130.34±60.62 (n=4785)	122.99±62.26^{F} (n=1072)	109.42±55.01 (n=3153)
HbA1c (%)	5.61±0.53^{C} (n=374)	5.5±0.49^{D} (n=737)	5.57±0.54^{G} (n=255)	5.71±0.54^{H} (n=317)	5.87±0.7^{B} (n=2633)	5.74±0.66 (n=3700)	6.01±0.73 (n=771)	5.93±0.67 (n=2199)
DBP (mm/Hg)	86.77±21.86 (n=208)	90.54±25.96 (n=281)	109.72±31.92 (n=54)	115.02±28.54 (n=54)	91.91±23.23^{B} (n=823)	88.15±23.7 (n=1523)	105.75±29.05 (n=125)	106.58±27.34 (n=309)
SBP (mm/Hg)	125.09±26.39^{A} (n=207)	113.09±29.75^{D} (n=278)	100.75±27.42 (n=55)	88.89±21.01^{H} (n=57)	125.66±31.37 (n=827)	121.83±28.65 (n=1544)	114.96±29.83 (n=130)	108.06±32.95 (n=320)

Data presented as mean± SD and statistical comparisons between different study groups based on sex at birth and weight status. Comparisons include A) normal weight White men vs. White women $p < 0.05$, B) obese White men vs. White women $p < 0.05$, C) normal weight White men vs. obese White men $p < 0.05$, and D) normal weight White women vs. obese White women $p < 0.05$, E) normal weight B/AA men vs. B/AA women $p < 0.05$, F) obese B/AA men vs. B/AA women $p < 0.05$, G) normal weight B/AA men vs. obese B/AA men $p < 0.05$, and H) normal weight B/AA women vs. obese B/AA women $p < 0.05$. Abbreviations: BMI: body mass index; kg: kilograms; m: meters; cm: centimeters; mg: milligrams; dL: deciliters; mm: millimeters; Hg: mercury; WC: waist circumference; HC: hip circumference; TG: triglycerides; DBP: diastolic blood pressure; and SBP: systolic blood pressure

3.2. Association of MCU SNPs with obesity in the AoU cohort

Association analyses revealed no significant associations between the 11 MCU SNPs and increased obesity in the White study participants suggesting, decreased risk for obesity (Table 2). SNPs rs2121094, rs7092031, rs2121097, rs7081970, rs3009550, and rs3009554 were significantly associated with decreased risk of obesity in both White men and women. Interestingly, rs6415912, rs6480644, and rs9416029 showed association with decreased probability of obesity development.

Three MCU SNPs in B/AA men revealed evidence of association with increased susceptibility to obesity: rs34072881 (OR: 1.30; 95% CI: 1.12-1.50; -value: 0.0004), rs3009554 (OR:1.68; 95% CI:1.53-1.86; P<0.0001), and rs9416029 (OR: 1.46; 95% CI:1.33-1.59; P<0.0001). In the B/AA women, 6 MCU SNPs rs3009556 (OR:1.06; 1.00-1.11; P-value: 0.035), rs6415912 (OR:1.07; 1.02-1:12; P-value: 0.007), rs6480644 (OR:1.07; 1.01-1.12; P-value: 0.011), rs9416029 (OR: 1.60; 95% CI: 1.48-1.73; P<0.0001), rs3009550 (OR: 1.08; 95% CI: 1.05-1.12; P<0.0001), and rs3009554 (OR: 1.38; 95% CI: 1.27-1.50; P<0.0001) was significantly associated with obesity, indicating an increased probability of developing obesity (Table 2). The SNPs rs9416029 (B/AA men: OR=1.46; B/AA women: OR=1.60) and rs3009554 (B/AA men: OR=1.68; B/AA women: OR=1.38) exhibitied significan associatedwith increased risk of obesity in both B/AA menand women.

Table 2 Association of MCU SNPs with obesity in the AoU cohort

SNP	Alleles	White Men		White Women		B/AA Men		B/AA Women	
		OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value
rs34072881	C/T	--	--	--	--	1.30(1.12-1.50)	0.0004*	0.77(0.69-0.86)	<0.0001*
rs3009556	T/C	--	--	--	--	0.75(0.70-0.80)	<0.0001*	1.06(1.00-1.11)	0.035*
rs6415912	A/G	--	--	0.94(0.91-0.98)	0.002*	0.79(0.74-0.84)	<0.0001*	1.07(1.02-1.12)	0.007*
rs6480644	C/T	--	--	0.94(0.90-0.97)	0.0004*	0.79(0.74-0.84)	<0.0001*	1.07(1.01-1.12)	0.011*
rs2121094	G/A	0.89(0.84-0.95)	0.0002*	0.89(0.86-0.92)	<0.0001*	0.74(0.69-0.79)	<0.0001*	--	--
rs7092031	G/T	0.89(0.84-0.95)	0.0002*	0.88(0.85-0.92)	<0.0001*	0.70(0.66-0.75)	<0.0001*	--	--
rs2121097	A/T	0.89(0.84-0.95)	0.0002*	0.89(0.85-0.92)	<0.0001*	0.70(0.66-0.75)	<0.0001*	--	--
rs7081970	C/T	0.80(0.75-0.85)	<0.0001*	0.87(0.84-0.90)	<0.0001*	0.72(0.67-0.76)	<0.0001*	--	--
rs9416029	A/G	--	--	0.89(0.86-0.92)	<0.0001*	1.46(1.33-1.59)	<0.0001*	1.60(1.48-1.73)	<0.0001*
rs3009550	G/A	0.80(0.75-0.85)	<0.0001*	0.86(0.83-0.90)	<0.0001*	0.93(0.90-0.97)	0.0006*	1.08(1.05-1.12)	<0.0001*
rs3009554	G/A	0.90(0.85-0.95)	0.0004*	0.89(0.86-0.92)	<0.0001*	1.68(1.53-1.86)	<0.0001*	1.38(1.27-1.50)	<0.0001*

Abbreviations: SNP: single nucleotide polymorphism; MAF: minor allele frequency; CI: confidence interval. The odds ratios and corresponding p-values were calculated using logistic regression adjusting for age and HbA1c levels. p was significant at a value of <0.05. * Statistical significance.

3.3. Association of MCU SNPs with BMI in the AoU cohort

The association of 11 MCU SNPs with BMI was also explored in the AoU cohort (Table S3). Multiple linear regression models identified that the 11 MCU variants were not associated with BMI (P-values range: 0.099-0.955). Interestingly, in White men and women all MCU SNPs revealed lower BMI (β range: -0.026 - -0.059). Conversely, in B/AAs, 9 MCU SNPs (rs34072881, rs6415912, rs6480644, rs2121094, rs7092031, rs2121097, rs7081970, rs9416029, and rs3009554) were observed with higher BMI (β range: 0.003-0.063) (Table S3).

3.4. Association of MCU SNPs with waist circumference in the AoU cohort

As shown in Table 3 , only 1 SNP, rs3009554, associated with increased waist circumference (β = 0.244; P=0.026). We observed differences in beta coefficients with regard to self-reported race. Five MCU SNPs (rs34072881, rs6415912, rs9416029, rs3009550, and rs3009554) were observed with decreased waist circumference (β range: -0.030 - -0.062) in White participants, however these SNPs were observed with increased waist circumference (β range: 0.006-0.244) in B/AA study participants (Table S4). In both White men and women MCU SNPs rs3009556, rs6480644, rs2121094, rs7092031, rs2121097, and rs7081970 were observed with decreased waist circumference (β range: -0.003 - -0.050) (Table S4). Differences in the beta coefficients with respect to sex at birth were oberserved in B/AA study participants. There are 4 SNPs (rs3009556, rs6480644, rs2121097, and rs7081970) that revealed increased waist circumference in B/AA men (β range 0.009-0.078) and decreased waist circumference (β range: -0.001 - -0.040) in B/AA women (Table S4).

3.5. Association of MCU SNPs with hip circumference in the AoU cohort

Association analyses between MCU SNPs and hip circumference revealed 4 SNPs (rs6415912, rs6480644, rs2121094, and rs7092031) were signifcantly associated with decreased hip circumference in B/AA men (Table 3). Nominal associations were observed with rs2121097 and rs7081940 and decreased hip circumference in B/AA men (P value >0.052; Table 3). In contrast, these 6 SNPs were observed with increased hip circumference in B/AA women (β range: 0.031-0.049) (Tables 3 and S5).

In White participants three SNPs (rs3009556, rs6480644, and rs6415912) and in B/AA participants rs34072881, rs9416029, and rs3009554 were observed with increased hip circumference (β range: 0.002-0.121;Table S5). Furthermore, seven SNPs (rs2121094, rs7092031, rs2121097, rs7081970, rs9416029, rs3009550, and rs3009554) were observed with decreased hip circumference (β range: -0.007 - -0.021) in the White participants (Table S5).

Table 3: Association of MCU SNPs with waist and hip circumference from the AoU cohort

Obesity Trait	SNP	Alleles	White Men		White Women		B/AA Men		B/AA Women	
			Estimate(β)	**P-value**	**Estimate(β)**	**P-value**	**Estimate(β)**	**P-value**	**Estimate(β)**	**P-value**
WC	rs3009554	G/A	-0.062(0.052)	0.238	-0.046(0.041)	0.269	0.244(0.108)	0.026*	0.139(0.116)	0.233
HC	rs6415912	A/G	0.041(0.040)	0.307	0.022(0.029)	0.442	-0.111(0.054)	0.042*	0.047(0.052)	0.365

HC	rs6480644	C/T	0.041(0.040)	0.307	0.024(0.029)	0.405	-0.111(0.054)	0.042*	0.049(0.052)	0.342
HC	rs2121094	G/A	-0.007(0.040)	0.865	-0.019(0.029)	0.513	-0.109(0.053)	0.040*	0.049(0.052)	0.472
HC	rs7092031	G/T	-0.007(0.040)	0.865	-0.021(0.029)	0.472	-0.109(0.053)	0.040*	0.031(0.051)	0.549
HC	rs2121097	A/T	-0.007(0.040)	0.865	-0.021(0.029)	0.472	-0.103(0.053)	0.052	0.045(0.051)	0.377
HC	rs7081970	C/T	-0.007(0.040)	0.865	-0.020(0.028)	0.493	-0.103(0.053)	0.052	0.045(0.051)	0.377

Abbreviations: SNP: single nucleotide polymorphism; MAF: minor allele frequency; WC: waist circumference; HC: hip circumference. A linear regression was performed with an additive model; waist and hip circumference for adjusted age and HbA1c levels. The associations' results are estimate, standard error (β), and the corresponding p-value. p was significant at a value of <0.05. * Statistical significance.

4. Discussion

This is the first analysis of the MCU gene with obesity traits in two racially diverse cohorts from the AoU Research Program. We explored cross-sectional analyses to determine the relationship between eleven polymorphisms in the MCU gene with anthropometric measurements and obesity risk in White and B/AA participants.

Obesity is a polygenic disease in which the genetic basis of body weight regulation is complex [18]. Our interest in the MCU gene was due to the established link between obesity and dysregulated organelle calcium influx and the important role for MCU in regulation of mitochondrial calcium homeostasis [14]. Associations found in this study are significant by determining genetic risk factors of obesity predisposition in a diverse cohort of study participants.

Our study revealed MCU SNPs significantly associated with increased waist circumference and decreased hip circumference in B/AA men of the AoU cohort. We found 3 MCU SNPs in B/AA men: rs34072881 (OR: 1.30; 95% CI: 1.12-1.50;), rs3009554 (OR:1.68; 95% CI:1.53-1.86), and rs9416029 (OR: 1.46; 95% CI:1.33-1.59 significantly associated with obesity, increasing the probability of developing obesity. Obesity is a major risk for hypertension [29]. B/AA participants were more hypertensive relative to the White men and women. These significant association between MCU SNPs and obesity in B/AA men could be due to their hypertensive state. Studies have shown that MCU is upregulated in pressure overload in the heart [30]. Further studies are required to elucidate the underlying mechanisms.

The 11 selected MCU genetic variants spanned the base-pair coordinates chr10:72696113-72851477 three of which (rs6415912, rs6480644, and rs3009550) occur within the enhancer regulatory region [23]. The potential impact of these 3 MCU SNPs in this regulatory region could disrupt transcription factor recognition and binding to the enhancer region [24]. Further, these alterations to enhancer activity could modify MCU gene expression. MCU SNPs, rs6415912 and rs6480644, were significantly associated with decreased hip circumference in B/AA men (Table 3). Altered MCU gene expression could be a potential diagnostic marker for adiposity in B/AA men.

This study found that the minor alleles were either protective or associated with an increased probability of developing obesity. Nine MCU SNPs, the minor alleles are protective against obesity in White women, with OR between 0.86-0.94. Whereas six of the eleven MCU SNPs: rs3009556 (OR:1.06; P-value =0.035), rs6415912 (OR:1.07; P-value =0.007), rs6480644 (OR:1.07; P-value=0.011), rs9416029 (OR: 1.60; P<0.0001), rs3009550 (OR: 1.0), and rs3009554 (OR: 1.38) were significantly associated with obesity in B/AA women. These findings suggests that these MCU SNPs may be a potential determinant factor for developing obesity in B/AA women. For 6 MCU SNPs, the minor alleles are protective against obesity in White men, with OR between 0.80-0.90. No previous studies, to our knowledge, have reported these associations.

B/AA women have the highest prevalence of obesity in the United States [28]. MCU SNPs within the enhancer regulatory region, rs3009550 and rs6415912, were significantly

associated with obesity in B/AA women in the AoU cohort. MCU expression and/or activity alteration could be a potential clinical biomarker to show propensity for obesity development in B/AA women.

However, our analyses did not reveal a significant association between MCU SNPs and higher BMI in any of the study population. Remarkably, reduced β values for MCU SNPs were shown in White men and women and were associated with lower BMI and decreased waist circumference. Whereas increased β values for MCU SNPs observed in B/AA men and women were associated with higher BMI. Furthermore, increased β values were observed with increased hip circumference in B/AA women. These findings could potentially be due to epigenetics such as environmental exposures (stress, smoking, lifestyle, etc.). Further studies are needed to elucidate the molecular mechanisms of these observed differences.

This work suggests that mutation in the MCU gene is a causative factor for obesity. This strong association between MCU and obesity could potentially be due to the role of MCU in mitochondrial oxidative metabolism and insulin resistance [19]. Previous studies have shown that imbalanced mitochondrial oxidative metabolism results in obesity and diabetes [22]. MCU allows calcium entry into the mitochondrial matrix which promotes mitochondrial oxidative metabolism. Moreover, MCU plays an essential role in glucose-induced insulin secretion [19]. Furthermore, previous studies have demonstrated altered mitochondrial calcium flux in adipocytes may play a role in lipid accumulation and obesity [31-32]. Thus, alterations in MCU function can impair mitochondrial oxidative metabolism, lipid metabolism, and insulin secretion which are prominent features of obesity.

After Bonferroni correction, one would consider a P-value ≤0.0045 as significant evidence for association. After Bonferroni correction SNPs rs9416029, rs3009550, and rs3009554 in B/AA women and SNPs rs9416029, rs3009554, and rs34072881 in B/AA men remained significant.

There are glaring health disparities in the quality of obesity care that disproportionately affect racial and ethnic minorities, specifically B/AA [26]. Genetic ancestry is defined as the genetic information we inherit from our ancestors [25]. This testing has been useful in assessing predicted disease risk. The MCU SNP, rs34072881, is a synonymous variant; strikingly in this study we found that this SNP was exclusively present in the B/AA participants. The reported allele frequencies in dbSNP are T=0.1947 in Africans and T=0.00042 in Europeans. This strongly suggests that rs34072881 of the MCU gene is inherited in those with higher African ancestry. However, these findings require further confirmation in other cohorts with balanced stratification of White and B/AA study participants.

Commented [LT1]: What is the frequency in Africans and Whites in dbSNP?

While the findings in this study are insightful, our study is without limitations, including sparse existing literature on MCU genetic variants and transcriptional regulation. Additionally, having more of a balance in the stratification of the population between the normal weight and obese study groups could potentially assist with identifying more associations between SNPs and obesity. Relying solely on BMI as a measure of adiposity without considering body fat mass composition might mask underlying associations. Additionally, we did not account for potential confounding effects of genetic admixture.

5. Conclusions

In this study, we found a novel association between MCU genetic variants with obesity in adults from the AoU Research Program. Specifically, we observed that the minor alleles allele G of rs9416029, allele A of rs3009554, and allele T of rs34072881 were associated with obesity in B/AA men. Additionally, our study revealed that allele G of rs9416029, allele C of rs3009556, allele G of rs6415912, allele T of rs6480044, allele A of rs3009550, and allele A of rs3009554 are associated with obesity in B/AA women. These findings suggest that these MCU SNPs may play a significant role in the development of obesity in B/AA adults.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Table S1: Frequency of Selected MCU SNPs in the AoU cohort; Table S2: Association of MCU SNPs with obesity in the AoU cohort; Table S3: Association of MCU SNPs with BMI in the AoU cohort; Tables S4: Association of MCU SNPs with waist circumference in the AoU cohort; Table S5: Association of MCU SNPs with hip circumference in the AoU cohort.

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References

1. Li, M., et al., Trends in body mass index, overweight and obesity among adults in the USA, the NHANES from 2003 to 2018: a repeat cross-sectional survey. BMJ Open, 2022. 12(12): p. e065425.
2. Wang, Y., et al., Has the prevalence of overweight, obesity and central obesity levelled off in the United States? Trends, patterns, disparities, and future projections for the obesity epidemic. International Journal of Epidemiology, 2020. 49(3): p. 810-823.
3. Kim, D.D. and A. Basu, Estimating the Medical Care Costs of Obesity in the United States: Systematic Review, Meta-Analysis, and Empirical Analysis. Value Health, 2016. 19(5): p. 602-13.
4. Wang, Y., et al., Will all Americans become overweight or obese? estimating the progression and cost of the US obesity epidemic. Obesity (Silver Spring), 2008. 16(10): p. 2323-30.
5. Bick, A.G., et al., Genomic data in the All of Us Research Program. Nature, 2024.
6. Denny, J.C., et al., The "All of Us" Research Program. N Engl J Med, 2019. 381(7): p. 668-676.
7. García-Solís, P., et al., Fat mass obesity-associated (FTO) (rs9939609) and melanocortin 4 receptor (MC4R) (rs17782313) SNP are positively associated with obesity and blood pressure in Mexican school-aged children. British Journal of Nutrition, 2016. 116(10): p. 1834-1840.
8. Dougkas, A., et al., The impact of obesity-related SNP on appetite and energy intake. British Journal of Nutrition, 2013. 110(6): p. 1151-1156.
9. Gueorguiev, M., et al., Association Studies on Ghrelin and Ghrelin Receptor Gene Polymorphisms With Obesity. Obesity, 2009. 17(4): p. 745-754.
10. Prakash, J., et al., Association of FTO rs9939609 SNP with Obesity and Obesity- Associated Phenotypes in a North Indian Population. Oman Med J, 2016. 31(2): p. 99-106.
11. de Mello, A.H., et al., Mitochondrial dysfunction in obesity. Life Sciences, 2018. 192: p. 26-32.
12. Foskett, J.K. and B. Philipson, The mitochondrial Ca(2+) uniporter complex. J Mol Cell Cardiol, 2015. 78: p. 3-8.
13. Li, C., et al., Implications of MCU complex in metabolic diseases. The FASEB Journal, 2023. 37(8): p. e23046.

14. Arruda, A.P. and G.S. Hotamisligil, Calcium Homeostasis and Organelle Function in the Pathogenesis of Obesity and Diabetes. Cell Metab, 2015. 22(3): p. 381-97.
15. Abdi Beshir, S., et al., A narrative review of approved and emerging anti-obesity medications. Saudi Pharm J, 2023. 31(10): p. 101757.
16. Ramirez, A.H., et al., The All of Us Research Program: data quality, utility, and diversity. medRxiv, 2020: p. 2020.05.29.20116905.
17. Chang, E. and J. Mostafa, The use of SNOMED CT, 2013-2020: a literature review. J Am Med Inform Assoc, 2021. 28(9): p. 2017-2026.
18. Hinney, A. and J. Hebebrand, Polygenic obesity in humans. Obes Facts, 2008. 1(1): p. 35-42.
19. D'Angelo, D. and R. Rizzuto, The Mitochondrial Calcium Uniporter (MCU): Molecular Identity and Role in Human Diseases. Biomolecules, 2023. 13(9).
20. GraphPad Prism version 9.4.0 [software]. San Diego, CA: GraphPad Software, Inc.; 2023.
21. Chen, Y., et al., Prevalence of Testing for Diabetes Among US Adults With Overweight or Obesity, 2016-2019. Prev Chronic Dis, 2023. **20**: p. E116.
22. Dai, W. and L. Jiang, Dysregulated Mitochondrial Dynamics and Metabolism in Obesity, Diabetes, and Cancer. Frontiers in Endocrinology, 2019. **10**.
23. Martin, F.J., et al., Ensembl 2023. Nucleic Acids Research, 2022. **51**(D1): p. D933-D941.
24. Yang, M.G., et al., Characterization of sequence determinants of enhancer function using natural genetic variation. eLife, 2022. **11**: p. e76500.
25. Lewis, A.C.F., et al., Getting genetic ancestry right for science and society. Science, 2022. **376**(6590): p. 250-252.
26. Washington, T.B., et al., Disparities in Access and Quality of Obesity Care. Gastroenterol Clin North Am, 2023. **52**(2): p. 429-441.
27. Hamilton, et al. (2011) The PhenX Toolkit: Get the Most From Your Measures. American Journal of Epidemiology, 174(3), 253-60.
28. Lofton, H., et al., Obesity among African American people in the United States: A review. Obesity (Silver Spring), 2023. 31(2): p. 306-315.
29. Shariq, O.A. and T.J. McKenzie, Obesity-related hypertension: a review of pathophysiology, management, and the role of metabolic surgery. Gland Surg, 2020. 9(1): p. 80-93.
30. Wang, P., et al., Elevated MCU Expression by CaMKIIδB Limits Pathological Cardiac Remodeling. Circulation, 2022. 145(14): p. 1067-1083.
31. Jia, M., et al., Deletion of the mitochondrial calcium uniporter in adipose tissue promotes energy expenditure and alleviates diet-induced obesity. Molecular Metabolism, 2024. 80: p. 101873.
32. Wright, L.E., et al., Increased mitochondrial calcium uniporter in adipocytes underlies mitochondrial alterations associated with insulin resistance. Am J Physiol Endocrinol Metab, 2017. 313(6): p. E641-e650.