

# Copy Number Variations Associated With Obesity-Related Traits in African Americans: A Joint Analysis Between GENOA and HyperGEN

Wei Zhao<sup>1</sup>, Nathan E. Wineinger<sup>2,3</sup>, Hemant K. Tiwari<sup>2</sup>, Thomas H. Mosley<sup>4</sup>, Ulrich Broeckel<sup>5</sup>, Donna K. Arnett<sup>6</sup>, Sharon L.R. Kardia<sup>1</sup>, Edmond K. Kabagambe<sup>6</sup> and Yan V. Sun<sup>1,7</sup>

Obesity is a highly heritable trait and a growing public health problem. African Americans (AAs) are a genetically diverse, yet understudied population with a high prevalence of obesity (BMI >30 kg/m<sup>2</sup>). Recent studies based upon single-nucleotide polymorphisms (SNPs) have identified genetic markers associated with obesity. However, a large proportion of the heritability of obesity remains unexplained. Copy number variation (CNV) has been cited as a possible source of missing heritability in common diseases such as obesity. We conducted a CNV genome-wide association study of BMI in two African-American cohorts from Genetic Epidemiology Network of Arteriopathy (GENOA) and Hypertension Genetic Epidemiology Network (HyperGEN). We performed independent and identical association analyses in each study, then combined the results in a meta-analysis. We identified three CNVs associated with BMI, obesity, and other obesity-related traits after adjusting for multiple testing. These CNVs overlap the *PARK2*, *GYP A*, and *SGCZ* genes. Our results suggest that CNV may play a role in the etiology of obesity in AAs.

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## INTRODUCTION

Obesity is a major public health problem, particularly among African Americans (AAs). In 2008, the prevalence of obesity among AAs in the United States was 37.3% in adult men and 49.6% in adult women, whereas the US national prevalence was 32.2% and 35.5% in adult men and women, respectively (1). Obesity increases the likelihood of developing a variety of chronic diseases including diabetes, hypertension, high cholesterol, stroke, heart disease, cancers, and arthritis (2), leading to excess mortality and morbidity (3) and exerting a severe economic burden on the healthcare system (4). Environmental factors such as excessive energy intake and sedentary lifestyle are known to contribute to obesity. However, genetic factors have been found to strongly modulate an individual's susceptibility to obesity. Twin and family studies have estimated the heritability of BMI to range from 40 to 70% (5,6). However, genome-wide association studies (GWAS) based upon single-nucleotide polymorphisms (SNPs) have only accounted for a modest proportion of the total genetic variation—a common finding known as missing heritability (7,8). Structural variants such as copy number variations (CNVs) have been suggested as a potential source of this missing heritability (7,8).

CNVs have been reported to be associated with human disease, disorders (9–12), and quantitative gene expression levels (13,14) that, in some cases, are likely to have functional effects (14,15). A recent study demonstrated that the white patients with onset of obesity before 10 years of age had significantly more large, rare deletions than controls (16). This finding was replicated in obese whites regardless of the age of onset (17). However, obese AAs were found to possess less burden of rare CNVs, measured by the total span of CNVs per individual and average length of CNVs, compared to AA controls (18). A genome-wide association study (GWAS) of obesity in children found eight rare CNVs that were exclusive to obese white and African-American children (19). A study on BMI in Chinese identified a CNV region which overlaps a gene-regulating energy homeostasis (20). This finding was replicated in whites (21). These studies suggest that CNVs may play an important role in the genetic architecture of obesity. A large scale survey of CNVs is needed to further understand the genetics of obesity in AAs.

We conducted a CNV genome-wide association study on BMI and other obesity-related traits in two established African-American cohorts: the Genetic Epidemiology

<sup>1</sup>Department of Epidemiology, School of Public Health, University of Michigan, Ann Arbor, Michigan, USA; <sup>2</sup>Department of Biostatistics, University of Alabama at Birmingham, Birmingham, Alabama, USA; <sup>3</sup>Scripps Translational Science Institute, Scripps Health, San Diego, California, USA; <sup>4</sup>Department of Medicine, University of Mississippi Medical Center, Jackson, Mississippi, USA; <sup>5</sup>Department of Pediatrics and Medicine and Human and Molecular Genetics Center, Medical College of Wisconsin, Milwaukee, Wisconsin, USA; <sup>6</sup>Department of Epidemiology, University of Alabama at Birmingham, Birmingham, Alabama, USA; <sup>7</sup>Department of Epidemiology, Rollins School of Public Health, Emory University, Atlanta, Georgia, USA. Correspondence: Yan V. Sun (yvsun@emory.edu)

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Network of Arteriopathy (GENOA) and Hypertension Genetic Epidemiology Network (HyperGEN) from the National Heart, Lung and Blood Institute (NHLBI) Family Blood Pressure Program. Participants in both studies were genotyped on the Affymetrix 6.0 array—a platform which includes probes specifically designed for CNV analysis. We found three CNVs that were significantly associated with BMI and obesity-related traits after correcting for multiple testing. We also examined three previously reported CNVs linked to obesity (21–23), but found no evidence of association.

## METHODS AND PROCEDURES

### Sample

The GENOA study is a community-based study of hypertensive sibships that aims to identify genes influencing blood pressure (24). AA subjects were enrolled in the field center in Jackson, MS. Sibships were recruited who met the eligibility requirement: at least two adults were clinically diagnosed with essential hypertension before age 60. Other siblings were invited to participate regardless of affection status. Data was collected in two phases: between 1995 and 2000, 1,854 subjects were examined; then between 2000 and 2004, 1,482 of these participants were re-examined. The GENOA study was approved by the institutional review boards of the participating institutions, and each participant provided written informed consent.

The HyperGEN study is a family based cross-sectional study designed to identify genetic contributors to hypertension (25). AA men and women were enrolled from centers in Birmingham, AL and Forsyth County, NC. In the first recruitment phase, sibships were recruited who met eligibility requirements: probands with onset of hypertension by age 60 and one or more hypertensive siblings who were willing to participate in the study (1995–2000). In the second phase, the offspring of the hypertensive siblings were recruited (2000–2003). At present, 1,224 AA subjects have been enrolled in HyperGEN and have provided the necessary phenotypic and genetic data. The HyperGEN study was approved by the institutional review board of the participating institutions, and each participant provided written informed consent.

### CNV analysis

Study participants in GENOA ( $N = 1,355$ ) and HyperGEN ( $N = 1,224$ ) were genotyped using the Affymetrix Genome-wide Human SNP Array 6.0 platform. Genetic samples were excluded if they had an overall SNP call rate <95% or sex mismatch between genotypic and phenotypic measurement based upon quality control implementations. HyperGEN samples who had a disproportionately large number of copy number

polymorphisms (>25%) or were genotyped in small batches (16 or less subjects per batch) were also excluded. Following these quality control procedures, 1,263 AAs from GENOA and 1,026 AAs from HyperGEN remained for the current study (92 and 198 were excluded from GENOA and HyperGEN, respectively). The Human Genome 18 reference build was used for probe localization.

The GENOA study used the Affymetrix Genotyping Console 3.0.1 to generate a reference genome by using all 1,263 raw intensity files (CEL files) from the Human SNP Array 6.0 platform (Affymetrix Genotyping Console 3.0.1 User Manual). The boundaries of the common CNV segments were determined using the predefined CNV regions (26). Genotype calls for these CNVs were determined using the Canary algorithm (26). Any CNV call whose confidence score was <0.8 was excluded from the analysis (lower scores indicate more uncertainty), resulting in a 90% call rate. In total, we identified 1,130 CNV regions. Among these, 24 regions were monomorphic (normal type for all subjects) and were excluded from the analyses.

The HyperGEN study also used the Human SNP Array 6.0 platform to generate raw intensity files. CNVs were called using the Canary application within Birdsuite software, Version 1.5.5 (27). Samples were analyzed by batch to eliminate batch effects (28). CNV genotype calls with confidence values >0.1 were removed from the analysis (higher scores indicate more uncertainty), resulting in a 92% call rate. In total, we identified 1,285 CNV regions. Among these, 84 regions were monomorphic and were excluded from the analyses. The chromosomal boundaries and the copy number state in each study were exported and used in the association analyses of BMI and related traits.

The Genotyping Console and the Birdsuite software have different implementations of the same Canary algorithm. Therefore, the confidence scores from the two software packages have different ranges for high-quality CNV call. The confidence scores were used to exclude CNV calls with poor confidence. We chose the threshold to have ~10% overall missingness in both analysis datasets.

### Statistical analysis

We used a linear mixed effect model to test for CNV–BMI associations in each identified CNV region in each study population. This model included CNV, age, age-squared, and sex as fixed effect covariates and family as a random effect to adjust for familial correlations within the families. CNV was treated as an additive trait, using the integer CNVs obtained from Canary. Principal component analysis was used to adjust for population stratification (29). The first 10 PCs based on 762,766 autosomal SNPs (MAF >0.01 and call rate >95%) from GENOA samples were calculated and incorporated into the mixed model as fixed effect covariates. Similarly, the first 10 PCs based on 823,728 autosomal SNPs from HyperGEN samples were included as covariates in the HyperGEN analysis. In extracting PCs, SNPs were removed from the

**Table 1 Basic descriptive statistics of the samples**

Trait	GENOA			HyperGEN		
	Total ( $N = 1,263$ )	Male ( $N = 398$ )	Female ( $N = 865$ )	Total ( $N = 1,026$ )	Male ( $N = 337$ )	Female ( $N = 689$ )
Age (years), mean (SD)	58.0 (10.07)	59.3 (9.62)	57.4 (10.22)	44.3 (13.43)	43.1 (13.63)	45.0 (13.29)
Weight (kg), mean (SD)	88.3 (18.21)	90.3 (17.06)	87.4 (18.66)	90.9 (23.55)	92.3 (21.21)	90.4 (24.61)
Height (cm), mean (SD)	168.8 (8.94)	177.9 (6.47)	164.5 (6.39)	167.6 (8.90)	176.4 (6.81)	163.3 (6.26)
BMI, kg/m <sup>2</sup> , mean (SD)	31.1 (6.49)	28.5 (4.95)	32.3 (6.76)	32.4 (8.12)	29.6 (6.26)	33.8 (8.56)
Waist circumference (cm), mean (SD)	103.4 (16.25)	100.3 (12.10)	104.8 (17.67)	102.6 (18.71)	99.7 (16.57)	104.0 (19.51)
Hip circumference (cm), mean (SD)	113.2 (14.11)	105.6 (10.38)	116.7 (14.25)	114.4 (16.35)	107.9 (12.76)	117.7 (16.95)
Obese (BMI $\geq 30$ kg/m <sup>2</sup> )	640 (50.7%)	128 (32.2%)	512 (59.2%)	570 (55.7%)	136 (40.5%)	434 (63.1%)

GENOA, Genetic Epidemiology Network of Arteriopathy; HyperGEN, Hypertension Genetic Epidemiology Network.

calculation if they exhibited Mendelian inheritance errors, missingness >1%, MAF <1%, or failure of Hardy–Weinberg equilibrium in founders (HWE *P* value <0.001).

Associated CNVs were examined with other obesity-related traits: weight, hip circumference, and waist circumference. Identical linear mixed models were fit to perform the analyses on these continuous traits. We also stratified the sample into obese (BMI ≥30 kg/m<sup>2</sup>) and nonobese (BMI <30 kg/m<sup>2</sup>). The relationship between CNV and obesity status was examined using generalized estimating equations.

An inverse variance based meta-analysis was carried out using METAL software (30) to combine the results from GENOA and HyperGEN. This approach calculated a weighted sum effect size, where the effect size for each study was weighted by the estimated SEs. False discovery rate was calculated to adjust for multiple testing and a false discovery rate *q* value of 0.1 was set as the threshold for significance.

All statistical analyses were performed with R statistical environment version 2.9.0 from R Project (<http://www.r-project.org/>) and METAL (30).

## RESULTS

Basic descriptive statistics of the sample from each study population are summarized in **Table 1**. The GENOA and HyperGEN studies have similar sample sizes and phenotypic measurements on the traits we examined.

CNVs identified in this study were classified into three categories: deletion (copy number is <2), duplication (copy number is >2) and mixture type (observed copy numbers are less and more than 2). Among the 1,106 CNVs called in GENOA, 186 CNVs were mixed type, 750 CNVs were deletion type and 170 were duplication type. Similarly, among the 1,201 CNVs called in HyperGen, 221 CNVs were mixed type, 779 CNVs were deletion type and 201 were duplication type. There were 782 CNVs whose call types are consistent between these two studies, which suggest high quality CNV calls. Among these CNVs, 566 were deletion type, 95 duplication type and 121 mixed type. In this study, we focused on previously reported CNV regions with relatively common frequency in the HapMap sample. We did not observe any large CNVs (length >500 kb) with frequency lower than 1% in both GENOA and HyperGEN AA populations.

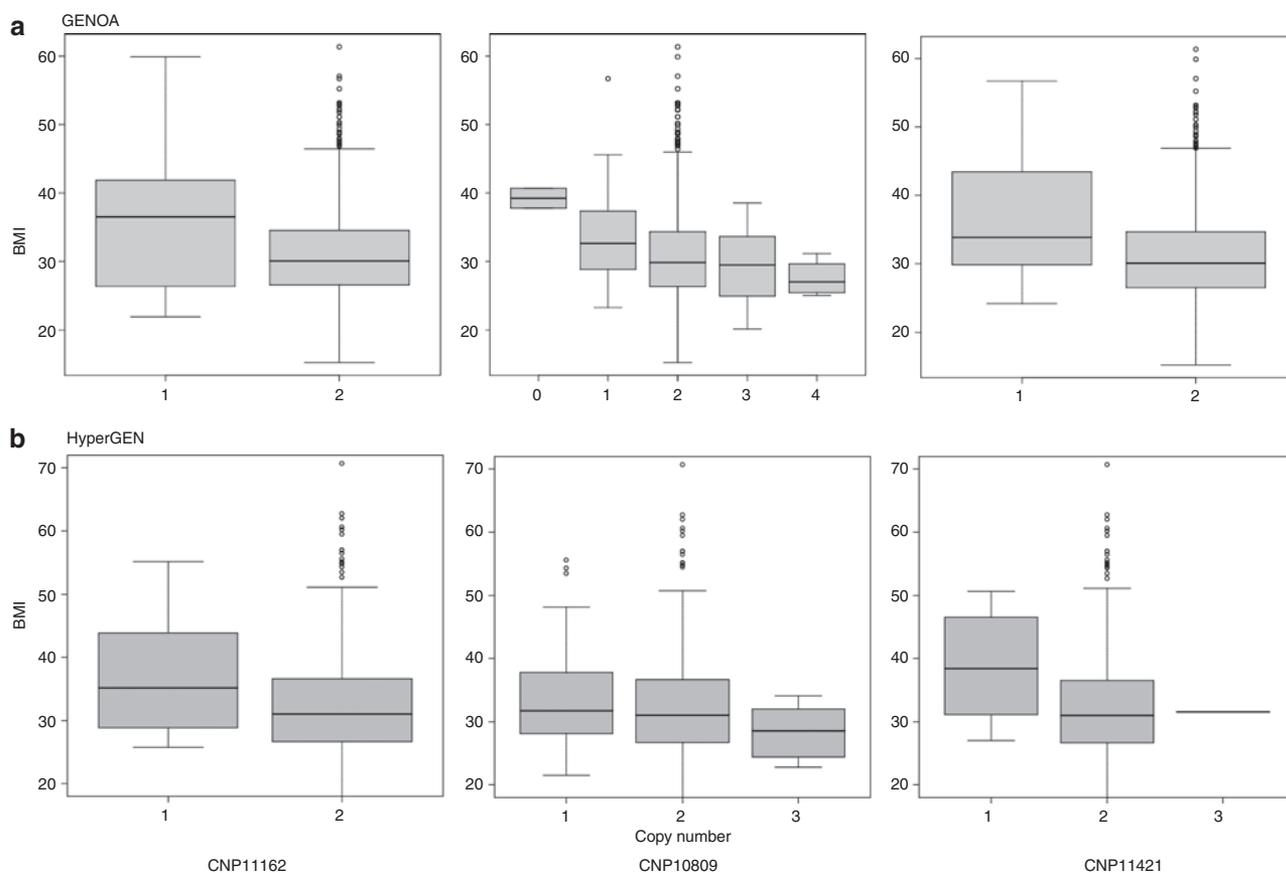
There were a total of 1,037 CNVs that were shared between GENOA and HyperGEN. Among the 1,037 CNVs that were included in the meta-analysis, 55 CNV regions had *P* value <0.05 in the meta-analysis. After adjusting for multiple testing, three CNVs had *q* value <0.1: CNP11162, CNP10809, and CNP11421 (**Table 2**). CNP11162 is located on chromosome 6, 162,416,281 to 162,423,724 bp with 1.7% of deletion; CNP10809 is located on chromosome 4, 145,220,925 to 145,232,498 bp with 4.2% of deletion and 1.7% of duplication; and CNP11421 is located on chromosome 8, 14,553,275 to 14,559,579 bp with 1.2% of deletion and 0.04% of duplication. The percentage of deletion (0 and 1 copies) and duplication (3 and 4 copies) were the percentage of individuals who carried loss or gain among all the individuals from both GENOA and HyperGEN. All the three CNVs included deletions. Although CNP10809 was mixture type, a greater number of deletions than duplications were observed in this region (82 individuals had loss and 34 individuals had gain). CNP11421 in HyperGEN was mixture type as well with only one individual who had gain. All of

**Table 2** CNVs associated with BMI

CNV	Chr	Start position	End position	N	GENOA			HyperGEN			Meta analysis				
					Type	β	SE	P value	N	Type	β	SE	P value	FDR q value	
CNP11162	6	162,416,281	162,423,724	1,263	Deletion	-4.05	1.39	0.0036	1,021	Deletion	-5.93	2.24	0.0081	0.0001	0.052
CNP10809	4	145,220,925	145,232,498	1,008	Mixture <sup>a</sup>	-2.31	0.63	0.0003	938	Mixture <sup>a</sup>	-1.75	1.32	0.1712	0.0001	0.052
CNP11421	8	14,553,275	14,559,579	1,258	Deletion	-4.31	1.89	0.0229	1,023	Mixture <sup>a</sup>	-6.12	2.05	0.0026	0.0002	0.070

CNV, copy number variation; GENOA, Genetic Epidemiology Network of Arteriopathy; HyperGEN, Hypertension Genetic Epidemiology Network.

<sup>a</sup>Observed copy numbers can be less or more than 2 (i.e., some individuals had either deletion or amplification creating mixture of individuals with deletion, amplification, or normal)



**Figure 1** The relationship of BMI and the three significant copy number variations (CNVs) (CNV11162, CNV10809, and CNV11421). (a) Box plots of BMI with different copies of CNVs in Genetic Epidemiology Network of Arteriopathy (GENOA); (b) box plots of BMI with different copies of CNVs in Hypertension Genetic Epidemiology Network (HyperGEN).

the three CNV regions show negative association with BMI, suggesting deletions in these regions may increase the risk of obesity. **Figure 1** shows how BMI changes over different copies of CNVs in each study population.

Association analyses of these three CNVs with obesity, weight, hip circumference, and waist circumference were also conducted. Results are included in **Table 3**. As expected, these CNVs were found to be associated with most of the other obesity-related traits. The number of each type of CNVs in obese and nonobese groups is shown in **Table 4**.

We also examined the three previously reported CNV regions associated with obesity: CNP59 located on chromosome 1, 72,528,701 to 72,535,958 bp; CNP2150 located on chromosome 16, 19,853,151 to 19,874,863 bp, and CNP1732 located on chromosome 11, 55,130,608 to 55,209,585 (21–23). In our study, we found no evidence of association with these three CNVs: the  $P$  value for CNP59 was 0.367 in GENOA and 0.255 in HyperGEN; the  $P$  value for CNP2150 was 0.420 in GENOA and 0.594 in HyperGEN; and the  $P$  value for CNP1732 was 0.406 in GENOA and 0.005 in HyperGEN. Previous reports suggest low copy number in CNP1732 is correlated with obesity in children (21). However, although we found CNP1732 to be significantly associated with BMI in HyperGEN, the association was in the opposite direction: lower copy number is associated with lower BMI. A meta-analysis examining these CNVs

did not show evidence of an association with BMI ( $P$  value = 0.150, 0.336, and 0.346 for CNV59, CNV2150, and CNV1732, respectively). Furthermore, we looked at the number of each CNV type in the obese group and the nonobese group. In GENOA, the number of deletions for CNP59, CNP2150 and CNP1732 were 31, 140, and 150 in the obese group compared to 38, 137, and 131 in the nonobese group. In HyperGEN, there were 97, 92, and 113 deletions in the obese group compared to 82, 71, and 107 in the nonobese group, respectively. There was no evidence of an enrichment of CNV in either group.

## DISCUSSION

In this study, we performed genome-wide association analyses between CNVs and BMI in two AA populations. A meta-analysis suggested CNP11162, CNP10809, and CNP11421 were significantly associated with BMI. We performed additional association analyses of those CNVs with other obesity-related traits including weight, waist circumference, hip circumference, and dichotomized obesity, and found consistently significant associations. These variants overlap with genes *PARK2* (Parkinson protein 2, E3 ubiquitin protein ligase), *GYP A* (Glycophorin A), and *SGCZ* (Sarcoglycan, Zeta), respectively.

*PARK2* encodes for parkin, a ubiquitin ligase. Recessive mutations in the *PARK2* gene have been found in familial

**Table 3 Association of BMI-associated CNVs and other obesity-related traits**

CNV	GENOA					HyperGEN					Meta analysis
	N	Type	$\beta$	SE	P value	N	Type	$\beta$	SE	P value	P value
<i>Weight (kg)</i>											
CNP11162	1,263	Deletion	-8.71	4.02	0.0305	1,021	Deletion	-18.49	6.56	0.0048	0.0009
CNP10809	1,008	Mixture <sup>a</sup>	-5.08	1.82	0.0055	938	Mixture <sup>a</sup>	-3.75	3.88	0.3197	0.0034
CNP11421	1,258	Deletion	-11.42	5.48	0.0375	1,023	Mixture <sup>a</sup>	-21.02	6.04	0.0005	0.0001
<i>Hip circumference (cm)</i>											
CNP11162	1,263	Deletion	-4.05	1.39	0.0036	1,021	Deletion	-11.23	4.47	0.0120	0.0004
CNP10809	1,008	Mixture <sup>a</sup>	-2.31	0.63	0.0003	938	Mixture <sup>a</sup>	-3.14	2.64	0.2234	0.0001
CNP11421	1,258	Deletion	-4.31	1.89	0.0229	1,023	Mixture <sup>a</sup>	-13.62	4.07	0.0007	0.0005
<i>Waist circumference (cm)</i>											
CNP11162	1,263	Deletion	-8.00	3.60	0.0265	1,021	Deletion	-11.98	5.20	0.0202	0.0017
CNP10809	1,008	Mixture <sup>a</sup>	-4.60	1.64	0.0052	938	Mixture <sup>a</sup>	-5.71	3.05	0.0568	0.0008
CNP11421	1,258	Deletion	-8.85	4.92	0.0723	1,023	Mixture <sup>a</sup>	-11.61	4.70	0.0125	0.0024
<i>Obesity (BMI <math>\geq 30</math> kg/m<sup>2</sup>)</i>											
CNP11162	1,263	Deletion	-0.66	0.45	0.1409	1,021	Deletion	-0.91	0.77	0.2291	0.06203
CNP10809	1,008	Mixture <sup>a</sup>	-0.80	0.23	0.0004	938	Mixture <sup>a</sup>	-0.26	0.45	1.000	0.0006
CNP11421	1,258	Deletion	-0.83	0.62	0.1804	1,023	Mixture <sup>a</sup>	-1.23	0.80	0.0898	0.0454

CNV, copy number variation; GENOA, Genetic Epidemiology Network of Arteriopathy; HyperGEN, Hypertension Genetic Epidemiology Network.

<sup>a</sup>Observed copy numbers can be less or more than 2 (i.e., some individuals had either deletion or amplification creating mixture of individuals with deletion, amplification, or normal).

**Table 4 The frequency table of CNVs in obese group vs. nonobese group**

CNV	Copy number	GENOA			HyperGEN			Total		
		N	Obese	Nonobese	N	Obese	Nonobese	N	Obese	Nonobese
CNP11162	1	1,263	15	7	1,021	11	6	2,284	26	13
	2		625	616		557	447		1182	1063
CNP10809	0	1,008	2	0	938	0	0	1,946	2	0
	1		35	15		17	13		52	28
	2		450	478		501	401		951	879
	3		10	14		2	4		12	18
	4		1	3		0	0		1	3
CNP11421	1	1,258	8	3	1,023	14	3	2,281	22	6
	2		630	617		554	451		1,084	1,068
	3		0	0		1	0		1	0

CNV, copy number variation; GENOA, Genetic Epidemiology Network of Arteriopathy; HyperGEN, Hypertension Genetic Epidemiology Network.

Parkinson's disease (31), which was characterized by dopamine degeneration in substantial nigral pathway. Animal studies confirmed that knockout of this gene in mice disrupted dopaminergic transmission in striatal area (32,33). Dopamine is known to play an important role in modulating reward sensitivity, conditioning, and high-level cognitive control, which are all involved in the regulation of food intake (34) and therefore have a potential role in obesity. In one study, palatable food was shown to release dopamine in the dorsal striatum that is highly correlated with the level of pleasure subjects reported from eating the food (35). There is evidence from human

imaging studies suggesting that obese individuals might have impairments in the dopaminergic pathway (36,37). Thus, it is possible that a deletion in part of *PARK2* gene leads to deficit in dopamine transmission that is involved in homeostatic regulation of food intake, and results in excessive food intake and obesity. Most recently, *PARK2* knockout mice were found to resist body weight gain when they were exposed to a high fat diet during the age of 12–18 weeks, which suggests that this gene is involved in the regulation of fat intake (38). More interestingly, a recent study found an intronic SNP in the *PARK2* gene associated with levels of several serum amino acids that

are directly involved in metabolic pathway (39). The putative functional role of *PARK2* on serum metabolites may assist further understanding of the relationship between the deletion in *PARK2* gene and obesity-related traits.

*GYP A* is a gene that bears the antigenic determinants for the MN and Ss blood groups (40), and *SGCZ* encodes a protein that is part of sarcoglycan complex which bridges the inner cytoskeleton and the extracellular matrix (41). Neither of these two genes has been reported to be associated with any obesity-related traits.

In a recent large scale GWAS, two CNV regions on chromosome 1 and 16 were reported to be associated with BMI in whites (22,23). Another recent family based GWAS reported one CNV region on chromosome 11 that was associated with early onset of extreme obesity (21). We identified CNVs overlapping with these reported regions (CNP59 overlaps with the chromosome 1 region, CNP2150 overlaps with the chromosome 16 region and CNP1732 overlaps with the chromosome 11 region) and specifically examined whether these CNVs were associated with BMI in our study cohorts. However, we were unable to replicate these associations with BMI or other obesity-related traits. The frequencies of the three reported CNVs on chromosome 1, 16, and 11 (all of them are deletion polymorphisms) were 62, 13, and 28% in the reported studies of whites (21–23) whereas the frequencies of the overlapping CNVs (CNP59, CNP2150, and CNP1732) were 12, 21, and 22% in our study cohorts. Also, the large sample sizes (32,387 and 249,796 participants) or the family design of the reported studies increased the power to detect small effect sizes. Our combined study of 2,289 AAs may have limited power to detect effects of that size. For example, if we assume the frequency of the non-normal variants is 0.3 and effect size is 0.17, the power of our study is only 0.14 at an  $\alpha$  level of 0.05.

We identified three CNVs that were associated with BMI and obesity in AA populations. While our results should be interpreted within the context of obesity in hypertensive families, we have highlighted a potential causal pathway in one of these genes (*PARK2*) that may lead to dysfunction in brain rewarding and cognitive control regulating food intake, and thus result in excessive or compulsive food intake and obesity. As CNVs in our reported genes have not been previously found to be associated with BMI in studies based on white populations, our findings reinforce the need to stratify or account for population differences in genetic studies, particularly when considering traits like obesity that exhibit differing patterns among populations.

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#### DISCLOSURE

The authors declared no conflict of interest.  
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