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## Ethnic differentiation of copy number variation on chromosome 16p12.3 for association with obesity phenotypes in European and Chinese populations

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### Abstract

**OBJECTIVE**—Genomic copy number variations (CNVs) have been strongly implicated as important genetic factors for obesity. A recent genome-wide association study identified a novel variant, rs12444979, which is in high linkage disequilibrium with CNV 16p12.3, for association with obesity in Europeans. The aim of this study was to directly examine the relationship between the CNV 16p12.3 and obesity phenotypes, including body mass index (BMI) and body fat mass.

**SUBJECTS**—Subjects were a multi-ethnic sample, including 2286 unrelated subjects from a European population and 1627 unrelated Han subjects from a Chinese population. Body fat mass was measured using dual energy X-ray absorptiometry.

**RESULTS**—Using Affymetrix Genome-Wide Human SNP Array 6.0, we directly detected CNV 16p12.3, with the deletion frequency of 27.26 and 0.8% in the European and Chinese populations, respectively. We confirmed the significant association between this CNV and obesity (BMI:  $P=1.38 \times 10^{-2}$ ; body fat mass:  $P=2.13 \times 10^{-3}$ ) in the European population. Less copy numbers were associated with lower BMI and body fat mass, and the effect size was estimated to be 0.62 (BMI) and 1.41 (body fat mass), respectively. However, for the Chinese population, we did not observe significant association signal, and the frequencies of this deletion CNV are quite different between the European and Chinese populations ( $P<0.001$ ).

**CONCLUSION**—Our findings first suggest that CNV 16p12.3 might be ethnic specific and cause ethnic phenotypic diversity, which may provide some new clues into the understanding of the genetic architecture of obesity.

### Keywords

CNV; 16p12.3; BMI; body fat mass; association

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### CONFLICT OF INTEREST

The authors declare no conflict of interest.

## INTRODUCTION

Obesity is a major public health problem, resulting in increased risk for multiple disorders, including type 2 diabetes, cardiovascular disease and some forms of cancer.<sup>1,2</sup> Although excessive energy intake and diminished physical activity contribute to the increasing prevalence of obesity, previous studies have demonstrated a strong genetic component to obesity risk, with heritability estimated of ~40–70%.<sup>3,4</sup> Body mass index (BMI) is the most widely used phenotype to evaluate obesity due to its ease of measurement. Besides BMI, body fat mass can also be used as a complementary phenotype to characterize obese status. Identifying genetic determinants of these phenotypes could lead to a better understanding of the pathogenesis of obesity.

In the past years, genome-wide association studies (GWASs) have successfully identified a number of loci for obesity, including *FTO*, *MC4R* and so on.<sup>5–8</sup> Although most of the findings of GWASs are highly reproducible, they explain only a small portion of heritability (<10%) of obesity collectively.<sup>9</sup> This situation suggests that the missing heritability might in part be explained by additional genes or different types of genetic variants, such as copy number variations (CNVs). CNV is a common type of genomic variability with size of 1 kb to several Mb. CNVs have been demonstrated to be related to a series of complex diseases, such as autism,<sup>10</sup> schizophrenia<sup>11</sup> and osteoporosis.<sup>12</sup> Several CNV regions have also been reported for obesity and BMI, including 16p11.2,<sup>13,14</sup> 10q11.22(ref.15) and 11q11,<sup>16</sup> which implicate that CNVs have an important role in predisposing to obesity. Recently, two large-scale GWASs on obesity have detected two SNPs, rs2815752 and rs12444979, which are in strong linkage disequilibrium with two CNVs, 1p31.1 (tagged by rs2815752)<sup>17</sup> and 16p12.3 (tagged by rs12444979).<sup>18</sup> CNV 1p31.1 has been successfully confirmed for obesity by another group.<sup>16</sup> However, for CNV 16p12.3, the result for association with obesity is still controversial.<sup>16</sup> Replication of genetic associations in additional populations by independent group is necessary to evaluate a positive finding and determine its generality. Therefore, the aim of this study was to directly examine the relationship between the CNV 16p12.3 and obesity phenotypes, including BMI and body fat mass. Our samples included not only Whites, but also Chinese, to see whether this CNV is common or ethnicity-specific.

## MATERIALS AND METHODS

### Subjects

The study was approved by the Institutional Review Board or Research Administration of Xi'an Jiaotong University, Hunan Normal University, Creighton University and University of Missouri-Kansas City. Signed informed consent documents were obtained from all study participants before entering the study.

### European sample

The European sample consisted of 2286 unrelated adults living in the US Midwestern area. All of the subjects were normal healthy subjects of Northern European origin and defined by a comprehensive suite of exclusion criteria.<sup>19</sup> Briefly, subjects with chronic diseases and conditions involving vital organs (heart, lung, liver, kidney and brain) and severe endocrine, metabolic or nutritional diseases that might affect fat metabolism were excluded from this study.

BMI was calculated as body weight (kg) divided by the square of height (m). Weight was measured in light indoor clothing without shoes, using a calibrated balance beam scale, and height was measured using a calibrated stadiometer. Body fat mass was measured using dual energy X-ray absorptiometry by Hologic 4500 DEXA machines (Hologic Inc., Bedford, MA, USA). The short-term reproducibility (coefficient of variation) of BMI and fat mass

measurements is on average 0.2 and 1.1%, respectively. Measurement of body fat mass by DEXA is considered to be highly accurate and gold standard. The correlation between BMI and fat mass was 0.85 ( $P < 0.01$ ) in this sample.

### Chinese sample

The Chinese sample consisted of 1627 unrelated subjects. The subjects were recruited from Midwestern Chinese Han adults living in Xi'an and Changsha cities. The exclusion criteria was same as with European sample. The measurements of BMI and body fat mass were obtained by the same approach as those adopted in the European sample.

### Genotyping

Genomic DNA was extracted from peripheral blood leukocytes using standard protocols. For all the samples, SNP genotyping was performed using Genome-Wide Human SNP Array 6.0 (Affymetrix, Santa Clara, CA, USA) according to the Affymetrix protocol, which has been described in our previous publication.<sup>20</sup> Only samples with a minimum call rate of 95% were included. Because of efforts of repeat experiments, all samples met this criteria, and the final mean call rate reached a high level of 98.93% for the European sample and 98.96% for the Chinese sample, respectively.

### CNV detection

Common CNVs were identified using the CANARY algorithm implemented in the Birdsuite software,<sup>21</sup> which utilized a previously defined copy number polymorphism (CNP, namely CNV with frequency greater than 1%) map based on HapMap samples.<sup>22</sup> CNV 16p12.3 was denoted by CNP 2150 according to the CNP map.

### Statistical analyses

Before association analyses, principal component analysis implemented in EIGENSTRAT<sup>23</sup> was used to correct for potential population stratification that may lead to spurious association results. The first 10 principal components emerging from the EIGENSTRAT analyses, along with sex and age, were used as covariates to adjust the raw BMI or fat mass in each sample. Association analyses were performed by linear regression for the adjusted residuals.

## RESULTS

The basic characteristics of the study subjects are presented in Table 1. We summarized the association results for CNP 2150 with BMI and body fat mass in Table 2. According to the CNP map, CNP 2150 is located between 19 853 151 and 19 874 863 bp on chromosome 16 spanning ~21 kb (named CNV 16p12.3). For the European sample, we detected 46 subjects (2.02%) with a homozygous deletion, 577 subjects (25.24%) with a heterozygous deletion (1 copy) and 1663 subjects (72.74%) with wild type (2 copies). The prevalence of the deletion polymorphism was concordant with that expected from the Hardy–Weinberg equilibrium test ( $P = 0.676$ ). We were able to confirm the association between this CNV and BMI ( $P = 1.38 \times 10^{-2}$ ) in the European sample. We also found a more significant association signal between this CNV and body fat mass, with a  $P$ -value of  $2.13 \times 10^{-3}$ . As shown in Figure 1, less copy numbers were significantly associated with lower BMI and body fat, and the effect size ( $\beta$ ) was estimated to be 0.62 (BMI) and 1.41 (body fat mass) for each copy number, respectively. In addition, as this CNV is in high linkage disequilibrium with the tagging SNP rs12444979 ( $r^2 = 0.96$ ), we also examined associations between rs12444979 and obesity phenotypes. Significant associations were observed both for BMI ( $P = 0.010$ ) and body fat mass ( $P = 0.002$ ).

For the Chinese sample, we found that the frequency of this deletion CNV was different from that in the European sample ( $P < 0.001$  by  $\chi^2$ -test). We only detected 13 subjects (0.8%) with a heterozygous deletion. Most of the subjects were wild type with two copy numbers (99.2%). We didn't observe significant association between this CNV, and BMI and body fat mass (Table 2). For the tagging SNP rs12444979, there is no polymorphism in the Chinese sample.

## DISCUSSION

A recent large-scale GWAS identified a novel variant, rs12444979, which is in high linkage disequilibrium with CNV 16p12.3, for association with obesity.<sup>18</sup> The deletion allele was tagged by the T allele of rs12444979 with the allele frequency of 0.13. This study provided an indirect evidence for the association between CNV 16p12.3 and obesity, as the identification of CNV was based on the tagging SNP. In our study, we successfully detected CNV 16p12.3 by using the Affymetrix Genome-Wide Human SNP Array 6.0, which is designed for the detection of common CNVs, and directly confirmed the significant association between this CNV and obesity phenotypes in the European population. Our results demonstrated the validity of the initial finding and further supported the potential contribution of CNV 16p12.3 to the pathogenesis of obesity.

The statistical power of our study is estimated by using the program Genetic Power Calculator (<http://pngu.mgh.harvard.edu/~purcell/gpc/qtlassoc.html>). We set the significance level at  $P = 0.05$ . Assuming that a marker has a minor frequency of 0.05 and is in strong linkage disequilibrium ( $D' = 0.9$ ) with a functional mutation that accounts for 1% variation of a phenotype, our European and Chinese samples can achieve >95% statistical power, which is large enough to detect a genetic variant. However, we could not replicate the association between CNV 16p12.3 and obesity in the Chinese population, which revealed an ethnic differentiation for this CNV. One possible explanation for such ethnic difference could be that the frequencies of this CNV are quite different between the European and Chinese populations ( $P < 0.001$ ). The copy number deletion of CNV 16p12.3 is much common in the European population with the deletion frequency of 27.26%, which may contribute to the overall risk of obesity in Europeans; whereas, only 0.8% of the Chinese subjects have this deletion CNV. Our findings supported that it is necessary to evaluate associations in populations of different ancestries, especially for CNV analyses, as the genomic variation is greater when compared across ethnicities.

CNV 16p12.3 is a 21-kb deletion that locates ~50 kb upstream of the gene *GPRC5b* (G protein-coupled receptor, family C, group 5, member B). The protein encoded by this gene is a member of the type-3 G protein-coupled receptor family. This protein may mediate the cellular effects of retinoic acid on the G protein signal transduction cascade. The direct connection between this protein and the biology of obesity phenotypes is still unknown. Our results may provide an important entry point for the future investigation.

In conclusion, we confirmed the recently found association between CNV 16p12.3 and obesity phenotypes in the European population, which highlights the importance of this CNV for obesity. Future functional studies are needed to elucidate its detailed role and uncover the new insight into the biology of obesity. We first suggest that this CNV is ethnic specific, as it has no effect on obesity in the Chinese population. The development and application of future therapies and interventions should consider the ethnicity of subjects.

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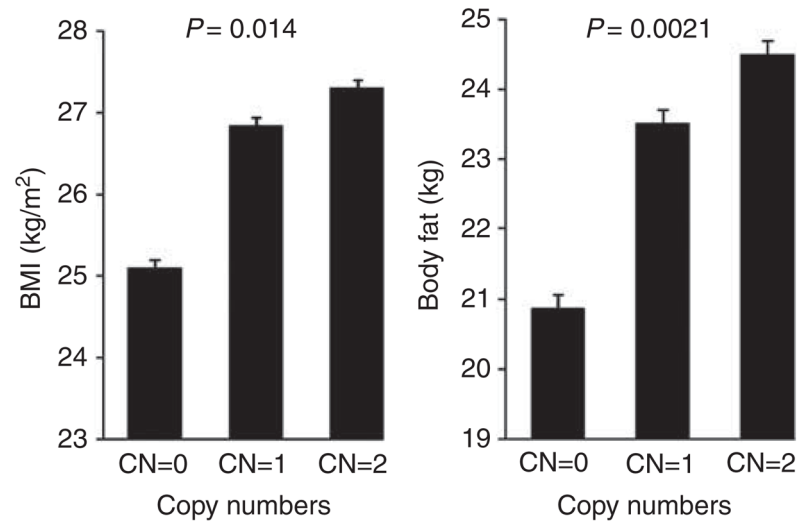
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**Figure 1.**  
Phenotype differences for CNV 16p12.3 in the European sample.

**Table 1**

Basic characteristics of the study subjects

Trait	European sample	Chinese sample
Number	2286	1627
Age (years)	51.37 (13.76)	34.49 (13.24)
Weight (kg)	75.27 (17.54)	60.12 (10.48)
Height (cm)	166.35 (8.47)	164.25 (8.16)
Female/male	1727/558	825/802
BMI (kg m <sup>-2</sup> )	27.14 (5.75)	22.21 (3.03)
Body fat (kg)	24.17 (10.63)	14.02 (5.44)

Abbreviation: BMI, body mass index. Data are shown as mean (s.d.).



**Table 2**

Association results for CNV 16p12.3 with obesity phenotypes in European and Chinese samples

<i>Sample</i>	<i>Copy numbers (N)</i>		<i>HWE</i>	<i>P-value</i>		
	<i>N = 0</i>	<i>N = 1</i>		<i>N = 2</i>	<i>BMI</i>	<i>Body fat mass</i>
European	46	577	1663	0.676	1.38 × 10 <sup>-2</sup>	2.13 × 10 <sup>-3</sup>
Chinese	0	13	1614	0.862	0.993	0.613

Abbreviations: BMI, body mass index; HWE, Hardy-Weinberg equilibrium.