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## A family-based association study identified *CYP17* as a candidate gene for obesity susceptibility in Caucasians

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### Abstract

The cytochrome P450c17 $\alpha$  gene (*CYP17*) encodes a key biosynthesis enzyme of estrogen, which is critical in regulating adipogenesis and adipocyte development in humans. We therefore hypothesized that *CYP17* is a candidate gene for predicting obesity. In order to test this hypothesis, we performed a family-based association test to investigate the relationship between the *CYP17* gene and obesity phenotypes in a large sample comprising 1873 subjects from 405 Caucasian nuclear families of European origin recruited by the Osteoporosis Research Center of Creighton University, USA. Both single SNPs and haplotypes were tested for associations with obesity-related phenotypes, including body mass index (BMI) and fat mass. We identified three SNPs to be significantly associated with BMI, including rs3740397, rs6163, and rs619824. We further characterized the linkage disequilibrium structure for *CYP17* and found that the whole *CYP17* gene was located in a single-linkage disequilibrium block. This block was observed to be significantly associated with BMI. A major haplotype in this block was significantly associated with both BMI and fat mass. In conclusion, we suggest that the *CYP17* gene has an effect on obesity in the Caucasian population. Further independent studies will be needed to confirm our findings.

### Keywords

*CYP17*; Body mass index; Obesity; Association study

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### Conflict of interest

All the authors declare that they have no conflicts of interest.

## INTRODUCTION

The prevalence of obesity is increasing throughout the world, making it a worldwide public health problem (Khan and Bowman, 1999). Obesity is characterized by excessive body fat that adversely affects health and is a major risk factor for type 2 diabetes mellitus, coronary heart disease, hypertension, excess mortality, and some forms of cancer (Kopelman, 2000). It is estimated that the obese population in developed countries has more than doubled over the last decade (James, 2004).

Many studies have shown that estrogen plays an important role in obesity. Some of them indicate that menopause-induced estrogen deficiency is associated with increased abdominal obesity (Tchernof et al., 1998) and higher total cholesterol in plasma (Maynar et al., 2001). Estrogen replacement therapy can prevent menopause-induced gains in adipose tissue mass (Gambacciani et al., 2001). Mattiasson et al. (2002) found decreased intra-abdominal and intra-pelvic fat compartments in postmenopausal women after one year of estrogen treatment. Puder et al. (2006) found that serum estradiol level was inversely associated with central fat accumulation in women with regular menstrual cycles. The cytochrome P450c17 $\alpha$  gene (*CYP17*) plays an important role in the biosynthesis of estrogen. The *CYP17* gene mediates the early steps of endogenous estrogen biosynthesis by converting pregnenolone and progesterone to androgen and estrogen precursors. However, no study has explored the relationship between the *CYP17* gene and obesity.

In this study, we aimed to test for associations between polymorphisms in *CYP17* and obesity susceptibility in a large Caucasian sample of 1873 subjects from 405 nuclear families.

## MATERIAL AND METHODS

### Subjects

The study was approved by the Creighton University Institutional Review Board in USA. All the study subjects were Caucasians of European origin and signed informed-consent documents before they entered the study in the Osteoporosis Research Center of Creighton University to search for genes underlying common human complex traits, including obesity and osteoporosis, etc. The detailed design and recruitment procedures were published previously (Deng et al., 2002). A total of 405 nuclear families were recruited with 1873 subjects, including 740 parents, 744 daughters and 389 sons. Among these, 341 families were composed of both parents and at least one offspring. In the remaining 64 families, there were at least two children with either one or no parent. The average family size was  $4.62 \pm 1.78$  (mean  $\pm$  SD, standard deviation), ranging from 3 to 12, and there were 1512 sibling pairs in total. For each subject, information on age, gender, medical history, and family history was acquired.

### Phenotype measurement

Obesity phenotypes included body mass index (BMI) and body fat mass. BMI was calculated as the ratio of total body weight divided by the square of height (in units of kg/m<sup>2</sup>). Weight was measured in light indoor clothing, using a calibrated balance beam

scale, and height was measured using a calibrated stadiometer. Fat mass was measured by a Hologic 4500 dual-energy X-ray absorptiometry (DXA) scanner (Hologic Corporation, Waltham, MA, USA). The measurement precision of BMI as reflected by the coefficient 0.2%. The coefficient of variation for fat mass was 1.2%. for artifacts and confounding features. Subjects with significant excluded from the analysis.

### SNP selection and genotyping

For each subject, genomic DNA was extracted from whole blood using a commercial isolation kit (Genra Systems, Minneapolis, MN, USA) following the procedure detailed in the kit. SNPs were selected mainly according to public databases such as dbSNP (<http://www.ncbi.nlm.nih.gov/SNP/>) and SNPper (<http://snpper.chip.org/bio/snpper-enter>). We selected 7 SNPs in *CYP17* based on the following criteria: 1) validation status, especially in Caucasians; 2) an average density of 1 SNP per 4 kb; 3) degree of heterozygosity, i.e., minor allele frequencies >0.05; 4) functional relevance and importance, and 5) reported to dbSNP by various sources. Seven SNPs were successfully genotyped using the high-throughput BeadArray SNP genotyping technology from Illumina Inc. (San Diego, CA, USA). The average rate of missing genotype data reported by Illumina is ~0.05%.

### Statistical analyses

We used PedCheck (O'Connell and Weeks, 1998) to check Mendelian consistency of SNP genotype data within families before analysis. Any inconsistent genotypes were removed. The error checking option embedded in Merlin (Abecasis et al., 2002) was run to identify and disregard the genotypes flanking excessive recombinants, further reducing genotyping errors. Hardy-Weinberg equilibrium for each SNP was tested in parents using the PEDSTATS procedure implemented in Merlin. Haplotype analyses were performed by the Haploview software (Barrett et al., 2005) to generate graphical representation of linkage disequilibrium (LD) structure as measured by  $D'$ . The Box-Cox transformation was used for converting the raw phenotypic data to normal distribution by Minitab (State College, PA, USA).

For single- and multiple-marker haplotype transmission, the program FBAT was used (<http://www.biostat.harvard.edu/fbat/fbat.htm>). This program is based on the original transmission disequilibrium test, in which alleles transmitted to affected offspring are compared with the expected distribution of alleles among offspring (Ewens and Spielman, 1995). All FBAT analyses assumed an additive model. The statistical analyses were performed on the transformed data using age and gender as covariates that significantly affect the obesity phenotypes. Permutated P values were obtained through 10,000 permutations to account for the multiple haplotypes within each block by the haplotype version of FBAT (HBAT) (Horvath et al., 2004).

## RESULTS

The basic characteristics of the studied samples are presented in Table 1. Among all the 1873 subjects, 262 females (125 mothers, 137 daughters) and 193 males (119 fathers, 74 sons) were obese (BMI  $\geq 30$  kg/m<sup>2</sup>).

We performed single-SNP association analyses. The results are summarized in Table 2. For BMI, significant associations were 0.016, rs6163 ( $P = 0.018$ ) and rs619824 ( $P = 0.039$ ). For fat mass, only one SNP showed a marginal association signal (rs619824:  $P = 0.058$ ).

We further characterized the LD structure for *CYP17* and conducted haplotype association analyses. The 7 SNPs we tested spanned a ~23-kb genetic region within or around the *CYP17* gene, with average marker distance of 1 SNP per 3.9 kb. These 7 SNPs included 1 promoter marker, 1 exonic marker, 2 intronic markers, and 3 3'-untranslated region markers. Only 1 haplotype block was identified in the whole *CYP17* gene (Figure 1). Table 3 summarizes the haplotype association results. After 10,000 permutations, the whole block of *CYP17* was significantly associated with BMI (permutation  $P$  value = 0.019). For the single haplotype, we also found a major haplotype, ACCTCGC, with the frequency of 0.571, to be significantly associated both with BMI ( $P = 0.013$ ) and fat mass ( $P = 0.021$ ).

## DISCUSSION

In this study, we explored the relationship between the estrogen-related gene *CYP17* and obesity-related phenotypes in a large Caucasian sample using family-based association analysis. We identified 3 SNPs and a haplotype in *CYP17* to be significantly associated with BMI, suggesting that the *CYP17* gene may have potential influence on variation of BMI and the pathogenesis of obesity.

In the pathway of estrogen biosynthesis, *CYP17* catalyzes the conversion of 17-hydroxypregnenolone to dehydropregnenolone (DHEA). DHEA was marketed as a weight-loss pill for years. Some studies found that DHEA could enhance muscle strength and muscle mass. Morales et al. (1998) treated 16 older people for six months using 100 mg oral DHEA daily and observed that DHEA decreased fat mass and increased both knee muscle strength and lumbar back strength. Villareal and Holloszy (2006) found that DHEA replacement had the beneficial effect of enhancing the increases in muscle mass and strength induced by heavy resistance exercise in elderly individuals. Moreover, many studies proved that DHEA could help reduce excessive fat storage (Hansen et al., 1997; Han et al., 1998; Villareal and Holloszy, 2004). A possible mechanism is that DHEA reduces abdominal fat by activating the peroxisome proliferator activated receptor, which can up-regulate the transcription of fatty acid transport proteins that facilitate fatty acid entry into cells, as well as the expression of enzymes involved in the  $\beta$ -oxidation of fatty acids (Villareal and Holloszy, 2004). The close relationship between DHEA and obesity partially supported our observed association between *CYP17* and obesity.

The transcription of *CYP17* is regulated by cyclic AMP (cAMP) and steroidogenic factor-1 (SF-1) (Lund et al., 1997; Dammer et al., 2007). SNP rs6163, located in exon 1, is close to the major cAMP response elements (-243 to -225 bp) and an SF-1 binding site (-80 to -40 bp) (Demeter et al., 1996; Bischof et al., 1998). Previous studies found that the mutation or deletion of exon 1 induced the declined activities of cytochrome P450c17 $\alpha$  (Yanase et al., 1989; Laflamme et al., 1996). Ahlgren et al. (1992) found exon 4 and exon 6 mutations in a cytochrome P450c17 $\alpha$ -deficient patient. SNP rs3740397, located in intron 5, is very close to exon 6. The SNPs of *CYP17* are all located in one block and thus in strong LD. Therefore,

rs6163 and rs3740397 may be related to the regulation of gene expression of *CYP17* and the activity of cytochrome P450c17 $\alpha$ .

In summary, we are the first to suggest the potential effect of one key estrogen biosynthesis gene, *CYP17*, on BMI variation, which might have important implications for understanding the mechanism involved in the pathology of obesity. Our findings could be a starting point for follow-up molecular functional studies.

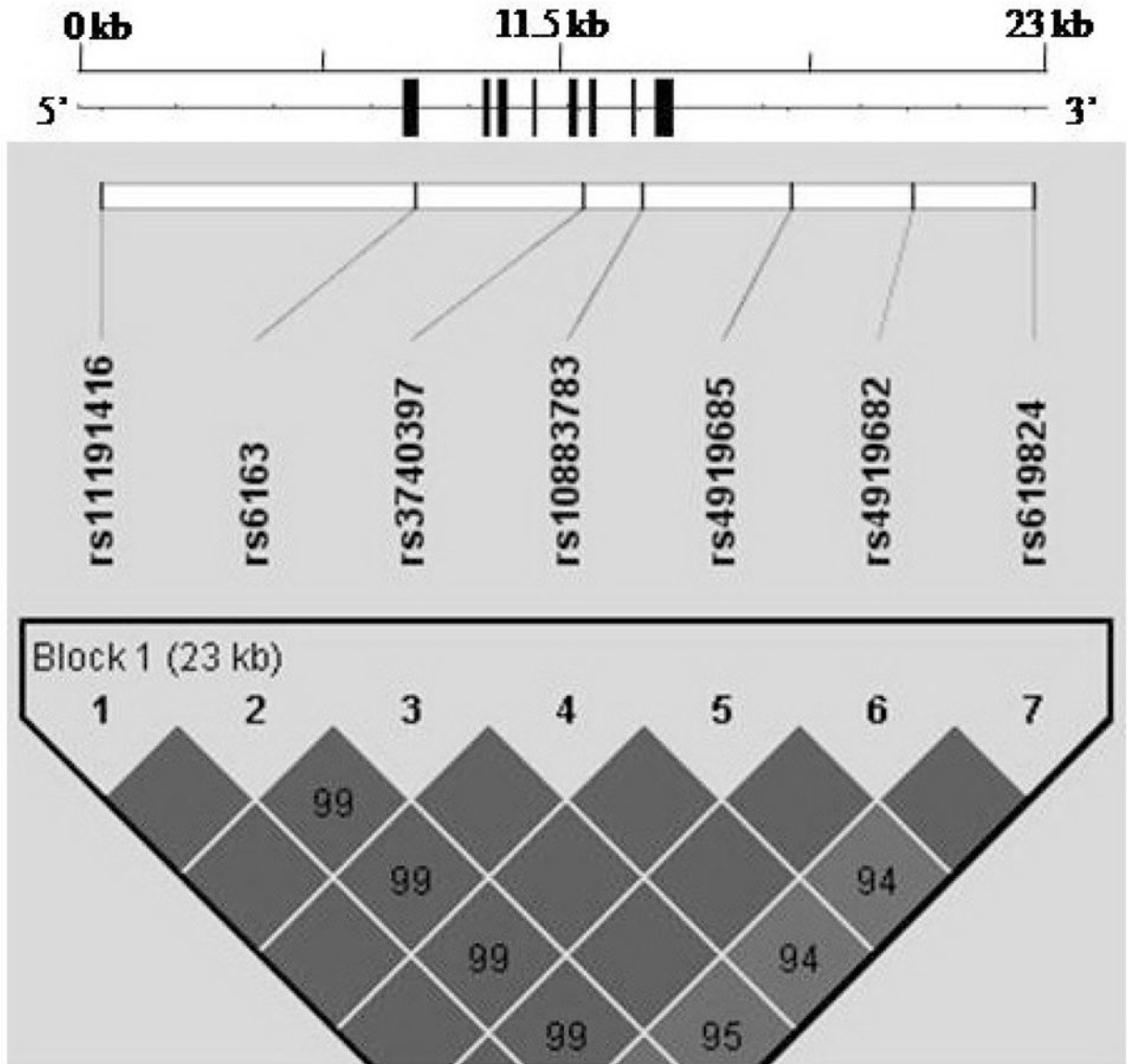
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**Figure 1.**  
Gene structure and linkage disequilibrium pattern for the *CYP17* gene.

**Table 1**

Descriptive characteristics of the study subjects from 405 nuclear families.

	<b>Mother (N = 380)</b>	<b>Father (N = 360)</b>	<b>Daughter (N = 744)</b>	<b>Son (N = 389)</b>
Age (years)	62.4 ± 10.4	62.8 ± 10.7	37.7 ± 10.3	36.0 ± 10.9
Height (m)	1.62 ± 0.06	1.77 ± 0.07	1.65 ± 0.06	1.80 ± 0.07
Weight (kg)	73.81 ± 15.21	90.30 ± 15.26	70.40 ± 16.42	87.60 ± 15.94
BMI (kg/m <sup>2</sup> )	28.04 ± 5.57	28.85 ± 4.42	25.77 ± 5.86	27.02 ± 4.39
Fat mass (kg)	28.70 ± 9.87	24.73 ± 7.90	24.44 ± 10.45	20.13 ± 8.11

Data are reported as means ± SD of the raw phenotypic values without adjustment for covariates; total sample size, N = 1873.



Table 2

Single-SNP association results for *CYP17* gene polymorphisms with body mass index (BMI) and fat mass.

No.	SNP ID	Physical position <sup>a</sup>	Alleles <sup>b</sup>	MAF	Location	BMI P value	Fat mass P value
1	rs11191416	104594906	A/C	0.094	Promoter	0.320	0.237
2	rs6163	104586914	C/A	0.397	Exon1	<b>0.018</b>	0.079
3	rs3740397	104582665	C/G	0.396	Intron5	<b>0.016</b>	0.083
4	rs10883783	104581142	T/A	0.301	Intron7	0.064	0.299
5	rs4919685	104577352	C/A	0.301	3' UTR	0.064	0.299
6	rs4919682	104574320	G/A	0.287	3' UTR	0.080	0.310
7	rs619824	104571278	C/A	0.442	3' UTR	<b>0.039</b>	0.058

<sup>a</sup>Positions are based on the NCBI web site (<http://www.ncbi.nlm.nih.gov/SNP/>).

<sup>b</sup>The second allele is the minor allele. MAF = minor allele frequency. Results with P values <0.05 are shown in bold.

**Table 3**

Haplotype association results for *CYP17* gene polymorphisms with body mass index (BMI) and fat mass.

Block	Haplotype	Haplotype frequency <sup>a</sup>	BMI P value <sup>b</sup>	Fat mass P value
Block1	ACCTCGC	0.571	<b>0.013</b>	<b>0.021</b>
	AAGAAAA	0.269	0.050	0.160
	CAGTCGA	0.091	0.331	0.242
	ACCTCGA	0.054	0.484	0.844
			<b><i>0.019</i></b>	<b><i>0.086</i></b>

<sup>a</sup>We only listed the common haplotypes with estimated frequencies of more than 5%.

<sup>b</sup>Results with P values <0.05 are shown in bold. Permutation P value of the whole block is shown in bold italics.