

## RESEARCH ARTICLE

# DRD4 Gene Variant Associated With Body Mass: The National Longitudinal Study of Adolescent Health

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In this article we report a novel statistically significant association between the D4.7/D4.7 genotype of the DRD4 gene and the body mass of black and Hispanic participants in the National Longitudinal Study of Adolescent Health (Add Health). We investigated the role of the 48-bp repeat polymorphism of the dopamine receptor 4 gene (DRD4) in body mass regulation in 2,277 adolescents and young adults followed in 1995 (12–18 years old), 1996, and 2002 by Add Health. After the effects of age, sex, and ethnicity were adjusted, the D4.7/D4.7 genotype reduced the body mass index (BMI)-percentile score by 15 and 12.5, as compared to those with other genotypes, for African-Americans ( $P = 0.0047$ ) and Hispanic-Americans ( $P = 0.037$ ), respectively. Although the D4.7/D4.7 genotype was associated with a lower BMI-percentile score in white individuals compared to other genotypes, the difference was not significant. Individuals heterozygous for D4.7 did not differ from those with the other/other genotype. *Hum Mutat* 27(3), 236–241, 2006. © 2006 Wiley-Liss, Inc.

KEY WORDS: body mass; BMI; DRD4; obesity; dopamine receptor

## INTRODUCTION

The prevalence of adult overweight (defined as body mass index (BMI) = 25–29.9 kg/m<sup>2</sup>) and obesity (BMI > 30) has increased dramatically in the United States over the past 20–30 years. Over 65% of U.S. adults are considered overweight, and 31% are considered obese [Manson and Bassuk, 2003]. Alarming, this health problem is becoming common among children and adolescents: 16% of U.S. youth (12–19 years old) in 1999–2000 were classified as overweight [Ogden et al., 2002]. Obesity is associated with increased mortality and morbidity, and has well-documented associations with hypertension, metabolic syndrome, dyslipidemia, type-2 diabetes, coronary heart disease, osteoarthritis, stroke, and several cancers [Bray, 2000].

Our understanding of genetic effects on human weight regulation has increased enormously in recent years [Comuzzie, 2002; Comuzzie and Allison, 1998; Snyder et al., 2004]. Studies have consistently shown that 40–70% of the variation in obesity-related measures is heritable. Over 200 human quantitative trait loci (QTL) for obesity-related phenotypes have been identified, with 35 genomic regions implicated in at least two studies [Snyder et al., 2004]. Moreover, there are at least 15 candidate genes that are supported by a minimum of five independent studies.

Neurotransmitters in the brain have been targeted in the attempt to develop weight-control medications that regulate food intake [Bray and Tartaglia, 2000]. Several neurotransmitters (dopamine, GABA, norepinephrine, and serotonin), as well as peptides and amino acids, are involved in the regulation of food intake [Schwartz et al., 2000]. Among them, dopamine attracts particular interest because this neurotransmitter seems to regulate food intake by modulating food reward via the mesolimbic

circuitry of the brain [Balcioğlu and Wurtman, 1998; Martel and Fantino, 1996].

The purpose of the present study was to investigate the role of the 48-bp repeat polymorphism [c.744(ACCCGCGCCCCGCTCCCCAGGACCCCTGCGGCCCGACTGTGCGCC)2-11] of the dopamine receptor 4 gene (DRD4; MIM# 126452; HGNC# 3025; GDB# 127782; GenBank: NM\_000797.2) in the body mass of 2,277 adolescents and young adults who were followed in 1995, 1996, and 2002 by the National Longitudinal Study of Adolescent Health (Add Health) [Harris et al., 2003]. DRD4, which maps 11p15.5 spanning 3.4 kb, is one of the five types of dopamine receptors. A functional VNTR polymorphism was identified in the third exon in the DRD4 gene, the region coding for the third intracellular loop of the receptor [Van Tol et al., 1992]. The genetic variant is a 16 amino acid (48 bp) repeat polymorphism, which is repeated two to 11 times, with two (D4.2), four (D4.4), and seven (D4.7) repeats being the most common alleles [Van Tol et al., 1992; Lichter et al., 1993]. In vitro studies suggest that the exon III DRD4 7-repeat allele (D4.7) has decreased affinity for dopamine, and transmits weaker intracellular signals in comparison with other exon III alleles [Asghari et al., 1995].

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Pharmacological evidence implicates the *DRD4* gene in obesity. Clozapine, which binds with high affinity to *DRD4* [Van Tol et al., 1991], can lead to increased food consumption and subsequent weight gain, both of which are associated with increased leptin production [Bromel et al., 1998]. Two recent studies reported an association between the 7-repeat of the *DRD4* gene and obesity and binge eating in a sample of women with seasonal affective disorder (SAD) [Levitin et al., 2004a,b]. Wang et al. [2001] suggested that the 7-repeat allele promotes consumption of highly palatable foods, perhaps to compensate for sluggish dopamine levels. However, one study that assessed the relationship between the 16 amino acid repeat polymorphism and BMI found no association [Hinney et al., 1999].

## MATERIALS AND METHODS

### Subjects

The data source for the current analysis was Add Health, a school-based study of the health-related behaviors of 12–18-year-old adolescents who were in grades 7–12 in 1994–1995 in the United States [Harris et al., 2003]. The Add Health study is longitudinal, and adolescents were interviewed three times during a 7-year period in 1994–1995, 1995–1996, and 2001–2002. Add Health is based on a stratified sample of 80 high schools. The schools were stratified by region, urbanicity, school type (public, private, or parochial), ethnic mix, and size. For each high school selected, a feeder school (typically a middle school) was identified and recruited, yielding one school pair in each of 80 different communities. Because some schools spanned grades 7–12, we had 132 schools in our sample, each of which was associated with one of the 80 communities. Our analysis used the sibling sample (2,277 individuals) of Add Health because DNA measures were available only for this subset of the Add Health sample. Our sibling sample was composed of monozygotic twins, dizygotic twins, and full biological siblings. Our sample included 2,101, 2,147, and 2,275 measures from Waves I, II, and III, respectively, from a total of 2,277 individuals who contributed at least one measure to the analysis. Of the 2,277 individuals, 64%, 20%, and 16% were white, African-American, and Hispanic-American, respectively. The few Asians in the sample were deleted because no Asian is homozygous for D4.7. The correlation between siblings in the same family and the repeated measures over time for the same individual was addressed by the standard mixed model (see Analytical Strategies below). The Add Health Study obtained written informed consent from all participants, and the Institutional Review Board of the University of North Carolina, Chapel Hill, approved this study.

### Overweight and Obesity Measures

Our analysis used the standard body mass index ( $BMI = \text{kg}/\text{m}^2$ ) as an indicator of overweight and obesity. Body weight was measured and recorded to the nearest pound. Height was measured according to the standardized protocol. Despite its limitations, such as potential mistreatment of higher lean body mass as body fat, somewhat dissimilar developmental implications for boys and girls, and possible genetically-based body shapes and proportions, most leading scholars recommend the BMI as the most practical index for assessing overweight for adolescents and obesity in young adults, especially for large-scale, population-based studies [WHO, 1995; Cole et al., 2000; Himes and Dietz, 1994].

Add Health has collected three waves of overweight and obesity data. The height and weight measures were self-reported in Wave I, and both self-reported and measured in Waves II–IV. Our analysis used Wave-I self-reported data, and Waves II and III

measured weight and height data. Goodman et al. [2000] investigated self-reported measures collected by Add Health using measured data in Wave II as a standard. There was a strong correlation between measured and self-reported height (0.94), and between measured and self-reported weight (0.95). None of the demographic and socioeconomic status (SES) variables, including race, parental education, and household income, were significantly associated with differences in self-reported vs. measured BMI, except for gender. On average, girls underreport their BMI by 0.27 unit, and boys overreport their BMI by 0.03. Self-reported BMI correctly classified 96.2% of the respondents as to obesity status. In this case, girls were no more likely than boys to be misclassified as obese. Goodman et al. [2000] concluded that self-reported height and weight are valid measures for understanding teen obesity and its correlates, at least in the Add Health population.

We used the age- and sex-specific BMI percentile score (BMI-P), which is adjusted for the nonlinear effects of age and gender. The adjustment for age and sex is important for adolescent body mass data because of the rapid growth that occurs during childhood and adolescence. The adjustment is accomplished by transforming the raw BMI score into the age- and sex-specific percentile score based on the observed growth percentile curves for children, adolescents, and youth constructed by the Centers for Disease Control and Prevention/National Center for Health Statistics (CDC/NCHS) [Kuczumski et al., 2000] for the United States. These standard growth percentile curves are based on data from five national health examination surveys conducted from 1963 to 1994 and five supplementary data sources.

### DNA Preparation and Genotyping

In Wave III in 2002, DNA samples were collected from a subset of the Add Health sample. Genomic DNA was isolated from buccal cells at the Institute for Behavioral Genetics, University of Colorado (Smolen and Hewitt, [www.cpc.unc.edu/projects/addhealth/](http://www.cpc.unc.edu/projects/addhealth/)), using a modification of published methods [Lench et al., 1988; Meulenbelt et al., 1995; Spitz et al., 1996; Freeman et al., 1997]. The average yield of DNA was  $58 \pm 1 \mu\text{g}$ . The best overall measurement of the quality of the DNA samples is their utility in genotype determinations. All of the Wave III buccal DNA samples are of excellent quality and over the past 18 months have been used to assess nearly 48,000 genotypes that include short tandem repeats (STRs), variable number tandem repeats (VNTRs), and single nucleotide polymorphisms (SNPs). The zygosity of the twins was determined with 12 unlinked STRs.

The assay used to genotype for *DRD4* was a modification of the method of Sander et al. [1997]. The primer sequences used [Lichter et al., 1993] were as follows: forward, 5'-AGGACCCTCA TGGCCTTG-3' (fluorescently labeled), and reverse, 5'-GCGACT ACGTGGTCTACTCG-3'. Amplification products were resolved by 3.5% agarose gel electrophoresis and were visualized by staining with ethidium bromide. Fragment sizes were determined by comparison with molecular length standards. The numeric designation of each allele refers to the number of repeats it contains. This method results in PCR products of (in bp): 379 (D4.2), 9%; 427 (D4.3), 3%; 475 (D4.4), 65%; 523 (D4.5), 1%; 571 (D4.6), 0.8%; 619 (D4.7), 20%; 667 (D4.8), 0.9%; 715 (D4.9), 0.06%; and 763 (D4.10), 0.2%. To ensure genotypic control, each subject was analyzed twice with entirely separate PCRs and runs. The results were posted only if the two sets of results agreed. A series of  $\chi^2$  tests, each performed within a self-reported ethnic group (European-, African-, or Hispanic-American), revealed no deviation from the Hardy-Weinberg

equilibrium. Of the nine alleles in the polymorphism, the 4 repeat (65.3%) and 7 repeat (20.4%) accounted for 86% of the variants observed in our population. Our analysis focuses on these two alleles and the genotypes associated with these two alleles.

**Analytical Strategies**

We employed a two-step analytical strategy. First, we compared, in a contingency table, the means of BMI-P across *DRD4* genotypes, ethnic groups, and Add Health Waves. Second, we use the mixed regression model [Searle, 1971; Searle et al., 1992] to estimate the association between the *DRD4* genotypes and measures of body mass (BMI-P). We conducted the regression analysis for the all-ethnicity sample, as well as separately for the European-American, African-American, and Hispanic samples. Our sample consisted of twins and siblings, as well as the repeated measures of the same individual over different Waves, and these observations were not independent. The mixed model has long been established in the statistical literature for the analysis of data that are not independent. Several major statistical packages (e.g., SAS; SAS Institute Inc., www.SAS.com) include the mixed model as a standard procedure. The following equation describes the mixed model of BMI-P for the ethnicity-combined data

$$(BMI - P)_{jit(s)} = \beta_0 + \beta_1 black_{jt} + \beta His_{jt} + u_{j0(s)} + v_{ji} + e_{jit(s)}$$

where *j*, *i*, and *t* index the sibling cluster or pair, individual, and Add Health Waves, respectively; and *s* = *m*, *d*, or *f* indicates whether the sibling cluster or pair are MZ twins, DZ twins, or full biological siblings. Thus the model allows the random effect at the sibling cluster level and the measure level to vary by type of sibling cluster. Conditional on the random intercepts *u<sub>j0</sub>* and *v<sub>ji</sub>* at the sibling cluster level and individual level, the siblings and repeated measures were assumed to be independent. Age and sex were already adjusted in BMI-P. The models were estimated by SAS.

**RESULTS**

**Contingency Table Analysis**

Table 1 shows the mean BMI percentile score (BMI-P) and its standard deviation (SD) by *DRD4* genotype, ethnicity, and Add Health Wave. The Waves may be considered as a proxy for age, with respondents ranging in age from 12–18 at Wave I, 13–19 at

Wave II, and 19–25 at Wave III. We compared the mean BMI-P across three genotype groups: those with two copies of the 7-repeat (D4.7) allele, those with one copy, and those with no copy. The genotype prevalence is also provided. The frequency of those homozygous for D4.7 was higher for blacks (6%) and Hispanics (6%) than for whites (4%). The BMI-P has already been adjusted for age and sex. The mean BMI-P for the D4.7/D4.7 genotype appears to be substantially and consistently lower than those for the D4.7/other or other/other genotypes across all ethnic groups and all Add Health Waves. Statistical significance tests were not performed for the comparisons because standard tests are not valid due to the correlation among the siblings in the sample.

**Regression Analysis**

We tested the effect of genotype in a mixed model (Searle, 1971; Searle et al., 1992) framework controlling for the correlation among MZ twins, DZ twins, full siblings, and the repeated measures of each individual. The results on the fixed effects and their 95% confidence intervals are presented in Table 2 (estimated random parameters not shown). The other/other genotype category and white are used as references categories for genotype and ethnicity, respectively. The basic all-ethnicities model shows that the D4.7/D4.7 genotype has a statistically significant protective effect of -7.73 (P = 0.005) in the BMI-P score with the 95% confidence interval of (-13.1, -2.34); that is, after adjusting for the effects of age, sex, and ethnicity, individuals with the D4.7/D4.7 genotype are ranked 7.73 lower in the BMI percentile score than individuals with the other/other genotype. Individuals heterozygous for D4.7 do not differ from those with the other/other genotype. On average, African-Americans score 4.9 percentile points higher, and Hispanic-Americans score 7.56 percentile points higher than white Americans. To address the potential for population stratification, we conducted these analyses stratified by ethnicity. Among African-Americans and Hispanic-Americans, the D4.7/D4.7 genotype is associated with a 15-point (P = 0.0047) and 12.5-point (P = 0.037) reduction in BMI percentile score from participants with other *DRD4* genotypes, respectively. Although the D4.7/D4.7 genotypes was associated with a lower BMI-percentile score in white individuals compared to those with other genotypes, the difference was not significant.

TABLE 1. Mean BMI Percentile (Standard Deviation) by *DRD4* Genotype, Ethnicity, and Add Health Wave

DRD4 Genotypes	Genotype frequency	Wave I (n = 2101) <sup>a</sup>		Wave II (n = 2147) <sup>a</sup>		Wave III (n = 2275) <sup>a</sup>	
		Aged 12–18		Aged 13–19		Aged 19–25	
<b>All Ethnicities</b>							
Other/Other	0.61	58.78	(28.27)	57.38	(29.81)	66.28	(29.50)
D4.7/Other	0.34	58.79	(27.86)	56.88	(30.10)	66.93	(28.28)
D4.7/D4.7	0.047	52.02	(29.61)	47.09	(32.61)	61.32	(30.03)
<b>White</b>							
Other/Other	0.63	55.36	(28.27)	54.73	(29.81)	63.91	(29.54)
D4.7/Other	0.33	57.36	(27.94)	55.94	(30.64)	64.66	(28.67)
D4.7/D4.7	0.04	52.28	(30.42)	50.08	(33.44)	61.98	(30.64)
<b>Black</b>							
Other/Other	0.60	65.17	(26.34)	60.89	(28.84)	68.57	(30.06)
D4.7/Other	0.34	59.86	(27.98)	56.04	(29.37)	66.38	(28.71)
D4.7/D4.7	0.06	48.03	(28.67)	41.60	(29.80)	61.51	(30.00)
<b>Hispanic</b>							
Other/Other	0.57	65.72	(28.13)	64.46	(29.57)	73.50	(27.17)
D4.7/Other	0.33	62.72	(27.25)	61.17	(28.81)	75.62	(24.71)
D4.7/D4.7	0.06	55.97	(29.64)	46.63	(34.41)	59.55	(29.82)

<sup>a</sup>The individuals from Waves I, II, and III are largely identical.

TABLE 2. Regression Estimates of the Association of the 7-Repeat Allele (D4.7) of the Dopamine-4 Receptor (DRD4) with BMI Percentile Score

BMI-p	$(\hat{\beta} \text{ (95\%CI)})$			
	All ethnicities	White	Black	Hispanic
Intercept	<b>58.8 (57.0, 60.6)<sup>a</sup></b>	58.1 (56.1, 60.1)	65.5 (62.2, 68.7)	<b>66.8 (62.9, 70.6)</b>
Other/Other	–	–	–	–
Other/D4.7	0.35 (–2.04, 2.75)	1.67 (–1.39, 4.72)	–3.81 (–8.88, 1.26)	0.55 (–5.24, 6.34)
D4.7/D4.7	<b>–7.73 (–13.1, –2.34)</b>	–3.63 (–10.9, 3.68)	<b>–14.9 (–25.3, –4.61)</b>	<b>–12.5 (–24.26, –0.72)</b>
White Americans	–	–	–	–
African Americans	<b>4.90 (1.74, 8.06)</b>	–	–	–
Hispanic Americans	<b>7.56 (4.12, 11.0)</b>	–	–	–
Log likelihood	–29898.3	–18987.9	–6034.8	–4865.8
Number of persons	2,277	1,443	463	371
Number of measures	6,651	4,238	1,334	1,079

BMI-p, body mass index percentile score.

<sup>a</sup>Significant data cells are in bold.

To test whether our results were confounded by body weight increases in pregnant women, we conducted a separate round of analysis with all pregnant females excluded. Our results remained essentially the same. About 2% of our measures were taken when the female was pregnant. The percentages of the D4.7/D4.7 genotype among pregnant females (0.044) and nonpregnant females (0.046) are about the same.

### Population Stratification

We used three strategies to address the potential impact of population structure. First, in addition to estimating the association between the genotypes and body mass using the entire sample, we stratified our analysis by self-reported ethnicity (Tables 1 and 2). However, there may exist a “cryptic” population structure that is not well described by self-reported ethnicity [Pritchard et al., 2000; Helgason et al., 2005].

Our second strategy addressed this concern by taking advantage of the detailed categories of self-reported race/ethnicity in Add Health. Race/ethnicity identity was constructed from adolescents’ answers to the following questions: What is your race? Which category best describes your racial background? In keeping with the new U.S. Census policy, Add Health respondents were allowed to mark as many ethnicity categories as they felt applied to them [Harris et al., 2003]. About 7.5%, 3.5%, and 0% of African, Hispanic, and white (the primary ethnic categories that are self-identified) participants marked more than one category, respectively. Of those who marked more than one ethnic category, the large majority (86%) marked two ethnic categories and 12% marked three ethnic categories. We repeated our analyses after eliminating those individuals who designated themselves as multi-ethnic. The results remained essentially unchanged, with –14.3 ( $P = 0.0088$ ) for African-Americans compared to –14.9 ( $P = 0.0047$ ; Table 2), and –12.8 ( $P = 0.032$ ) for Hispanic-Americans compared to –12.5 ( $P = 0.037$ ; Table 2).

As a third strategy, we applied Allison et al.’s [1999] procedure to test for possible population stratification. Following the idea used in the development of sibship tests of linkage and association [Curtis, 1997; Boehnke and Langefeld, 1998; Spielman and Ewens, 1998], Allison et al. [1999] reasoned that the probabilities of genotypes of full siblings depended entirely on parental genotypes, and that controlling for the effects of sibship would be equivalent to controlling for parental genotypes. Indexing sibships by  $j$ , individuals by  $k$ , and genotypes by  $i$ , the procedure

can be written as a mixed model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + e_{ijk},$$

where  $\alpha_i$ , or the effect of genotype  $i$ , is assumed to be fixed;  $\beta_j$ , or the effect of sibship  $j$ , is assumed to be random; and  $(\alpha\beta)_{ij}$  is an interaction term specifying the dependence of the random effect of sibship on genotype. The conditioning on sibship in the model eliminates the possible confounding of population stratification. This model is a special case of the mixed model [Searle, 1971; Searle et al., 1992]. We implemented this procedure in SAS, and our results did not change (data not shown).

### DISCUSSION

Our data yielded a novel statistically significant association between the D4.7/D4.7 genotype of the *DRD4* gene and body mass in black and Hispanic participants of Add Health. The presence of the D4.7/D4.7 genotype is associated with a reduction of about 12–15 in the rank of the BMI percentile score for the African-American and Hispanic samples.

Excluding the current study, only limited data are available regarding the role of *DRD4* variants and body mass regulation. The results of the few studies that have been conducted are apparently contradictory [e.g., Hinney et al., 1999; Levitan et al., 2004a,b]. A recent German study [Hinney et al., 1999] did not detect any association between obesity and the 48-bp repeat polymorphism of the *DRD4* gene. However, in terms of ethnicity, the German population is much closer to our European-American sample than the African- or Hispanic-American samples, and in our European-American sample the association was not detected. In addition, the German sample consisted mainly of extremely obese children and adolescents, whereas ours is representative of the full range of body mass in a large population of children and adolescents in the United States. Moreover, whereas the German study did not examine the D4.7 homozygotes, they were the focus of our analysis.

Our findings are also in apparent contrast to those reported by Levitan et al. [2004a, 2004b], who reported a positive association of the 7 repeat of the *DRD4* gene and obesity and binge eating in a sample of women with SAD. However, their study was limited by a small sample size, since only 131 women with SAD, aged 18–65 years (vs. our age range of 12–25 years), who met DSM-IV criteria for major depression with a winter seasonal pattern were examined. The typical SAD patient is a premenopausal woman

with marked craving for high-carbohydrate/high-fat foods and significant weight gain during winter depressive episodes. The two studies also used significantly different BMI measures. Levitan et al. [2004a,b] used a maximal lifetime BMI recalled through a question about maximum weight achieved from age 16 onward with corresponding height, whereas our study used weight and height measures obtained at the time of the interview. The two sets of results must be compared with caution until the major differences between the samples are understood.

The “reward deficiency syndrome” hypothesis [Blum et al., 1996] is often invoked in association studies examining the role of the dopaminergic system. The hypothesis in the context of obesity research suggests that individuals with low intrinsic dopamine activity in the brain reward pathways tend to compensate by using various reinforcing behaviors, including increased food consumption [Comings and Blum, 2000] or risk-taking behavior (in the case of novelty seeking). However, most tests of the reward-deficiency syndrome hypothesis used polymorphisms in the dopamine D2 receptor gene (*DRD2*) rather than those in *DRD4*. Biochemical analyses of *DRD4* protein variants have demonstrated that the D4.7 protein has a blunted response for cAMP reduction, requiring a threefold increase in dopamine concentration for reductions comparable to the D4.4 protein [Asghari et al., 1995]. Thus, the inhibitory neurons utilizing the *DRD4* D4.7 receptor would require increased dopamine for “optimal” function [Swanson et al., 2000].

Recently Ding et al. [2002] and Wang et al. [2004] developed a more general framework for interpreting the differential effects of the various variants in the 48 bp VNTR in *DRD4*. They sequenced the entire *DRD4* locus from 103 individuals homozygous for D4.2, D4.4, or D4.7 variants of the VNTR using individuals of African, European, Asian, North and South American, and Pacific Island ancestry. While the D4.4/D4.4 homozygotes displayed little linkage disequilibrium (LD) over the region examined, the D4.7/D4.7 individuals exhibited dramatically stronger LD surrounding the D4.7 allele. Moreover, the evidence appeared to suggest that the selection acts at the D4.7 VNTR itself, rather than at an adjacent site. They proposed that the D4.4 allele has been the most common allele throughout most of early human history, and that the D4.7 allele started as a rare mutation about 40,000–50,000 years ago. However, instead of viewing the D4.7 as a “defective” allele, they suggested that the D4.7 allele led to behaviors that were selected for in certain environments. As a result, the D4.7 allele increased in frequency and existed as a balanced (rather than a purely directional spreading) polymorphism. This framework contrasts the hypothesis of reward deficiency syndrome, which views the D4.7 variant as a suboptimal allele, and provides a more general framework in which the empirical findings involving the human dopamine receptor D4 (*DRD4*) gene locus can be interpreted.

We were only able to investigate one polymorphism in the *DRD4* gene. An alternative explanation for our findings is that other functional variants within the *DRD4* gene or in other genes on chromosome 11 are in LD with the *DRD4* variants evaluated herein. However, it is biologically plausible that the functional D4.7 repeat variants directly affect body mass regulation. Our study is also limited because the D4.7/D4.7 genotype was relatively rare in our study population (especially in white participants), and it is possible that some of our findings are attributable to chance. For this reason, it is important for these findings to be replicated in a much larger population-based study.

The absence of an association between the D4.7/D4.7 genotype and BMI-P in the European-American sample suggests that

environmental factors may be at work. Proximate factors such as physical activity, sedentary behavior, and dietary patterns, as well as underlying environmental factors (e.g., SES, level of parental education, and contextual factors such as neighborhood walkability and physical activity facilities/resources) may explain the differences among the European-, African-, and Hispanic-Americans in our findings. We hypothesize that the European-American participants might have compensated for the absence of the D4.7/D4.7 genotype by increasing their physical activity, reducing sedentary behavior, and consuming a healthier diet. In future work we will focus on incorporating these proximate and underlying factors into the genetic model we have developed.

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## REFERENCES

- Allison DB, Heo M, Kaplan N, Martin ER. 1999. Development of sibling-based tests of linkage in the presence of association for quantitative traits that do not require parental information. *Am J Hum Genet* 64:1754–1764.
- Asghari V, Sanyal S, Buchwaldt S, Paterson A, Jovanovic V, Van Tol HH. 1995. Modulation of intracellular cyclic AMP levels by different human dopamine D4 receptor variants. *J Neurochem* 65:1157–1165.
- Balcioglu A, Wurtman RJ. 1998. Effects of phentermine on striatal dopamine and serotonin release in conscious rats: in vivo microdialysis study. *Int J Obes Relat Metab Disord* 22:325–328.
- Blum K, Cull JG, Braverman ER, Comings DE. 1996. Reward deficiency syndrome. *American Scientist* 84:132–145.
- Boehnke M, Langefeld CD. 1998. Genetic association mapping based on discordant sib pairs: the discordant-alleles test. *Am J Hum Genet* 62:950–961.
- Bray GA. 2000. Overweight, mortality, and morbidity. In: Bouchard C, editor. *Physical activity and obesity*. Champaign, IL: Human Kinetics. p 31–53.
- Bray GA, Tartaglia LA. 2000. Medicinal strategies in the treatment of obesity. *Nature* 404:672–677.
- Bromel T, Blum WF, Ziegler A, Schulz E, Bender M, Fleischhaker C, Remschmidt H, Krieg JC, Hebebrand J. 1998. Serum leptin levels increase rapidly after initiation of clozapine therapy. *Mol Psychiatry* 3:76–80.
- Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. 2000. Establishing a standard definition for child overweight and obesity worldwide: international survey. *BMJ* 320:1–6.
- Comings DE, Blum K. 2000. Reward deficiency syndrome: genetic aspects of behavioral disorders. In: Uylings HBM, Van Eden CG, DeBruin JCP, Feenstra MGP, Pennatz CMA, editors. *Progress in Brain Research*. 126:325–341.
- Comuzzie AG, Allison DB. 1998. The search for human obesity genes. *Science* 280:374–1377.

- Comuzzie AG. 2002. The emerging pattern of the genetic contribution to human obesity. *Best Pract Res Clin Endocrinol Metab* 16:611–621.
- Curtis D. 1997. Use of siblings as controls in case-control association studies. *Ann Hum Genet* 61:319–333.
- Ding YC, Chi HC, Grady DL, Morishima A, Kidd JR, Kidd KK, Flodman P, Spence MA, Schuck S, Swanson JM, Zhang YP, Moyzis RK. 2002. Evidence of positive selection acting at the human dopamine receptor D4 gene locus. *Proc Natl Acad Sci USA* 99:309–314.
- Freeman B, Powell J, Ball D, Hill L, Craig I, Plowmin R. 1997. DNA by mail: an inexpensive and noninvasive method for collecting DNA samples from widely dispersed populations. *Behav Genet* 27:251–257.
- Goodman E, Hinden BR, Khandelwal S. 2000. Accuracy of teen and parental reports of obesity and body mass index. *Pediatrics* 106:52–58.
- Harris KM, Florey F, Tabor J, Bearman PS, Jones J, Udry JR. 2003. The national longitudinal study of adolescent health: research design. [www.cpc.unc.edu/projects/addhealth/design](http://www.cpc.unc.edu/projects/addhealth/design).
- Helgason A, Yngvadottir B, Hrafnkelsson B, Gulcher J, Stefansson K. 2005. An Icelandic example of the impact of population structure on association studies. *Nat Genet* 37:90–95.
- Himes JH, Dietz WH. 1994. Guidelines for overweight in adolescent preventive services: recommendations from an expert committee. *Am J Clin Nutr* 59:307–316.
- Hinney A, Schneider J, Ziegler A, Lehmkuhl G, Poustka F, Schmidt MH, Mayer H, Siegfried W, Remschmidt H, Hebebrand J. 1999. No evidence for involvement of polymorphisms of the dopamine D4 receptor gene in anorexia nervosa, underweight, and obesity. *Am J Med Genet* 88:594–597.
- Kuczmarowski RJ, Ogden CL, Grummer-Strawn LM, Flegal KM, Guo SS, Wei R, Mei Z, Curtin LR, Roche AF, Johnson CL. 2000. CDC growth charts: United States. *Adv Data* 314:1–27. [www.cdc.gov/growthcharts](http://www.cdc.gov/growthcharts).
- Lench N, Stanier P, Williamson R. 1988. Simple non-invasive method to obtain DNA for gene analysis. *Lancet* 1:1356–1358.
- Levitan RD, Masellis M, Lam RW, Muglia P, Basile VS, Jain U, Kaplan AS, Tharmalingam S, Kennedy SH, Kennedy JL. 2004a. Childhood inattention and dysphoria and adult obesity associated with the dopamine D4 receptor gene in overeating women with seasonal affective disorder. *Neuropsychopharmacology* 29:179–186.
- Levitan RD, Masellis M, Basile VS, Lam RW, Kaplan AS, Davis C, Muglia P, Mackenzie B, Tharmalingam S, Kennedy SH, Macciardi F, Kennedy JL. 2004b. The dopamine-4 receptor gene associated with binge eating and weight gain in women with seasonal affective disorder: an evolutionary perspective. *Biol Psychiatry* 56:665–669.
- Lichter JB, Barr CL, Kennedy JL, Van Tol HH, Kidd KK, Livak KJ. 1993. A hypervariable segment in the human dopamine receptor D4 (DRD4) gene. *Hum Mol Genet* 2:767–773.
- Manson JE, Bassuk SS. 2003. Obesity in the United States: a fresh look at its high toll. *JAMA* 289:229–230.
- Martel P, Fantino M. 1996. Mesolimbic dopaminergic system activity as a function of food reward: a microdialysis study. *Pharmacol Biochem Behav* 53:221–226.
- Meulenbelt I, Droog S, Trommelen GJ, Boomsma DI, Slagboom PE. 1995. High-yield noninvasive human genomic DNA isolation method for genetic studies in geographically dispersed families and populations. *Amer J Hum Genet* 57:1252–1254.
- Ogden CL, Flegal KM, Carroll MD, Johnson CL. 2002. Prevalence and trends in overweight among US children and adolescents, 1999–2000. *JAMA* 288:1728–1732.
- Pritchard JK, Stephens M, Donnelly P. 2000. Inference of population structure using multilocus genotype data. *Genetics* 155:945–959.
- Sander T, Harms H, Dufeu P, Kuhn S, Rommelspacher H, Schmidt LG. 1997. Dopamine D4 receptor Exon III alleles and variation of novelty seeking in alcoholics. *Am J Med Genet* 74:483–487.
- Schwartz NW, Woods SC, Porte D Jr, Seeley RJ, Baskin DG. 2000. Central nervous system control of food intake. *Nature* 404:661–671.
- Searle SR. 1971. *Linear models*. New York: Wiley.
- Searle SR, Casella G, McCulloch C. 1992. *Variance components*. New York: Wiley.
- Snyder EE, Walts B, Pérusse L, Chagnon YC, Weisnagel SJ, Rankinen T, Bouchard C. 2004. The human obesity gene map: the 2003 update. *Obes Res* 12:369–439.
- Spielman RS, Ewens WJ. 1998. A sibship test for linkage in the presence of association: the sib transmission/disequilibrium test. *Am J Hum Genet* 62:450–458.
- Spitz E, Moutier R, Reed T, Busnel MC, Marchaland C, Roubertoux PL, Carlier M. 1996. Comparative diagnoses of twin zygosity by SSLP variant analysis, questionnaire, and dermatoglyphic analysis. *Behav Genet* 26:55–64.
- Swanson JM, Oosterlaan J, Murias M, Schuck S, Flodman P, Spence MA, Wasdell M, Ding YC, Chi HC, Smith M, Mann M, Carlson C, Kennedy JL, Sergeant JA, Leung P, Zhang YP, Sadeh A, Chen C, Whalen CK, Babb KA, Moyzis R, Posner MI. 2000. Attention deficit/hyperactivity disorder children with a 7-repeat allele of the dopamine receptor D4 gene have extreme behavior but normal performance on critical neuropsychological tests of attention. *Proc Natl Acad Sci USA* 97:4754–4759.
- Van Tol HH, Bunzow JR, Guan HC, Sunahara RK, Seeman P, Niznik HB, Civelli O. 1991. Cloning of the gene for a human dopamine D4 receptor with high affinity for the antipsychotic clozapine. *Nature* 350:610–614.
- Van Tol HH, Wu CM, Guan HC, Ohara K, Bunzow JR, Civelli O, Kennedy J, Seeman P, Niznik HB, Jovanovic V. 1992. Multiple dopamine D4 receptor variants in the human population. *Nature* 358:149–152.
- Wang GJ, Volkow ND, Logan J, Pappas NR, Wong CT, Zhu W, Netusil N, Fowler JS. 2001. Brain dopamine and obesity. *Lancet* 357:354–357.
- Wang E, Ding YC, Flodman P, Kidd JR, Kidd KK, Grady DL, Ryder OA, Spence MA, Swanson JM, Moyzis RK. 2004. The genetic architecture of selection at the human dopamine receptor D4 (DRD4) gene locus. *Am J Hum Genet* 74:931–944.
- WHO. 1995. World Health Organization Expert Committee. Physical status, the use and interpretation of anthropometry. WHO technical report series no. 854.