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The Dopamine D_2 Receptor (DRD2) as a Major Gene in Obesity and Height[1]

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Dopamine plays a major role in the regulation of appetite and growth hormone. Dopaminergic agonists suppress appetite and dopamine D_2 receptor antagonists enhance it. We examined the hypothesis that allelic variants of the DRD2 locus may be associated with weight and height. Sarkar and Sommer described two DRD2 polymorphisms that could be haplotyped by PCR. For weight, the mean Z score (National Center for Health Statistics) for 208 subjects without haplotype 4 was 0.086 versus 0.557 for 280 subjects with haplotype 4, $P = 0.0003$. Two separate sets of subjects were studied and these results were significant for both, providing an internal replication. For height, the mean Z score for 164 subjects without haplotype 4 was 0.1677 versus 0.6885 for 219 subjects with haplotype 4, $P < 0.00001$. These and other data suggest that the 4 haplotype is in linkage disequilibrium with allelic variants of the DRD2 gene that play a major role in the regulation of weight (obesity) and height, and may serve as a risk factor in late-onset non-insulin-dependent diabetes mellitus (NIDDM).

Over 30 million U.S. adults are obese (1) and obesity is associated with an increased risk for non-insulin-dependent diabetes mellitus (NIDDM), hypertension, and cardiovascular and other diseases. A conservative estimate of the cost of obesity to society in 1986 was 39 billion dollars (1).

Adoption studies have shown that obesity has a strong genetic influence (2–6). In a study of weight (corrected for height, i.e., body mass index or BMI, weight in kg/(height in m)^2) of children separated from their mothers at birth, the correlation between weight of the adult daughters to their biological mothers was significant while there was no correlation with the weight of the mother who adopted them (2). Studies of identical and fraternal twins (7) show an intrapair correlation coefficient for BMI of 0.66 and 0.7 for female and male identical twins

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reared apart. The corresponding figures for fraternal female and male twins were 0.25 and 0.15. Thus, both twin and adoption studies suggest the presence of one or more genes that play a major role in regulating weight.

Dopamine plays a major role in the regulation of appetite (8). The most effective drugs used to suppress appetite contain dextroamphetamine, a dopaminergic agonist, and a major side effect of dopamine D_2 receptor antagonists, such as haloperidol, is marked weight gain. Not only do addictive drugs, such as cocaine and amphetamines affect the dopaminergic reward pathways of the brain (9), but food does also (10). Endogenous opioids are increased in obesity (11,12) and dopamine plays a role in the regulation of opioid levels (13,14). Growth hormone is also regulated by dopaminergic neurons. Because of these interactions we have sought to determine if variants of the DRD2 locus play a role in the control of weight and height.

Civelli and colleagues cloned the human dopamine D_2 gene in rats (15) and humans (16) and described a *Taq*I A polymorphism at the 3′ end of the DRD2 gene (16). In 1990 Blum and colleagues (17) reported that of 35 severe alcoholics, 69% carried the *Taq* A1 allele, versus only 20% of 35 controls. Some studies have verified this observation, others have not. We observed significant increases in the prevalence of the A1 allele in a number of disorders in which defects in dopamine metabolism have been implicated, including Tourette syndrome, attention deficit hyperactivity disorder (ADHD), autism, and post-traumatic stress disorder (18). The higher frequency of the A1 allele in patients with full Tourette syndrome compared to mild cases or controls has been confirmed in two other studies (19,20). Since cocaine and amphetamines cause a much greater perturbation of the mesolimbic, mesocortical dopaminergic reward pathways than alcohol, we investigated the possibility that the A1 allele might be better correlated with polysubstance abuse than alcoholism per se. This proved to be the case (21) and two other studies have reported similar findings (22; Noble and Blum, personal communication).

Since the subjects studied by Blum *et al.* (17) were deceased, they were able to directly examine the dopamine D_2 receptor density in the caudate nucleus. This showed that the A1 allele was associated with a significant decrease in receptor [^{3}H]spiperone B_{max}, even in the nonalcoholic controls (23).

Because the *Taq* A1 allele is located outside and 3′ to the coding sequence of the DRD2 gene, one of the intriguing aspects of the DRD2 studies has been the assumption that the A1 allele might be in rather poor linkage disequilibrium with the presumptive mutations actually causing the variations in the dopamine D_2 receptor density. There has thus been considerable interest in other variants of the DRD2 gene. Sarkar and Summer (24,25) reported the presence of two new variants in the center of the DRD2 locus, one being a G vs T substitution, the other within an exon a C vs T (His^{313}). Since these were only 241 base pairs apart it was possible to include them both in the same allele-specific polymerase chain reaction (PCR). This allowed the identification of four haplotypes. Haplotype 3 was present mostly in Orientals. In non-Orientals this resulted in three haplotypes and six genotypes. Using the same DNA samples on deceased subjects studied by Noble *et al.* (23) we have observed a significant decrease in B_{max} for subjects

carrying the 1 haplotype and an increase in the K_d of subjects with the 24 and 44 genotypes (26), suggesting the presence of at least two mutant alleles at the DRD2 locus.

We explored the possibility that the different haplotypes of the DRD2 locus might be associated with differences in mean weight and height for three reasons. (a) The previous studies have suggested the presence of physiologically important alleles of the DRD2 locus played a role in the function of the dopaminergic reward pathways with a significant increase in the prevalence of the D_2A1 of D_2B1 allele in severe drug abuse (21,22; Blum *et al.*, personal communication). The same pathway plays a role in the regulation of food intake (10). (b) Our studies of DRD2 haplotypes showed that since the 1 haplotype was in partial linkage disequilibrium with the D_2A1 allele and the 4 haplotype was in partial linkage disequilibrium with the D_2D2 allele, haplotyping would allow a further subdivision of D_2D2A2 individuals. In our previous study, 100% of 20 obese subjects were D_2D2A2 homozygotes. While the numbers were small, one explanation was that obesity was associated with a second physiologically important allele of the DRD2 locus in linkage disequilibrium with the 4 haplotypes. (c) Growth hormone is a dopaminergically regulated pituitary hormone that plays a role in the control of both weight and height.

METHODS

A total of 516 subjects were haplotyped, one group from the Jerry L. Pettis V.A. Hospital (Loma Linda, CA) and a second group from the City of Hope National Medical Center (Duarte, CA). The studies at both centers were approved by the IRB. The V.A. subjects consisted of 174 patients on the addiction treatment ward, 117 hospital controls, and 20 subjects participating in a smoking cessation clinic. All subjects were unrelated. The City of Hope subjects consisted of 204 individuals from the City of Hope Tourette syndrome/ADHD clinic. In both groups there was only a partial overlap with the subjects reported previously in our studies of the D_2A1 allele. All weights and heights were based on information obtained on the initial visit before the administration of medications. A group of 25 obese subjects (more than 100 pounds overweight) was specifically studied. These individuals were obtained from a weight loss clinic (27). Since weight and height measurements were not available on all subjects, the numbers in the tables are less than 516. Haplotyping for the two groups was performed in separate laboratories (Dr. Comings and Dr. Flanagan).

To allow comparisons between subjects independent of age, race, and sex, we utilized the data from the National Center for Health Statistics. This permitted us to determine the Z score for each person for weight and height and thus to examine the mean Z scores for subjects with different DRD2 genotypes.

Haplotyping was performed by the double allele-specific procedure of Sarkar and Sommer (24). The major modifications were the use of Perfect Match and the substitution of Tween 20 and NP40 in place of formamide. The buffer was 10 mM Tris, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.05% Tween 20, 0.05% NP40, 2.0 μl Perfect Match, and 0.5 μM primers per 100 μl reaction mixture. Of this 25 μl was added to the reaction vial and 0.25 μg of genomic DNA was added.

TABLE 1A
Mean *Z* Score for Weight by DRD2 Genotype

Genotype	*N*	Mean	SD
11	19	0.112	1.37
12	75	−0.031	1.33
22	110	0.160	1.12
14	60	0.357	1.15
24	149	0.534	1.44
44	71	0.774	1.92
Total	484		

Note. *F* ratio (ANOVA) = 3.41, $P = 0.005$. For 398 Caucasians only, *F* ratio = 5.71, $P < 0.0001$.

The thermal cycler was set to 95°C for 4 min, then cycles of 1 min at 95°C, 2 min at 50°C, and 3 min at 72°C for a total of 30 cycles. At the end of cycling the amplified DNA was examined by electrophoresing in 3% agarose. Four different reaction mixtures were required for haplotyping.

Direct sequencing of the DRD2 gene (25) identified a G vs T polymorphism present in intron 5 (region IV) of the DRD2 gene. The G variant was present in 73% of Caucasians and T was present in 27%. Sequencing also identified a C vs T polymorphism present in an exon (His^{313}), also in region IV. The C variant was present in 69% of Caucasians and the T was present in 31%. The unique feature of these two polymorphisms was that they were only 241 base pairs apart. This allowed haplotyping to be done by using allele-specific primers on both ends of the PCR fragment (24). This produced four haplotypes as follows:

Haplotype	G→T	C→T
1	T	C
2	G	C
3	T	T
4	G	T

In this manuscript when a single chromosome is referenced it is called haplotype, i.e., haplotype 4. Then both chromosomes are referenced, it is called genotype, i.e., genotype 24. A simple technique was available for double checking the haplotyping results. Oligomers with the sequence TCTCCACTGCACTCCT and CATGCCTCAGTGACAT (25) allowed amplification of the site of the C→T polymorphism. This could then be cut with *Nco*I when the polymorphism was T. It is not cut when it was C.

RESULTS AND DISCUSSION

Weight. Table 1A gives the results for weight. Subjects with genotypes 11, 12, and 22 had lower mean *Z* scores (−0.031 to +0.160) than those with genotypes 14, 24, and 44 (0.357 to 0.774), $P = 0.005$. When examined (Table 1B) on the basis of carrying or not carrying a 4 haplotype, $P = 0.0003$. When restricted to

TABLE 1B
Mean Z Score for Weight by DRD2 Haplotype-4 vs Non-4

Genotype	N	Mean	SD
11,12,22	204	0.086	1.22
14,24,44	280	0.557	1.53

Note. F ratio (ANOVA) = 13.28, P = 0.0003. For 398 Caucasians only, F ratio = 18.14, $P <$ 0.0001.

Caucasians the P values were <0.0001 for stratification by genotype and <0.00001 for haplotype 4 versus non-4.

Bray *et al.* (28) have described five obesity risk groups based on BMI. A comparison of the obesity risk groups in the haplotype 4 and non-4 groups is shown in Table 2A. This comparison indicates, in general, a progressive increase in the percentage of subjects carrying the 4 haplotype from class 0 (normal weight) to class 4 (very high risk obese), P = 0.0057. The percentage of subjects in class 0 who carried the 4 haplotype was 54.8 versus 87.5% for those in class 4. The results were even more significant when restricted to Caucasians (P = .0018).

We also examined the dichotomous stratification into haplotype 4 vs non-4 for various ranges of Z scores (Table 2B). Here again there was a trend for a progressive increase in the percentage of subjects carrying haplotype 4 with increasing Z scores, P = 0.0065. Again when restricted to Caucasians only, the results were also significant, P = 0.00066.

Since the study was performed with two separate sets of subjects it allowed us to have one set serve as a replication sample. The results were significant in both groups. For example, the comparison by ANOVA of the average Z score for six different genotypes gave a P = 0.009 for the 265 V.A. subjects, and a P = 0.025 for the 197 City of Hope subjects. In the V.A. sample the mean Z score for the 109 subjects without haplotype 4 was −0.1229 versus 0.338 for the 157 with a 4

TABLE 2A
Percentage of Subjects with DRD2 Haplotype 4 in Different Obesity Risk Classes Based on Body Mass Index (28)

Obesity risk class	BMI	Haplotype 4		Non-4 haplotype	Total
		N	%	N	N
0	≤25	149	54.8	123	272
1	26–30	78	57.4	58	136
2	31–35	37	69.8	16	53
3	36–40	7	58.3	5	12
4	>40	14	87.5	2	16
	Total				489

Note. Mantel–Haenszel χ^2 = 7.64, P = 0.0057. For 392 Caucasians only, Mantel–Haenszel χ^2 = 9.77, P = 0.0018.

TABLE 2B
Percentage of Subjects with DRD2 Haplotype-4 in Different *Z* Score Classes for Weight

Z score	Haplotype 4		Non-4 haplotype	Total
	N	%	*N*	*N*
≤1.0	28	52.8	25	53
−1.1–0.0	91	50.8	88	179
0.1–1.0	85	61.6	53	138
1.1–2.0	36	57.1	27	63
2.1–3.0	17	77.3	5	22
>3.1	13	76.5	4	17
Total				489

Note. Mantel–Haenszel $\chi^2 = 7.41$, $P = 0.0065$. For 387 Caucasians only, Mantel–Haenszel $\chi^2 = 11.59$, $P = 0.00066$.

haplotype. In the City of Hope samples the mean *Z* score for the 85 subjects without haplotype 4 was 0.331 versus 0.802 for the 113 with a 4 haplotype. Our subjects tended to show a predominance of individuals with a *Z* score of over 0 because of a purposeful selection of a number of overweight individuals. An additional potential contributing factor is that the study, on which the national data set that we used was based, was carried out in the mid-1960s and 1970s, and the present subjects were studied in 1991–1992. However, even if 1990 population means are now higher this would only affect our mean values and would have no effect on the relative results.

Obesity is commonly associated with hyperinsulinemia, insulin resistance, and impaired glucose tolerance (29–32), all of which represent potent risk factors for the development of type II diabetes and cardiovascular disease (33–35). Because of this we examined the random blood glucose in 80 Caucasian subjects. The mean for 35 subjects without the haplotype 4 was 95.11, SD was 9.4, while the mean for 45 haplotype 4 carriers was 110.7 and SD was 32.3, $F = 7.58$, $P = 0.007$.

Height. Table 3A gives these results for height. Subjects with genotypes 11 and 12 had lower mean *Z* scores (−0.1417 to −0.0297) than those with genotypes

TABLE 3A
Mean *Z* Score for Height by DRD2 Genotype ($N = 383$ Adults)

Genotype	*N*	Mean	SD
11	15	−0.1417	0.68
12	59	−0.0297	1.30
22	90	0.3428	1.04
14	43	0.472	0.91
24	121	0.674	0.98
44	55	0.889	1.03

Note. *F* ratio (ANOVA) = 6.59, $P < 0.0001$. For 309 Caucasians only, *F* ratio = 5.70, $P < 0.0001$.

TABLE 3B
Mean Z Score for Height by DRD2 Haplotype-4 vs Non-4

Genotype	N	Mean	SD
11,12,22	164	0.168	1.13
14,24,44	219	0.688	0.99

Note. F ratio (ANOVA) = 22.92, $P < 0.0001$. For 297 Caucasians only, F ratio = 19.62, $P < 0.0001$.

14, 24, and 44 (0.472 to 0.889, $P < .0001$). When examined on the basis of not-carrying or carrying the 4 haplotype (Table 3B), the mean Z scores were 0.168 vs 0.688, respectively, $P < 0.0001$. On the basis of carrying a 1 versus a 4 haplotype (Table 3C), the mean Z scores were -0.045 vs 0.741, respectively, $P < 0.0001$. When classified by five different ranges of z scores (Table 4) there was a progressive increase in the percentage of subjects carrying a 4 haplotype from 28% for the shortest subjects to 70.6% for the tallest. In each of these comparisons the results were also significant when restricted to Caucasians.

The study of height was restricted to adults 16 years of age or older. When we examined children, the results were complex and complicated by whether the subjects were pre- or postpubertal. After we have tested several hundred additional children, these results will be reported separately. Since many of the City of Hope subjects were children, there were fewer adults to form a comparison to the V.A. group. However, the trends were the same for both groups, and for haplotype 1 versus 4 both were significant (V.A. set: $N = 189$, F ratio = 22.43, $P < 0.0001$; COH set: $N = 61$, F ratio = 5.41, $P = 0.025$).

Since these results for weight and height were highly significant for both the total group and for a subset restricted to Caucasians, race was not a factor. While one might argue that ethnic variations in haplotype frequency within the Caucasian group might explain these findings, we feel this is unlikely for two reasons. First, the ethnic origin was ascertained and in the majority of our subjects it was so diverse that 4 to 12 different ethnic backgrounds were present in each individual. Second, we obtained the same trends in both groups of subjects.

We do not feel that inclusion of subjects with various psychiatric disorders in the series influenced the results since a significant proportion of the Loma Linda samples were controls. In addition, such an effect would require a very complex set of assumptions. For example, the 4 haplotype would have to be significantly

TABLE 3C
Mean Z Score for Height by DRD2 Haplotype-11, 12 vs 24, 44

Genotype	N	Mean	SD
11,12	74	−0.045	1.20
24,44	176	0.741	1.00

Note. F ratio (ANOVA) = 28.39, $P < 0.0001$. For 191 Caucasians only, F ratio = 24.59, $P < 0.0001$.

TABLE 4
Percentage of Total Adult Subjects with DRD2 Haplotype-4 in Different Z Score Classes for Height

Z score	Haplotype 4		Non-4 haplotype	Total
	N	%	N	N
≤1.0	7	28.0	185	25
−1.1–0.0	46	42.2	63	109
0.1–1.0	92	64.8	50	142
1.1–2.0	60	70.6	25	85
>2.1	12	70.6	5	17
Total	378			

Note. Mantel–Haenszel $\chi^2 = 25.14$, $P < .00001$. For 296 Caucasians only, Mantel–Haenszel $\chi^2 = 21.35$, $P < .00001$.

increased in subjects with Tourette syndrome, ADHD, drug addiction, and alcoholism, each of these disorders would have to be associated with obesity, and the obesity would have to be independent abnormalities of the DRD2 locus. Nonetheless, additional replication studies using a random set of epidemiologically samples individuals are planned.

Independent of these caveats, these observations suggest that haplotype 4 is in linkage disequilibrium with allelic variants of the DRD2 gene which play a major role in appetite, obesity, and height. This is consistent with the known importance of dopamine in these areas. Since obesity is such a major risk factor for the development of Type II or non-insulin-dependent diabetes mellitus, studies of DRD2 haplotypes in diabetic subjects are in progress.

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