D_2 Dopamine Receptor Gene and Obesity

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(Accepted 30 November 1993)

The prevalence of TaqI A D_2 dopamine receptor (DRD2) alleles was determined in 73 obese women and men. In this sample with a mean body mass index of 35.1, the A1 (minor) allele of the DRD2 gene was present in 45.2% of these nonalcohol, nondrug abusing subjects. The DRD2 A1 allele was not associated with a number of cardiovascular risk factors examined, including blood lipids (cholesterol, high-density lipoprotein [HDL]- and low-density lipoprotein [LDL]-cholesterol, and triglycerides). However, phenotypic factors characterized by the presence of parental history and postpuberty onset of obesity as well as carbohydrate preference were associated with obese subjects carrying the A1 allele. The cumulative number of these three factors was positively and significantly ($p < .0002$) related to A1 allelic prevalence. The data showing an association of the minor allele of the DRD2 gene with phenotypic characteristics suggest that this gene, located on q22-q23 region of chromosome 11, confers susceptibility to a subtype of this disorder. © *1994 by John Wiley & Sons, Inc.*

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Part of this study was presented at the 1993 annual meeting of the American Society of Human Genetics, New Orleans [Noble, E. P., Noble, R. E., Ritchie, T., Grandy, D. K., & Sparkes, R. S. (1993). *The American Journal of Human Genetics* 53(Suppl.), 17, 1993].

International Journal of Eating Disorders, Vol. 15, No. 3, 205–217 (1994)

CCC 0276–3478/94/030205–13

Obesity, a heterogeneous and widely prevalent disorder, has for long been considered to be essentially determined by environmental factors (Stunkard, 1988). However, recent family, twin, and adoption studies (Bray, 1981; Laskarzewski et al., 1983; Sims, 1990; Stunkard et al., 1986; Zonta, Jayakar, Bosisio, Galante, & Pennetti, 1987) are pointing to heredity as also an important contributor to the development of obesity. Thus, a significant correlation is found in the body mass index (BMI) of adoptees and their biological but not of their adoptive parents and siblings (Stunkard et al., 1986). Moreover, the topographical distribution of fat has also been found to have hereditary components. In studies of families (Bouchard, Perusse, Leblanc, & Theriault, 1988) and monozygotic twins (Bouchard, Tremblay, Després, Nadeau, & Lupien, 1990), internal fat is found to be influenced more by heredity than is the amount of subcutaneous fat.

If a diathesis toward obesity is in part determined by heredity, then it should have a molecular genetic representation. Given the complex array of metabolic systems that contribute to obesity, it would not be unexpected that several genes will be implicated in this disorder. Indeed, already, polymorphisms in the genes for apolipoprotein-B (Rajput-Williams et al., 1988), apolipoprotein-E (Fumeron et al., 1988; Pouloit et al., 1990), low density lipoprotein (LDL) receptor (Zee, Griffiths, & Morris, 1992), glucocorticoid receptor (Weaver, Hitman, & Kopelman, 1991), and insulin (Weaver, Kopelman, & Hitman, 1991) have been associated with obesity.

Food, like a variety of reinforcing substances such as alcohol and other drugs of abuse, when consumed can produce euphoria or pleasure. Although the precise localization and specificity of the reinforcing properties of these substances are under debate, there is general accord that they are manifested in the dopaminergic reward pathways of the brain (For reviews see Hoebel, 1985; Koob, 1992; Wise & Rompre, 1989). Evidence that the dopaminergic system may be implicated in obesity is suggested from studies showing the effectiveness of amphetamine-like drugs in weight loss (Scoville, 1975). However, the abuse potential of these drugs has limited their use. Furthermore, neuroleptics, which block the D_2 dopamine receptor (DRD2), have been shown to lead to body weight gain in clinical (Caffey, 1961; Doss, 1979) and animal studies (Baptist, Parada, & Hernandez, 1987). In view of observations suggesting that obesity is in part determined by heredity and because the dopaminergic system may be involved in eating behavior, the question raised herein is whether a dopamine (DA) receptor gene is implicated in some forms of obesity. In the present report, the prevalence of *Taq*I A DRD2 alleles was determined in obese subjects. Moreover, the relationships of these alleles to anthropomorphic and metabolic parameters as well as to parental history and onset of obesity and food preference were ascertained.

METHODS

Patients

Female and male obese subjects were recruited to participate in a long-term dexfenfluramine weight reduction study at the Cathedral Hill Obesity Clinic in San Francisco, California. Subjects had to be between 18–65 years of age and in good general physical and mental health. Institutional Review Board approval was obtained for this study, and informed consent was signed by the subjects after the nature of the procedures and maintenance of confidentiality were explained to them.

Inclusion criterion for the present study was a BMI (weight [kg]/height $[M]^2$) ≥ 28.0 for

women and men, which is above the recommended National Center for Health Statistics levels (27.3 for women and 27.8 for men) for obesity (National Institutes of Health Consensus Development Panel on the Health Implications of Obesity, 1985). Exclusion criteria were: pregnant or lactating women or women of child-bearing potential who were not using medically accepted means of contraception; obesity of endocrine origin (e.g., Cushing's disease, Stein-Leventhal, or hypothyroidism syndromes); and history of anorexia nervosa, bulimia, alcoholism, or drug abuse.

At the first visit, to ensure eligibility, subjects were screened with medical history and physical examination, and informed consent was obtained from all the participants. They were weighed in light clothing without shoes to the nearest 0.1 kg and their heights were recorded. Waist circumference was measured at the level of the umbilicus, using a measuring tape with the subject in mid-expiratory position. Hip circumference was recorded over the widest part of the hip region, and the waist-hip ratio (waist/hip $\times$ 100) was calculated. After a 5-min rest, blood pressure (measured with a mercury sphygmomanometer on the right arm) and pulse were determined as part of a general physical examination.

Venous blood was obtained after an overnight fast for routine chemical as well as lipid (cholesterol, high-density lipoprotein [HDL]- and low-density lipoprotein [LDL]-cholesterol, and triglycerides) analysis. A sample of blood (10 ml) in tubes containing ethylenediaminetetraacetic acid (EDTA) was also collected for molecular genetic analysis.

Through interview, history of anorexia nervosa, bulimia, alcoholism, and drug abuse was noted. Furthermore, through administration of questionnaires, family (mother's and father's) history of obesity, the participants' onset of obesity (childhood [before puberty], adolescent [after puberty], and adulthood [after age 18 years]), and their food preference (carbohydrates, proteins, fats, or foods in general) were also obtained. Food preference was further validated by personal interview with each patient wherein the three categories of foods (carbohydrates, proteins, and fats) and examples of types of each (e.g., carbohydrates: sweets and starches) were clearly delineated. The fourth choice, food in general, was indicated if the patient liked all three food categories.

DNA Analysis

Genomic DNA was extracted from the blood sample (Old, 1986) and subsequently used as template for the polymerase chain reaction (PCR; Saiki et al., 1988). The primers 5014 and 971 were used to amplify a 310 bp fragment spanning the polymorphic *Taq*I A site of the DRD2 gene (Grandy et al., 1989). The sequence for the 5014 primer was 5′-CCgtcgaCCCTTCCTGAGTGTCATCA-3′ and for the 971 primer was 5′-CCgtcgaCGGCTGGCCAAGTTGTCTA-3′ (lowercase letters code for *Sal*I site). The primer sequences were provided by one of us (D.K.G.) and they were synthesized by Oligos Etc. Inc. (Wilsonville, OR).

Amplification was carried out in 100-μl reactions using 1 μg of genomic DNA and 2.5 units of Ampli*Taq* DNA polymerase (Perkin Elmer) in a standard reaction cocktail containing 200 μM of each of the four dNTPs, 1.5 mM $MgCl_2$, and the recommended buffer provided by the manufacturer (Perkin Elmer). After an initial denaturation step at 94°C for 5 min, DNA was amplified in three-step cycles as follows: denaturation at 94° for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 30 s using the Perkin Elmer GeneAmp 9600 thermocycler. After 35 cycles, a final extension step at 72°C for 5 min was used.

A 10-μl aliquot was removed and analyzed by gel electrophoresis in a 2.5% agarose gel

containing ethidium bromide and visualized under ultraviolet (UV) light. The expected 310 bp fragments were visualized with minimal background. Approximately 500 ng of the DNA was digested with 5 units of *Taq*I restriction enzyme (Boehringer-Mannheim Biochemical) at 65°C for 2 hr. The resulting products were analyzed by agarose gel electrophoresis as before. Allelic data were obtained on all but one subject (no PCR product). The A1/A2 genotype is revealed by three fragments: 310 bp, 180 bp, and 130 bp; the A2/A2 genotype is indicated by two fragments: 180 bp and 130 bp; and the A1/A1 genotype is shown by the uncleaved 310 fragment (Figure 1).

Statistical Analysis

Demographic, clinical, laboratory, interview, and questionnaire data were coded and entered into a computer data base. DRD2 allelic prevalence, obtained by personnel blinded to the aformentioned information, was also coded and the two data sets were merged for analyses. *t* tests were used to compare interval data, and chi-square statistic

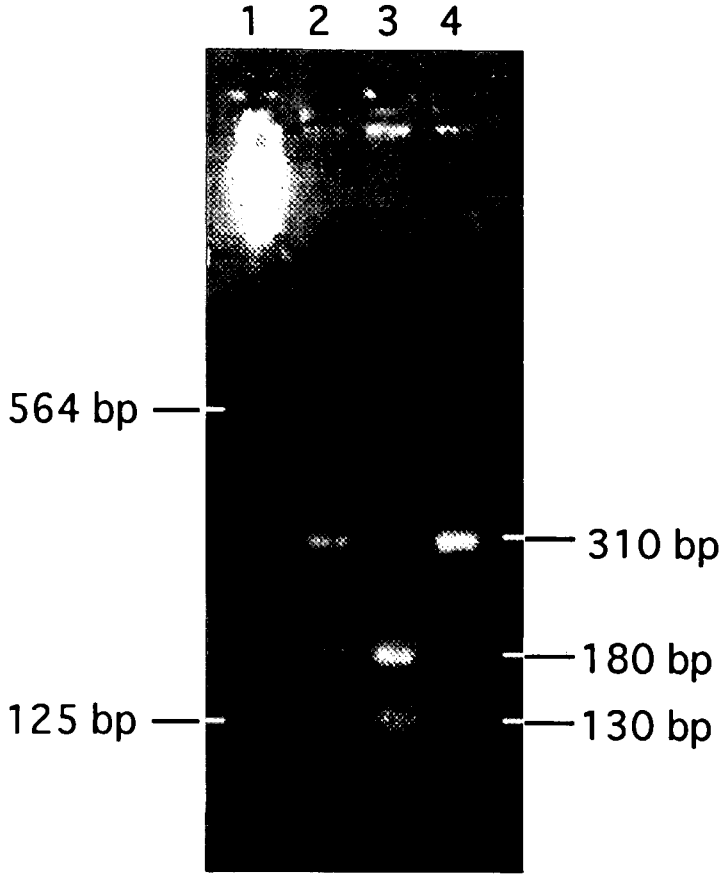

Figure 1. Identification of D_2 dopamine receptor mutation by allele-specific polymerase chain reaction. Lane 1, size marker (DNA cleaved with the restriction enzyme *Hind*III); lane 2, A1/A2 genotype (310 bp, 180 bp, 130 bp); lane 3, A2/A2 genotype (180 bp, 130 bp); lane 4, A1/A1 genotype (310 bp).

with Yates' correction for continuity (Siegel, 1956), as appropriate, was used for group comparisons of ordinal and nominal values. A chi-square linear trend analysis (Cochran, 1954) was used to test if increasing risk factors for obesity are associated with A1 allelic prevalence. A two-tailed p value of .05 was considered statistically significant.

RESULTS

Of a total of 80 patients who volunteered for the study, 7 were excluded from analysis: a man with a history of alcoholism, five women with a BMI <28.0, and a subject for whom no PCR product was obtained. Parental history of obesity was unknown in 3 subjects, onset of obesity was not available in 1 subject, and food preference data were available in only 47 subjects. The age (mean ± SE) of the 73 obese individuals was 37.2 ± 1.2 years. Age of the 33 subjects carrying the $A1^+$ allele (A1/A2 and A1/A1 genotypes) was 37.7 ± 1.6 years, and 36.8 ± 1.9 years for the 40 subjects carrying the $A1^-$ allele (A2/A2 genotype). The age difference between $A1^+$ and $A1^-$ allelic individuals was not significant ($p = .75$). The sample consisted of 57 women and 16 men. Of the 57 women, 24 had the $A1^+$ allele, whereas 33 had the $A1^-$ allele. Of the 16 men, 9 had the $A1^+$ allele, whereas 7 had the $A1^-$ allele. The difference in allelic distribution between the sexes, as expected (the DRD2 gene is localized on chromosome 11), was not significant ($\chi^2 = 0.52$, $p = .47$).

The present sample consisted of 42 non-Hispanic Caucasians of European descent, 10 Hispanics, 12 blacks, and 9 others (2 Asians, 2 Native Americans, 2 Filipinos, 2 Samoans, and 1 of mixed race), a distribution that roughly approximates the prevalence of these groups in the United States. Their *Taq*I A DRD2 genotypes are shown in Table 1. There was no significant difference in A1/A1, A1/A2, and A2/A2 genotypes among the present four groups of obese subjects studied ($\chi^2 = 8.50$, $p = .20$).

The relationship of 10 cardiovascular risk factors to DRD2 allelic prevalence in the obese subjects is shown in Table 2. None of the measured factors was significantly differentiated by their DRD2 allelic association. However, with the exception of triglyceride levels, all nine other risk factors were slightly worse in the $A1^+$ compared with the $A1^-$ allelic subjects.

The BMI of the A2/A2, A1/A2, and A1/A1 genotypes (mean ± SE) were: 34.9 ± 0.6 ($n = 40$), 34.8 ± 1.0 ($n = 27$), and 37.5 ± 1.5 ($n = 6$), respectively. The waist-hip ratio of the A2/A2, A1/A2, and A1/A1 genotypes (mean ± SE) were 81.7 ± 1.5 ($n = 40$), 82.4 ± 1.9 ($n = 27$), and 85.0 ± 3.7 ($n = 6$), respectively. Although the A1 homozygotes

Table 1. *Taq*I A D_2 dopamine receptor genotypes in obese subjects

Group	Genotypes		
	A1/A1	A1/A2	A2/A2
Non-Hispanic Caucasian ($n = 42$)	2	13	27
Hispanic ($n = 10$)	2	3	5
Black ($n = 12$)	1	8	3
Other ($n = 9$)[a]	1	3	5
Total ($n = 73$)	6	27	40

[a]Consisted of 2 Asians, 2 Native Americans, 2 Filipinos, 2 Samoans and 1 mixed race.

Table 2. Cardiovascular risk factors in obese subjects and their relationship to *Taq*I A D_2 dopamine receptor alleles

Measure	Total Subjects (n = 73)	$A1^+$ Subjects (n = 33)	$A1^-$ Subjects (n = 40)	Probability (two-tail)
Body mass index (kg/m^2)	35.1 ± 0.5	35.3 ± 0.8	34.9 ± 0.6	.70
Waist/hip × 100	82.2 ± 1.1	82.9 ± 1.7	81.7 ± 1.5	.60
Cholesterol (mg/dl)	198 ± 5	202 ± 7	194 ± 7.3	.41
Triglycerides (mg/dl)	133 ± 8	132 ± 11	134 ± 12	.89
HDL-Chol (mg/dl)	55.2 ± 1.5	54.2 ± 2.1	56.1 ± 2.2	.54
LDL-Chol (mg/dl)	120 ± 6	121 ± 6	119 ± 9	.80
Cholesterol/HDL-Chol	3.72 ± 0.12	3.90 ± 0.20	3.60 ± 0.15	.25
LDL-Chol/HDL-Chol	2.28 ± 0.13	2.36 ± 0.16	2.22 ± 0.19	.60
B.P. systolic (mm)	125 ± 2	126 ± 2	124 ± 2	.50
B.P. diastolic (mm)	83.0 ± 1.6	84.8 ± 1.5	81.6 ± 1.4	.11

Note. Values represent mean ± SE. HDL-Chol = high-density lipoprotein cholesterol; LDL-chol = low-density lipoprotein cholesterol; B.P. = blood pressure.

displayed higher values in these two measures compared with the A2 homozygotes and the heterozygotes, the differences between the relatively few A1 homozygotes and the other two genotypes were not statistically significant.

The relationship of DRD2 alleles to parental history of obesity in the present subjects is presented in Table 3. In obese subjects whose fathers and mothers were not obese, 31.0% carried the $A1^+$ allele. $A1^+$ allelic prevalence was 43.5% and 51.5% in subjects whose fathers and mothers, respectively, were obese. In subjects whose fathers and/or mothers were obese, 53.7% displayed the $A1^+$ allele; the difference in $A1^+$ allelic prevalence between this group and the group with negative parental history of obesity approached but did not achieve statistical significance ($\chi^2 = 2.67$, $p = .10$).

Table 3 also shows the relationship of DRD2 alleles to the age of onset of obesity. Subjects whose onset of obesity occurred when they were children, adolescents, and adults, respectively, had the following progressive increase in $A1^+$ allelic prevalence: 25.0%, 36.5%, and 56.4%, with $A1^+$ allelic prevalence being significantly higher ($\chi^2 = 4.41$, $p = .04$) in adult-onset than in child-onset obesity. Moreover, when the relationship of age of obesity onset to $A1^+$ allelic prevalence was ascertained using the Mantel-Haenszel test for linear association (Cochran, 1954), increasing age of onset was positively and significantly associated to $A1^+$ allelic classification ($\chi^2 = 5.42$, $p = .02$).

The relationship of food preference of obese subjects to their DRD2 allelic distribution is further shown in Table 3. Comparison made in allelic prevalence between subjects who prefer carbohydrates and subjects who prefer other foods (fats, proteins, or food in general) showed that 64.3% of the carbohydrate preferrers carried the $A1^+$ allele, whereas 21.1% of the subjects who preferred other foods carried this allele ($\chi^2 = 6.85$, $p = .009$).

Next, a determination was made of the relationship of the three phenotypic factors shown in Table 3 to $A1^+$ allelic prevalence. Factor scores on each obese subject were obtained by assigning a score of 1 for the presence of each of the following: parental history of obesity (father and/or mother obese), onset of obesity (adolescent or adult), and food preference (carbohydrate preferrers). Thus, scores ranging from 0 to 3 were obtained depending on the number of these factors present in each subject. Because there were only 2 subjects in the 0-factor group, their allelic data were combined with the subjects in the 1-factor group. $A1^+$ allelic prevalence in these various factor score cate-

Table 3. Relationship of *Taq*I A D_2 dopamine receptor alleles to parental history, onset of obesity, and food preference

Background Characteristics	$A1^+$	$A1^-$	$\%A1^+$	Significance
Parental history of obesity[a]				
Neither fathers nor mothers obese	9	20	31.0	—
Fathers obese	10	13	43.5	$\chi^2 = 0.40$, $p = .53$
Mothers obese	17	16	51.5	$\chi^2 = 1.88$, $p = .17$
Fathers and/or mothers obese	22	19	53.7	$\chi^2 = 2.67$, $p = .10$
Onset of obesity[b]				
Child	5	15	25.0	—
Adolescent	5	8	36.5	$\chi^2 = 0.19$, $p = .66$
Adult	22	17	56.4	$\chi^2 = 4.07$; $p = .04$
Food preference				
Carbohydrates	18	10	64.3	—
Other[c]	4	15	21.1	$\chi^2 = 6.85$, $p = .009$

[a]Comparison with neither fathers nor mothers obese. [b]Comparison with child-onset obesity. [c]Other includes proteins, fats, or food in general.

gories is shown in Figure 2. The $A1^+$ allele contributed to 9.1% in 0-1 factor group, 43.5% in the 2-factor group, and 84.6% in the 3-factor group. A significant difference in allelic prevalence was found among these three factor groups ($\chi^2 = 13.9$, $p = .001$). Furthermore, when the relationship of factor score to $A1^+$ allelic prevalence was determined using a linear association test (Cochran, 1954), increasing factor score was positively and significantly related to $A1^+$ allelic classification ($\chi^2 = 13.5$, $p = .0002$).

DISCUSSION

Eating is a highly reinforcing behavior as it provides not only necessary calories and nutrients for survival, but also feelings of gratification and pleasure. A variety of neurotransmitters/neuromodulators have been implicated in the control of feeding and satiety behaviors (for review see Bray, Ricquier, & Spiegelman, 1990). In this regard, the dopaminergic, adrenergic, and serotoninergic systems have received, by far, the greatest attention (Hoebel, Hernandez, Schwartz, Mark, & Hunter, 1989). However, it is not entirely clear which of the neuroactive systems acting in which discrete areas of the brain subserves which specific components of the complex behaviors associated with eating. Further, even less is known about how interactions and/or adaptations among these neuroactive systems ensue following chronic over- or underconsumption of food.

Neuroanatomical, neurophysiological, and neuropharmacological studies, in general, support the view that psychoactive substances of abuse exert their reinforcing properties in the dopaminergic reward system of the mesocorticolimbic pathway (for reviews see Koob, 1992; Wise, 1987). Neurochemical studies have also shown a commonality of actions, through the dopaminergic system, in the reinforcing properties of these substances. Thus, alcohol (Fadda, Argiolas, Melis, Serra, & Gessa, 1980; Imperato & Di Chiara, 1986), cocaine (Boja & Kuhar, 1989; Izenwasser, Werling, & Cox, 1990), and nicotine (Brazell, Mitchell, Joseph, & Gray, 1990; Imperato, Mulas, & Di Chiara, 1986), when consumed, raise DA levels and affect DA metabolism particularly in brain reward areas. There is also growing evidence that food similarly manifests its reinforcing effects through the dopaminergic pathways of the brain (for reviews see Hoebel, 1985; Wise, 1987). When rats ate (Heffner, Hartman, & Seiden, 1980) or when sucrose sham-feeding

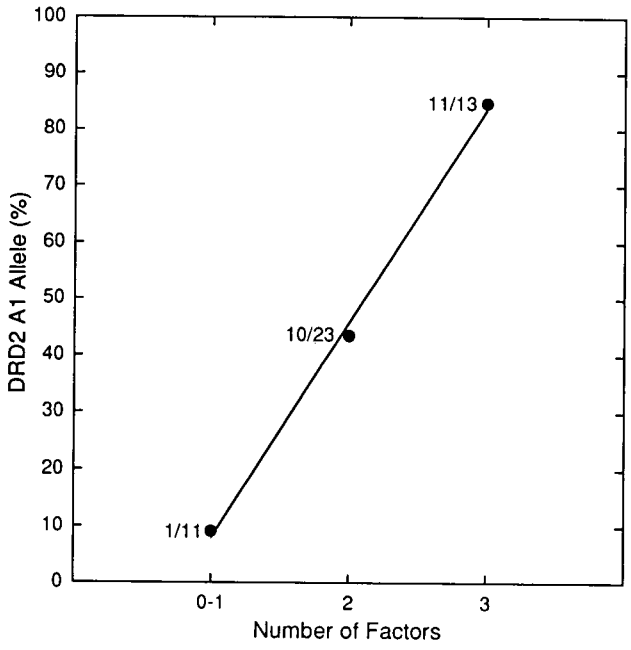

Figure 2. D_2 dopamine receptor A1 allele as a function of phenotypic factors in obese subjects. The factors include: parental history of obesity (presence of at least one obese parent), adolescent- or adult-onset of obesity, and carbohydrate preference. Linear trend analysis shows that increasing factor scores are positively and significantly associated with the prevalence of the D_2 dopamine receptor A1 allele ($\chi^2 = 13.5$, $df = 1$, $p = .0002$).

was used (Smith & Schneider, 1988), the dihydroxyphenylacetic acid to dopamine ratio (DOPAC/DA) increased in the hypothalamus (Heffner et al., 1980; Smith & Schneider, 1988) and in the nucleus accumbens (NAC) and amygdala (Heffner et al., 1980). Further, microdialysis studies performed in the NAC showed that following feeding, extracellular concentration of DA, DOPAC, and homovanillic acid (HVA) increased (Hernandez & Hoebel, 1988). Moreover, electrical stimulation of the perifornical lateral hypothalamus (LH), capable of inducing feeding, also increased extracellular DA, DOPAC, and HVA in the NAC (Heffner et al., 1980). Put together, the data suggest that LH stimulation by food activates the mesolimbic dopaminergic reward system, and that DA release in the NAC is involved in the reinforcement of eating behavior. Although the facilitatory or inhibitory role of serotonin and other neuroactive agents is not precluded in feeding behavior, the total current evidence does support an important role for the brain dopaminergic system in food reinforcement.

If a hereditary basis underlies some forms of obesity, could a gene in the dopaminergic system be involved in this disorder? Given the complex nature of obesity, the lack of a clear mode of transmission, and the fact that unlike mendelian traits, genetic variance for behavior rarely accounts for more than half the phenotypic variance (Plomin, 1990), the application of linkage analysis, as presently constituted, to obesity would be premature in this complex behavioral disorder (Freidman, Leibel, Bahary, Siegel, & Truett, 1991). Instead, as an initial step, we chose association studies, because a distinct advantage of such an approach is that no assumptions need to be made on the mode of inheritance, penetrance, and age of onset of this disorder.

Grandy et al. (1989) first described a restriction fragment length polymorphism (RFLP) with alleles (the less prevalent A1 and the more prevalent A2) identified within the DRD2 gene. This two-allele *Taq*I A RFLP was detected with the genomic phage clone λhD2G1, which contains exon 8 and the 3′ untranslated portion of the DRD2. Using RFLP of *Taq*I A digest of DNA isolated from alcoholic and nonalcoholic subjects, a number of recent studies have determined A1 allelic association with alcoholism. Several reviews of these studies have affirmed the presence of this association in alcoholism (Cloninger, 1991; Conneally, 1991; Noble, 1993; Uhl, Blum, Noble, & Smith, 1993) although one (Gelernter, Goldman, & Risch, 1993) claims a lack of association (however see Noble & Blum, 1993). Moreover, additional studies have also found the DRD2 gene to be implicated in other drugs of abuse (Comings, Comings, et al., 1991; Noble et al., 1993; Smith et al., 1992).

In the present study of nonalcoholic and nondrug abusing subjects who were significantly obese (mean BMI = 35.1), the prevalence of the DRD2 A1 allele was determined to ascertain whether or not there is an association of this allele with biological or behavioral manifestations in a certain type of obesity. Examination of several cardiovascular risk factors showed that none of these were significantly distinguished by their association with the $A1^+$ or the $A1^-$ allele. This would suggest that factors other than polymorphism at the DRD2 locus contributed to the risk for cardiovascular disease.

Although not significant, the A1/A1 genotype showed a higher trend for both BMI and waist-hip ratio when compared with the A2/A2 and the A1/A2 genotypes. This is interesting in view of previous observations showing the number of DRD2 in the brain to be lowest in subjects with the A1/A1 genotypes compared with those having the other two genotypes (Noble, Blum, Ritchie, Montgomery, & Sheridan, 1991). Moreover, a recent study of alcoholics found all subjects homozygous for the A1 allele to have the more severe form of alcoholism (Arinami et al., 1991). Although the evidence presented herein suggests that the more severe obese subjects were also homozygous for the A1 allele, the small sample size of the A1 homozygotes ($n = 6$) precluded a definitive conclusion.

As indicated earlier in the text, there is now growing and convincing evidence that hereditary factors are involved in obesity. The present data add to this corpus of knowledge in suggesting that the DRD2 gene is implicated in obesity. Specifically, obese subjects who had a negative parental history of obesity had a lower $A1^+$ allelic prevalence than subjects who had at least one parent obese (31.0 vs. 53.7%). Although this finding is supportive of a role of the DRD2 gene in obesity, it does not preclude the involvement of other genes and environmental factors in the present sample of obese subjects.

The onset of obesity was found to be related to DRD2 polymorphism. This is shown in the progressive increase of $A1^+$ allelic prevalence in child-, adolescent-, and adult-onset obesity: 25.0%, 36.5%, and 56.4%, respectively. The reasons are not clear why $A1^+$ allelic prevalence varies with the onset of obesity. However, it may be hypothesized that metabolic genes, such as those involved in the synthesis and disposition of carbohydrates, lipids, and hormones, may increase the risk for obesity early on in life, while the effect of DRD2 polymorphism on receptor expression in the maturing brain reward system may occur later on in life leading to obesity and other consumptory problems such as alcoholism and drug abuse. Further studies are needed to address this issue in addition to determining how environmental factors influence early- or late-onset obesity.

DRD2 allelic association was also found with food preference. Specifically, a more than three-fold and significantly higher prevalence of the $A1^+$ allele was observed in

those obese subjects who preferred carbohydrates than those who preferred other foods (64.3% vs. 21.1%). In this regard, previous studies on human eating habits have also shown a certain class of individuals who display a high demand for carbohydrates (Wurtman, 1987; Wurtman, Wurtman, Reynolds, Tasy, & Chew, 1987). This raises the issue of whether carbohydrates release brain DA more promptly or efficiently than other foods and hence produce a greater feeling of reward particularly in $A1^+$ allelic subjects. This differential reinforcement possibility based on DRD2 polymorphism clearly needs experimental validation; however, it does not preclude other aminergic and peptidergic mechanisms in the maintenance and other aspects of eating behavior.

The evidence presented herein showing the relationship of the A1 allele to three phenotypic factors (parental history and adolescent- and adult-onset of obesity and carbohydrate preference) found in the present obese subjects further supports a role of the DRD2 A1 in obesity when these individual factors are summed. A significant and strong positive linear trend was found between increasing factor score in the obese subjects and the presence of the A1 allele. That the DRD2 gene is implicated in obesity is also derived from a recent report (Comings, MacMurray, et al., 1991) showing that additional polymorphisms in the DRD2 gene are also associated with obesity.

The pathophysiological basis for these molecular genetic findings in obesity is as yet unclear. However, it has been suggested (Comings, Comings, et al., 1991) that the most likely explanation for the apparent relationship between increased expression of symptoms and the prevalence of the A1 allele is that either the mutation causing *Taq*I A polymorphism or a mutation in linkage disequilibrium with the *Taq*I A polymorphism is associated with a decrease in the function of the DRD2 gene. Evidence for such an effect has come from a study (Noble et al., 1991) showing a significant decrease in the number of DRD2 binding sites in brains of individuals carrying the A1 allele compared with those who did not. Although the total numbers of DRD2 differ in A1 and A2 allelic individuals, further studies are needed to confirm the link between *Taq*I A DRD2 polymorphism and functional activity of DRD2.

There are several limitations to the present study relevant to rendering a broad generalization. The sample size of obese men as well as of blacks and of other non-Caucasians was not large. Furthermore, the obese subjects were individuals who volunteered for treatment of their disorder. Because it is possible that treatment probands are more seriously affected with their weight problems, it is uncertain how concordant the present findings would be in obese subjects found in the general population. It is for these reasons that the present results must be approached with caution and future studies are needed to confirm or reject these initial findings.

In conclusion, this study of nonalcohol, nondrug abusing obese subjects, all drawn from the same geographic locale, found the prevalence of the DRD2 A1 allele to be unrelated to several cardiovascular risk factors studied. On the other hand, a unique phenotypic profile, characterized by the presence of parental history and post-puberty onset of obesity as well as carbohydrate preference, was observed in obese subjects carrying the A1 allele. If the present study is replicated and extended, it could suggest that besides metabolic genes that contribute to obesity, another factor that increases the risk for a certain type of obesity is the DRD2 gene.

The authors are grateful to Servier Amérique, Neuilly-sur-Seine, France for a grant in support of this study (R.E.N.) and to the Christopher D. Smithers Foundation, NY (E.P.N.). We also express appreciation for the outstanding efforts of A. Jaeger in the preparation of this manuscript.

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