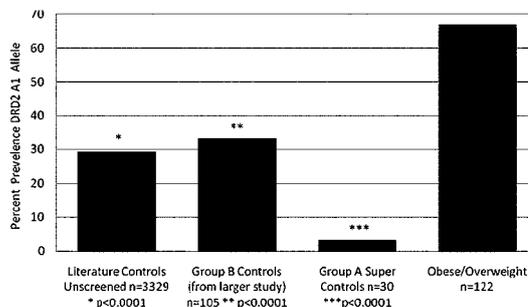


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Correlation of the *Taq1* dopamine D2 receptor gene and percent body fat in obese and screened control subjects: A preliminary report

5 Amanda L. C. Chen, Kenneth Blum,* Thomas J. H. Chen, John Giordano, B. William Downs, David Han, Debmayla Barh and Eric R. Braverman



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Correlation of the *Taq1* dopamine D2 receptor gene and percent body fat in obese and screened control subjects: A preliminary report†

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While there is a considerable body of literature correlating the role of dopaminergic genes and obesity, body mass index, body type, overeating, carbohydrate binging, energy expenditure and low dopamine D2 receptor (D2R) receptor density, there is a paucity of research concerning the dopamine D2 receptor gene (DRD2) variants and percent body fat. We report here the potential association of DRD2 genotypes and the percent fat phenotype. In this study we genotyped 122 obese/overweight (O/OW) Caucasian subjects and 30 non-obese Caucasian controls, screened to exclude substance abuse. The subjects were assessed for weight, body mass index (BMI; kg m⁻²) and percent body fat using dual energy X-ray absorptiometry (DEXA). The sample was separated into two independent groups; those with the *Taq1* A1 allele (A1/A1 or A1/A2) and those without the A1 allele (A2/A2). The controls had a normal range of body fat (25–31% for females and 18–25% for males). The O/OW subjects had a percent body fat value of over 32% for females and over 25% for males. For the O/OW subjects, the mean BMI was 29.3 ± 6.25 kg m⁻², mean body fat was 42.1 ± 7.5% and mean weight was 82.7 ± 21.7 kg. The DRD2 *Taq1* A1 allele was present in 67% of the O/OW subjects compared to 3.3% of super controls (A group), 33.3% of screened (for drug abuse and obesity) controls (B group) and unscreened literature controls 29.4% ($P \leq 0.001$). Comparing all cases with more than 34% body fat, utilizing logistic regression analysis, the DRD2 A1 allele accounts for 45.9% of the variance, which is statistically significant ($\chi^2 = 43.47$, degrees of freedom (df) = 1, $P < 0.0001$). These results are consistent with a role for the DRD2 gene in obesity, as measured by percent body fat as well as by weight and BMI.

Introduction

Overeating is a biogenetic condition that comes in many forms¹ and is inheritable.^{2–8} While there are certain environmental

factors which may reduce body fat, body mass and fat-free mass in both adults and children, including but not limited to, low-intensity, long-duration exercise, aerobic exercise combined with high-repetition resistance training and exercise programs combined with a behavioral-modification component, genetics may even play a more significant role.⁹

Compulsive eating disorders are likely to involve more than one defective gene because of their complexity.^{10–17} Given the considerable array of metabolic systems that contribute to overeating, it is not surprising that a number of neurochemical defects have been implicated. It is important to realize that carbohydrates cause the release of the pleasure-inducing brain chemical dopamine. There is general agreement that other pleasure-inducing substances such as alcohol and nicotine, like glucose, work through the dopaminergic pathways of the brain. This shows the common genetic thread of multiple addictions.¹⁸ There have been a number of investigations that have observed a significant association between an abnormal form of a gene known as the dopamine D2 receptor gene, and obesity; time of onset of obesity; carbohydrate preference or craving; high body mass index; co-morbid drug abuse; energy expenditure; hyperphagia (overeating);¹⁹ and low dopamine D2 receptors.¹ Since

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1 obesity and overeating including binge behavior is polygenic, many genes have been identified. Most of these genes are susceptibility genes. These genes are necessary, but not sufficient alone, to cause obesity.

5 In 1990, Blum and colleagues, using the *Taq1* polymorphism (gene variant) of the dopamine D2 receptor gene locus (DRD2), for the first time reported a strong association between a virulent form of alcoholism and the minor gene form (A1) of the DRD2 gene in this population.²⁰ Other studies further support an association of the A1 form of the DRD2 gene with substance abuse vulnerability and other compulsive behaviors.²¹ In this regard, the National Institute on Alcohol Abuse and Alcoholism recently reported data that strongly suggest that DRD2 is a susceptibility gene for substance abusers across multiple populations. Specifically, a haplotype block of 25.8 kb was highly associated with alcohol dependence and heroin addiction. Furthermore, a low number of dopamine D2 receptors suggests a hypodopaminergic function, as described by Eliot Gardner in a series of published works.²² When there is a paucity of dopamine receptors the person will be more prone to seek any substance (including glucose) or behavior that stimulates the dopaminergic system, as a form of self-healing. In this regard, we know that substances such as alcohol, cocaine, heroin, nicotine and glucose, as well as a number of behaviors like gambling and sex, preferentially release dopamine at the nucleus accumbens. In one study,¹ striatal dopamine D2 receptor availability was significantly lower in ten obese subjects compared to normal non-obese controls. In these obese subjects body mass index (BMI) correlated negatively with the measures of D2 receptors; the individuals with the lowest D2 values had the largest BMI.

30 Table 1 illustrates the role of dopaminergic genes in obesity and related behaviors. While we are making some progress in attempting to understand the cerebral mechanisms underlying the behaviors that lead to pathological overeating and obesity, dopamine, a neurotransmitter that modulates rewarding properties of food, is likely to be involved. To test the hypothesis that obese/overweight (O/OW) individuals having a high percent body fat compared to non-obese controls also have a high presence of the dopamine D2A1 allele, we measured percent

1 body fat and genotyped individuals for the presence or absence of the dopamine D2 receptor A1 allele.

5 Research methods and procedures

This study was conducted with IRB approval of both the Path Research Foundation (registration #IRB00002334) and the City of Hope National Medical Center, and all patients filled out and signed an approved consent form prior to entering this study. The genotyping was performed at the Department of Medical Genetics at the City of Hope National Medical Center, Duarte, California. The recruitment and characterization of the obese subjects was accomplished at the Health and Medical Research Foundation, San Antonio, Texas and the Sports Medicine Institute, Baylor College of Medicine, Houston, Texas. The recruitment and characterization of the well-screened controls were accomplished at the PATH Medical Research foundation, New York City, New York. The statistical analysis was performed in part at the University of Texas School of Public Health, San Antonio, Texas.

Subjects

25 **Controls.** In order to perform scientifically sound genetic association studies in a complex disease such as obesity, certain exclusion/inclusion criteria must be satisfied. Since it is now known that polymorphisms of the DRD2 gene (A1, B1, C1, the haplotype In6-Ex7 and other variants) are associated with a number of impulsive-addictive/compulsive disorders including severe alcoholism, polysubstance dependence, crack/cocaine dependence, smoking, obesity (BMI over 25), carbohydrate bingeing, conduct disorder, defense-style personality, schizoid/avoidant personality, violent crimes, pathological gambling, autism, Tourette syndrome, attention deficit disorder with or without hyperactivity (ADHD), severe withdrawal, depression, parental history of alcoholism, drug abuse, obesity, and inability to cope with anxiety; we decided to develop stringent non-Hispanic Caucasian controls.^{19,23} It is well known that there is a high rate of genetic polymorphisms in different ethnic groups (e.g. Hispanic, African Americans) thus it seemed parsimonious

Table 1 The role of dopaminergic pathways in obesity and related behaviors^a

DRD2 Receptors and psychoactive drugs

45 Certain anti-psychotic drugs increase feeding behavior and cause weight gain because they block dopamine D2 receptors and dopamine response
Drugs that stimulate either dopamine D1 and/or D2 receptors reduce overeating

Dopamine D2 receptors and gene

Dopamine stimulators normalize overeating, body fat and blood glucose levels by increasing serum dehydroepiandrosterone (DHEA).

There is evidence that BMI and even personality disorders (temperament) are directly correlated with the ability of dopamine to bind to its respective brain receptors.

50 Certain dopamine gene mutations associate with BMI.

The A1 form of the dopamine D2 receptor gene is associated with increase fat stores.

The A1 form of the dopamine D2 receptor gene is associated with a high BMI.

4 Using PET scans, obese individuals have been found to have fewer dopamine D2 receptors than lean controls.

5 Glucose causes a release of dopamine at brain sites.

5 Dopamine increases the release of glucose from blood cells (hepatocytes).

Other dopamine genes

55 Other genes, such as the dopamine 4 receptor, were also associated with obesity and a high BMI.

Both dopamine D1 and D2 receptors are linked to glucose metabolism and subsequent feeding behavior.

Certain dopamine genes form associates with low energy expenditure.

^a Source: see reviews Blum *et al.*^{19,20}

1 to carefully stratify accordingly. Specifically, these included
careful assessment of alcoholism, substance use disorder, family
history of chemical dependence, obesity, nicotine dependence
(smoking behavior), BMI over 25, carbohydrate binging, autism,
5 Tourettes, ADHD, mood disorders, personality (novelty
seeking) schizophrenia, movement disorders, migraine, patho-
logical gambling and post-traumatic stress. Thus, we carefully
stratified 184 individuals attending the Path Medical Clinic of
New York City. These patients were stringently assessed to
10 eliminate any impulsive, addictive (including carbohydrate
binging behavior and obesity, as defined by BMI and scale
weight) or compulsive behaviors including absence of both Axis
1 and Axis 2 diagnosis.

Computer analysis revealed a sub population that fit the above
exclusion/inclusion criteria. This sub-population consisted of
a total of 30 non-Hispanic Caucasian subjects (4 males and 26
females) with an average age of 44.8 (46.3% females and 43.4%
male) recruited from the total patient population of the Path
Medical Clinic in New York City. Both the BMI and body fat of
the 30 controls was within the normal range (BMI normal range
below 25 kg m⁻²; and having normal range of body fat between
25–31% for females and 18–25% for males). It is noteworthy that
over a five year period, we studied a total of 1506 subjects (71%
Caucasian, 1% black, 7% Hispanic, less than 1% Asian)
attending the Path Medical Clinic utilizing DEXA, and found
the average percent body fat to be 28.7.

Obese/overweight (O/OW) subjects. A total of 130 unrelated
non-Hispanic Caucasian O/OW subjects (BMI above 25 kg m⁻²
and percent body fat above 32%) were enrolled in the study, 122
(17 men and 105 women; mean (± S.E.) age, 42.3 years ± 8.8) of
whom completed the testing (93.8%). Subjects were recruited
from a variety of fitness and athletic clubs in San Antonio and
Houston, Texas, by fitness instructors and sales personnel who
provided information about the study to potential participants.
In order to ensure compliance, the fitness instructors were paid to
monitor the subjects as they progressed through the study to
ensure that the subjects reported their physical activity levels and
caloric intake, and completed the testing. All subjects were
asked to consult with their personal physician before giving
written informed consent. At the time of recruitment the selection
of the subjects was completely randomized, without any
knowledge of their DRD2 Genotype.

45 Dual energy X-ray absorptiometry

A number of studies have shown that DEXA can accurately
measure fat and lean content of skeletal mass with a typical
precision error for total body bone mineral content < 1%.²⁴
DEXA has also been shown to be a precise method for assessing
body composition in obese and non-obese subjects. DEXA
correlates highly with underwater weighing deuterium dilution
6 and total potassium. The reliability of DEXA makes it possible
to monitor the effects of relatively short-term dietary restrictions
and exercise on both regional and total body composition.

DEXA provides a three-compartment model of body
composition: fat, lean tissue mass and bone mineral content.
Measurements are made using a constant potential energy source
at 78 kVp and a K-edge filter (cerium) to achieve a congruent,

stable, dual-energy beam with effective energies of 40 to 70 keV.
The unit performs a series of transverse scans moving from head
to toe at 1 cm intervals; the area being scanned is approximately
60 × 200 cm. Data are collected for about 120 pixel elements per
transverse, with each pixel approximately 5 × 10 mm in size.
5 Total body measurements are completed in 10 to 20 min with
a scan speed of 16 cm s⁻¹, or in 20 min with a scan speed of 8 cm
s⁻¹. The rate value (ratio of low to high energy attenuation in soft
tissue) ranges from 1.2 to 1.4.

Genotyping

A total of 152 subjects were genotyped for the dopamine D2
receptor gene (30 stringent controls subjects, group A and 122 O/
OW subjects). All subjects were genotyped based on a neutral
identification number and read without knowledge of the indi-
vidual being typed.

Group B (105) control subjects, which had been previously
genotyped in the laboratory of David Comings as part of a larger
study, were screened for drug abuse and obesity. However, these
controls were not screened for all reward deficiency syndrome
(RDS) behaviors and were not screened with DEXA or geno-
typed in the present study.

Total genomic DNA was extracted from each coded blood
sample and aliquots were used for polymerase chain reaction
(PCR) analysis. The oligo-nucleotide primers, 5'-
CCGTCGACCCTTCCTGAGTGTTCATCA-3' and
5'CCGTCGACGGCTGGCCAAGTTGTCTA-3', were used to
amplify a 310 base pair (bp) fragment spanning the polymorphic
Taq1A site of the DRD2 gene. The D2A1 and D2A2 genotyping
was performed by a PCR technique.^{20,25,26} PCR was performed in
30 μL reaction mixtures containing 1.5 mM MgCl₂, 2 mM 2'-
deoxynucleotide-5'-triphosphates (dNTPs) 0.5 μM primers, 1 μg
of template DNA, 1.5 U of *Taq* polymerase (Boehringer Man-
nheim Corp., Indianapolis, IN), PCR buffer (20 mM tris-HCl
(pH 8.4) and 50 mM KCl. After an initial denaturation at 94 °C
for 4 min, the DNA was amplified with 35 cycles of 30 s at 94 °C,
30 s at 58 °C, and 30 s at 72 °C, followed by a final extension step
of 5 min at 72 °C. The PCR product was digested with 5 U of
Taq1 for 22 h at 65 °C for the *Taq1A* polymorphism. Digestion
products were then resolved on a 3% agarose gel (5 V cm⁻¹)
containing 0.65 μg ml⁻¹ ethidium bromide. There were three
DRD2 *Taq1A* genotypes: 1) the predominant homozygote, A2/
A2, which exhibits three restriction fragments of 180 and 130 bp;
2) the heterozygote, A1/A2, which exhibits three restriction
fragments of 310, 180, and 130 bp; and 3) the rare homozygote
A1/A1, which produces only the uncleaved 310 bp fragment.

Statistical analysis

Demographic, clinical, laboratory, interview and questionnaire
data were coded and entered into a computer database. DRD2
allelic prevalence, obtained by personnel blind to the aforemen-
tioned information, was also coded and the two data sets were
merged for analyses. *t*-Tests were used to compare interval data,
and chi-squared statistics with Yates' correction for continuity,²⁷
as appropriate, was used for group comparisons of ordinal and
nominal values. We examined the relative role of the DRD2 gene
in percent body fat. For the DRD2 gene we utilized univariate

logistic regression analysis with percent body fat as the dependent variable and the DRD2A1 allele, sex, and age as independent variables.

The rationale for imitating these experiments relates to the unknown involvement in percent body fat as a function of DRD2 polymorphisms, especially the DRD2 A1 allele. Due to a remarkable list of behaviors known to associate with RDS (phenotype) we decided to utilize what we have referred to as “super-controls”. This is the first time any laboratory carefully selected out all subclass RDS behaviors to serve as a control group. We also decided to utilize logistic regression analysis to determine the percent contribution of the DRD2 A1 allele as a predictor of percent body fat.

Results

Baseline characteristics for the 30 controls are provided in Table 2. The age (mean \pm S.E.) of the well-screened control individuals was 44.8 ± 7.1 years. The percent body fat (mean \pm S.E.) for these subjects was 28.4 ± 3.4 , the BMI (mean \pm S.E.) for these subjects was $22.4 \pm 2.9 \text{ kg m}^{-2}$ and the scale weight (mean \pm S.E.) for these controls was $61.3 \pm 6.7 \text{ kg}$.

Of the 130 subjects who were recruited for this study, only 8 failed to complete the final test: 1 subject became pregnant and was asked to withdraw from the study, 3 moved from the area, 1 was ill and 3 were lost to follow-up. Baseline characteristics for the 122 subjects who completed the study are provided in Table 2. The average age (years) of the O/OV participants was 42.3 ± 8.8 ; the mean percent body fat (%) for these subjects was 42.1 ± 7.5 ; mean BMI (kg m^{-2}) was 29.3 ± 6.25 and the scale weight (kg) was 82.7 ± 21.7 .

Using standard statistical analyses described above, we compared the “super” control (Group A) and O/OV groups with respect to age (A), scale weight (SW), percent body fat (BF) and BMI. The results obtained are as follows: A ($P < 0.15$), SW ($P < 0.001$), BF ($P < 0.001$), and BMI ($P < 0.001$) respectively (Fig. 1).

Insert graphics

It is noteworthy that the genotyping results show that compared to non-abusing and non-obese literature controls ($n = 3329$) carrying the DRD2 A1 allele at 29.4%²³ the defined 30 well-screened controls used in this study carried the DRD2 A1 allele at only 3.3%. However, the 122 O/OV subjects carried the DRD2 variant at 67%. Chi square analysis revealed a significant association with percent body fat compared to 30 super controls, ($\chi^2 = 38.5$, $df = 1$, $P < 0.0001$) (Fig. 2). Fig. 2 also shows the positive association of two other control data sets: 3329 unscreened population controls (at least $P < 0.0001$) and 105

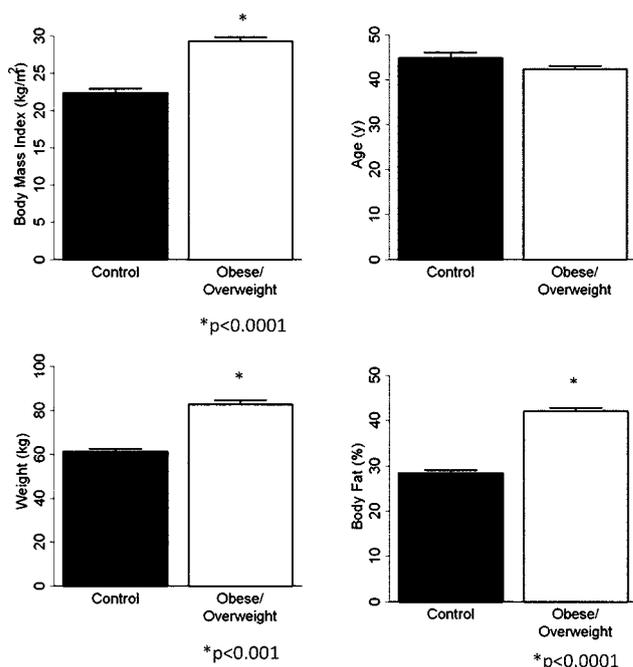


Fig. 1 Data for age, percent body fat as measured by DEXA, BMI and scale weight in super control group A and O/OV populations.

subjects (Control B) (at least $p < 0.0001$). Even more importantly and surprisingly, comparing all cases with more than 34% body fat utilizing logistic regression analysis, the DRD2 A1 allele accounts for 45.9% of the variance which is statistically significant ($\chi^2 = 43.47$, $df = 1$, $P < 0.0001$).

Discussion

While there is increasing evidence that genetic factors can influence differences in vulnerability to obesity, there still is controversy as to the exact genetic mechanism. Dopamine, acting through many receptors, can modulate the activity of neuronal reward pathways and thus effect a number of impulsive, addictive and compulsive behaviors, including overeating and carbohydrate bingeing. Over the last decade, a number of genetic variants have been associated with obesity and related substrates. Included in this list are CNS regulatory genes such as the OB (LEP) gene and the dopamine D2 receptor gene. In this regard a number of studies^{1,19,20} (see Table 1) have reported a positive association of the *Taq1* dopamine D2 receptor A1 allele and low density of D2 receptors in obesity, carbohydrate bingeing, parental history of obesity, comorbid substance use disorder (SUD) and energy expenditure.

Table 2 Mean (SD) baseline demographic data and DRD2 A1 allele percent prevalence for 152 subjects

Subjects (<i>n</i>)	Age (years)	Weight (kg)	Body fat (%)	BMI (kg m^{-2})	A1 allele (%)
Control subjects A ($n = 30$)	^a 44.8 \pm 7.1	^a 61.3 \pm 6.7	^a 28.4 \pm 3.4	^a 22.4 \pm 2.9	3.3
Control subjects B ($n = 105$)	36.5 \pm 6.9	60.5 \pm 6.1		21.9 \pm 2.9	33.4
O/OV Subjects ($n = 122$)	^a 42.3 \pm 8.8	^a 82.7 \pm 21.7	^a 42.1 \pm 7.5	^a 29.3 \pm 6.25	67.0

^a Source: Kaats *et al.*, *Current Therapeutic Research*, 1998, 59(6), 379–388.

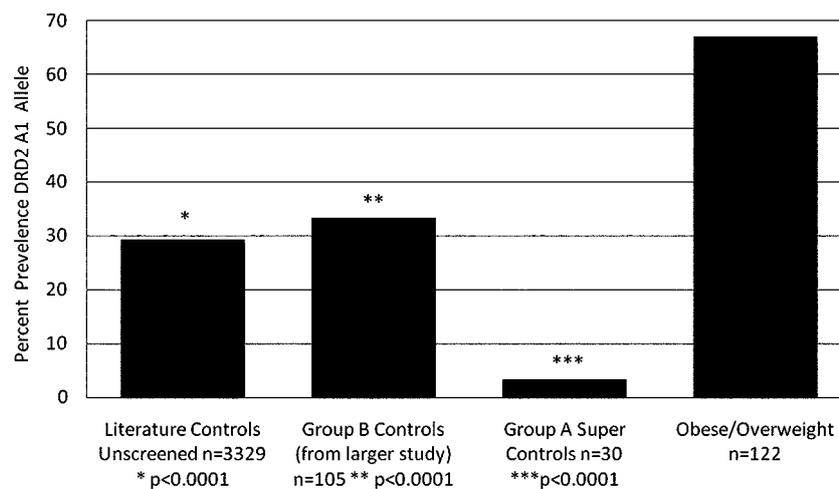


Fig. 2 Percent presence of *Taq1* dopamine D2 receptor A1 allele in population of unscreened controls ($n = 3329$); screened controls group B ($n = 105$); super screened controls group A ($n = 30$) and obese/overweight subjects ($n = 122$).

There are a number of plausible mechanisms that may contribute to these phenomena. The potential mechanisms involve: (1) the role of the DRD2 gene polymorphisms in obesity and related behaviors; (2) the role of the DRD2 mutations and energy expenditure; (3) the interaction of glucose and dopamine release and hyperphagia; (4) the role of dopamine and hyperglycemia; (5) the importance of using “super” normal controls in association studies; and (6) the relationship between personality, body type and dopaminergic genes.

In this population we found a very small number of individuals that carried the A1/A1 genotype in the O/O group (7/122) thereby not allowing any meaningful further statistical analysis of the data set. It is important to note that in association studies the phenomena known as **heterosis** may have impact on the interpretation of understanding allelic meaning. Molecular **heterosis** occurs when subjects heterozygous for a specific genetic polymorphism show a significantly greater effect (positive **heterosis**) or lesser effect (negative **heterosis**) for a quantitative or dichotomous trait than subjects homozygous for either allele. At a molecular level **heterosis** appears counterintuitive to the expectation that if the 1 allele of a two-allele polymorphism is associated with a decrease in gene expression, those carrying the 1/1 genotype should show the greatest effect, 1/2 heterozygotes should be intermediate, and 2/2 homozygotes should show the least effect. For many association studies positive heterosis occurs often, especially for the DRD2 gene.

Role of the DRD2 gene polymorphisms in obesity and related behaviors

Specifically, the reinforcing properties of food have also led to an examination of the involvement of DRD2 polymorphisms in obesity. Haplotype 4 (GT) of intron 6 and exon 7 of the DRD2 gene was found to be associated with an increased risk of obesity.²⁸ In another study, the DRD2 A1 allele was present in 45.2% of obese subjects,²⁹ a prevalence similar to that found in alcoholics, nicotine- and other drug-dependent subjects. In addition, the A1 allele was significantly associated with carbohydrate craving. Polymorphisms of the OB gene and the DRD2

A1 allele are each associated significantly with obesity,¹⁶ these two polymorphisms together accounted for about 20% of the variance in BMI, particularly in younger women. Another study has ascertained the relationship of the DRD2 A1 allele in obese subjects with and without comorbid substance use disorders.³⁰ In obese subjects, A1 allelic prevalence was significantly higher than in controls ($P < 10^{-4}$). Moreover, the progressive increase in comorbid substance use disorders in these obese subjects was positively related to increased A1 allelic prevalence ($P < 10^{-6}$). Furthermore, another case control study compared variants of the DRD2 gene in obese (BMI > 30) and non-obese control subjects.³¹ The DRD2 A1 allele was significantly higher in obese subjects compared to controls ($P = 0.002$) as was the DRD2 B1 allele ($P = 0.003$). The risk of obesity associated with the DRD2 A1 genotype was 3.48 compared to 4.55 for the DRD2 B1 genotype. Moreover, Thomas *et al.*³² assessed the *Taq1A* DRD2 alleles in 484 obese and 506 non-obese Chinese subjects. Obese subjects (using either BMI or waist-to-hip ratio criteria) had a significantly higher prevalence of the A1 allele ($P = 0.02$) and A1 allelic frequency ($P = 0.03$), than non-obese subjects. Finally, another study determined the association of an Nco1 polymorphism (C–T transition) in exon 6 of the DRD2 gene with hypertension.³³ Subjects with the TT genotype had significantly higher systolic blood pressure than subjects with the CT genotype ($P = 0.049$). Moreover, subjects with TT genotype had significantly higher diastolic blood pressure than either subjects with the CT or CC genotype ($P = 0.011$). The importance of the latter finding relates to the fact that there is strong evidence from epidemiological studies of a positive relationship between increased body weight and hypertension.³⁴

Role of the DRD2 mutations and energy expenditure

Two studies,^{35,36} assessed the role of other DRD2 mutations on weight and energy expenditure in Pima Indians. Individuals with a Cys-encoding allele had a higher BMI than those homozygous for the Ser311-encoding allele.³⁵ and furthermore, total energy expenditure and 24 h resting energy expenditure were lower in

1 homozygotes for the Cys311-encoding allele when compared to
heterozygotes and homozygotes for the Ser311-encoding allele.³⁶

5 The interaction of glucose and dopamine release and hyperphagia

Moreover, it is well known that pharmacological doses of the
glucose analogue, 2-deoxyglucose (2DG), cause acute glucose
deprivation and are associated with enhanced dopamine turn-
over in pre-clinical studies. In fact, lines of evidence indicate that
10 **12** a variety of metabolic stresses, including acute glucose depriva-
tion are associated with dopamine release. Using PET, Adler
*et al.*³⁷ found that 2DG administration enhanced synaptic
dopamine concentrations. The administration of 2DG is associ-
ated with significant striatal dopamine release even in healthy
volunteers. This data is important because it further ties glucose
levels to dopaminergic activity. Moreover, there is even a rela-
15 tionship between insulin levels and dopamine release in the
tuberoinfundibular neurons. The insulin effect is dependent on
Ca²⁺ ions and a protein kinase C Na⁺ – H⁺ exchange system.
Additionally, when there is lowered glucose in the brain leading
to cerebral global transient ischemia, monoamine release, espe-
20 cially dopamine, is inhibited. In this regard, Trugman *et al.*³⁸
showed D1 antagonists lowered glucose utilization by 24–28% in
the globus pallidus, entopeduncular nucleus, subthalamic nucleus,
substantia nigra and even the motor cortex, suggesting that
stimulation of the D1 receptor by endogenous dopamine
25 contributes to basal metabolism in these regions. In contrast
both D1 and D2 agonists increase glucose utilization. These
results suggest that feeding behavior is tied into the stimulation
of both D1 and D2 receptors and provides metabolic evidence
for the importance of D1 and D2 functional linkage in the brain,
30 which relates to hyperphagia or overeating.

35 Role of dopamine and hyperglycemia

Furthermore, the direct effects of dopamine on glucose release
from primary cultured rat hepatocytes were studied in Japan by
Shiroyama *et al.*³⁹ The authors investigated whether dopamine
has any direct effect on glucose release from hepatocytes through
the glycogenolytic and/or gluconeogenic pathways, and at the
40 same time determined the main type of adrenergic receptor
involved in glucose release. Dopamine caused the release of
glucose which was inhibited by the beta blocker, propranolol.
The authors conclude that dopamine has a direct effect on hep-
tocytes, increasing glucose release *via* both glycogenolytic and
45 gluconeogenic pathways, and is mediated by beta-adrenergic
receptors.

50 The importance of using “super” normal controls in association studies

We believe that this is the first report that provides direct genetic
evidence that the dopamine D2 receptor A1 allele (which has
been associated with fewer D2 receptors in humans)^{1,40} is posi-
55 **13** tively associated with increased percent body fat. We must
caution against any false notions that the DRD2 gene alone
causes fat storage, suggested by the high percent contribution
found in this particular study. The process of fat storage and the
role of genes is poorly understood, however, with confirming
data, our findings point to a significant involvement of percent

body fat in humans and dopamine functionality. It is plausible
that utilizing “super” controls will be important for future
association studies. In this regard, Neiswanger and Hill found
a strong association of the D2 A1 allele and alcoholism.⁴¹ Hill
suggested failures reported in the literature were due to poor
5 assessment of controls. They recommend the appropriate use of
“super” controls to more accurately assess a true phenotype,
especially important when studying complex behavioral
diseases.⁴² They also found evidence for linkage between the
dopamine D2 receptor gene and severe alcoholism, early onset,
10 physical dependence symptoms, and antisocial personality
disorder.⁴³ However, one caveat to the use of “super” controls
may be that they are not appropriate as these individuals do not
mirror the general population; thus these results should be
regarded as preliminary and warrant significant additional
15 research.

20 Relationship between personality, body type and dopaminergic genes

In a study by Yasuna⁴⁴ from Japan, personality is a behavioral
14 pattern, which differs among individuals. Fuemmeler *et al.*,⁴⁵
categorized personality variants according to the concept of
fundamental body types related to neurotransmitter genotypes.
Several lines of evidence suggest that the central dopamine
25 system may underlie the regulation of weight and personality
trait. In this study, the authors examined the dopamine D2
receptor (D2R) binding together with BMI and personality trait
on the temperament and character inventory in 16 subjects. The
data demonstrate a significant relation between D2R binding in
30 the amygdala, BMI and the personality trait of harm avoidance
(this is in agreement with other work by Blum *et al.* showing
significant association between D2RA1 variants and harm
avoidance⁴⁶). The authors conclude that variation of dopami-
nergic activity in the amygdala underlies the personality variants
35 related to body type.⁴⁷

It is noteworthy that family-based treatments show positive
relationships between parent and child weight losses. Epstein
*et al.*⁴⁸ showed a relationship between parent and child zBMI
change over 6 and 12 months ($r_s = 0.69, 0.77, p_s < 0.001$), and
40 concordance for the number of *Taq1* A1 alleles predicted the
similarity in parent and child weight loss at 6 ($P = 0.003$) and 12
($P = 0.025$) months. These results show concordance of the *Taq1*
A1 allele of the DRD2 between parents and children may be one
mechanism for the similar response to family-based treatments
45 within families.

Moreover certain D2 dopamine receptor *Taq1A* genotypes
(A1/A1, A1/A2) have been associated with obesity and substance
abuse. Barnard *et al.*⁴⁹ hypothesized that their presence would be
associated with reduced efficacy of dietary interventions in
50 individuals with type 2 diabetes. In fact, the A1 allele was highly
prevalent, occurring in 47% of white participants ($n = 49$), which
was significantly higher than the 29% prevalence previously
reported in non-diabetic whites ($P = 0.01$). As discussed earlier,
ethnicity is an important factor in dietary intake potentially
55 related to dopaminergic genotype. The A1 allele was found in
55% of black participants ($n = 44$). Black participants with A1
(+) genotypes had significantly greater mean body weight (11.2
kg heavier, $P = 0.05$) and greater intake of fat ($P = 0.002$),

1 saturated fat ($P = 0.01$), and cholesterol ($P = 0.02$) compared
with A2/A2 A1 (-) individuals. Among whites in the vegan
group, A1 (+) individuals reduced fat intake ($P = 0.04$) signifi-
cantly less than did A1 (-) individuals.

5 Interestingly, Simonen *et al.*⁵⁰ studied frequencies of a poly-
morphism in exon 6 of the DRD2 gene for the T and C alleles in
females, using a sports index to evaluate physical activity. The
TT homozygote in White women had 29–38% lower sports index
16 (F = 4.09, $P = .023$) and 27–33% lower work index (F = 6.23, $P =$
10 .004) than the CC homozygotes and CT heterozygotes. The
results suggest that DNA sequence variation in the DRD2 gene is
associated with physical activity levels among white women. In
a related study, Mathes *et al.*⁵¹ showed significantly greater
dopamine concentrations in dorsal striatum and nucleus
15 accumbens of running wheel (HR) mice compared to M16
(selected for obesity) and ICR (not selected for obesity) mice.
Microarray analysis showed significant gene expression differ-
ences between HR and M16 compared to ICR mice in both brain
areas, with changes revealed throughout the dopamine pathway
20 including D1 and D2 receptors, associated G-proteins (*e.g.*,
Golf), and adenylate cyclase (*e.g.*, *Adcy5*). The results suggest
that similar modifications within the dopamine system may
contribute to the expression of opposite phenotypes in mice,
demonstrating that alterations within central reward pathways
25 can contribute to both obesity and excessive exercise which may
be a competing phenotype.

In terms of energy expenditure as discussed earlier, Epstein
*et al.*⁵² observed that food reinforcement was greater in obese
than in non-obese individuals, especially in obese individuals
with the *Taq1* A1 allele. Energy intake was greater for individuals
high in food reinforcement and greatest in those high in food
reinforcement with the *Taq1* A1 allele. These data show that
individual differences in food reinforcement may be important
for obesity and that the DRD2 genotype may interact with food
35 reinforcement to influence energy intake. These results are not
surprising and are in agreement with Stice *et al.*⁵³ Weaker acti-
vation of the frontal operculum, lateral orbitofrontal cortex, and
striatum in response to imagined intake of palatable foods *versus*
imagined intake of unpalatable foods or water, predicted future
40 increases in body mass for those with the DRD2 *Tac IA* A1 allele
or the DRD4-7R allele. Data also suggest that for those lacking
these alleles, greater responsivity of these food reward regions
predicted future increases in body mass. Accordingly, this novel
prospective functional MRI (fMRI) study indicates that
45 responsivity of reward circuitry to food increases risk for future
weight gain, but that genes that impact dopamine signaling
capacity moderate the predictive effects, suggesting two quali-
tatively distinct pathways to unhealthy weight gain based on
genetic risk. Finally, Heber and Carpenter⁵⁴ suggested, upon
50 review of the literature, that there is increasing evidence that the
same brain reward circuits involved in perpetuating drug abuse
are involved in the hedonic urges and food cravings observed
clinically in overweight and obese subjects. A polymorphism of
the D2 dopamine receptor which renders it less sensitive to
55 dopamine stimulation has been proposed to promote self-stim-
ulatory behavior such as consuming alcohol, abusing drugs, or
binging on foods. It is important to determine how this poly-
morphism may interact with other well-known candidate genes
for obesity, including polymorphisms of the leptin receptor gene

and the opiomelanocortin gene. The complex interaction of poly-
genes and even control of glucose induced dopamine release
regulated by second messengers provide a novel mechanism. In
fact, Fulton *et al.*⁵⁵ recently demonstrated the ability of D2-AR
activation to increase Kv1.2 currents in co-transfected cells and
5 its reliance on G $\beta\gamma$ subunit signaling along with the physical
coupling of D2-AR and Kv1.2-containing channels in striatal
tissue.

Conclusions

In summary, we propose that while obesity is a polygenic
disorder, our findings indicate a putative important role of the
dopamine D2 receptor gene in morbid obesity, especially in high
risk populations.^{17,35} We therefore propose that these and other
findings will have important therapeutic implications, but at this
stage the present results should remain as a preliminary obser-
15 vation. While further confirming the work of Comings and
associates¹⁶ who found significant association of the DRD2A1
allele and body mass index accounting for only 2.8 percent of the
variance, our surprisingly high value of contribution to the
variance 45.5% percent body fat may support the use of more
stringent control selection in subsequent studies and warrants
20 further rigorous research.

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1 Authors Queries 1

Journal: FO

5 Paper: c1fo10089k 5

Title: Correlation of the *Taq1* dopamine D2 receptor gene and percent body fat in obese and screened control subjects: A preliminary report

10 Editor's queries are marked like this... **■**, and for your convenience line numbers are inserted like this... 5 10

Query Reference	Query	Remarks
15 1	[INFO-1] For your information: You can cite this paper before the page numbers are assigned with: (authors), Food Funct., DOI: 10.1039/c1fo10089k.	
20 2	Is the contents entry acceptable? The image can be in colour, at most 8 cm wide by 4 cm high (a CMYK, 600 dpi TIF file, preferably). A short descriptive text should also be supplied, highlighting the novelty of the work in no longer than 30 words. If the entry does not fit between the two horizontal lines, then please trim the text and/or the title.	
25 3	Please could you check that the p-values are correct here and throughout	
30 4	Please check that changes to the English in the section: Using PET scans, obese individuals have been found to have fewer dopamine D2 receptors than lean controls.; has not affected the meaning.	
35 5	Table 1: in the entry "Dopamine increases the release of glucose from blood cells (hepatocytes)." should 'blood cells' be 'liver cells'?	
40 6	The meaning of "DEXA correlates highly with underwater weighing Deuterium dilution and total potassium." is not clear - please clarify.	
45 7	Should "20 min" in "or in 20 min with a scan speed of 8 cm/s" be "40 min"?	
8	Please provide a citation for this work by Comings.	
9	"05 microM of primers" was changed to "0.5 microM of primers" Is this correct?	
50 10	Please check that changes to the English in this section have not affected the meaning.	
55 11	"carrying the 11 genotype..." was changed to "carrying the 1/2 genotype" for consistency with "A1/A1" genotypes.	
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14	Author names do not match- the first author of Reference 44 is Yasuna in the text and Yasuno in the Reference list. Please confirm which is correct.	
15	Please define: zBMI, rs and ps	
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