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Obesity has been presented in research literature as a polygenic or multiple gene disorder. Currently, 3 genes have been associated with obesity, dopamine receptor D2 (DRD2), dopamine transporter (DAT1), and dopamine beta hydroxylase (DBH). The primary objective of this study is to analyze the DRD2, DAT1 and DBH genes to determine if a correlation exists between certain allelic variations of these 3 genes and the body mass index of obese individuals.

We have developed an assay for the DRD2, DAT1 and DBH genes, utilizing polymerase chain reaction (PCR) technology. Within the DRD2 gene, 2 allelic variants have been identified, the A1 and A2 alleles. The A1 allele consists of a 310 bp fragment in which the Taq I restriction site has been deleted. The A2 allele consists of a 180 bp fragment and a 130 bp fragment. The presence of the A1 allele after enzyme digestion has shown a strong correlation to obesity in prior studies. With respect to the DAT1 gene, a VNTR of 40 bp's has been correlated to other disorders within the 'reward deficiency syndrome'. The fragment length identified most often is 440 or 480 bp, with 480 as the primary fragment in obesity. The DBH gene is similar to the DRD2 in that it also contains a Taq I restriction site. Two allelic variants are also identified, B1 and B2. The B1 allele contains no Taq I site and produces a 316 bp fragment while the B2 does cleave, exhibiting an 86 bp and a 230 bp fragment after enzyme digestion.

The presence of one or more of the aberrant alleles could be associated with and a predisposing factor to obesity.

OBESITY GENETICS: THE PREVALENCE

OF DRD2, DAT1 AND DBH GENES

IN THE OBESE INDIVIDUAL

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OBESITY GENETICS: THE PREVALENCE OF DRD2, DAT1 AND DBH GENES IN THE OBESE INDIVIDUAL

THESIS

Presented to the Graduate Council of the

Graduate School of Biomedical Sciences

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By

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Thank you for being there.

"If one advances confidently in the direction of their dreams and endeavors, to lead a life which they have imagined, they will be meet with success in common hours." Thoreau

TABLE OF CONTENTS

PAGE

ACKNOWLEDGMENTS iii

LIST OF TABLES v

LIST OF ILLUSTRATIONS vi

LIST OF ABBREVIATIONS / DEFINITIONS vii

CHAPTER

I. INTRODUCTION 01

II. PARTICIPANTS 13

III. EXPERIMENTAL PROCEDURES 15

IV. RESULTS/STATISTICAL ANALYSIS 35

V. DISCUSSION 46

VI. APPENDIX

A: IRB APPROVAL 50

B: HMRF DEMOGRAPHICS 55

C. MATERIALS AND METHODOLOGY 59

D. CONTROL GROUP DEMOGRAPHICS 66

E. SPSS DATA 69

VII. REFERENCES 100

LIST OF TABLES

Table 1	Possible human homologous regions to several mouse regions.....	04
Table 2	Reference list of previous dopamine studies	16
Table 3	Possible combined genotypes of DAT1, DRD2, DBH genes	34

LIST OF FIGURES

Figure 1	Reward Deficiency Syndrome	08
Figure 2	Outline of Dopamine pathways in the Brain	10
Figure 3	Dopamine Nerve pathways in the Brain	12
Figure 4	Human Chromosome 11	19
Figure 5	Gel Electrophoresis of DRD2 Gene	21
Figure 6	Schematic of the Synapse	24
Figure 7	Human Chromosome 5	26
Figure 8	Gel Electrophoresis of DAT1 Gene	28
Figure 9	Pathway of DBH Feedback Inhibition	29
Figure 10	Human Chromosome 9	31
Figure 11	Gel Electrophoresis of DBH Gene	33
Figure 12	Ranges and Averages Data	39
Figure 13	Data of Significant Observations of Ranking Obese Subjects	43
Figure 14	Data of Potential Obesity based on 3 digit code Classification	45

LIST OF ABBREVIATIONS / DEFINITIONS

bp	Base Pair
A	Adenine
T	Thymine
G	Guanine
C	Cytosine
DNA	Deoxyribonucleic Acid
BMI	Body Mass Index
LDL	Low Density Lipoprotein
DAT1	Dopamine Transporter
DRD2	Dopamine D2 Receptor
DBH	Dopamine Beta-Hydroxylase
PCR	Polymerase Chain Reaction
VNTR	Variable Number Tandem Repeat
DEXA	Dual Energy X-ray Absorptiometry
ANOVA	Oneway Analysis of Variance
IRB	Internal Review Board

Allele: One of the multiple possible forms of a gene that is found at a particular locus in the genome.

Annealing: The pairing of complementary single strands of DNA from a double helix.

Chromosome: Discrete unit of the genome carrying many genes. Each chromosome consists of a very long molecule of duplex DNA and an equal mass of proteins

Denaturation: Conversion from double stranded to the single stranded state. Most often accomplished by heating.

Dominant: Allele that determines the phenotype displayed in a heterozygote.

DNA Polymerase: An enzyme that catalyzes DNA synthesis from a template molecule and dNTP's

DNA Ligase: An enzyme that joins together two DNA molecules by forming a phosphodiester bond between the 3'-and-5' ends.

Electrophoresis: The separation of molecules using an electrical current, usually applied to a gel matrix. The gel matrix has a sieving effect which allows molecules to be separated on the basis of size.

Endonuclease: An enzyme that hydrolyzes phosphodiester bonds at internal locations.

Gene: A segment of DNA that encodes a single polypeptide, or protein.

Gene Expression: Transcription of RNA from a gene and translation of the RNA into a protein.

Genome: The entire collection of genetic information in an organism.

Genotype: The specific genes (which may or may not be expressed) that are present in an organism.

Heterzygote: An individual with different alleles at some particular locus.

Homozygote: An individual with the same allele corresponding loci on the homologous chromosome.

Markers: DNA, RNA or protein standards of known size which can be used to estimate the sizes of DNA, RNA or proteins in an electrophoretic gel.

Mutations: Any change (base deletions, insertions or substitutions) in a DNA sequence. If the mutation occurs within a gene, a change in the normal function of the expressed protein may result.

Peptide: Two or more amino acids covalently linked by a bond formed between the carboxy terminus of one amino acid and the amino terminus of the next.

Phenotype: The physical characteristics of an organism determined by its expressed genes.

Phosphotase: An enzyme that removes a phosphate group from a protein or nucleic acid substrate.

PCR: A repetitive process, usually aided by the action of a thermostable DNA polymerase, which copies a DNA template such that the number of copies increases exponentially. A typical procedure involves cycles of template denaturation, primer annealing and extension.

Primer: An oligonucleotide or short single-stranded nucleic acid which acts as a starting point for the synthesis of nucleic acids from a template.

Promoter: A region of the DNA to which RNA polymerase and accessory factors bind, allowing subsequent initiation of transcription.

Protein: A large molecule composed of one or more polypeptide chains which are made up of amino acids linked by peptide bonds in a defined sequence.

Receptor: A molecule which spans a membrane and, when bound by a single molecule, transmits information across that membrane.

Restriction Enzyme: An endonuclease that recognizes a specific DNA sequence and cleaves both DNA strands, either within the recognition site or at a defined distance from it.

Restriction Site: The specific DNA sequence to which a restriction enzyme binds.

Tandem Repeats: Multiple copies of the same sequence.

CHAPTER I

INTRODUCTION

The cause of obesity has been the focus of many researchers over the decades. Current statistics indicate over 30 million U.S. adults are obese with an estimated health care cost to the U.S. in 1996 at approximately thirty-nine billion dollars (Comings *et al*, 1993, Golditz, 1992). Additionally, Americans spent over 30 billion dollars in 1994 on 'fad' diets, books, tapes and other miracle weight loss aids (Baron, 1995).

The National Institute of Health Consensus Conference defines morbid obesity as a Body Mass Index (BMI) or Quetelet Index of 30 or more while overweight is generally considered to be a BMI of 25 - 29. Body Mass Index is more readily accepted as a means of defining obesity because it allows for integration of both height and weight proportions. BMI is obtained by the equation; weight divided by height squared ($W/H^{2)}$. Complications associated with severe obesity have been extensively studied and well documented. Obesity may result in pain in the lower back and feet, and serious disease states such as diabetes mellitus and hypertension that may lead to cardiovascular disease. While these ailments are serious, the affects of morbid obesity are far more than just physical. In many cases, severely obese individuals also suffer from mental pain and anguish, often resulting in low self esteem, anxiety and depression.

In the early stages of obesity research, concentration was on nutrition and environment as the most plausible contributing factors. Research found that obese parents, more often than not, had obese offspring (Skunkard, 1991, Sorensen *et al*, 1989). Did the parents have poor nutritional habits, did they simply overfeed themselves and their children, or is genetics and heredity involved? As early as 1923, at the Carnegie Institute of Washington, C.B. Davenport published data describing the role of inheritance as a factor in obesity (Davenport, 1923). In the late 1980's Skunkard reported significant correlations in the BMI of children and their biological parents

(Skunkard, 1988). However, a similar correlation did not exist between parents and their adopted children (Price *et al*, 1987, Sorensen *et al*, 1989).

Further research was done and expanded to include studies of maternal and fraternal twins reared in the same home and apart (Skunkard *et al*, 1990). This showed a closer correlation in BMI of monozygotic twins as compared to dizygotic twins with no difference in the circumstances under which they were raised. As a result, research into possible contributing factors of obesity began to shift away from environment and towards genetics and heredity.

Due to a high degree of homology in the genomes of mammalian species, it is possible to use rodent models to help identify genes potentially involved in the etiology of human obesity (Bouchard and Percisse, 1996). Claude Bouchard and Louis Percisse (1995, 1994) have numerous publications discussing their use of rodents in obesity studies. It has been found that mice can have five mutations found on five different chromosomes which can lead to obesity. Furthermore, the human homolog equivalents to the coding regions of the five mouse genes have been identified. Table 1 lists the mouse genes versus possible human homologs (Bouchard, 1995). The discovery of the similarities between mouse and human mutations have been paramount to the study of genetics surrounding human obesity.

Table 1:

Possible human homologous regions to several mouse regions.

Reprinted from Claude Bouchard, Genetics of Obesity: an update on molecular markers, International Journal of Obesity (1995) vol. 19, supp. 3.

TABLE 1

Locus	Transmission	Mouse chromosome	Human homologous chromosome
Diabetes (db)	Recessive	4	1p31-pter
Obese (ob)	Recessive	6	7q31
Tubbt (tub)	Recessive	7	11p15.1
Fat (fat)	Recessive	8	16q22-24
Yellow (Ay)	Dominant	2	20q13

Specifically, concentration of many molecular biologists and genetic epidemiologists has been on the mouse ob gene and its human OB homolog. Friedman and colleagues were successful in cloning and sequencing both the mouse and human OB genes in early 1991 (Friedman *et al*, 1991). Experiments demonstrated that a dramatic increase in body weight would result in mice who were genotyped as ob/ob homozygous for a single-gene mutation altering the expression of the ob gene. Current research surrounding the OB gene is focused on correlating an increased BMI and the presence of mutations in the OB gene. It has, however, long been suspected that obesity is far more intricate and complicated than to be limited to one genetic mutation. Polymorphisms in the genes for apolipoprotein-B, apolipoprotein-E and low density lipoprotein (LDL) receptors, glucocorticoid receptors, and insulin have also been associated with obesity (Nobel, 1994, Skunkard, 1991).

An American psychologist by the name of James Olds (1954) was performing experiments on laboratory rat brains when he mistakenly placed electrodes in the limbic system (Olds, 1969). He soon discovered that this area of the brain, stimulated when the rats pressed a lever, provided them with some kind of internal reinforcement or reward that they choose over everything else except sleep. Dr. Olds' mistake was the first of many efforts to begin establishing what brain regions and neurotransmitters were thought to be involved in the biological basis of chemical dependency. Although a variety of chemicals such as cocaine, opiates, alcohol, and now even food, appear to act on different areas of the brain by way of distinct mechanisms, the end result is the same; dopamine is released, thereby providing an internal reinforcement to the behavior (Koob and Bloom 1988). Based on this evidence and the conclusion of other research projects, it appears that dopamine is the primary neurotransmitter involved in the pleasure seeking reward system.

Research done by Scoville in 1975 also implicated the dopaminergic pathway as a possible site for a genetic relationship to obesity by showing the effectiveness of amphetamine-like drugs in weight loss. Food, like a variety of reinforcing substances such as alcohol and drugs, when consumed, can produce euphoria or pleasure resulting in stimulation of the dopaminergic pathway (Nobel, 1994). Kenneth Blum, *et al* (1996) coined the phrase 'reward system' of the brain to describe the pleasure associated with certain behaviors such as consumption of food and alcohol (Blum, *et al* 1996, Skunkard, 1991). Figure 1 demonstrates this 'reward system'. Although the precise location and specificity of the reinforcement remains debated, there is general consensus that they are manifested in the dopaminergic pathways of the brain (Nobel, 1994). Figure 2 represents the schematic of the dopamine pathway anatomy. There are three major dopaminergic pathways of the brain: Nigrostriatal which passes from the substantia nigra to the caudate and putamen (striatum). When defective, there is a decrease in muscle movement as observed in Parkinson's disease. Mesolimbic passes from the ventral tegmental area in the mesencephalon to the limbic system and the Mesocortical which passes from the ventral tegmental area to the frontal lobe and limbic cortex. Abnormalities in either the mesolimbic and mesocortical pathways often results in difficulty with attention and emotion including addictive behaviors. Figure 3 exhibits these pathways. Furthermore, compounds which block the D2 dopamine receptor have been shown to lead to an increase in body weight (Nobel, 1994).

By showing a positive correlation of certain genotypes to obesity, this study allows future generations to be aware of their propensity towards obesity and make decisions regarding their lifestyle and potentially the circumstances under which they raise their children, accordingly.

FIGURE 1

While other genes playing a role in interrelated disorders are still to be identified, the concept of a "Reward Deficiency Syndrome" unites addictive, impulsive and compulsive behaviors and may explain for the first time the way in which simple genetic anomalies give rise to complex aberrant behavior. Kenneth Blum *et al*, 1996

FIGURE 1

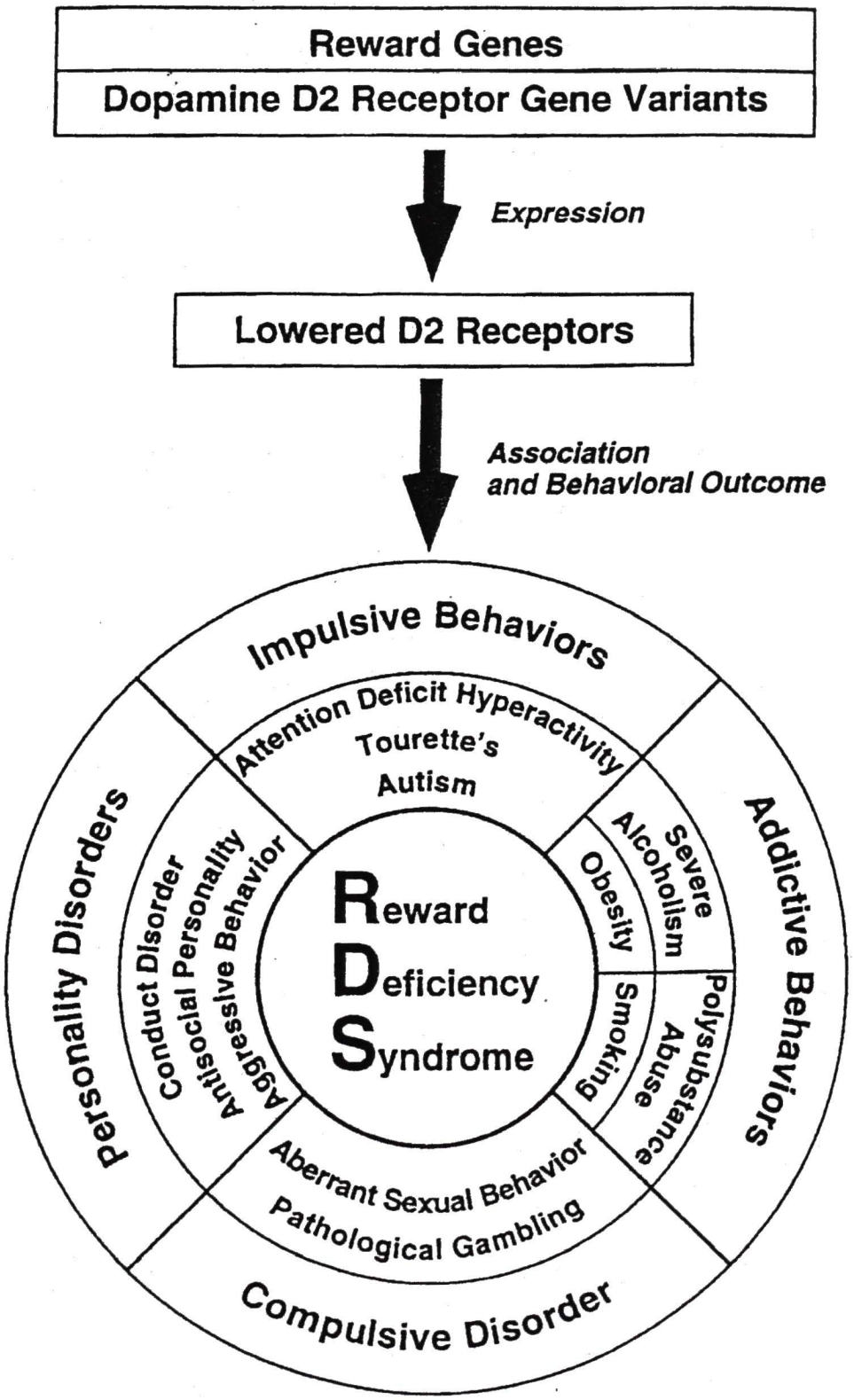

Reward Genes
Dopamine D2 Receptor Gene Variants
Expression
Lowered D2 Receptors
Association
and Behavioral Outcome
Impulsive Behaviors
Attention Deficit Hyperactivity
Tourette's
Autism
Addictive Behaviors
Severe
Alcoholism
Obesity
Polysubstance
Abuse
Smoking
Personality Disorders
Conduct Disorder
Antisocial Personality
Aggressive Behavior
Reward
Deficiency
Syndrome
Aberrant Sexual Behavior
Pathological Gambling
Compulsive Disorder

FIGURE 2

Outline of the dopamine pathways in the brain

The dopamine pathways of the brain. From L. Heimer, The human Brain and Spinal Cord, Springer-Verlag, New York, 1983

FIGURE 2

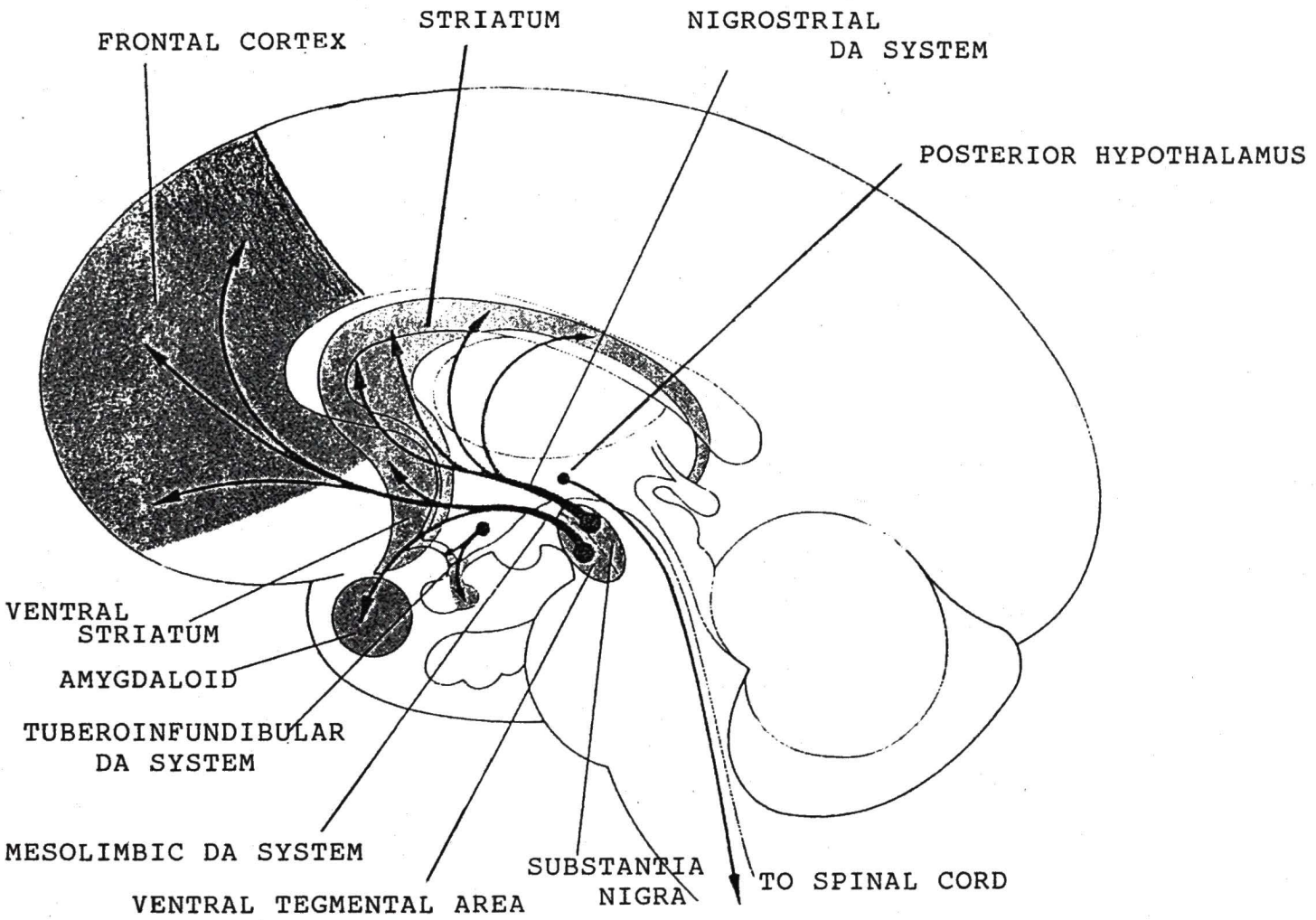

FRONTAL CORTEX
STRIATUM
NIGROSTRIAL
DA SYSTEM
POSTERIOR HYPOTHALAMUS
VENTRAL
STRIATUM
AMYGDALOID
TUBEROINFUNDIBULAR
DA SYSTEM
MESOLIMBIC DA SYSTEM
VENTRAL TEGMENTAL AREA
SUBSTANTIA
NIGRA
TO SPINAL CORD

FIGURE 3

Dopamine Nerve Pathways in the Brain:

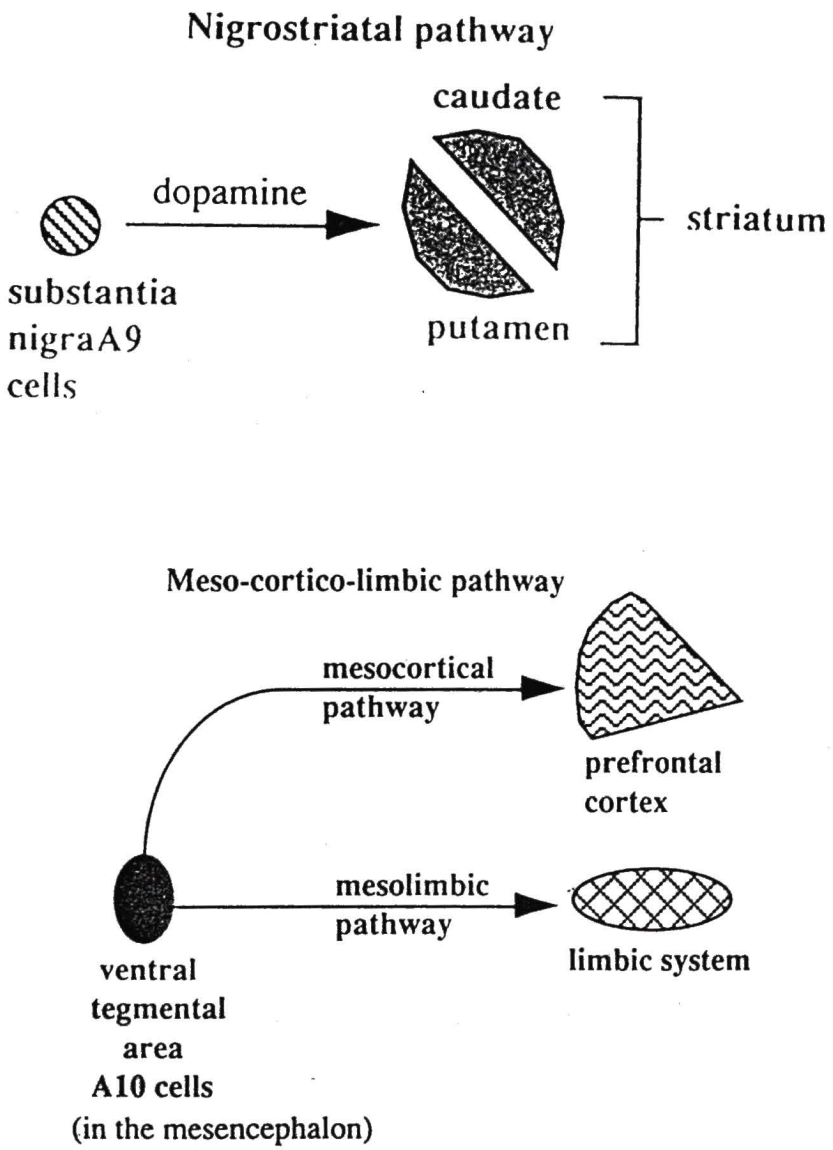
Nigrostriatal

Mesolimbic

Mesocortical

Reprinted from Tourette Syndrome and Human Behavior, David E. Comings, Hope Press, 1990

FIGURE 3


Nigrostriatal pathway
caudate
dopamine
striatum
substantia
nigraA9
cells
putamen
Meso-cortico-limbic pathway
mesocortical
pathway
prefrontal
cortex
mesolimbic
pathway
limbic system
ventral
tegmental
area
A10 cells
(in the mesencephalon)

CHAPTER II

PARTICIPANTS

This study, approved by the Internal Review Board Council of the University of North Texas Health Science Center (See Appendix A), was conducted on 106 obese individuals and 100 control individuals.

Buccal swabs from 106 obese subjects, determined to have a BMI greater than 30% for females and 25% for males were received from the HEALTH & MEDICAL RESEARCH FOUNDATION, 4900 Broadway, Suite 200 San Antonio, Texas 78209, under the direction of Dr. Gilbert Kaats. BMI for these individuals was determined using a variety of techniques available so that an average could be obtained allowing for more accurate BMI results. Each individual received a body composition (% body fat and lean mass) and bone densities using Dual Energy X-ray Absorptiometry (DEXA) technology. The DEXA unit used is a Lunar DPX X-ray Bone Densitometer, an FDA registered device used for scanning the whole body, upright and lateral spine, femur, and forearm to determine bone mineral density. Underwater displacement testing as well as five part measurements of the arms, thighs and abdomen were also used.

Of the original 106 samples, 16 were excluded from the study because their reported BMI was not within the guidelines acceptable for this project. Additionally, 12 were excluded due to failure of one or more genes to amplify. Therefore, of the original 106 samples forwarded by HMRF, 78 were ultimately used in statistical reporting. This study was conducted as a 'double blind study' as the BMI, bone density and all other information concerning the individuals was unknown to myself prior to completion of the project. Once genotyping was completed it was

forwarded to Dr. Kaats at HMRF in exchange for the appropriate demographic information needed for statistical analysis.

Formulating an experimentally sound 'control' group proved to be more difficult than expected and this will be addressed in the discussion. The control group consisted of 100 individuals who were asked to participate due to the visual appearance of being thin. It is now apparent that a person can have the appearance of average weight yet still have a BMI greater than 25 or 30 % depending on sex. Additionally, any persons with a family history of eating disorders , neuropsychiatric problems or addictions had to be excluded and these questions were posed to each control individual. It was later concluded that the responses given by some individuals could have been less than honest. These individuals would still be allowed in the control group but under false assumptions causing invalid data to be included without my knowledge. It was therefore necessary to perform statistical comparisons between the studies control group as well as literature controls. See Appendix B and D for dopaminergic profiles.

CHAPTER III

EXPERIMENTAL PROCEDURES

Experimental procedures involved the collection and isolation of genomic DNA; amplification of the DNA by PCR; restriction endonuclease digestion; gel electrophoresis and staining to determine the dopamine profile by way of certain DNA fragment lengths indicative of the three loci of particular interest; Dopamine Transporter Gene (DAT1), Dopamine Receptor D2 Gene (DRD2), and Dopamine Beta Hydroxylase Gene (DβH). Refer to Appendix C for details of materials and methodology.

The decision to genotype obese and control individuals for DAT1, DRD2, and DβH was based on numerous studies linking at least one or more of these genes to a variety of neuropsychiatric disorders as well as alcohol and drug abuse. The theory presented is that food and carbohydrates are addictive by way of similar neurotransmitter pathways as that of alcohol and other pleasure seeking addictions. Since it is practical to assume one single gene is not solely responsible for all addictions, but rather a collaboration or linkage of genes, I chose to observe the genotypes of individuals for the predominate dopaminergic genes previous studies have correlated to addictions or other neuropsychiatric disorders. Multiple references sited (Table 2).

TABLE 2

INVESTIGATOR	YEAR	POLYMORPHIC LOCI	RESULT
Blum, et al.	1990	DRD2 - A1	Positive
Comings, et al	1991	DRD2 - A1	Positive
Noble, et al	1991	DRD2 - A1	Positive
Parsian, et al	1991	DRD2 - A1	Positive
Johnson, et al	1992	DRD2 - A1	Positive
Amadeo, et al	1993	DRD2 - A1	Positive
Li, et al	1994	DAT1	Positive
Blum, et al	1995	DAT1	Positive
Comings, et al	1995	DAT1	Positive
Cook, et al	1995	DAT1	Positive
[Muramatsu / Higuchi	1995	DAT1	Positive
Blum, et al	1997	DBH	Positive
Comings, et al	1997	DBH	Positive

Grandy (1989) used rat brain cDNA previously isolated and identified by Bunzow (1988) as a probe to isolate human D2 receptor DNA from a human genomic library. The genomic clone isolated, 1hD2G1, had a 1.6 kb fragment that encoded the last 64 amino acids of the human D2 receptor and 1.2 kb of the 3' non-coding sequence. It was found that the fragment occurred only once in the human genome. It was Oliver Civelli with the Vollum Institute in Oregon who successfully cloned and sequenced the DRD2 gene, which is localized to the q22-q23 segment of chromosome 11 (Grandy, *et al*, 1989) (See Figure 4). The D2 receptor is one of five physiologically distinct dopamine receptors (D1-D5). Previous studies established the presence of the D2 receptor to the system of the brain which controls emotional responses including reinforcement related to many addictive behaviors.

The DRD2 gene has two allelic variants that are distinguished by the presence or absence of a *Taq 1* restriction endonuclease site. The A2 allele containing this *Taq 1* restriction site is found to be present in 76% of the general population. The A1 allele, which lacks the *Taq 1*, site is found in approximately 24% of the population. Research by Blum, et. al. has shown that the occurrence of the homozygous A1/A1 genotype is present in 85% of individuals purported to be severe alcoholics (Blum *et al*, 1996; Blum, 1994; Blum *et al*, 1996; Blum, 1994).

Amplification and electrophoresis of individual DNA samples results in the presence of a 310 base pair (bp) fragment. Contained within the 310 bp fragment is the polymorphic *Taq 1* site. The A1 allele contains a single nucleotide change, from T-A, eliminating the *Taq 1* restriction site. The *Taq 1* enzyme therefore does not cut the amplified product so that the presence of a 310 bp band in an individual is indicative of the A1 allele, considered to be the abnormal genotype. Under normal conditions, the presence of the *Taq 1* site results in the presence of a 180 bp fragment and 130 bp fragment. The presence of these two fragments indicates the presence of the A2 allele or that which is most predominant in the general population. Figure 5 exhibits the possible band lengths for the DRD2 gene.

FIGURE 4

Human Chromosome 11 identifying the q22-q23 segments as the D2 Receptor Locus

The probe necessary to identify the q22-q23 segment was first discovered by Oliver Civelli at the Oregon Health Sciences University in Portland in 1989.

FIGURE 4

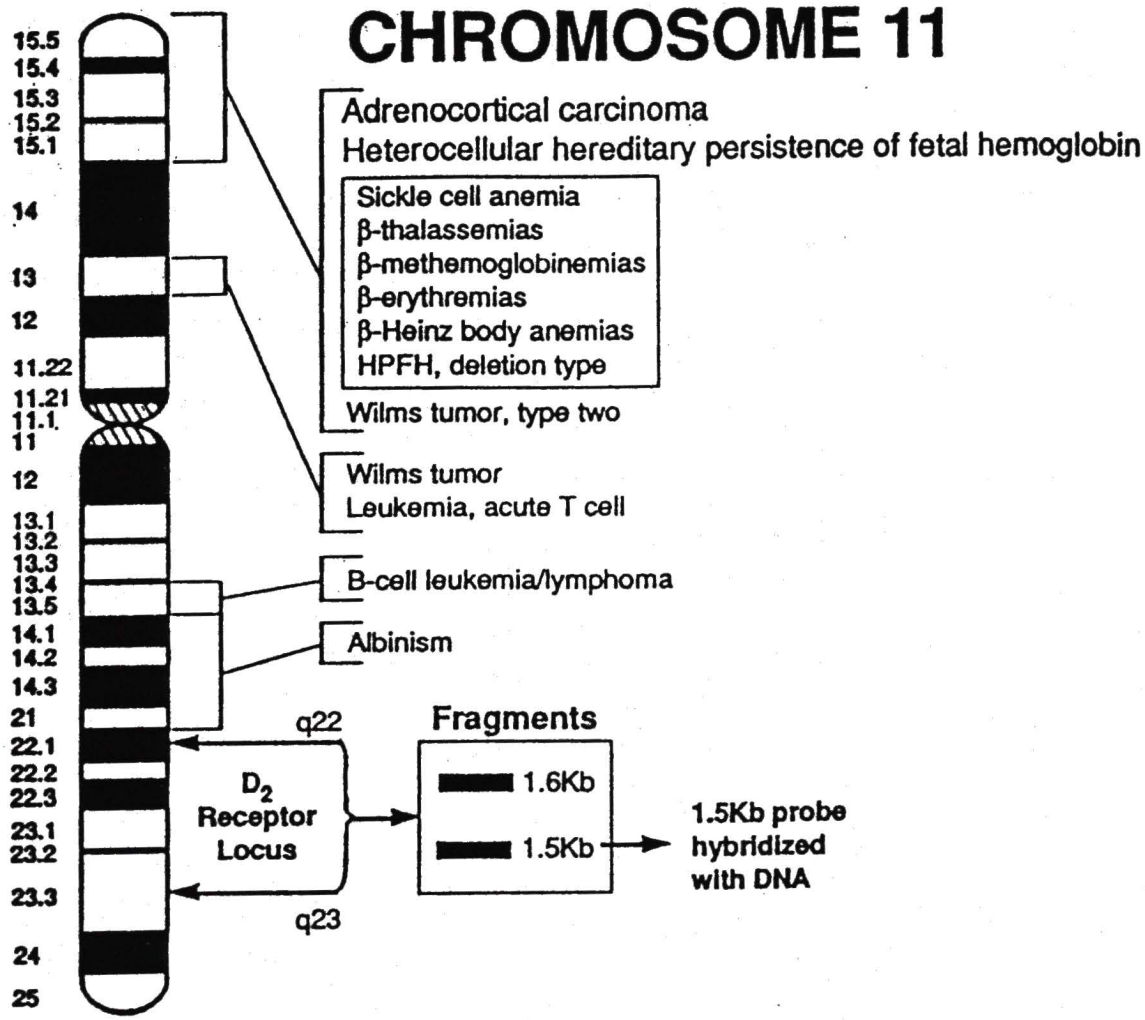

CHROMOSOME 11
15.5
15.4
15.3
15.2
15.1
14
13
12
11.22
11.21
11.1
11
12
13.1
13.2
13.3
13.4
13.5
14.1
14.2
14.3
21
22.1
22.2
22.3
23.1
23.2
23.3
24
25
Adrenocortical carcinoma
Heterocellular hereditary persistence of fetal hemoglobin
Sickle cell anemia
β-thalassemias
β-methemoglobinemias
β-erythremias
β-Heinz body anemias
HPFH, deletion type
Wilms tumor, type two
Wilms tumor
Leukemia, acute T cell
B-cell leukemia/lymphoma
Albinism
q22
D2 Receptor Locus
q23
Fragments
1.6Kb
1.5Kb
1.5Kb probe hybridized with DNA

FIGURE 5

Gel electrophoresis of the bands present in the Dopamine D2 Receptor Gene

Legend:

A1 = 310 bp fragment = abnormal

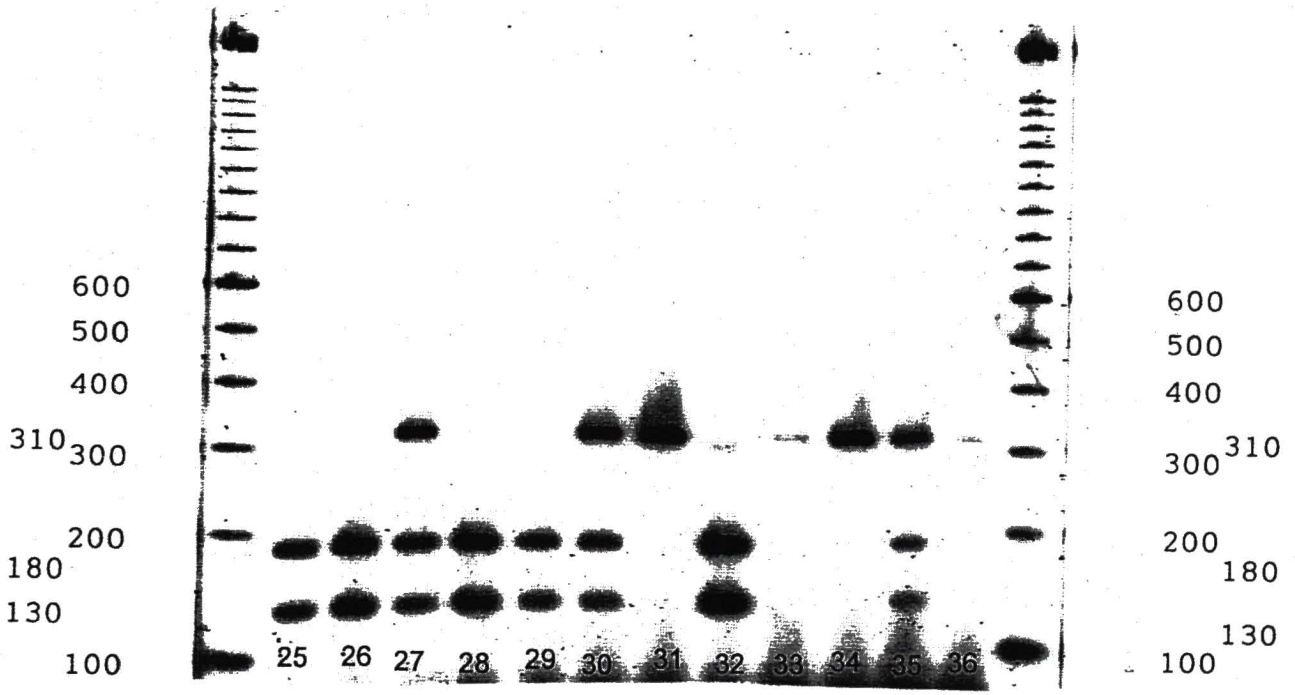
A2 = 130 bp and 180 bp fragments = normal

A1A1 = homozygous abnormal

A1/A2 or A2/A1 = heterozygous abnormal

A2/A2 = homozygous normal

FIGURE 5



LANE 25	A2/A2	LANE 31	A1/A1
LANE 26	A2/A2	LANE 32	A2/A2
LANE 27	A1/A2	LANE 33	NDA
LANE 28	A2/A2	LANE 34	A1/A1
LANE 29	A2/A2	LANE 35	A1/A2
LANE 30	A1/A2	LANE 26	NDA

The dopamine transporter gene is responsible for directing the re-uptake of dopamine from the synaptic cleft back into the presynaptic neuron from which it was released. Figure 6 is a schematic of this process. The DAT1 gene was cloned and sequenced by Vandenbergh and associates in 1992 and mapped to chromosome 5p15.3. See Figure 7 Sequence analysis identifies the presence of a polymorphic site within the 3' untranslated region of the DAT 1 gene. The polymorphism has been shown to result in a 40 bp variable number tandem repeat (VNTR) sequence. The most common allelic variation detected in DAT 1 corresponds to the allele having 10 repeats or 9 repeats. Following PCR using primers specific for the 3' untranslated region of the DAT1 gene, allelic variations are detected by gel electrophoresis. The presence of the 480 bp fragment is associated with individuals having the 10 allele, whereas the presence of the 440 bp fragment has been associated with the 9 allele. Refer to Figure 8 for examples of potential band length of the DAT1 gene. However, studies indicate that only when the homozygous 10/10 genotype is present has there been a link to various disorders including Tourette Syndrome, ADHD, ADD, and other neurologically-based problems (Comings, 1994, Blum *et al*, 1996, Uhl *et al*, 1993, Comings *et al*, 1991, Cook *et al*, 1995, Nobel *et al*, 1994).

FIGURE 6

Schematic drawing of the synapse. Reprinted from C. Stevens, The Neuron. Copyright 1979 by Scientific American, Inc.

FIGURE 6

Schematic of the synapse indicatinf the vesicles of neurotransmitter released into the cleft by way of the DAT1 gene

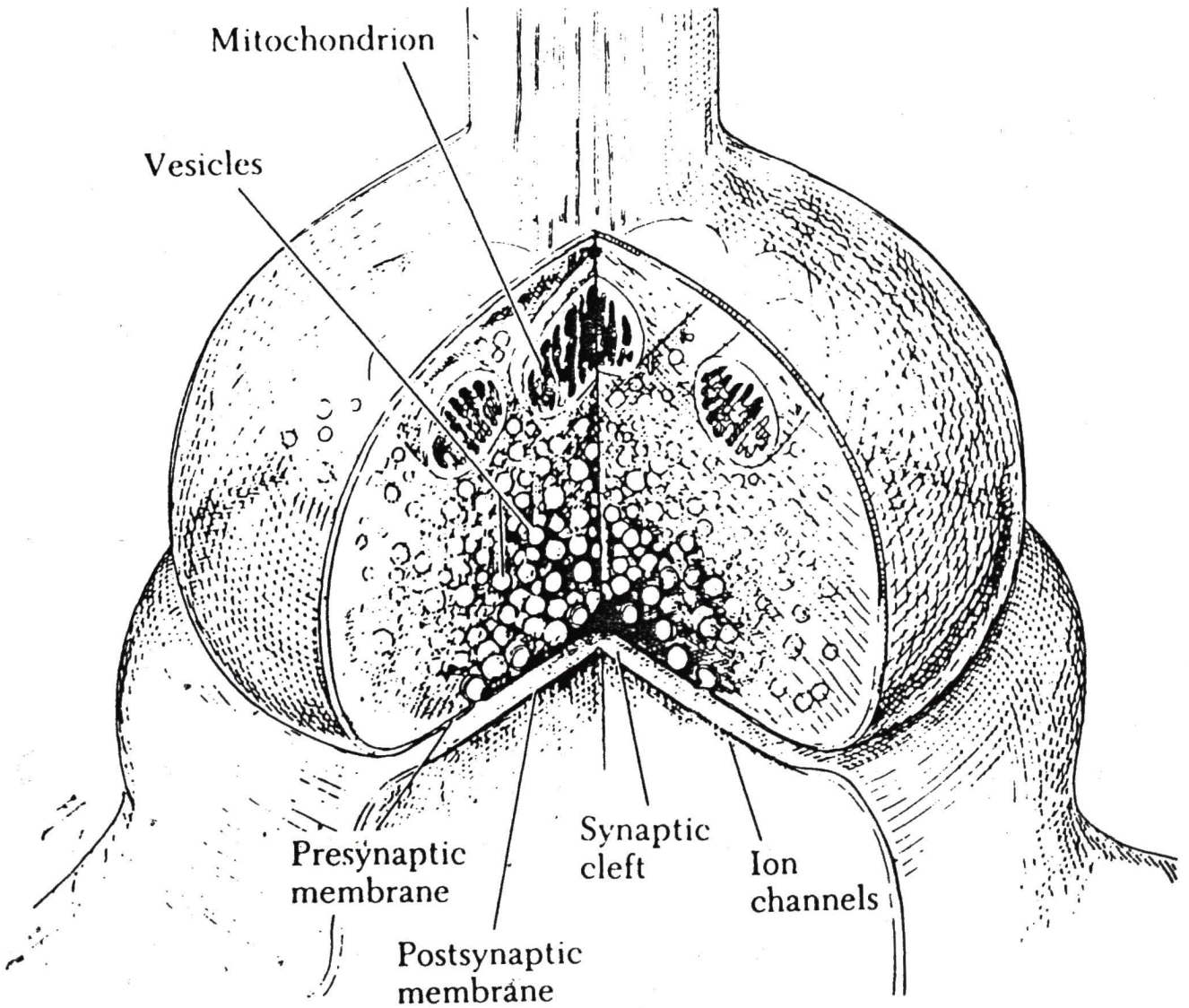


FIGURE 7

Human Chromosome 5 showing the q15.3 segment associated as the DAT 1 locus.

Cloning and subsequent sequencing of the DAT 1 gene occurred in 1992 by Vandenbergh and associates

FIGURE 7

Chromosome 5

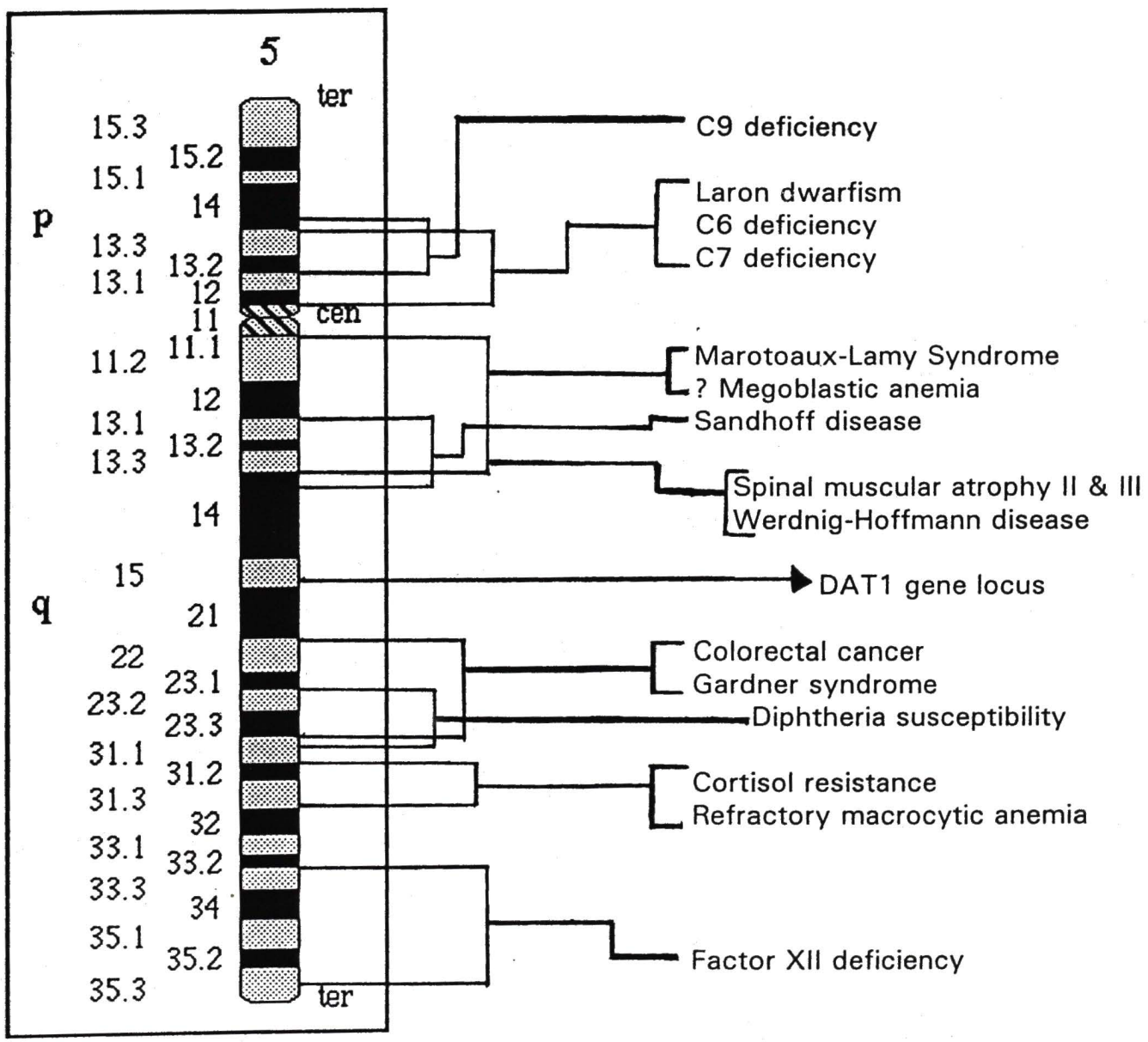


FIGURE 8

Gel electrophoresis of the possible bands associated with the Dopamine Transporter Gene

Legend:

The DAT1 gene is associated with a 40 bp VNTR, the most common occurrences are the 9 and 10 repeats.

440 bp = 9 repeats = normal

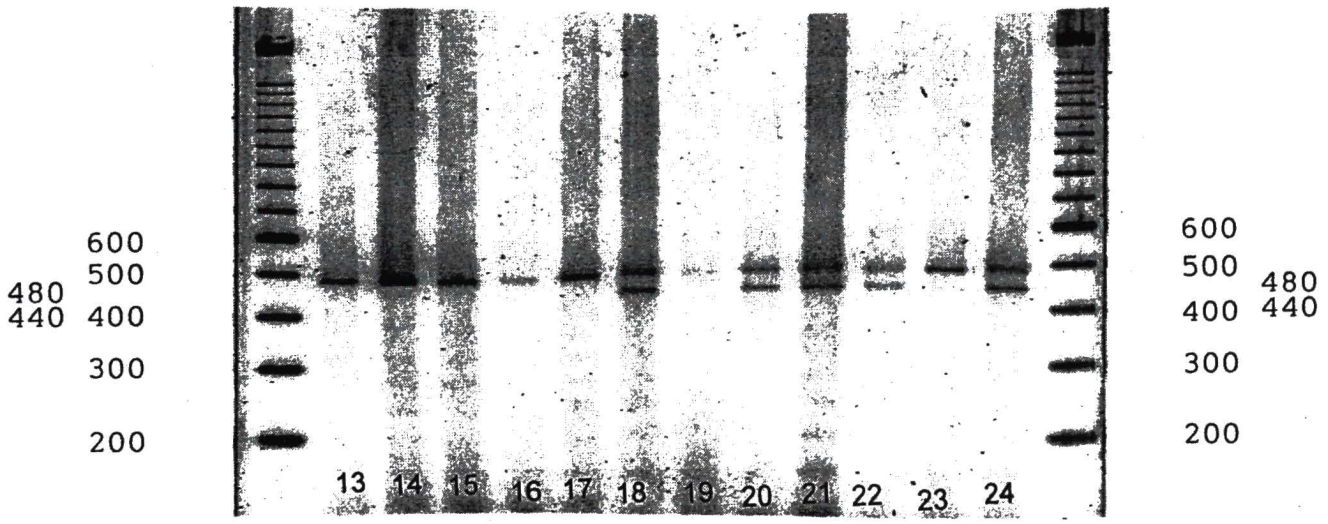
480 bp = 10 repeats = abnormal

9/9 = homozygous normal

9/10 or 10/9 = heterozygous normal

10/10 = homozygous abnormal

FIGURE 8



LANE 13	10/10
LANE 14	10/10
LANE 15	10/10
LANE 16	NDA
LANE 17	10/10
LANE 18	9/10
LANE 19	NDA
LANE 20	9/10
LANE 21	9/10
LANE 22	9/10
LANE 23	10/10
LANE 24	9/10

Dopamine Beta Hydroxylase (DβH) is an enzyme, located in the sympathetic nerve terminals and released into circulation where it catalyzes the conversion of dopamine to norepinepherine. Through feedback inhibition, norepinepherine inhibits tyrosine hydroxylase, which in turn inhibits the production of dopamine and norepinepherine (Comings *et al*, 1996). In experimental animals, the inhibition of DβH activity results in a decrease in norepinepherine levels which releases the inhibition of tyrosine hydroxylase ultimately resulting in the excessive production of dopamine. See Figure 9 for the pathway in which a disturbance of DBH causes feedback inhibition on the formation of dopamine. This increase in dopamine production has been associated with a variety of conditions, including pleasure seeking (Comings *et al*, 1996). The DβH gene is localized to chromosome 9q34 and contains two allelic variants, the B1 and B2, distinguished by the presence or absence of a *Taq 1* restriction site. Chromosome 9 see Figure 10. Following amplification by PCR methods, under 'normal' conditions, a 316 bp fragment will result. Contained within the 316 bp fragment is the polymorphic *Taq 1* site. Within the B1 allele, a single nucleotide change has eliminated the *Taq 1* restriction site, therefore the *Taq* enzyme is unable to cut the amplified product. The presence of the 316 band after gel electrophoresis is indicative of the B1 allele. Following restriction digestion of the amplified product and gel electrophoresis, the presence of an 86 bp fragment and a 230 bp fragment indicates the presence of the B2 allele. Refer to Figure 11 for band lengths.

Figure 9

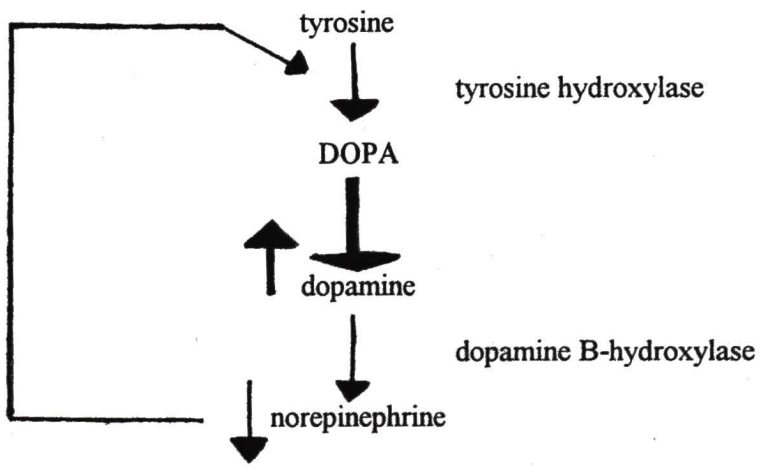


FIGURE 10

Human chromosome 9 identifying the q34 segment as the locus for the DBH gene

FIGURE 10

Chromosome 9

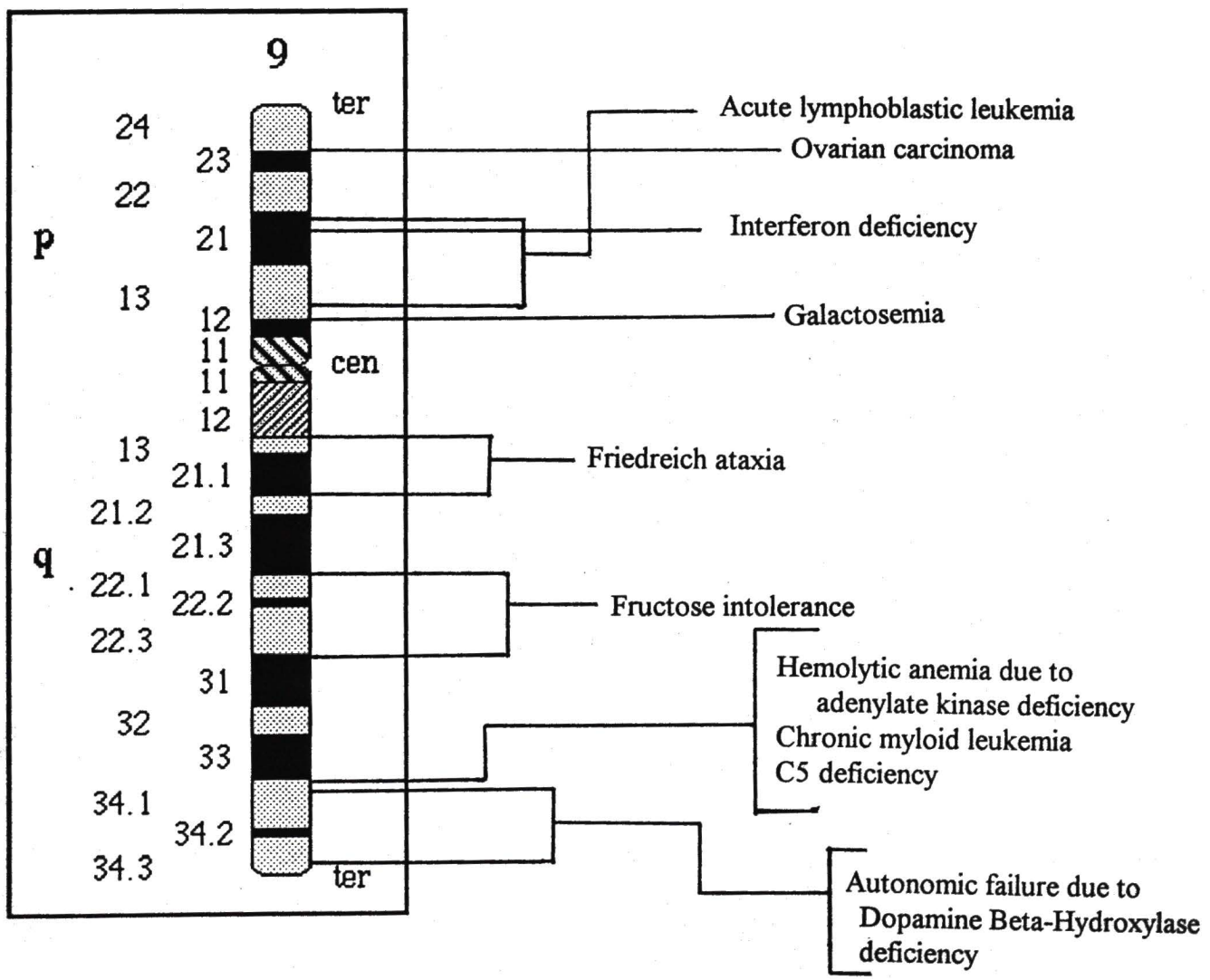


FIGURE 11

Gel Electrophoresis of the bands associated with Dopamine Beta-Hydroxylase

Legend:

B1 = a 316 bp fragment = abnormal

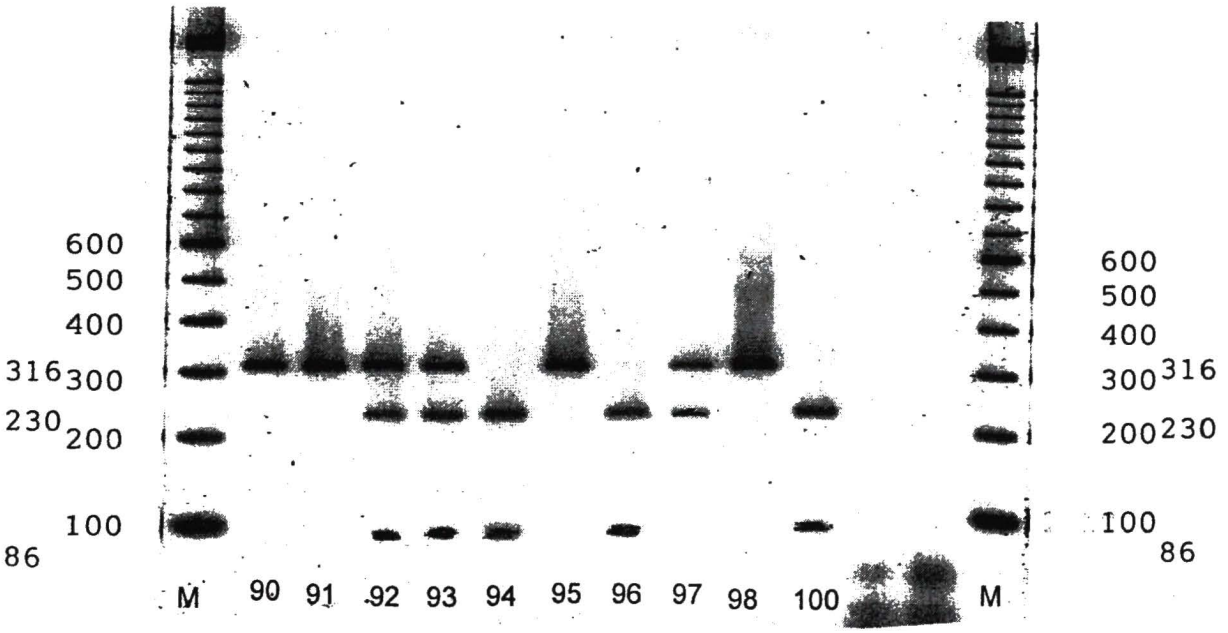
B2 = an 86 bp and 230 bp fragments = normal

B1/B1 = homozygous abnormal

B1/B2 or B2/B1 = heterozygous abnormal

B2/B2 = homozygous normal

FIGURE 11



LANE 90	B1/B1	LANE 95	B1/B1
LANE 91	B1/B1	LANE 96	B2/B2
LANE 92	B1/B2	LANE 97	B1/B2
LANE 93	B1/B2	LANE 98	B1/B1
LANE 94	B2/B2	LANE 100	B2/B2

TABLE 3

Possible genotypes of the DRD2, DAT1 and DβH Genes combined:

DRD2 Genotype	DAT1 Genotype	DbH Genotype
A1/A1	480/480 (10/10)	B1/B1
A1/A2	480/440 (10/9)	B1/B2
A2/A2	440/440 (9/9)	B2/B2

CHAPTER IV

RESULTS / STATISTICAL ANALYSIS

The genotypes as well as other demographic data was analyzed in a variety of data systems and fashions to determine if a positive association or trend existed that might lend itself to predict obesity based on genetic makeup. As is often the case, some data programs as well as trends showed significance whereas some systems showed none at all. All analyses are presented herein so that personal conclusions may be made without bias although discussions of the various trends will follow in the remaining chapter.

% Body Fat

The sample of obese subjects in a weight loss program were separated into two independent groups depending upon their percent body fat. Divisions were made above and below 28% and 34% depending on gender to represent obese and morbidly obese. The proportion above and below these obesity cut-off points were tested against one another for any association with the presence of either homozygous or heterozygous genotypes. The chi-square statistics were used to test for differences in the proportions of alleles between different groups for each gene separately. Any p values less than 0.05 are considered to indicate a statistically significant difference in the allelic proportions between groups. None of these comparisons proved to be statistically significant.

The three allelic pair combinations were then tested for any mean differences between mean percent body fat for each gene separately. One-way Analysis of Variance (ANOVA) was used to test for statistical differences between the means and for any linear increasing or decreasing trend for the alleles of each gene. Again, any p value less than 0.05 are considered to indicate a

statistically significant difference or linear trend between the allelic percent body fat means. None of the One-way ANOVA trends were significant.

The information about what are considered dominant or 'active' alleles within each of the three genes was combined by summing the DRD2 A1A1, A1A2; DAT1 10/10, 09/10 and DβH B1/B1 in the manner of Comings, 1998. Differences or linear trends in the means of the percent body fat for groups defined by the number of 'active' alleles ranging from 0-5 were tested using the One-way ANOVA. Again, any p values less that 0.05 are considered statistically significant and none were.

The sample of obese subjects was selected for only those above 28% body fat and were characterized as moderately to morbidly obese. The allelic pattern of the DRD2 and DAT1 genes of this group were compared against literature controls with A1A1 and A1A2 vs A2A2 for the DRD2 gene and 10/10 vs 09/10 and 09/09 for the DAT1 gene. Chi-square statistical analysis were used to test for differences in the proportions of alleles between obese group and literature controls for each gene separately. The DRD2 alleles were statistically significant. The chi-square was 38.5 with 1 degree of freedom and a p value less than 0.001. The chi-square analysis for DAT1 was not statistically significant. Refer to Appendix E for complete SPSS Data.

Ranges / Averages

As previously stated, current data supports the theory that homozygosity of the 10/10 allele for DAT1 is significant for other addictive behaviors. Whereas, either homozygosity or heterozygosity of the aberrant allele in DRD2 and DβH has been reported as significant in other addictive behaviors. This corresponds to either an A1/A1 or A1/A2 for DRD2 and B1/B1 or B1/B2 for DβH. It was found, that by averaging the BMI of the obese samples, the highest average was found to be in the homozygous abnormal genotype for each of the three genes. This trend remained consistent as the second highest BMI average was for the heterozygous abnormal genotype, leaving the lowest average BMI corresponding to the homozygous normal genotype. Even more promising is that out of a possible 78 obese individuals the highest percentage corresponded to the genotype current literature supports is abnormal. See Figure 12

FIGURE 12

Figure 12 represents the Ranges and averages data showing the positive trends in the obese individuals BMI opposed to the genotypes.

FIGURE 12

DAT1

GENOTYPE	AVERAGE BMI	% OBESE INDIV. WITH GENOTYPE
9/9	X	1.28%
10/9 or 9/10	42.38	52.56%
10/10	**44.46**	**46.15%**

X = one single individual therefore no average

DRD2

GENOTYPE	AVERAGE BMI	% OBESE INDIV. WITH GENOTYPE
A1/A1	**45.86**	**6.41%**
A1/A2 or A2/A1	**44.91**	**58.97%**
A2/A2	40.96	34.62%

DBH

GENOTYPE	AVERAGE BMI	% OBESE INDIV. WITH GENOTYPE
B1/B1	**46.23**	**3.85%**
B1/B2 or B2/B1	**44.8**	**60.26%**
B2/B2	43.58	35.90%

Potential

Using SPSS each obese individual and each control individual was assigned a 3 digit number consistent with genotype. The legend is as follows:

1= Homozygous **abnormal**

2= Heterozygous **abnormal (DRD2 and DBH only)**

3= Homozygous normal

DAT1:	**1= 10/10**	2= 9/10 or 10/9	3= 9/9
DRD2:	**1= A1/A1**	**2= A1/A2 or A2/A1**	3= A2/A2
DBH:	**1=B1/B1**	**2=B1/B2 or B2/B1**	3= B2/B2

The placement of the number is as follows: 100 position = DAT1

010 position = DRD2

001 position = DBH

Example: A person with this genotype: 10/10 A1/A2 B2/B2 would be assigned a three digit code of 123. The hundreds position indicating the DAT1 gene and a 1 specific for the 10/10 genotype, the 10's position indicates the DRD2 genotype, in this case a 2 which represents A1/A2 or A2/A1 and the last digit or ones position shows the DβH and for this individual it is a 3 therefore the person is B2/B2 for the DβH gene.

After the genotype had been established of all obese and control individuals they were assigned their own three digit code and the data input into the SPSS analysis system. See Appendix D for all codes. By averaging the % body fat found in each possible three digit code, the following observations were made: 63 obese persons out of 78 were found to be within 6 specific codes out of the possible 27. This means 81% of all obese subjects had only 22% of the possible codes making this a statistically significant finding as indicated by Figure 13. Further observations

found that 66% (4/6) of the six significant codes were ranked as the highest potential for morbid obesity based on average BMI. The other two significant codes were found to be indicative of borderline morbid obesity and none of the significant codes were within the lowest potential BMI indicating obesity. Based on this information a person with a three digit code of 122, 123, 222 or 233 has a high potential of being a morbidly obese individual. A person with a three digit code of 223 or 232 has a borderline potential of being morbidly obese. Any other three digit combination would not be a significant indicator for morbid obesity. See Figure 14.

FIGURE 13

Data indicating the significant observations of the ranking and considerations of the obese subjects

FIGURE 13

RANKING AND CONSIDERATIONS OF OBESE SUBJECTS

3 DIGIT CODE	BMI	# OF INDIVIDUALS
233	48.23	6
122	45.11	16
123	44.90	7
222	44.78	14
223	43.69	8
232	40.08	12

* Average BMI Group 1: 46.29
* Average BMI Group 2: 41.46

CONSIDERATIONS:

~ BMI range 44.5 +	High Potential
~ BMI range 40.0 - 44.4	Borderline
~ BMI less than 39	Low Potential

FIGURE 14

Data indicating the potential for obesity based on classification using 3 digit coding

FIGURE 14

DATA

CODE	BMI	#	% of Individuals
112	52.30	2	
221	51.25	2	
233	48.23	6	62% (48/78)
212	46.50	1	
122	45.11	16	
123	44.70	7	**High Potential**
222	44.48	14	
223	43.79	8	
133	43.55	5	33% (26/78)
113	40.40	1	
232	40.08	12	**Borderline**
132	39.20	2	
312	37.80	1	5% (4/78)
131	36.20	1	**Low Potential**

CHAPTER V

DISCUSSION

This thesis project set out to show if a significant correlation existed between morbidly obese individuals and their genetic dopamine profile. As has been stated, the dopaminergic pathway has exhibited significantly positive results with other neuropsychiatric disorders. The most well understood is the research of Dr. Ken Blum in alcoholism as well as Dr. Comings research with Tourette Syndrome. The thought that dopamine played a role in alcohol addiction surfaced in the 1980's with the first of many publications on the subject soon thereafter, in the early 1990's. The *Taq I* A1 allele of the DRD2 gene was the first of the dopaminergic genes to be explored. With success in demonstrating a strong association with DRD2, the spectrum was broadened and scientists began looking at the DAT1 gene, and later the DBH gene. Early research seemed to focus on each of these genes individually. Was there a positive correlation between one gene and one behavior? Comings *et al* was the first scientist to perform addictive and subtractive research with all three genes together. His area of speciality though, is Tourette Syndrome and some conduct disorder problems. Comings *et al* (1996) published the first article ever to combine the three dopaminergic genes.

In 1996, Dr. Blum coined the phrase 'reward deficiency syndrome'. Dr. Blum and others believe that an inborn chemical imbalance can exist that alters the intercellular signaling in the brain's reward process and could supplant an individual's feeling of well being with anxiety, anger or a craving of a substance that can alleviate the negative emotions. This chemical imbalance manifests itself as one or more behavioral disorders such as substance abuse, smoking and compulsive overeating or obesity.

Dr. Blum is a researcher and adjunct faculty member at the University of Texas Medical School - San Antonio. It was here that he began an association with Dr. Gilbert Kaats, whose main interest is medications for the obese individual. It is through Dr. Blum and his acquaintance with Dr. Kaats that I came to choose obesity as the focus of my Master's Thesis. It was decided that all three genes, each having already been shown by other research to be correlated to disorders of the reward system, should be explored for possible correlations between the presence of the aberrant alleles and obese individuals. A project scope that had not yet been done.

The mechanism behind an obese individual deriving pleasure and positive feedback from the intake of carbohydrates is similar to the pleasure an alcoholic receives from the consumption of alcohol. With this similarity in mind, and more than a decade of research, most of which exhibited positive findings, on the relationship between the Taq 1 A1 allele and alcoholism I began this research project expecting to find similar results with respect to obese individuals.

Studies concerning the DAT1 gene are far more limited in number and scope than those of the DRD2 gene, but have exhibited some positive findings with respect to the prevalence of the homozygous genotype. However, these findings have been limited to TS, ADHD and sexual and oppositional defiant disorders. With these findings there was a high potential for significant correlations to exist between the 40 bp repeat polymorphism in the obese subjects compared to the prevalence found in control subjects.

Studies correlating the presence of the Taq I B1 allele of the DBH gene in those with neuropsychiatric disorders is by far the most unknown of all the dopaminergic genes. Only recently have scientists begun to explore the potential of the DBH gene and therefore not much is known with respect to its application or pertinence in these disorders.

Overall, it was expected that there would be a significant presence of the aberrant genotypes of all three genes in the morbidly obese individuals when compared to the prevalence in the control population. The outcome was far from what was originally expected but there were

errors in the control group which may account for the inconsistent findings when statistical analysis was performed. As was stated earlier the greatest difficulty in accessing the significance was with the control group. It was not until the project was completed and the genotypes obtained was it realize that the control individuals used were not appropriately screened and therefore the results erroneous. It was decided to use current literature controls for statistical comparison. This proved acceptable for the DRD2 gene but not for the DAT1 or DBH genes. For these two genes research itself was limited and the controls used were screened for various other disorders not specifically obesity.

Statistical analysis was conducted on the original control group without any significance found. This left the possibility that the aberrant genes are more commonplace than originally thought or there is a positive history of one or more of the neuropsychiatric behaviors in the control individual's life lending to the presence of the abnormal genotype. Either way, the findings were of no statistical consequence. The only association that proved to be statistically relevant was the prevalence of the DRD2 gene in the obese group compared to the that of the literature controls. The chi-square analysis showed it to be 38.5 with 1 degree of freedom and a p value less than 0.001.

Although the control group caused problems with statistical analysis, there were some significant trends found within the obese group themselves and I continue to ascertain that there would be a significant finding between the presence of the aberrant genotypes of the three dopaminergic genes in morbidly obese as compared to non-obese individuals if a proper control group were established. With the magnitude that obesity affects the U.S. population of this country, it is a certainty that research will continue, and future studies will either prove or disprove this theory. With respect to other future studies, there are two important issues that will need to be addressed. First, if any one of the three dopaminergic polymorphisms are of themselves a major gene in the etiology of obesity. If so, they would be expected to show linkage in family

studies. Therefore, such studies are certain to be conducted in the near future. Secondly, ethnicity should be an issue in any future studies, as is the case with many other addictive disorders there are definite differences between those of varying ethnic backgrounds. This project did not take ethnicity or sex into consideration in either the [illegible] studies, as is the case with many other addictive disorder

Remember that obesity affects over 30 million US adults and is costing more than 39 billion dollars annually in health care. Add to that the additional 30 billion dollars spent on fad diets and other weight loss products and the numbers are staggering. Evermore so pressing is the side affects associated with the newer market diet pills, sometimes causing death. I sincerely hope that the genetics of obesity can be unraveled and that changes can be made to help those who are obese.

APPENDIX A

Approved IRB Human Subject Questionnaire

INFORMED CONSENT AUTHORIZATION TO PARTICIPATE IN A RESEARCH PROJECT

TITLE: **DEVELOPMENT OF A SIMPLE, NON-INVASIVE MOLECULAR DIAGNOSTIC TEST FOR ATTENTION DEFICIT HYPERACTIVITY DISORDER (ADHD)**

INSTITUTION: University of North Texas Health Science Center at Fort Worth

PATIENT NAME: _____

I. NATURE AND PURPOSE OF THIS STUDY:

I understand that I am being asked to take part in a research study, involving a very simple, non-invasive procedure called buccal swab isolation. This procedure involves rubbing the inside of my cheek with a Q-tip swab to collect cells that will be used to obtain DNA.

II. PARTICIPATION IN THE STUDY:

I understand that to participate in this study I must be of legal age or obtain parental/legal guardian consent.

III. PROCEDURES TO BE FOLLOWED:

Clinical psychological testing will be performed by trained personnel at their preferred location. Molecular diagnostic testing will be performed in the DNA Systems Lab, Department of Pathology, UNTHSCFW. The procedure is as follows: A total of four swab samples will be collected per individual. Each swab will be taken with twenty five strokes on the inside of the cheek (buccal cavity), two swabs from the left cheek and two swabs from the right cheek. Only one person will be swabbed at a time, therefore, not allowing any mistakes. Each swab is allowed to air dry approximately one

minute, and then placed in an envelope labeled with the subjects name, age, sex, date collected, and the person's name who collected the swabs. The swabs will be mailed to UNTHSCFW for processing and analysis.

IV. BENEFITS, RISKS, AND ALTERNATIVE TREATMENTS:

I understand that my disorder may or may not be improved by the testing I receive. I understand that I will be told about any changes in the way this study is done, or about any new information such as increased risks that may affect my willingness to participate in the study. I understand that, as with any new procedure, there may be risks that may occur during or afterwards that we cannot predict.

V. VOLUNTARY PARTICIPATION:

I understand that my participation in this study is voluntary. I am free to withdraw my consent and discontinue my participation at any time, and as a result of this, I will not be penalized or lose any of the benefits to which I am otherwise entitled. I understand that I may also be removed from this study without my consent.

VI. COSTS:

I understand that my participation in this study is free of charge. I understand that I will not be paid. During this study, all psychological examinations, buccal swab isolations, and study tests will be at no cost to me. I understand that the University of North Texas Health Science Center at Fort Worth assumes no responsibility for my participation in this study.

VII. PROBLEMS OR QUESTIONS:

Should any problems or questions arise with regard to my rights, I can contact Jerry C. McGill, Ph.D., Chairman, Institutional Review Board, University of North Texas Health Science Center at Fort Worth,

(817) 735 - 2561 for information. In addition, I can contact Dr. Arthur Eisenberg at (817) 735 - 5014 or pager (817) 671 - 2438 or Dr. Mark Sherman at (817) 735 - 2397 of pager (817) 671 - 1984 to answer questions about the procedure or any other related questions.

VIII. CONFIDENTIALITY:

I have read, or have had read to me in my first language, the above information. The consent and the meaning of this information has been explained to me. I hereby voluntarily consent and offer to take part in this study. I understand that all records related to this study will be made available to the Institutional Review Board at the University of North Texas Health Science Center at Fort Worth. I understand that information containing my records will be kept as confidential as possible. The results of this research project may be presented at meetings or in publications, however, my identity will not be revealed.

I have read the above information about this study and have been able to express questions and concerns which have been satisfactorily answered by Drs. Eisenberg or Sherman. I understand the purpose of the study as well as the potential risks that are involved.

I HAVE RECEIVED A COPY OF THIS INFORMED CONSENT AGREEMENT.

Subject's Name (Printed)	Age	Subject's Signature	Date

Witness' Name (Printed)	Witness' Signature	Date

Signature of Person Obtaining Consent
(if other than Investigator)

Investigator's Signature

If subject is a minor or has a court appointed representative, the following signature lines are required:

Signature of Legally Responsible Representative	Relationship to Subject

APPENDIX B

Dopamine Profiles and 3 Digit Codes

Obese Subjects

HMRF OBESITY SAMPLE PROFILES

	DAT1	DRD2	DBH	3 Digit Code	Misc.	% Body Fat
1	10/9	A1/A2	B1/B2	XXX	DISCARD	32.8
2	10/9	A1/A2	B1/B2	222		45
3	10/9	A1/A2	XX	XXX	DISCARD	54.2
4	10/9	A1/A2	B2/B2	223		36.8
5	10/10	A1/A2	B1/B2	122		36.5
6	10/10	A1/A2	B1/B2	122		53.6
7	10/10	A1/A2	B1/B2	122		33.5
8	10/10	A1/A2	B1/B2	XXX	DISCARD	32.1
9	10/9	A2/A2	B1/B2	232		40.1
10	10/10	A1/A1	B2/B2	113		40.4
11	10/9	A2/A2	B1/B2	232		40.9
12	10/10	A2/A2	B1/B1	131		36.2
13	10/9	A1/A2	B2/B2	223		36.5
14	10/9	A1/A2	B1/B2	222		51.3
15	10/10	A1/A2	B1/B2	XXX	DISCARD	29.4
16	10/9	A1/A2	B1/B2	222		44.3
17	10/10	A1/A2	B1/B2	122		43.3
18	10/9	A1/A2	B1/B1	221		52.2
19	10/10	A1/A2	B2/B2	123		44.7
20	10/10	A1/A2	B1/B2	XXX	DISCARD	28.4
21	10/10	A1/A2	B1/B2	122		50.3
22	10/9	A1/A2	B2/B2	233		53.4
23	10/10	A1/A2	B2/B2	123		51.1
24	10/10	A1/A2	B1/B2	122		47.1
25	10/10	A1/A2	B1/B2	122		46.2
26	10/9	A1/A1	B2/B2	XXX	DISCARD	28.2
27	10/9	A1/A2	B1/B2	222		46.1
28	10/9	A1/A2	B1/B2	222		48.6
29	10/10	A1/A2	B1/B2	122		50.8
30	10/10	A1/A2	B2/B2	XXX	DISCARD	24.2
31	10/10	A1/A2	B2/B2	123		43.6
32	10/10	A1/A2	B1/B2	122		51.3
33	10/9	A1/A2	B1/B2	222		36.9
34	9/9	A1/A1	B1/B2	312		37.8

35	10/10	A1/A2	B1/B2	122		39.3
36	10/10	A1/A2	B2/B2	123		41.5
37	10/10	A1/A2	B1/B2	122		43.1
38	10/10	A1/A1	B2/B2	133		48.3
39	10/10	A1/A2	B2/B2	XXX	DISCARD	24.3
40	10/10	A1/A1	B1/B2	112		47.3
41	10/9	A2/A2	B1/B2	XXX	DISCARD	25.9
42	10/9	A1/A2	B2/B2	223		37.4
43	10/10	A1/A2	B1/B2	122		38.5
44	10/9	A1/A2	B1/B1	221		50.3
45	10/9	A1/A2	B1/B2	222		48.7
46	10/9	A1/A2	B2/B2	XXX	DISCARD	34.8
47	10/10	A2/A2	B2/B2	133		42.2
48	10/10	A2/A2	B2/B2	133		39.2
49	10/9	A2/A2	B1/B2	XXX	DISCARD	26.9
50	10/9	A2/A2	B1/B2	232		39.1
51	10/9	A1/A2	B2/B2	223		42.1
52	10/10	A1/A2	B1/B2	122		36.6
53	10/10	A1/A2	B1/B2	122		46.3
54	10/10	A1/A2	B2/B2	123		41.5
55	10/9	A1/A2	B2/B2	223		50.9
56	10/10	A1/A1	B1/B2	112		57.3
57	10/10	A1/A2	B2/B2	123		47.2
58	9/9	A1/A2	XX	XXX	DISCARD	37.4
59	10/10	A2/A2	B1/B2	XXX	DISCARD	34.4
60	10/9	A1/A2	B1/B2	222		46.7
61	10/9	A1/A2	B1/B2	222		44.4
62	10/9	A1/A2	B1/B2	222		40.5
63	10/9	A2/A2	XX	XXX	DISCARD	47.1
64	10/10	A1/A2	B1/B2	XXX	DISCARD	30.1
65	10/10	A1/A2	XX	XXX	DISCARD	28.9
66	10/9	A2/A2	B1/B2	232		42.6
67	10/9	A2/A2	B1/B2	232		36.1
68	10/9	A2/A2	B1/B2	232		31.2
69	10/9	A2/A2	B1/B2	232		36.1
70	10/10	A2/A2	B2/B2	133		43.1
71	10/9	A2/A2	B2/B2	233		44.6
72	10/9	A1/A2	B1/B2	222		47.7
73	10/9	A1/A2	B2/B2	223		46.8

74	10/10	A2/A2	B2/B2	133		44.6
75	10/9	A1/A2	XX	XXX	DISCARD	52.5
76	10/9	A1/A2	B1/B2	222		47.3
77	10/9	A2/A2	B1/B2	232		40.8
78	10/9	A2/A2	B1/B2	232		54.1
79	10/10	A2/A2	XX	XXX	DISCARD	26.8
80	10/9	A2/A2	XX	XXX	DISCARD	49.3
81	10/9	A2/A2	B1/B2	232		44.4
82	10/9	A2/A2	B1/B2	232		30.4
83	10/10	A2/A2	B1/B2	132		30.5
84	10/9	A1/A1	B1/B2	212		46.5
85	10/9	A2/A2	B1/B2	XXX	DISCARD	29.5
86	10/9	A1/A2	B1/B2	XXX	DISCARD	31.1
87	10/9	A1/A2	B1/B2	222		30.8
88	10/9	A2/A2	B2/B2	233		56.1
89	10/9	A2/A2	B2/B2	233		41.1
90	10/9	A2/A2	B2/B2	233		55.1
91	10/9	A2/A2	B2/B2	233		46.2
92	10/10	A1/A2	B2/B2	123		43.4
93	10/10	A1/A2	B2/B2	XXX	DISCARD	27.2
94	10/9	A2/A2	B2/B2	233		46.5
95	10/9	A2/A2	B1/B2	232		45.3
96	10/10	A1/A2	B1/B2	122		41.2
97	10/10	A2/A2	B1/B2	132		47.9
98	10/10	A1/A2	B1/B2	122		54.3
99	10/9	A1/A2	XX	XXX	DISCARD	50.6
100	10/9	A1/A2	B2/B2	223		46.5

APPENDIX C

Materials and Methodology

A. DNA ISOLATION

1. Label 500 µL microcentrifuge tubes and place swab in the appropriate tube.
2. Cut or break the stem off the swab and discard.
3. Make a master mix of Protein Lysis Buffer / 0.5% SDS / Proteinase K as follows:
   297 mL PLB/SDS
   3 mL Proteinase K (10mg/µL)
   Multiply by the number of samples +1.
4. Add 300 µL of the master mix solution to each tube containing the head of the buccal swab.
5. Close the tube and incubate for 2 hours in a 55° C heat block.
6. After incubation, invert the tube and tap lightly to remove liquid from the bottom tip of the tube and then cut the tip off the tube.
7. Place the 500 µL tube in a labeled 2 ml screw-cap microcentrifuge tube and centrifuge at 13,000 rpm for 30 seconds.
8. Repeat steps 6 and 7 with the second sample from the participant, the 2 ml tube will now contain approximately 600 µL of solution.
9. Add 300 µL of 7.5 M lithium chloride ($LiCl^2$) to the tubes, vortex vigorously and place on ice 10 minutes.
10. Centrifuge tubes at 13,000 rpm for ten (10) minutes to pellet proteins and 'trash' material at the bottom.
11. Pour the supernatant into a clean, labeled tube and discard the tube with the protein pellet.
12. Add 1 mL of 100% ethanol (EtOH), vortex vigorously and incubate at room temperature for a minimum of 30 minutes.
13. Centrifuge tubes at 13,000 rpm for 5 minutes to pellet DNA.
14. Carefully pour off the 100% EtOH supernatant, add 1 ml of 70% ethanol, vortex to wash the DNA. Centrifuge at 13,000 rpm for 5 minutes to re-pellet DNA.
15. Carefully pour off the 70% EtOH supernatant
16. Place the tube in the speed vac with no top and allow the DNA to dry to a white pellet.
17. Resuspend the DNA pellet in 100 µL of TE^{-4} buffer and store in 4° C refrigerator.

B. IMMOBILIZING DNA SAMPLES ON NYLON MEMBRANES

1. Prepare DNA standards of 1, 2, 4, 10, 20, 40, and 80 ng in polypropylene tubes. Place 90 µL 0.5 M NaCl, 0.5 M NaOH into each tube. Select DNA standards for the detection range desired. Add 10 µL of prediluted standard DNA solution to each tube and mix well. Incubate 5 minutes at room temperature to denature the DNA.

2. Prepare the sample DNAs. Place 97.5 µL of 0.5 M NaOH, .5 M NaCl in one polypropylene tube for each sample. Add 2.5 µL of the sample DNA and mix well. Incubate 5 minutes at room temperature.

3. Prewet a piece of Biodyne A nylon membrane for 5 minutes in 2X SSC. Place the pre-wetted membrane in a vacuum slot blot apparatus.

4. Load the entire volume of each sample into separate wells and allow the vacuum to pull the samples slowly through the membrane (air pressure 10 - 12 in Hg).

5. Rinse each well with an additional 400 µL 0.5 M NaOH, 0.5 NaCl, allowing the vacuum to pull the liquid through the membrane.

6. Remove the membrane from the slot blot apparatus. Neutralize the membrane by rinsing in 0.2 M Tris (pH 7.5), 2X SSC for 5 minutes. Immediately bake 30 minutes at 80° C and UV crosslink with 0.15 Joules/cm^2 254 nm light to fix the DNA to the membrane.

C. HYBRIDIZING THE MEMBRANE WITH HUMAN DNA PROBE

1. Place the Biodyne membrane in a clean container and equilibrate with 0.1 ml of ACES 2.0 Hybridization Solution at 50° C for each cm^2 of membrane area (approximately 15 ml/membrane). Incubate for 20 minutes at 50° C on a rocker or shaking water bath to prehybridize the membrane.

2. Prewarm 0.05 ml of hybridization solution to 50° C for each cm^2 of membrane area. Add 0.5 µL Human DNA probe per ml of hybridization solution. Approximately 7.5 µL of probe to 15 ml of hybe solution. Human DNA probe used is *D17Z1*. Pour out the prehybridization solution and add the hybridization solution containing the probe. Incubate for an additional 20 minutes on the rocker at 50° C.

3. After hybridization, wash the membrane 2 times for 10 minutes each in a 1:5 dilution of ACES 2.0 wash buffer concentrate at 50° C in a volume of approximately 90 ml, while on the rocker.

4. Wash the membrane 2 more times, 5 minutes each in ACES 2.0 1X final wash buffer in a volume of approximately 50 ml at room temperature, on the rocker.

5. Immediately continue to the DNA detection process, do not let membrane dry!

D. DETECTING HYBRIDIZATION WITH ATTOPHOS

1. After the final wash, pour out the wash buffer. Make sure to remove as much of the solution as possible without damaging the membrane.
2. Using sterile pipet, pipet 10 ml of Attophos onto the membrane making sure it is completely covered. Soak the membrane 5 minutes while rocking on room temperature rocker on full range, slow motion.
3. Drain off as much solution as possible and place the membrane in a plastic development folder.
4. Heat seal all sides of the development folder except one. This allows you to then squeeze out excess solution and seal the remaining side.
5. Store the sealed folder in the dark at room temperature for approximately 6 hours (overnight).
6. Quantitate the amount of DNA using the Fluorimager. See next set of procedures.

E. DNA QUANTITATION PROCEDURE USING FLUORIMAGER

1. Place the membrane on the glass plate and insert into the fluorimager.
2. Scan the membrane and allow image to come onto the screen. Work with the computer controls to obtain the clearest image of the membrane possible.
3. Using the computer controls, select box tool to make first box around the first control.
4. Go back to the arrow button, select it and then go back to second control.
5. Hold down the mouse button and select box, move from the first box to the next while holding down the mouse button. Use shift V to make a new box around the next object. Continuing this until all of the specimens results have been boxed and numbered.
6. Return to the first box, select 'analysis' then background correction then local medium close box.
7. Return to 'analysis', select volume report setup, click okay, close box
8. Return to 'analysis' menu, select volume report, click on report. This will bring up an embedded excel spreadsheet. Close analysis window.
9. Double click in the excel worksheet to bring it to full working size.
10. Insert one column to the left of the first column, type in the standards in nanograms
11. Select the column with the standards, hold control key down and select the volumes that correspond to the standards then release the control key.
12. Go to insert menu and select 'chart' and 'on this sheet'.
13. Select cross hairs for the chart, approximately 2 x 3.
14. Chart wizard will come up. There will be windows 1-5:
    click next
    xy scatter for chart type
    click next
    select xy for chart format
    click next
    click next
    Type in x axis = ng DNA and y axis = volume rfu
    click finish
15. A curve is then plotted, should be as linear as possible
16. Double click in the gray area within the chart
17. Click on the plotted line within the chart until it turns yellow
18. Go to insert menu, select trendline then linear
19. Click on options menu, select display equation on this chart then click okay. This produces the equation in the format y=mx + b.
20. Use the numbers given to insert into this equation: ng unknown =(C8-b)/m
21. Type the above for the first sample not a control, hit enter select this box and copy all the way down through the unknown samples. When done do also for controls. The controls, if the graph is linear, should equal the amount of standards used.
22. Select page set up and print.

The nanograms of DNA established through this quantitative procedure will be used to determine the amount of each sample to use when amplifying the DNA via PCR techniques.

F. POLYMERASE CHAIN REACTION (PCR) FOR DNA AMPLIFICATION FOR DAT1 AND DRD2

To determine the dopamine profile of each individual, DNA will be extracted from cells of the buccal cavity, purified and amplified by Polymerase Chain Reaction (PCR) techniques, an alternative to cloning. The process of PCR allows for the replication and amplification of a small amount of genomic DNA in a short period of time. PCR is accomplished by denaturing double stranded DNA into single strands by excess heat. Synthetic oligonucleotides complementary to the 3' ends of the DNA segment of interest are added to serve as primers for the DNA chain synthesis. When a temperature resistant enzyme called *Taq Polymerase* is added with deoxynucleotides, the synthesis or copy of the genomic DNA can be completed. This process is continued for a pre-determined number of cycles amplifying the DNA by a factor of two for each cycle. So, with the completion of twenty cycles, the DNA sequence between the primers has been replicated and amplified more than a millionfold. Using PCR technology, I will be able to amplify enough DNA from cells scraped off the mucosal lining of the buccal cavity, to digest for the three different genes. Run the samples on a gel and determine the genotype.

1. Combine the following reagents for a 'master mix'. Amount is determined by the number of samples + 1.

10X PCR Buffer	2.5 µL
DAT Primer	1.25 µL
DRD2 Primer #1	1.25 µL
DRD2 Primer #2	1.25 µL
dNTP mix	0.5 µL
Taq Polymerase	0.125 µL
Sterile Water	Varies (Total reaction volume = 25 µL)
DNA	1 µL

DNA

(diluted if necessary so that 10-50 ng/µL is present, determined by previous quant. procedure)

2. Add the DNA to a microcentrifuge tube for the Perkin Elmer 9600 Thermal Cycler.

3. Add 24 µL of the 'master mix' to each tube with DNA.
4. Mix samples well and place in the Perkin Elmer9600 Thermal Cycler and perform PCR via the following parameters:

95° C	2 minutes	01 cycle
94° C	30 seconds	
63° C	30 seconds	32 cycles
72° C	45 seconds	
72° C	10 minutes	01 cycle
15° C	HOLD	01 cycle

5. PCR is complete, remove 5 µL of the sample, undigested, and set aside. Move on to the Taq digestion phase.

G. TAQ DIGESTION FOR THE DRD2 AND DAT BANDS

1. Using the same tube from the PCR reaction, which now contains 20 μL, add the following:

10X Buffer E	5.00 μL
Taq 1	1.00 μL
Sterile water	24.00 μL
PCR product	20 μL
Total Reaction Volume =	50 μL

2. Incubate the samples in the Perkin Elmer 9600 Thermal Cycler at 65° C for 2 hours
3. Proceed to gel electrophoresis and detection steps.

H. POLYMERASE CHAIN REACTION (PCR) FOR DNA AMPLIFICATION FOR DβH GENE

1. Combine the following reagents for a 'master mix'. Amount is determined by the number of samples + 1.

10X PCR Buffer	2.5 μL
DbH Primer	1.00 μL
dNTP mix	0.5 μL
Taq Polymerase	0.125 μL
Sterile Water	Varies (Total reaction volume = 25 μL)
DNA	1 μL

(diluted if necessary so that 10-50 ng/μL is present, determined by the previous quantitation procedure)

2. Add the DNA to a microcentrifuge tube for the Perkin Elmer 9600 Thermal Cycler.
3. Add 24 μL of the 'master mix' to each tube with DNA.
4. Mix samples well and place in the Perkin Elmer9600 Thermal Cycler and perform PCR via the following parameters:

94° C	2 minutes	01 cycle
94° C	30 seconds	
50° C	30 seconds	30 cycles
72° C	45 seconds	
15° C	HOLD	01 cycle

5. PCR is complete, remove 5 μL of the sample, undigested, and set aside. Move on to the Taq digestion phase.

I. TAQ 1 DIGESTION FOR THE DβH BANDS

1. Using the same tube from the PCR reaction, which now contains 20 mL, add the following:

10X Buffer E	5.00 μL
Taq 1	1.00 μL
Sterile water	24.00 μL
PCR product	20 μL
Total Reaction Volume =	50 μL

2. Incubate the samples in the Perkin Elmer 9600 Thermal Cycler at 65° C for 2 hours

3. Proceed to gel electrophoresis and detection steps.

J. AGAROSE GEL ELECTROPHORESIS OF AMPLIFICATION PRODUCTS

1. Prepare a 3% NuSieve 3:1 agarose gel by adding 3.0 gm of agarose to 100ml of 1X TAE buffer. Mark the liquid level in the container, then heat in microwave to dissolve the agarose. Add deionized water to make up for any volume lost to evaporation.
2. Cool the agarose to 55° C. Pour the agarose into the gel tray, make certain the tray is level. Insert the appropriate gel comb and allow to set for 30 minutes.
3. Prepare the samples by mixing 8 μL of sample with 2 μL of 5X Loading Dye.
4. Once the gel is set, remove the comb and place in electrophoresis box. Cover with 1X TAE Buffer to a depth that covers the gel.
5. Load the samples and a 100 bp molecular weight marker
6. Set the voltage at approximately 100 volts and run for 1 hour.
7. After electrophoresis, stain the gel in 1X TAE containing 0.5 mg/ml SYBR-Green. Gently rock for 20 minutes at room temperature. Remove the SYBR-Green solution and wash the gel thoroughly in deionized water.
8. Scan the gel with the fluorimager using the 570 filter and 900 PMT voltage.
9. Using the controls on the fluorimager, adjust the contrast of the image to its best quality and print.
10. Determine and record the genotype.
11. Repeat steps 1-11 once for DAT1 and DRD2 analysis and once for DβH analysis.

APPENDIX D

Dopamine Profiles and 3 Digit Codes
Control Group

OBESITY CONTROL SAMPLES

	DAT1	DRD2	DBH	3 Digit Code	Misc.	% Body Fat
1	10/10	A1/A2	B2/B2	123		Unknown
2	10/10	A2/A2	B2/B2	133		Unknown
3	10/10	A1/A1	B2/B2	113		Unknown
4	10/9	A2/A2	B1/B1	231		Unknown
5	10/9	A2/A2	B1/B1	231		Unknown
6	10/10	A2/A2	B1/B2	132		Unknown
7	10/10	A2/A2	B1/B2	132		Unknown
8	10/10	A2/A2	B2/B2	133		Unknown
9	10/10	A1/A2	B1/B2	122		Unknown
10	10/10	A2/A2	B1/B1	131		Unknown
11	10/9	A2/A2	B1/B1	231		Unknown
12	XX	XX	B1/B2	XXX	DISCARD	Unknown
13	10/10	A1/A2	B2/B2	123		Unknown
14	10/10	A2/A2	B1/B1	131		Unknown
15	10/10	A1/A2	B1/B2	122		Unknown
16	10/10	A2/A2	B1/B2	132		Unknown
17	10/10	A2/A2	B1/B1	131		Unknown
18	10/9	A1/A2	B1/B2	222		Unknown
19	10/9	A2/A2	XX	XXX	DISCARD	Unknown
20	10/9	A1/A2	B1/B2	222		Unknown
21	10/9	A2/A2	B2/B2	233		Unknown
22	10/9	A1/A2	B2/B2	223		Unknown
23	10/10	A2/A2	B2/B2	133		Unknown
24	10/9	A2/A2	B2/B2	133		Unknown
25	10/10	A2/A2	B1/B2	132		Unknown
26	10/9	A2/A2	B2/B2	233		Unknown
27	10/10	A1/A2	XX	XXX	DISCARD	Unknown
28	10/10	A2/A2	B1/B2	132		Unknown
29	10/9	A2/A2	B1/B2	232		Unknown
30	10/10	A1/A2	B1/B2	122		Unknown
31	10/10	A1/A1	B2/B2	113		Unknown
32	10/10	A2/A2	XX	XXX	DISCARD	Unknown
33	10/10	A1/A1	B1/B2	112		Unknown

34	10/9	A1/A1	B1/B2	212		Unknown
35	10/10	A1/A2	B1/B2	122		Unknown
36	10/9	A1/A2	B2/B2	223		Unknown
37	10/10	A1/A2	B1/B2	122		Unknown
38	10/10	A2/A2	B2/B2	133		Unknown
39	10/10	A1/A2	B1/B2	122		Unknown
40	XX	XX	B1/B2	XXX	DISCARD	Unknown
41	10/9	A1/A2	B2/B2	223		Unknown
42	10/9	A2/A2	B2/B2	233		Unknown
43	10/10	A2/A2	B1/B2	132		Unknown
44	10/10	A2/A2	B1/B1	131		Unknown
45	10/9	A2/A2	B1/B2	232		Unknown
46	10/10	A2/A2	B1/B2	132		Unknown
47	10/9	A2/A2	B2/B2	233		Unknown
48	10/10	A2/A2	B2/B2	133		Unknown
49	10/10	A2/A2	XX	XXX	DISCARD	Unknown
50	10/10	A1/A1	B2/B2	113		Unknown

APPENDIX E

SPSS Data

Crosstabs

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
A1 ALLELE * % Fat @ 28%	103	70.1%	44	29.9%	147	100.0%
A1 ALLELE * % Fat @ 34%	103	70.1%	44	29.9%	147	100.0%
A1 ALLELE * Morbidly Obese	103	70.1%	44	29.9%	147	100.0%
DAT 1010 allele * % Fat @ 28%	103	70.1%	44	29.9%	147	100.0%
DAT 1010 allele * % Fat @ 34%	103	70.1%	44	29.9%	147	100.0%
DAT 1010 allele * Morbidly Obese	103	70.1%	44	29.9%	147	100.0%
DBH * % Fat @ 28%	94	63.9%	53	36.1%	147	100.0%
DBH * % Fat @ 34%	94	63.9%	53	36.1%	147	100.0%
DBH * Morbidly Obese	94	63.9%	53	36.1%	147	100.0%

A1 ALLELE * % Fat @ 28%

Crosstab

			% Fat @ 28%		
			< 28% fat	>= 28% fat	Total
A1 ALLELE	A2A2	Count	3	31	34
		% within A1 ALLELE	8.8%	91.2%	100.0%
		% within % Fat @ 28%	50.0%	32.0%	33.0%
		% of Total	2.9%	30.1%	33.0%
	A1A2	Count	3	60	63
		% within A1 ALLELE	4.8%	95.2%	100.0%
		% within % Fat @ 28%	50.0%	61.9%	61.2%
		% of Total	2.9%	58.3%	61.2%
	A1A1	Count		6	6
		% within A1 ALLELE		100.0%	100.0%
		% within % Fat @ 28%		6.2%	5.8%
		% of Total		5.8%	5.8%
Total		Count	6	97	103
		% within A1 ALLELE	5.8%	94.2%	100.0%
		% within % Fat @ 28%	100.0%	100.0%	100.0%
		% of Total	5.8%	94.2%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1.058[a]	2	.589
Likelihood Ratio	1.344	2	.511
Linear-by-Linear Association	1.044	1	.307
N of Valid Cases	103		

a. 3 cells (50.0%) have expected count less than 5. The minimum expected count is .35.

Risk Estimate

	Value
Odds Ratio for A1 ALLELE (A2A2 / A1A2)	a

a. Risk Estimate statistics cannot be computed. They are only computed for a 2*2 table without empty cells.

A1 ALLELE * % Fat @ 34%

Crosstab

			% Fat @ 34%		
			< 34% fat	>= 34% fat	Total
A1 ALLELE	A2A2	Count	7	27	34
		% within A1 ALLELE	20.6%	79.4%	100.0%
		% within % Fat @ 34%	35.0%	32.5%	33.0%
		% of Total	6.8%	26.2%	33.0%
	A1A2	Count	12	51	63
		% within A1 ALLELE	19.0%	81.0%	100.0%
		% within % Fat @ 34%	60.0%	61.4%	61.2%
		% of Total	11.7%	49.5%	61.2%
	A1A1	Count	1	5	6
		% within A1 ALLELE	16.7%	83.3%	100.0%
		% within % Fat @ 34%	5.0%	6.0%	5.8%
		% of Total	1.0%	4.9%	5.8%
Total		Count	20	83	103
		% within A1 ALLELE	19.4%	80.6%	100.0%
		% within % Fat @ 34%	100.0%	100.0%	100.0%
		% of Total	19.4%	80.6%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	.064[a]	2	.968
Likelihood Ratio	.065	2	.968
Linear-by-Linear Association	.062	1	.803
N of Valid Cases	103		

a. 2 cells (33.3%) have expected count less than 5. The minimum expected count is 1.17.

Risk Estimate

	Value
Odds Ratio for A1 ALLELE (A2A2 / A1A2)	a

a. Risk Estimate statistics cannot be computed. They are only computed for a 2*2 table without empty cells.

A1 ALLELE * Morbidly Obese

Crosstab

			Morbidly Obese		
			MALES LT 28% AND FEMALES LT 34%	MALES GE 28% AND FEMALES GE 34%	Total
A1 ALLELE	A2A2	Count	3	31	34
		% within A1 ALLELE	8.8%	91.2%	100.0%
		% within Morbidly Obese	25.0%	34.1%	33.0%
		% of Total	2.9%	30.1%	33.0%
	A1A2	Count	8	55	63
		% within A1 ALLELE	12.7%	87.3%	100.0%
		% within Morbidly Obese	66.7%	60.4%	61.2%
		% of Total	7.8%	53.4%	61.2%
	A1A1	Count	1	5	6
		% within A1 ALLELE	16.7%	83.3%	100.0%
		% within Morbidly Obese	8.3%	5.5%	5.8%
		% of Total	1.0%	4.9%	5.8%
Total		Count	12	91	103
		% within A1 ALLELE	11.7%	88.3%	100.0%
		% within Morbidly Obese	100.0%	100.0%	100.0%
		% of Total	11.7%	88.3%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	.478[a]	2	.787
Likelihood Ratio	.482	2	.786
Linear-by-Linear Association	.473	1	.492
N of Valid Cases	103		

a. 2 cells (33.3%) have expected count less than 5. The minimum expected count is .70.

Risk Estimate

	Value
Odds Ratio for A1 ALLELE (A2A2 / A1A2)	a

a. Risk Estimate statistics cannot be computed. They are only computed for a 2*2 table without empty cells.

DAT 1010 allele * % Fat @ 28%

Crosstab

			% Fat @ 28%		
			< 28% fat	>= 28% fat	Total
DAT 1010 allele	0909	Count		2	2
		% within DAT 1010 allele		100.0%	100.0%
		% within % Fat @ 28%		2.1%	1.9%
		% of Total		1.9%	1.9%
	1009	Count	2	52	54
		% within DAT 1010 allele	3.7%	96.3%	100.0%
		% within % Fat @ 28%	33.3%	53.6%	52.4%
		% of Total	1.9%	50.5%	52.4%
	1010	Count	4	43	47
		% within DAT 1010 allele	8.5%	91.5%	100.0%
		% within % Fat @ 28%	66.7%	44.3%	45.6%
		% of Total	3.9%	41.7%	45.6%
Total		Count	6	97	103
		% within DAT 1010 allele	5.8%	94.2%	100.0%
		% within % Fat @ 28%	100.0%	100.0%	100.0%
		% of Total	5.8%	94.2%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1.185[a]	2	.553
Likelihood Ratio	1.290	2	.525
Linear-by-Linear Association	1.169	1	.280
N of Valid Cases	103		

a. 4 cells (66.7%) have expected count less than 5. The minimum expected count is .12.

Risk Estimate

	Value
Odds Ratio for DAT 1010 allele (0909 / 1009)	a

a. Risk Estimate statistics cannot be computed. They are only computed for a 2*2 table without empty cells.

DAT 1010 allele * % Fat @ 34%

Crosstab

			% Fat @ 34%		
			< 34% fat	>= 34% fat	Total
DAT 1010 allele	0909	Count		2	2
		% within DAT 1010 allele		100.0%	100.0%
		% within % Fat @ 34%		2.4%	1.9%
		% of Total		1.9%	1.9%
	1009	Count	9	45	54
		% within DAT 1010 allele	16.7%	83.3%	100.0%
		% within % Fat @ 34%	45.0%	54.2%	52.4%
		% of Total	8.7%	43.7%	52.4%
	1010	Count	11	36	47
		% within DAT 1010 allele	23.4%	76.6%	100.0%
		% within % Fat @ 34%	55.0%	43.4%	45.6%
		% of Total	10.7%	35.0%	45.6%
Total		Count	20	83	103
		% within DAT 1010 allele	19.4%	80.6%	100.0%
		% within % Fat @ 34%	100.0%	100.0%	100.0%
		% of Total	19.4%	80.6%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1.221[a]	2	.543
Likelihood Ratio	1.590	2	.452
Linear-by-Linear Association	1.104	1	.293
N of Valid Cases	103		

a. 2 cells (33.3%) have expected count less than 5. The minimum expected count is .39.

Risk Estimate

	Value
Odds Ratio for DAT 1010 allele (0909 / 1009)	a

a. Risk Estimate statistics cannot be computed. They are only computed for a 2*2 table without empty cells.

DAT 1010 allele * Morbidly Obese

Crosstab

			Morbidly Obese		
			MALES LT 28% AND FEMALES LT 34%	MALES GE 28% AND FEMALES GE 34%	Total
DAT 1010 allele	0909	Count		2	2
		% within DAT 1010 allele		100.0%	100.0%
		% within Morbidly Obese		2.2%	1.9%
		% of Total		1.9%	1.9%
	1009	Count	5	49	54
		% within DAT 1010 allele	9.3%	90.7%	100.0%
		% within Morbidly Obese	41.7%	53.8%	52.4%
		% of Total	4.9%	47.6%	52.4%
	1010	Count	7	40	47
		% within DAT 1010 allele	14.9%	85.1%	100.0%
		% within Morbidly Obese	58.3%	44.0%	45.6%
		% of Total	6.8%	38.8%	45.6%
Total		Count	12	91	103
		% within DAT 1010 allele	11.7%	88.3%	100.0%
		% within Morbidly Obese	100.0%	100.0%	100.0%
		% of Total	11.7%	88.3%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1.044[a]	2	.593
Likelihood Ratio	1.262	2	.532
Linear-by-Linear Association	1.013	1	.314
N of Valid Cases	103		

a. 2 cells (33.3%) have expected count less than 5. The minimum expected count is .23.

Risk Estimate

	Value
Odds Ratio for DAT 1010 allele (0909 / 1009)	a

a. Risk Estimate statistics cannot be computed. They are only computed for a 2*2 table without empty cells.

DBH * % Fat @ 28%

Crosstab

			% Fat @ 28%		
			< 28% fat	>= 28% fat	Total
DBH	B2B2	Count	3	31	34
		% within DBH	8.8%	91.2%	100.0%
		% within % Fat @ 28%	60.0%	34.8%	36.2%
		% of Total	3.2%	33.0%	36.2%
	B1B2	Count	2	55	57
		% within DBH	3.5%	96.5%	100.0%
		% within % Fat @ 28%	40.0%	61.8%	60.6%
		% of Total	2.1%	58.5%	60.6%
	B1B1	Count		3	3
		% within DBH		100.0%	100.0%
		% within % Fat @ 28%		3.4%	3.2%
		% of Total		3.2%	3.2%
Total		Count	5	89	94
		% within DBH	5.3%	94.7%	100.0%
		% within % Fat @ 28%	100.0%	100.0%	100.0%
		% of Total	5.3%	94.7%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1.369[a]	2	.504
Likelihood Ratio	1.446	2	.485
Linear-by-Linear Association	1.339	1	.247
N of Valid Cases	94		

a. 4 cells (66.7%) have expected count less than 5. The minimum expected count is .16.

Risk Estimate

	Value
Odds Ratio for DBH (B2B2 / B1B2)	[a]

a. Risk Estimate statistics cannot be computed. They are only computed for a 2*2 table without empty cells.

DBH * % Fat @ 34%

Crosstab

			% Fat @ 34%		
			< 34% fat	>= 34% fat	Total
DBH	B2B2	Count	5	29	34
		% within DBH	14.7%	85.3%	100.0%
		% within % Fat @ 34%	26.3%	38.7%	36.2%
		% of Total	5.3%	30.9%	36.2%
	B1B2	Count	14	43	57
		% within DBH	24.6%	75.4%	100.0%
		% within % Fat @ 34%	73.7%	57.3%	60.6%
		% of Total	14.9%	45.7%	60.6%
	B1B1	Count		3	3
		% within DBH		100.0%	100.0%
		% within % Fat @ 34%		4.0%	3.2%
		% of Total		3.2%	3.2%
Total		Count	19	75	94
		% within DBH	20.2%	79.8%	100.0%
		% within % Fat @ 34%	100.0%	100.0%	100.0%
		% of Total	20.2%	79.8%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	2.068[a]	2	.356
Likelihood Ratio	2.682	2	.262
Linear-by-Linear Association	.367	1	.545
N of Valid Cases	94		

a. 2 cells (33.3%) have expected count less than 5. The minimum expected count is .61.

Risk Estimate

	Value
Odds Ratio for DBH (B2B2 / B1B2)	[a]

a. Risk Estimate statistics cannot be computed. They are only computed for a 2*2 table without empty cells.

DBH * Morbidly Obese

Crosstab

			Morbidly Obese		
			MALES LT 28% AND FEMALES LT 34%	MALES GE 28% AND FEMALES GE 34%	Total
DBH	B2B2	Count	4	30	34
		% within DBH	11.8%	88.2%	100.0%
		% within Morbidly Obese	36.4%	36.1%	36.2%
		% of Total	4.3%	31.9%	36.2%
	B1B2	Count	7	50	57
		% within DBH	12.3%	87.7%	100.0%
		% within Morbidly Obese	63.6%	60.2%	60.6%
		% of Total	7.4%	53.2%	60.6%
	B1B1	Count		3	3
		% within DBH		100.0%	100.0%
		% within Morbidly Obese		3.6%	3.2%
		% of Total		3.2%	3.2%
Total		Count	11	83	94
		% within DBH	11.7%	88.3%	100.0%
		% within Morbidly Obese	100.0%	100.0%	100.0%
		% of Total	11.7%	88.3%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	.416[a]	2	.812
Likelihood Ratio	.765	2	.682
Linear-by-Linear Association	.050	1	.824
N of Valid Cases	94		

a. 3 cells (50.0%) have expected count less than 5. The minimum expected count is .35.

Risk Estimate

	Value
Odds Ratio for DBH (B2B2 / B1B2)	a

a. Risk Estimate statistics cannot be computed. They are only computed for a 2*2 table without empty cells.

Oneway

Descriptives

begin % fat

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
B2B2	34	.419294	.04877E-02	1.380E-02	.391211	.447378	.2420	.5500
B1B2	57	.412002	.89895E-02	1.046E-02	.391043	.432960	.2630	.5440
B1B1	3	.463000	.62612E-02	4.980E-02	.248715	.677285	.3640	.5220
Total	94	.416267	.93772E-02	8.187E-03	.400009	.432525	.2420	.5500

Test of Homogeneity of Variances

begin % fat

Levene Statistic	df1	df2	Sig.
.317	2	91	.729

ANOVA

begin % fat

			Sum of Squares	df	Mean Square	F	Sig.
Between Groups	(Combined)		7.900E-03	2	3.950E-03	.622	.539
	Linear Term	Unweighted	5.266E-03	1	5.266E-03	.829	.365
		Weighted	5.190E-05	1	5.190E-05	.008	.928
		Deviation	7.849E-03	1	7.849E-03	1.236	.269
Within Groups			.578	91	6.352E-03		
Total			.586	93			

Post Hoc Tests

Homogeneous Subsets

begin % fat

Student-Newman-Keuls[a,b]

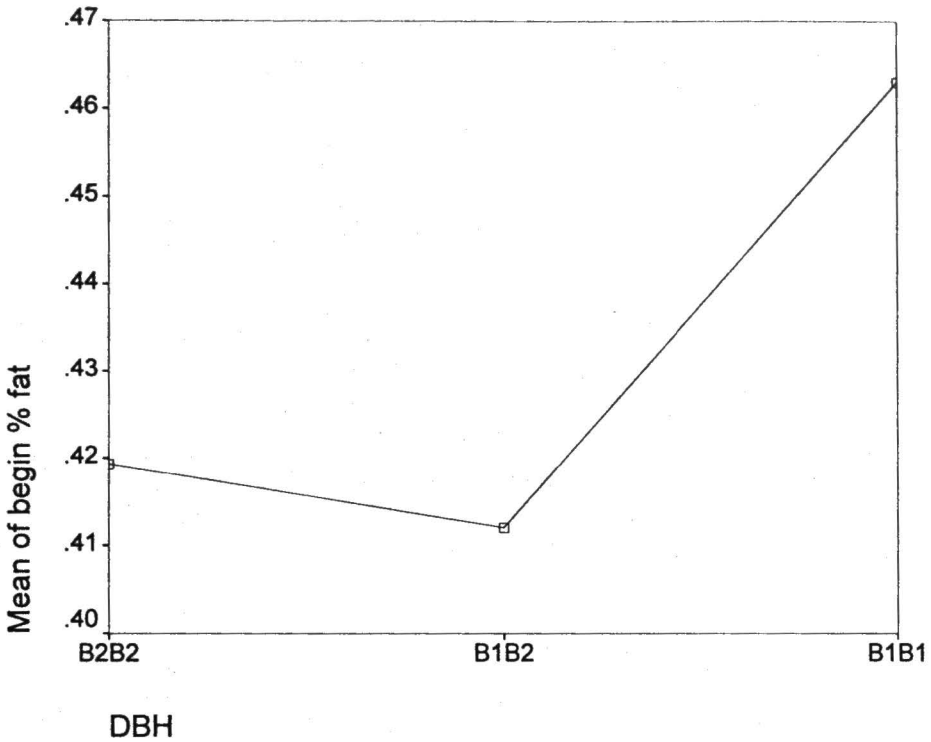
DBH	N	Subset for alpha = .05
		1
B1B2	57	.412002
B2B2	34	.419294
B1B1	3	.463000
Sig.		.415

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 7.889.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Means Plots



Oneway

Descriptives

begin % fat

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
A2A2	34	.407412	.91214E-02	1.357E-02	.379805	.435019	.2630	.5500
A1A2	63	.424637	.14509E-02	1.026E-02	.404123	.445150	.2420	.5440
A1A1	6	.424667	.81899E-02	3.600E-02	.332117	.517216	.2860	.5390
Total	103	.418952	.06564E-02	7.947E-03	.403189	.434716	.2420	.5500

Test of Homogeneity of Variances

begin % fat

Levene Statistic	df1	df2	Sig.
.119	2	100	.888

ANOVA

begin % fat

			Sum of Squares	df	Mean Square	F	Sig.
Between Groups	(Combined)		6.760E-03	2	3.380E-03	.515	.599
	Linear Term	Unweighted	1.518E-03	1	1.518E-03	.231	.632
		Weighted	5.621E-03	1	5.621E-03	.856	.357
		Deviation	1.139E-03	1	1.139E-03	.173	.678
Within Groups			.657	100	6.568E-03		
Total			.664	102			

Post Hoc Tests

Homogeneous Subsets

begin % fat

Student-Newman-Keuls[a,b]

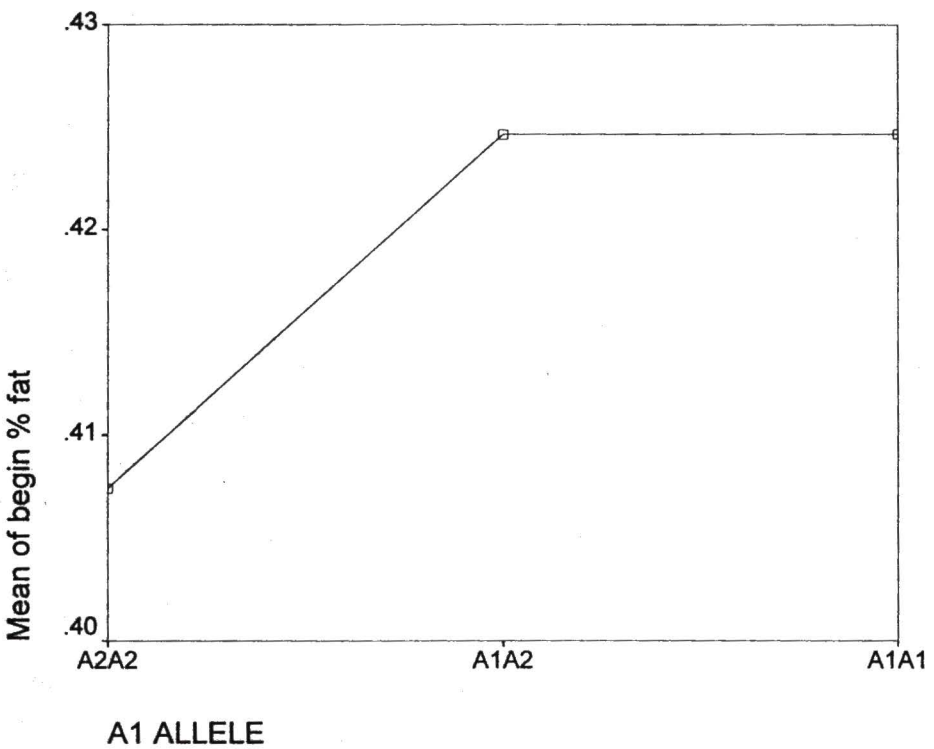
A1 ALLELE	N	Subset for alpha = .05
		1
A2A2	34	.407412
A1A2	63	.424637
A1A1	6	.424667
Sig.		.838

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 14.154.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Means Plots



Oneway

Descriptives

begin % fat

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
0909	2	.376000	.82843E-03	2.000E-03	.350588	.401412	.3740	.3780
1009	54	.428372	.89995E-02	1.075E-02	.406810	.449935	.2630	.5500
1010	47	.409957	.34704E-02	1.218E-02	.385450	.434465	.2420	.5440
Total	103	.418952	.06564E-02	7.947E-03	.403189	.434716	.2420	.5500

Test of Homogeneity of Variances

begin % fat

Levene Statistic	df1	df2	Sig.
2.327	2	100	.103

ANOVA

begin % fat

			Sum of Squares	df	Mean Square	F	Sig.
Between Groups	(Combined)		1.228E-02	2	6.142E-03	.943	.393
	Linear Term	Unweighted	2.212E-03	1	2.212E-03	.340	.561
		Weighted	3.868E-03	1	3.868E-03	.594	.443
		Deviation	8.417E-03	1	8.417E-03	1.292	.258
Within Groups			.651	100	6.513E-03		
Total			.664	102			

Post Hoc Tests

Homogeneous Subsets

begin % fat

Student-Newman-Keuls[a,b]

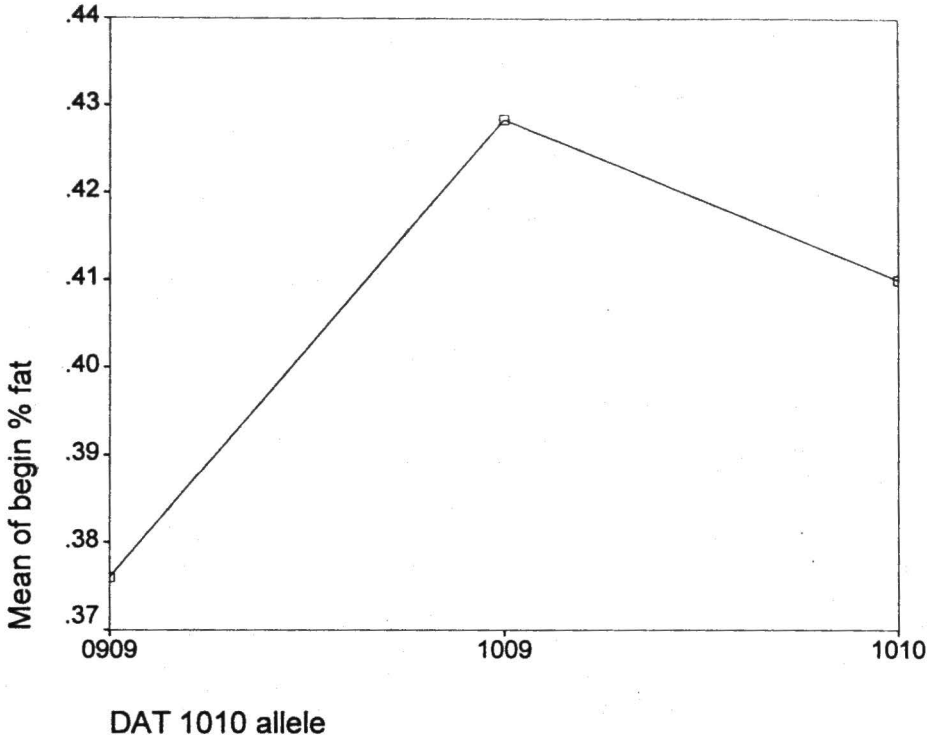
DAT 1010 allele	N	Subset for alpha = .05
		1
0909	2	.376000
1010	47	.409957
1009	54	.428372
Sig.		.528

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.558.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Means Plots



Frequencies

Statistics

		DBH	A1 ALLELE	DAT 1010 allele	GENE_SUM
N	Valid	94	103	103	83
	Missing	53	44	44	64

Frequency Table

DBH

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	B2B2	34	23.1	36.2	36.2
	B1B2	57	38.8	60.6	96.8
	B1B1	3	2.0	3.2	100.0
	Total	94	63.9	100.0	
Missing	System	53	36.1		
Total		147	100.0		

A1 ALLELE

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	A2A2	34	23.1	33.0	33.0
	A1A2	63	42.9	61.2	94.2
	A1A1	6	4.1	5.8	100.0
	Total	103	70.1	100.0	
Missing	System	44	29.9		
Total		147	100.0		

DAT 1010 allele

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	0909	2	1.4	1.9	1.9
	1009	54	36.7	52.4	54.4
	1010	47	32.0	45.6	100.0
	Total	103	70.1	100.0	
Missing	System	44	29.9		
Total		147	100.0		

GENE_SUM

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	1.00	6	4.1	7.2	7.2
	2.00	28	19.0	33.7	41.0
	3.00	25	17.0	30.1	71.1
	4.00	22	15.0	26.5	97.6
	5.00	2	1.4	2.4	100.0
	Total	83	56.5	100.0	
Missing	System	64	43.5		
Total		147	100.0		

Frequencies

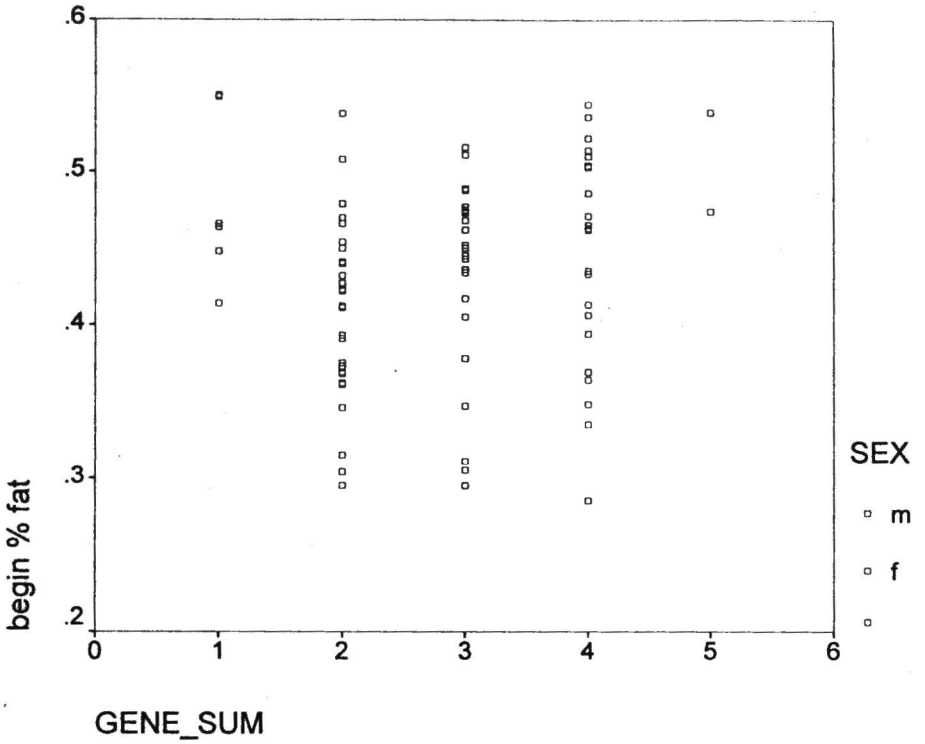
Statistics

begin % fat

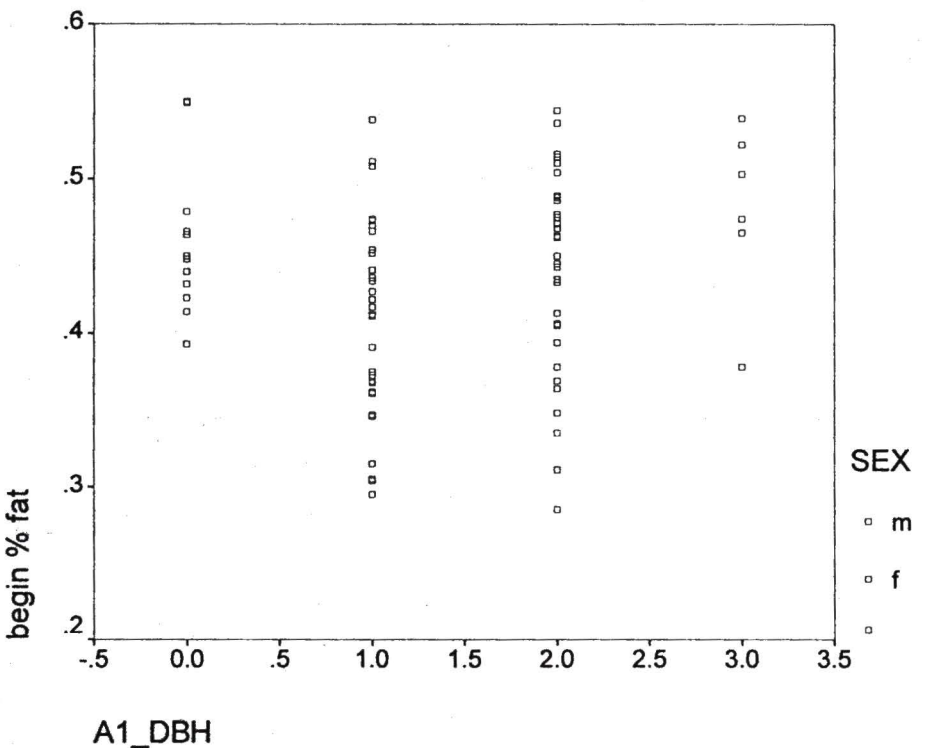
N	Valid	132
	Missing	15

begin % fat

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	.2420	1	.7	.8	.8
	.2440	1	.7	.8	1.5
	.2630	1	.7	.8	2.3
	.2700	1	.7	.8	3.0
	.2710	1	.7	.8	3.8
	.2730	1	.7	.8	4.5
	.2850	1	.7	.8	5.3
	.2860	1	.7	.8	6.1
	.2950	2	1.4	1.5	7.6
	.3040	1	.7	.8	8.3
	.3050	1	.7	.8	9.1
	.3060	1	.7	.8	9.8
	.3100	1	.7	.8	10.6
	.3110	2	1.4	1.5	12.1
	.3150	1	.7	.8	12.9
	.3210	1	.7	.8	13.6
	.3240	1	.7	.8	14.4
	.3280	1	.7	.8	15.2
	.3310	1	.7	.8	15.9
	.3350	2	1.4	1.5	17.4
	.3450	1	.7	.8	18.2



Graph



Oneway

Descriptives

begin % fat

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1.00	6	.481833	.56288E-02	2.271E-02	.423454	.540212	.4140	.5500
2.00	28	.412964	.35630E-02	1.201E-02	.388317	.437611	.2950	.5380
3.00	25	.429844	.25430E-02	1.251E-02	.404028	.455660	.2950	.5160
4.00	22	.443727	.17178E-02	1.529E-02	.411929	.475525	.2850	.5440
5.00	2	.506500	.59619E-02	3.250E-02	.35483E-02	.919452	.4740	.5390
Total	83	.433435	.69621E-02	7.350E-03	.418813	.448057	.2850	.5500

Test of Homogeneity of Variances

begin % fat

Levene Statistic	df1	df2	Sig.
.478	4	78	.752

ANOVA

begin % fat

			Sum of Squares	df	Mean Square	F	Sig.
Between Groups	(Combined)		3.912E-02	4	9.779E-03	2.322	.064
	Linear Term	Unweighted	2.335E-03	1	2.335E-03	.554	.459
		Weighted	3.280E-03	1	3.280E-03	.779	.380
		Deviation	3.584E-02	3	1.195E-02	2.836	.043
Within Groups			.329	78	4.212E-03		
Total			.368	82			

Post Hoc Tests

Homogeneous Subsets

begin % fat

Student-Newman-Keuls[a,b]

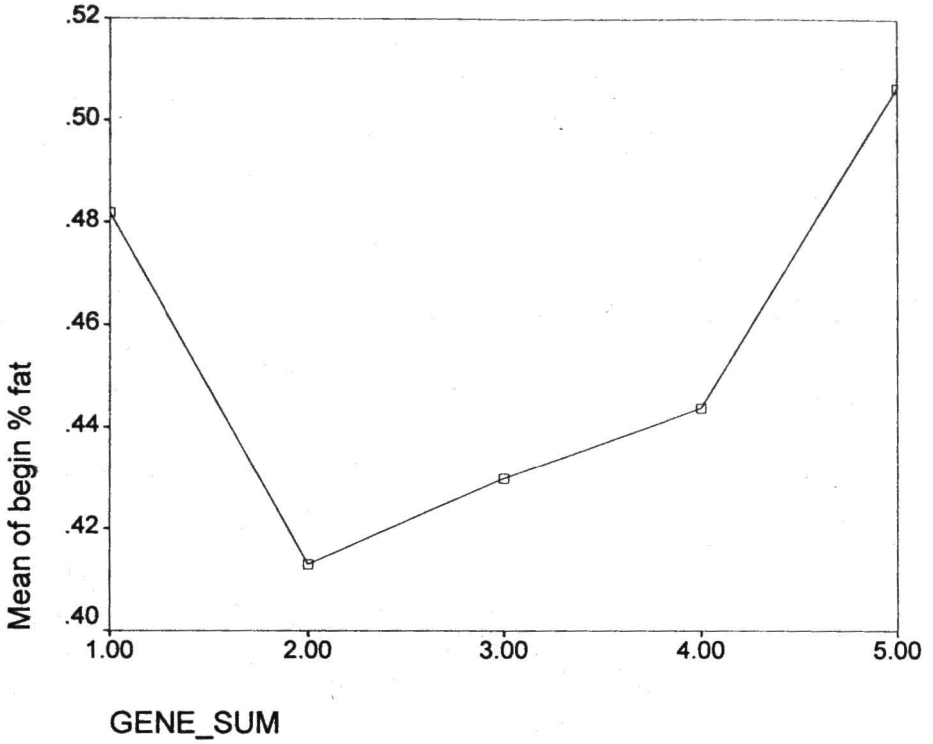
GENE_SUM	N	Subset for alpha = .05
		1
2.00	28	.412964
3.00	25	.429844
4.00	22	.443727
1.00	6	.481833
5.00	2	.506500
Sig.		.087

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.347.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Means Plots



Oneway

Descriptives

begin % fat

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
.00	12	.459000	.84524E-02	1.399E-02	.428215	.489785	.3930	.5500
1.00	33	.409303	.86278E-02	1.195E-02	.384969	.433637	.2950	.5380
2.00	32	.439972	.55435E-02	1.159E-02	.416341	.463603	.2850	.5440
3.00	6	.480167	.73391E-02	2.341E-02	.419993	.540340	.3780	.5390
Total	83	.433435	.69621E-02	7.350E-03	.418813	.448057	.2850	.5500

Test of Homogeneity of Variances

begin % fat

Levene Statistic	df1	df2	Sig.
1.064	3	79	.369

ANOVA

begin % fat

			Sum of Squares	df	Mean Square	F	Sig.
Between Groups	(Combined)		4.153E-02	3	1.384E-02	3.353	.023
	Linear Term	Unweighted	3.836E-03	1	3.836E-03	.929	.338
		Weighted	3.854E-03	1	3.854E-03	.934	.337
		Deviation	3.768E-02	2	1.884E-02	4.563	.013
Within Groups			.326	79	4.128E-03		
Total			.368	82			

Post Hoc Tests

Homogeneous Subsets

begin % fat

Student-Newman-Keuls[a,b]

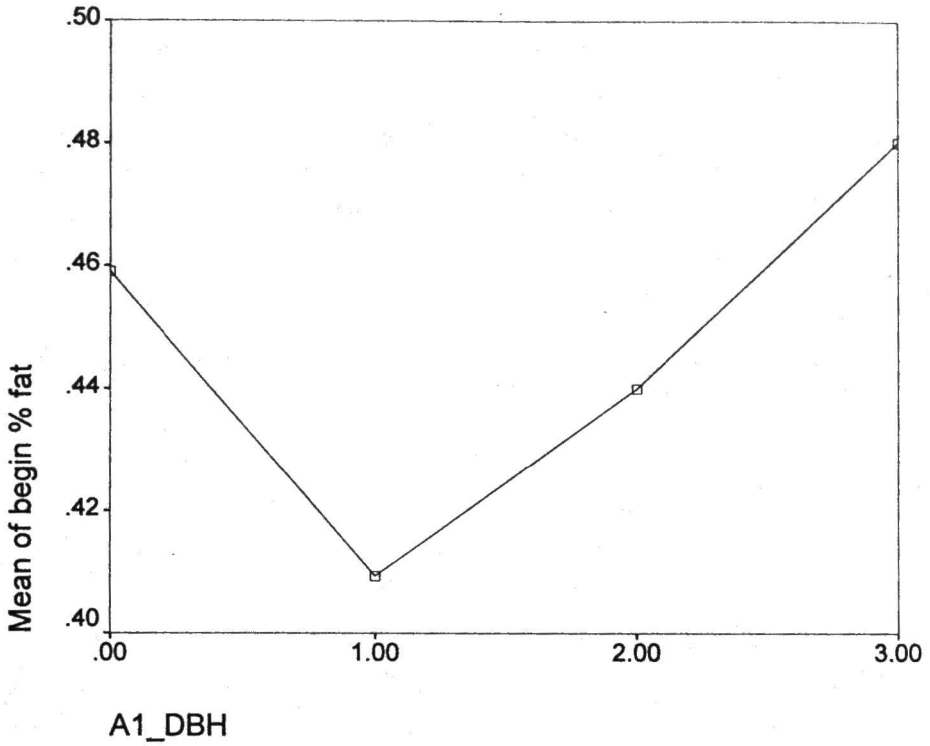
		Subset for alpha = .05	
A1_DBH	N	1	2
1.00	33	.409303	
2.00	32	.439972	.439972
.00	12	.459000	.459000
3.00	6		.480167
Sig.		.129	.258

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 12.839.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Means Plots



Frequencies

Statistics

DBH

N	Valid	94
	Missing	53

DBH

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	B2B2	34	23.1	36.2	36.2
	B1B2	57	38.8	60.6	96.8
	B1B1	3	2.0	3.2	100.0
	Total	94	63.9	100.0	
Missing	System	53	36.1		
Total		147	100.0		

Crosstabs

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
A1 allele * GROUP	127	100.0%	0	.0%	127	100.0%

A1 allele * GROUP Crosstabulation

			GROUP		
			Super Controls	Obese	Total
A1 allele	A2A2	Count	29	31	60
		% within A1 allele	48.3%	51.7%	100.0%
		% within GROUP	96.7%	32.0%	47.2%
		% of Total	22.8%	24.4%	47.2%
	A1A1 or A1A2	Count	1	66	67
		% within A1 allele	1.5%	98.5%	100.0%
		% within GROUP	3.3%	68.0%	52.8%
		% of Total	.8%	52.0%	52.8%
Total		Count	30	97	127
		% within A1 allele	23.6%	76.4%	100.0%
		% within GROUP	100.0%	100.0%	100.0%
		% of Total	23.6%	76.4%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	38.493[b]	1	.000		
Continuity Correction[a]	35.941	1	.000		
Likelihood Ratio	45.352	1	.000		
Fisher's Exact Test				.000	.000
Linear-by-Linear Association	38.190	1	.000		
N of Valid Cases	127				

a. Computed only for a 2x2 table

b. 0 cells (.0%) have expected count less than 5. The minimum expected count is 14.17.

Risk Estimate

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for A1 allele (A2A2 / A1A1 or A1A2)	61.742	8.039	474.187
For cohort GROUP = Super Controls	32.383	4.549	230.543
For cohort GROUP = Obese	.524	.410	.671
N of Valid Cases	127		

Logistic Regression

```
Dependent Variable..   GROUP

Beginning Block Number  0.  Initial Log Likelihood Function

-2 Log Likelihood   138.85775

* Constant is included in the model.


Beginning Block Number  1.  Method: Enter

Variable(s) Entered on Step Number
1..        GENE       A1 allele

Estimation terminated at iteration number 6 because
Log Likelihood decreased by less than .01 percent.

 -2 Log Likelihood          93.505
 Goodness of Fit           126.999
 Cox & Snell - R^2            .300
 Nagelkerke - R^2             .452

                    Chi-Square     df Significance

 Model                  45.352      1         .0000
 Block                  45.352      1         .0000
 Step                   45.352      1         .0000

Classification Table for GROUP
The Cut Value is .50
                                Predicted
                          Super Controls       Obese        Percent Correct
                                       S       I       O
Observed                  +---------------+---------------+
   Super Controls      S  I         0      I       30      I      .00%
                          +---------------+---------------+
   Obese               O  I         0      I       97      I   100.00%
                          +---------------+---------------+
                                                 Overall    76.38%

---------------------- Variables in the Equation -----------------------

Variable            B        S.E.      Wald     df      Sig        R     Exp(B)

GENE(1)       -4.1230      1.0401   15.7122      1     .0001   -.3142      .0162
Constant       4.1896      1.0075   17.2913      1     .0000
```

Crosstabs DBH ----------

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Ilele * GROUP	140	100.0%	0	.0%	140	100.0%

Ilele * GROUP Crosstabulation

			GROUP		
			Super Controls	Obese	Total
Ilele	2 2	Count	24	31	55
		% within Ilele	43.6%	56.4%	100.0%
		% within GROUP	47.1%	34.8%	39.3%
		% of Total	17.1%	22.1%	39.3%
	1,2 or 2,2	Count	27	58	85
		% within Ilele	31.8%	68.2%	100.0%
		% within GROUP	52.9%	65.2%	60.7%
		% of Total	19.3%	41.4%	60.7%
Total		Count	51	89	140
		% within Ilele	36.4%	63.6%	100.0%
		% within GROUP	100.0%	100.0%	100.0%
		% of Total	36.4%	63.6%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	2.032[b]	1	.154		
Continuity Correction[a]	1.552	1	.213		
Likelihood Ratio	2.019	1	.155		
Fisher's Exact Test				.208	.107
Linear-by-Linear Association	2.018	1	.155		
N of Valid Cases	140				

a. Computed only for a 2x2 table

b. 0 cells (.0%) have expected count less than 5. The minimum expected count is 20.04.

Risk Estimate

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for Ilele (2 2 / 1,2 or 2,2)	1.663	.824	3.355
For cohort GROUP = Super Controls	1.374	.891	2.118
For cohort GROUP = Obese	.826	.628	1.086
N of Valid Cases	140		

Logistic Regression for DBH----------

Dependent Variable.. GROUP

Beginning Block Number 0. Initial Log Likelihood Function

-2 Log Likelihood 183.63639

* Constant is included in the model.

Beginning Block Number 1. Method: Enter

Variable(s) Entered on Step Number
1.. GENE llele

Estimation terminated at iteration number 3 because
parameter estimates changed by less than .001

-2 Log Likelihood	181.617
Goodness of Fit	140.000
Cox & Snell - R^2	.014
Nagelkerke - R^2	.020

	Chi-Square	df	Significance
Model	2.019	1	.1553
Block	2.019	1	.1553
Step	2.019	1	.1553

Classification Table for GROUP
The Cut Value is .50

		Predicted		
		Super Controls S	Obese O	Percent Correct
Observed				
Super Controls	S	0	51	.00%
Obese	O	0	89	100.00%
			Overall	63.57%

---------- Variables in the Equation ----------

Variable	B	S.E.	Wald	df	Sig	R	Exp(B)
GENE(1)	-.5087	.3581	2.0183	1	.1554	-.0100	.6013
Constant	.7646	.2330	10.7708	1	.0010		

Crosstabs DAT 1010 ----------

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
llele * GROUP	188	100.0%	0	.0%	188	100.0%

Ilele * GROUP Crosstabulation

			GROUP		
			Super Controls	Obese	Total
Ilele	2 2	Count	57	54	111
		% within Ilele	51.4%	48.6%	100.0%
		% within GROUP	62.6%	55.7%	59.0%
		% of Total	30.3%	28.7%	59.0%
	1,2 or 2,2	Count	34	43	77
		% within Ilele	44.2%	55.8%	100.0%
		% within GROUP	37.4%	44.3%	41.0%
		% of Total	18.1%	22.9%	41.0%
Total		Count	91	97	188
		% within Ilele	48.4%	51.6%	100.0%
		% within GROUP	100.0%	100.0%	100.0%
		% of Total	48.4%	51.6%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.942[b]	1	.332		
Continuity Correction[a]	.676	1	.411		
Likelihood Ratio	.944	1	.331		
Fisher's Exact Test				.374	.205
Linear-by-Linear Association	.937	1	.333		
N of Valid Cases	188				

a. Computed only for a 2x2 table

b. 0 cells (.0%) have expected count less than 5. The minimum expected count is 37.27.

Risk Estimate

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for Ilele (2 2 / 1,2 or 2,2)	1.335	.745	2.393
For cohort GROUP = Super Controls	1.163	.853	1.585
For cohort GROUP = Obese	.871	.661	1.148
N of Valid Cases	188		

Logistic Regression DAT 1010

Dependent Variable.. GROUP

Beginning Block Number 0. Initial Log Likelihood Function

-2 Log Likelihood 260.43182

* Constant is included in the model.

Beginning Block Number 1. Method: Enter

Variable(s) Entered on Step Number
1.. GENE llele

Estimation terminated at iteration number 2 because
Log Likelihood decreased by less than .01 percent.

-2 Log Likelihood	259.488
Goodness of Fit	188.000
Cox & Snell - R^2	.005
Nagelkerke - R^2	.007

	Chi-Square	df	Significance
Model	.944	1	.3313
Block	.944	1	.3313
Step	.944	1	.3313

Classification Table for GROUP
The Cut Value is .50

		Predicted		
		Super Controls S	Obese O	Percent Correct
Observed				
Super Controls	S	57	34	62.64%
Obese	O	54	43	44.33%
			Overall	53.19%

---------- Variables in the Equation ----------

Variable	B	S.E.	Wald	df	Sig	R	Exp(B)
GENE(1)	-.2889	.2979	.9407	1	.3321	.0000	.7491
Constant	.2348	.2295	1.0471	1	.3062		

CHAPTER VII

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