Genetic Dissection of Atypical Antipsychotic–Induced Weight Gain: Novel Preliminary Data on the Pharmacogenetic Puzzle

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Atypical antipsychotics such as clozapine represent a significant improvement over typical antipsychotics in the treatment of schizophrenia, particularly regarding extrapyramidal symptoms. Despite their benefits, use is limited by the occurrence of adverse reactions such as sedation and weight gain. This article provides a comprehensive review and discussion of obesity-related pathways and integrates these with the known mechanisms of atypical antipsychotic action to identify candidate molecules that may be disrupted during antipsychotic treatment. Novel preliminary data are presented to genetically dissect these obesity pathways and elucidate the genetic contribution of these candidate molecules to clozapine-induced weight gain. There is considerable variability among individuals with respect to the ability of clozapine to induce weight gain. Genetic predisposition to clozapine-induced weight gain has been suggested. Therefore, genetic variation in these candidate molecules may predict patient susceptibility to clozapine-induced weight gain. This hypothesis was tested for 10 genetic polymorphisms across 9 candidate genes, including the serotonin 2C, 2A, and 1A receptor genes (HTR2C/2A/1A); the histamine H_1 and H_2 receptor genes (H1R/H2R); the cytochrome P450 1A2 gene (CYP1A2); the β_3 and α_{1a}-adrenergic receptor genes (ADRB3/ADRA1A); and tumor necrosis factor α (TNF-α). Prospective weight gain data were obtained for 80 patients with schizophrenia who completed a structured clozapine trial. Trends were observed for ADRB3, ADRA1A, TNF-α, and HTR2C; however, replication in larger, independent samples is required. Although in its infancy, psychiatric pharmacogenetics will in the future aid clinical practice in the prediction of response and side effects, such as antipsychotic-induced weight gain, and minimize the current "trial and error" approach to prescribing.

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ANTIPSYCHOTICS AND WEIGHT GAIN

The pharmacologic treatment of schizophrenia continues to present a therapeutic challenge for clinicians. Although both classes of antipsychotics, typical and atypical, offer some degree of efficacy, it is clear that they do not ameliorate all symptoms of the disease. A significant drawback to the treatment of schizophrenia with antipsychotics is the occurrence of adverse reactions. Typical antipsychotics can cause sexual dysfunction and induce movement side effects, including extrapyramidal symptoms and tardive dyskinesia. Novel atypical antipsychotics offer a number of tolerability benefits over the traditional typical antipsychotics, principally regarding extrapyramidal symptoms. However, the differential binding profile between the 2 classes, in terms of both the variety of receptors that the antipsychotics have affinity for and the range of affinities for each receptor, contributes to differences demonstrated with respect to the side effect profile. Although atypical antipsychotics have a lower incidence of motor side effects, their use is hindered by side effects such as weight gain and sedation. Given the current increase in popularity of the novel atypical antipsychotics, a particularly pressing issue has become the prevalent side effect of weight gain. This side effect is often ignored because it is associated with a common and "normal" presentation compared with other antipsychotic side effects. However, it is clear that this side effect can undermine

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compliance, inclining patients to experience a relapse, and may also lead to significant psychological distress and medical morbidity and mortality.

Most of the novel antipsychotics cause a considerable amount of weight gain,[1] and individuals with schizophrenia are as obese or more obese than individuals without schizophrenia.[2] Several atypical antipsychotics can cause clinical obesity, as defined by a body mass index (BMI; calculated weight in kilograms divided by height in meters squared) > 30 kg/m^2. Obesity is a chronic disorder and represents a serious threat to public health. Specifically, weight gain and obesity have been associated with significant morbidity and mortality from diseases such as type 2 diabetes mellitus, hypertension, coronary artery disease, cerebrovascular disease, respiratory dysfunction, gallbladder disease, osteoarthritis, and some types of cancer (e.g., colon, breast, endometrial, prostate, gallbladder).[3] Mortality rates are slightly elevated in people who are marginally overweight (BMI = 25–30 kg/m^2), while these rates increase dramatically in the clinically obese (BMI > 30 kg/m^2), particularly those with excessive visceral abdominal fat stores.[4] Most alarming is evidence that the prevalence of clinical obesity in the United States has increased from 12.0% to 17.9% between 1991 and 1998; this is a trend that has been noted in other countries as well.[5]

In addition to these threats to health and longevity, clinical obesity caused by atypical antipsychotics can lead to psychological distress and cause patients to discontinue their medications. Weight gain can affect self-esteem, and patients have indicated that this side effect is the most distressing, surpassing both sedation and sexual dysfunction.[6] Although there is a lack of specific studies looking at the effects of weight gain on compliance, clinically it is understood that weight gain negatively affects compliance and can increase the risk of relapse. Furthermore, particularly in Western societies, the existence of negative attitudes toward obesity may place patients at further disadvantage in terms of reintegration into society. It has been shown that biases toward obesity can lead to social disadvantages in obtaining employment, a residence, an education, and a spouse.[3,6] These findings, along with the serious health implications associated with weight gain, demonstrate that the common antipsychotic side effect of weight gain is a critical clinical as well as public health concern that needs to be addressed.

PSYCHIATRIC PHARMACOGENETICS AND PHARMACOGENOMICS

The treatment of schizophrenia presents clinicians with a therapeutic challenge, and often a "trial and error" approach to prescribing is applied. Clinicians often find themselves changing the prescribed antipsychotic or titrating the dose to maximize efficacy and minimize side effects. Patients clearly respond differently to the same recommended dose of a particular antipsychotic, with some responding adequately to treatment, others showing little response to treatment, and others developing toxic adverse reactions. There appears to be considerable variability among patients in terms of both the efficacy of antipsychotics to alleviate psychosis as well as their propensity to develop an antipsychotic side effect such as weight gain (i.e., not all treated patients actually gain weight, and the extent of the weight gain varies considerably from patient to patient). This side effect occurs in only a proportion of predisposed patients. It is likely that this variability in patient propensity to gain weight is determined by a combination of genetic and environmental factors. The area of psychiatric pharmacogenetics seeks to merge the fields of genetics and pharmacology to try to predict the clinical effects of the medication. The final outcome of the research is to identify, before treatment, those patients for whom the medication will be most efficacious as well as those patients who will or will not present with a side effect, ultimately leading to a reduction in drug-related morbidity and mortality.

Pharmacogenetics refers to the effect of inheritance on interindividual variations in outcomes to xenobiotics and drugs.[7] These variations may involve interindividual differences in therapeutic efficacy or adverse drug reactions. Pharmacogenetic research is a well-established discipline with an almost 40-year history.[8] This research uses many different strategies that start with identification of candidate proteins that are pertinent to the action or metabolism of a given drug. Examples of such proteins are biotransformation enzymes, drug receptor or transporter molecules, and signal-transducing protein complexes. Genetic variation within the genes that encode these candidate proteins is then analyzed using molecular genetic and statistical techniques to determine its contribution to variation in drug response and/or side effects.

Pharmacogenomics is defined as the compilation of comprehensive information about genomic sequences using techniques such as gene mapping, sequencing, statistical genetics, and expression analysis.[7] This information is then applied to the identification of genomic "hot spots" and subsequently to the discovery of susceptibility loci contributing to interindividual variation in drug response and side effect profiles.[9,10] Recently, Nebert[11] noted that the 2 terms, *pharmacogenetics* and *pharmacogenomics*, have been used interchangeably, but differences can be drawn in that pharmacogenomics emphasizes the development of novel drugs based on newly discovered genes from the genome project.

The aim of both pharmacogenomic and pharmacogenetic studies is to determine the impact of genetic variation or polymorphisms on the interindividual differences in drug outcomes, with the ultimate goal of predicting the patient's response to medication and/or propensity to develop side effects.[10] The difference between these 2 related fields lies in their methodological approach. In particular,

Table 1. Atypical Antipsychotic–Induced Weight Gain: A Complex Phenotype

Biological and genetic factors
Liver pharmacokinetics
Brain pharmacodynamics
Adipose endocrine function
White adipose tissue
Brown adipose tissue
Pancreatic insulin and diabetes
Demographic factors
Age
Ethnicity
Sex
Response to drug treatment
Weight before drug treatment
Environmental factors
Smoking
Dieting
Nutrition
Exercise habits
Emotional changes
Increased food availability
High-fat diet
Socioeconomic status

pharmacogenetics is based on a priori hypotheses regarding candidate genes, while pharmacogenomics uses techniques that screen markers across the entire genome to identify chromosomal regions of interest. In the future, techniques such as DNA chip microarray polymorphism detection and genotyping will become increasingly important in these rapidly expanding fields.[12] The recent completion of the Human Genome Project will complement and advance work in pharmacogenomics by providing the complete location and sequence variants within the human genome. In addition, this same information will be known for the human genes that may be of particular interest in pharmacogenetics.

Although there is a rich history of pharmacogenetic research, the area of psychiatric pharmacogenetics is a relatively young field, with most research conducted in the last decade. The foundation for psychiatric pharmacogenetic research regarding antipsychotics has been laid by studies of clozapine response (comprehensively reviewed in Masellis et al.[13] and Arranz et al.[14]). Much progress has also been made regarding the pharmacogenetics of antipsychotic adverse reactions, and most promising have been the multiple replications of an association between typical antipsychotic–induced tardive dyskinesia and a genetic variant of the dopamine D_3 receptor gene.[15–18] Surprisingly, very little research has been conducted investigating pharmacogenetic predictors of the antipsychotic side effect of weight gain. To date, there has been a single published study[19] that briefly mentioned a lack of association between a genetic polymorphism in the 5-HT_{2C} receptor gene and clozapine-induced weight gain.

In general, pharmacogenetic phenotypes such as antipsychotic-induced weight gain fit the definition of a genetic "complex trait" as proposed by Lucek and Ott[20]; as such, trying to uncover genetic contributors to these phenotypes is much more difficult than for simple single-gene Mendelian traits. With complex traits, multiple genes are usually involved; with each interacting with intricate patterns (additive, epistatic, or heterogeneity) and differing extent of contribution, the mode of inheritance is unclear (dominant or recessive transmission), and there are multiple environmental and demographic factors that can influence the phenotype. When studying these pharmacogenetic phenotypes, it is important to be aware of the myriad of biological, environmental, and demographic factors that can influence the phenotype. Within the biological realm, it is imperative to investigate both pharmacodynamic factors (i.e., genetic variation in the central and peripheral antipsychotic target receptors) and the often-underestimated pharmacokinetic factors, including genetic variation in the enzymes that metabolize the antipsychotics. A patient expressing a particular enzyme variant for rapid metabolism may quickly reduce the antipsychotic concentration below the therapeutic plasma level; on the other hand, a patient with a slowly metabolizing version of this enzyme may accumulate toxic drug concentrations, predisposing to an adverse reaction such as weight gain. In terms of environmental and demographic factors, clearly environmental aspects such as patient diet, smoking, and exercise habits can influence the expression of a side effect such as weight gain. In addition, demographic variables such as female sex, certain ethnicities (e.g., African American), younger age, and low pretreatment (baseline) weight/fat content may increase the propensity and extent of weight gain during treatment with atypical antipsychotics.[21,22] When possible, it is important to include these factors into studies that try to uncover genetic contributors to control for their possible confounding effects. Table 1 summarizes the potential contributors.

The purpose of this article is to provide a brief review of the most significant findings regarding obesity pathways and research, speculate on the possible mechanisms through which an atypical antipsychotic may interfere with these pathways to cause weight gain, provide novel preliminary data from our research team that attempt to dissect these putative pathways using clozapine as a model to try to identify genetic variation that may, in the future, be used to predict those patients who will or will not gain weight while being treated, and introduce a field of study that may profoundly influence the current state of clinical prescribing in psychiatry by minimizing the "trial and error" approach.

CLOZAPINE AS A MODEL OF ATYPICAL ANTIPSYCHOTIC–INDUCED WEIGHT GAIN

Clozapine, 8-chloro-11-(4-methyl-1-piperazinyl)-5*H*-dibenzo [*b*,*e*] [1,4] diazepine, is a member of the dibenzodiazepine class of antipsychotic drugs. Clozapine,

the prototype atypical antipsychotic, remains the most effective agent for the treatment of patients with refractory schizophrenia; in recent years, it has gained much popularity as a first-line treatment. Among the atypical antipsychotics, clozapine and olanzapine appear to have the greatest weight gain liability.[1] Clozapine has affinities ranging from low to high for many receptors from multiple neurotransmitter systems. These include the dopamine D_1–D_5; serotonin 5-$HT_{1A/1D}$, 5-$HT_{2A/2C}$, 5-HT_3, 5-HT_6, and 5-HT_7; histaminergic H_1–H_3; muscarinic $M_{1–5}$; $GABA_A$; and $\alpha_{1–2}$-adrenergic and $\beta_{1–3}$-adrenergic receptors. (For a review of the K_i values of clozapine for these receptors, see Ashby and Wang[23] and Bymaster et al.[24]). Olanzapine, a thienobenzodiazepine with antipsychotic effects, displays a similar binding profile to clozapine, with high receptor affinity binding in vitro at serotonin 5-$HT_{2A/2C}$, dopamine D_1 and D_4, muscarinic $M_{1–5}$, α_1-adrenergic, and histaminergic H_1 receptors.[25] Differences in receptor-binding profiles among the various antipsychotics as well as differences in affinity for these receptors and differing drug metabolism may contribute to the varying weight gain liabilities associated with the antipsychotics.

Reviewing the literature, Leadbetter et al.[26] found that 13% to 85% of patients treated with clozapine had an associated increase in weight. Umbricht et al.[27] found that the cumulative incidence of all patients reaching 20% or more overweight, representing a significant long-term health risk, was > 50%. Bromel et al.[28] demonstrated that 75% of their patients treated with clozapine reported an increased appetite and desire to eat, with some patients reporting binge-eating episodes. Clearly, some patients gain weight while others do not when treated with clozapine. Genetic predisposition to the ability of clozapine to induce weight gain has been suggested,[27,29] and there is ample evidence demonstrating that body weight, metabolism, and feeding behavior are influenced by genetic factors.[30,31]

Several studies investigating the relative contributions of genetic and environmental factors in regulation of body weight have indicated that as much as 70% of the variability in human body weight can be accounted for by genetic factors; however, each study notes that the issue is complex and that polygenic gene-gene, gene-environment interactions, with pleiotropic and nonpleiotropic effects, are likely to be involved.[32–35] Figure 1 attempts to integrate the various proposed mechanisms and pathways involved in weight regulation, satiety, basal metabolism, lipogenesis/lipolysis, and energy intake/expenditure, highlighting both the central nervous system (CNS) pathways in addition to peripheral pathways. Throughout this article, Figure 1 will be revisited to gain an appreciation for the complexities of what has already been discovered regarding the obesity-regulating pathways and then to hopefully demonstrate that some antipsychotics, such as clozapine, may hypothetically interfere with these pathways to cause the weight gain seen during treatment. It is hypothesized that genetic variation in these pathways, particularly those that are directly influenced by antipsychotics, may explain why some patients are predisposed to gaining weight while others seem to be protected from this side effect.

METHOD

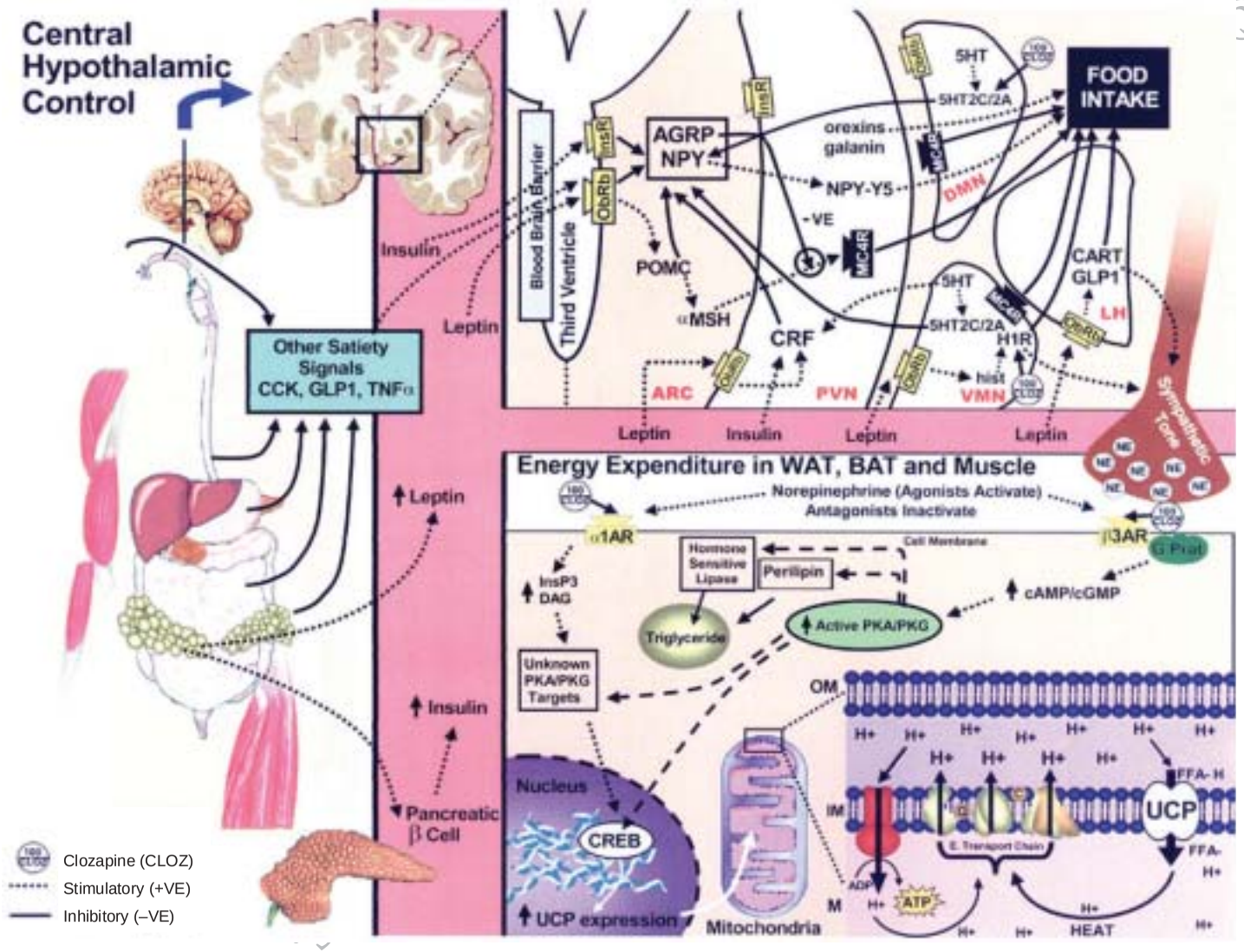
Clinical Sample

A sample of 80 patients with DSM-III-R diagnoses of schizophrenia was prospectively assessed for clozapine-induced weight gain. Patients were selected in the United States from the clinics of Herbert Y. Meltzer, M.D., and Jeffrey A. Lieberman, M.D. During treatment with clozapine, the weight of these patients in kilograms was prospectively assessed at baseline and 6 weeks. The phenotype used for the genetic analysis was a simple delta change score (6-week weight minus baseline weight). Table 2 summarizes the demographic characteristics of our sample.

Genetic Association Strategy Statistics

For each candidate gene variant/polymorphism, the patients were grouped according to genotype; for each of these groups, the mean value for the quantitative phenotype of weight change in kilograms from baseline to 6 weeks of treatment with clozapine was computed. The mean values for each genotype category were then compared statistically using an analysis of covariance (ANCOVA). The use of this parametric ANCOVA statistic is more powerful than the traditional nonparametric χ^2 case-control/Fisher exact designs that have been used in the past. From a statistical perspective, the parametric ANCOVA F statistic does not require as large a sample size to detect a significant association as would the traditional nonparametric case-control χ^2/Fisher exact tests and as such is more powerful. This method does not require control subjects because the variation in the continuous measure provides internal control. There is also no loss of information that inherently occurs when patients are categorized using weight gainers and non–weight gainers. With categorical designs, an extreme weight gainer is treated no differently than a patient who is slightly over the threshold. This type of analysis is also advantageous in that it allows for the incorporation of demographic and environmental factors into the analysis and thus allows control for the putative confounding effects of these additional variables. We were able to correct for possible demographic confounders such as sex, ethnicity, pretreatment baseline weight, and responder/nonresponder status by including them as covariates in the analysis. It is acknowledged that a better phenotype such as waist circumference or waist-to-hip ratio could have been used and that several environmental factors may be involved and should also be included as covariates; however, this information was not available for this sample. The assumptions of the ANCOVA statistic were tested to determine the appropriateness of the sample. The assumption of

Figure 1. Putative Antipsychotic Disruption of Central and Peripheral Obesity-Related Pathways[a]



[a]Abbreviations:
ADP = adenosine diphosphate
AGRP = agouti-related protein
αMSH = α-melanocyte-stimulating hormone
α1AR = α_1-adrenergic receptor
ARC = arcuate nucleus
ATP = adenosine triphosphate
BAT = brown adipose tissue
β3AR = β_3-adrenergic receptor
cAMP = cyclic adenosine monophosphate
C = cytochrome C
CART = cocaine and amphetamine–regulated transcript
CCK = cholecystokinin
cGMP = cyclic guanosine monophosphate
CREB = cyclic AMP response element–binding protein transcription factor
CRF = corticotropin-releasing factor
DAG = diacylglycerol
DMN = dorsomedial nucleus
E. = Electron (Transport Chain)
FFA-H = free fatty acid
FFA-H^+ = oxidized free fatty acid
5HT = serotonin
GLP1 = glucagon-like peptide
G.Prot = G protein
hist = histamine
H1R = histamine H_1 receptor
IM = inner mitochondrial membrane
InsP3 = inositol triphosphate
InsR = insulin receptor
LH = lateral hypothalamic area
M = mitochondrial matrix
MC4R = melanocortin 4 receptor
NE = norepinephrine
NPY = neuropeptide Y
ObRb = leptin receptor
OM = outer mitochondrial membrane
POMC = pro-opiomelanocortin
PKA = protein kinase A
PKG = protein kinase G
PVN = paraventricular nucleus
Q = cytochrome Q
TNFα = tumor necrosis factor α
UCP = uncoupling protein
+VE = stimulatory
–VE = inhibitory
VMN = ventromedial nucleus
WAT = white adipose tissue

equal variance between each genotypic class was tested using the Levene test for homogeneity of variances. The Kolmogorov-Smirnov test was used to test for normal distributions in each genotypic category. A χ^2 test was used to test whether the distribution of genotypes was in accordance with Hardy-Weinberg equilibrium. A Tukey HSD test for post hoc comparisons was used to determine where any differences in mean existed among the genotypic categories. A difference between genotypic categories may indicate a putative role for the particular genetic variant in the observed interindividual variability in the clozapine-induced weight gain phenotype. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS; Chicago, Ill.), version 10.0. Our group has pioneered the use of this method in the genetic analysis of "complex traits" and has now uncovered 2 genetic risk loci (the dopamine D_3 receptor gene and the cytochrome P450 1A2 gene) that contribute to the prediction of typical neuroleptic-induced tardive dyskinesia.[15,36,37] We have also developed novel statistics to determine the specific ways in which combinations of genes interact to produce a given phenotype.[38,39]

OBESITY AND WEIGHT REGULATION RESEARCH

Obesity and Energy Homeostasis

Obesity results when energy balance tips toward the side of chronic excess in energy intake rather than toward the side of energy expenditure (Figure 2). Several biological/genetic and environmental/acquired factors influence feeding control, energy efficiency, and fat accumulation/adipogenesis, resulting in this shift in energy balance. One must initially gain an appreciation for the genetic products associated with these biochemical processes to speculate on possible disruptions caused by antipsychotic medications.

In terms of feeding control, several central and peripheral pathways exist that govern sensations of satiation and hunger. Appetite regulation involves both short-term response pathways, such as those signals that regulate satiation after a meal or hunger from gustatory or olfactory messages (e.g., cholecystokinin, glucagon-like peptide GLP-1), and long-term response pathways, including those that signal the state of energy reserves (e.g., peripheral adipose tissue size signals, leptin, insulin).

Collectively, data from several research paradigms converge and suggest that atypical antipsychotic–induced weight gain and obesity result from multiple neurotransmitter/receptor interactions with resultant strong alterations in appetite and feeding behavior, although metabolic changes also contribute. Patients treated with clozapine generally report that they are unable to control their appetite even after eating a full meal. Satiety signals arise in a variety of areas, including the olfactory and gustatory tracts, esophagus, stomach, liver, and intestines, and are processed centrally in the hypothalamus, which contributes to the regulation and maintenance of an individual's homeostatic body weight. Therefore, it is possible that some antipsychotics may disturb satiety processing in the hypothalamus by binding to receptors involved in weight and satiety regulation. Consequently, genetic differences in these candidate receptors that have affinity for clozapine and are expressed in the hypothalamus are prime candidates to be investigated when trying to uncover genetic determinants of clozapine-induced changes in satiety and subsequent weight gain.

Much less has been postulated regarding the potential disruption of energy efficiency and adipogenesis by antipsychotic treatment. Energy efficiency refers to the degree with which an individual actually utilizes and stores the energy supplied in food that is ingested. Thermogenesis and resting metabolic rate are implicated in energy efficiency concerns, and clearly some individuals have greater resting metabolic rates than other people and dissipate food energy as heat rather than using or storing this energy as fat for later use. Thermogenesis is mediated by mitochondrial uncoupling proteins (UCP1–3), which channel food energy to the dissipation of heat rather than being used to create usable energy (i.e., adenosine triphosphate [ATP]).

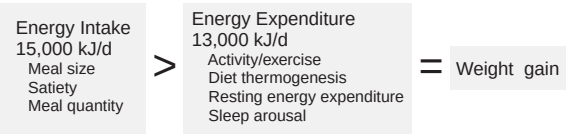
Adipogenesis is the process through which preadipocytes differentiate into fat-storing adipocytes. Increases in both the number of adipocytes and in the amount of fat

Table 2. Demographic Characteristics of the Patients Used in This Current Study (2 Clinical Research Facilities)[a]

Characteristic	Meltzer[a] (N = 64)	Lieberman[a] (N = 16)	Total Sample (N = 80)
Age (y),[b] mean ± SD	33.3 ± 8.8	32.0 ± 6.4	33.1 ± 8.35
Gender,[c] N			
Male	44	8	52 (65%)
Female	20	8	28 (35%)
Ethnicity,[d] N			
Caucasian	45	13	58 (72%)
African American	19	3	22 (28%)
Smoking status, N			
Smokers	26	0	26 (32%)
Nonsmokers	12	0	12 (15%)
Unknown	26	16	42 (53%)

[a]Unpublished data from Herbert Y. Meltzer, M.D., and Jeffrey A. Lieberman, M.D.
[b]F = 0.32, df = 2,78; p = .57 (age vs. clinical site).
[c]χ^2 = 0.16, df = 1, p = .69 (gender vs. clinical site).
[d]χ^2 = 0.38, df = 1, p = .54 (ethnicity vs. clinical site).

Figure 2. Cause for Obesity Occurrence[a]



[a]Obesity occurs when energy intake chronically exceeds energy expenditure.

Table 3. Summary of Selected Endogenous Molecules and Their Effects on Energy Homeostasis Pathways[a]

Symbol	Molecule	Effect of ↑ Levels on Energy Homeostasis	Putative Atypical Antipsychotic (AA) Interaction
Ob, Lep	Leptin	↓ food intake, ↓ adipogenesis, ↑ EE, ↓ weight gain	Indirect increases seen with AA Rx, AA may affect leptin sensitivity
Ins	Insulin	↓ food intake, ↑ EE, ↓ weight gain	Indirect increases seen with AA Rx, with subsequent ↓ insulin sensitivity
NPY	Neuropeptide Y	↑ food intake, ↓ EE, ↑ weight gain	Indirect: AAs modulate NPY expression, inconsistencies regarding directionality
POMC	Pro-opiomelanocortin	↓ food intake, ↓ adipogenesis, ↑ EE, ↓ weight gain	?
α MSH	α Melanocyte-stimulating hormone	↓ food intake, ↓ adipogenesis, ↑ EE, ↓ weight gain	?
β END	β Endorphin	↑ food intake, ↑ weight gain	?
MC4R	Melanocortin 4 receptor	Agonism causes ↓ food intake, ↓ adipogenesis, ↑ EE, ↓ weight gain	?
AGRP	Agouti-related protein	↑ food intake, ↓ EE, ↑ weight gain	?
MCH	Melanin-concentrating hormone	↑ food intake, ↑ weight gain	?
CART	Cocaine and amphetamine–regulated transcript	↓ food intake, ↑ EE, ↓ weight gain	?
CRF	Corticotropin-releasing factor	↓ food intake, ↑ EE, ↓ weight gain	?
PPAR-γ	Peroxisome proliferation–activated receptor γ	↑ differentiation of fibroblasts or preadipocytes into mature adipocytes, ↑ adipogenesis, ↑ insulin sensitivity, ↑ weight gain	Indirect: AAs ↑ TNF-α, which interacts with PPAR-γ
UCP1–3	Uncoupling protein	↑ in peripheral thermogenesis, ↑ EE, ↓ weight gain	Indirect: AAs ↓ UCP expression via antagonism of adrenergic receptors
CCK	Cholecystokinin	↓ food intake, ↓ weight gain	? Indirect: CCK expression ↑ following clozapine Rx
GLP-1	Glucagon-like peptide	↓ food intake, ↓ weight gain	?
OREX	Orexin A/B	↑ food intake, ↓ EE, ↑ weight gain	?
GAL	Galanin	↑ food intake, ↓ EE, ↑ weight gain	?
OXT	Oxytocin	↓ food intake, ↑ EE, ↓ weight gain	? Indirect: clozapine causes ↑ oxytocin
5-HT	Serotonin	↓ food intake, ↓ weight gain	See $5\text{-HT}_{2C/1A/2A}$ below
5-HT_{2C}	Serotonin 2C receptor	Antagonism causes ↑ food intake, ↑ weight gain	Direct antagonism
5-HT_{2A}	Serotonin 2A receptor	Antagonism causes ↑ food intake, ↓ EE, ↑ weight gain	Direct antagonism
5-HT_{1A}	Serotonin 1A receptor	Agonism causes ↑ food intake, ↓ EE, ↑ weight gain	Direct partial agonism by clozapine
H1R	Histamine H_1 receptors	Antagonism causes ↑ food intake, ↓ EE, ↑ adipogenesis, ↑ weight gain	Direct antagonism
NE	Norepinephrine	↓ adipogenesis, ↑ EE, ↓ weight gain	See β3AR and α1AR below
β3AR	β_3-Adrenergic receptor	Antagonism causes ↑ adipogenesis, ↓ lipolysis, ↓ insulin sensitivity, ↓ EE, ↑ weight gain	Direct antagonism
α1AR	α_1-Adrenergic receptor	Antagonism causes ↑ adipogenesis, ↓ EE, ↑ weight gain	Direct antagonism
M3R	Muscarinic M_3 receptor	Agonism causes ↑ food intake, ↑ adipogenesis, ↓ EE, ↑ weight gain	Direct partial agonism by clozapine
TNF-α	Tumor necrosis factor α	Slight increases cause ↑ differentiation of preadipocytes into mature adipocytes, ↑ adipogenesis, ↓ insulin sensitivity, ↑ weight gain; "supraphysiologic" increases cause cachexia , ↑ proteolysis	Indirect increases seen with AA Rx

[a]Other abbreviations and symbols: EE = energy expenditure, Rx = treatment, UCP = uncoupling protein, ↑ = increased, ↓ = decreased, ? = possible or unknown.

stored within these adipocytes can influence an individual's tendency to gain weight. Peroxisome proliferator–activated receptors have been implicated as potential contributors to the number of adipocytes. Other factors in this system include differential substrate oxidation (e.g., lipids and carbohydrates), adipose tissue metabolism, fat storage and energy expenditure due to physical activity, diet-induced thermogenesis, and resting processes (see Figure 2).

A more detailed account of the aforementioned gene products and the biochemical pathways involved in feeding control, energy efficiency, and fat accumulation/adipogenesis will be provided in the subsequent sections. Table 3 provides a concise summary of these products and their effects on energy homeostasis. Antipsychotics may hypothetically interfere with all of these pathways to cause the side effect of weight gain, and this will be further discussed in the genetic dissection section of this report.

Central Regulation and the Hypothalamus

The regulation of energy balance requires a number of CNS structures that must integrate multiple neural, endocrine, and metabolic signals to assess the immediate and long-term biological need for energy, to generate or inhibit conscious experiences of satiety or hunger, and

subsequently to elicit the appropriate behavioral, autonomic, and endocrine responses. Several higher brain regions are involved in energy homeostasis, although a multitude of classical studies that have used lesioning, ablation, and electrical stimulation techniques implicate the hypothalamus as a major "feeding control center" for energy homeostasis. The hypothalamus has privileged access to the circulation or portal system through the third ventricle and, thus, is able to receive input as well as generate responses via both circulatory and neuronal means.

The neuroanatomical structures of the hypothalamus that have received the most attention regarding energy homeostasis are the lateral hypothalamic area, which expresses melanin-concentrating hormone, the orexins (hypocretins), and a high density of neuropeptide Y (NPY) receptors (Y_1, Y_5); the arcuate nucleus (ARC), which is associated with a specialized portion of the blood-brain barrier that allows the transport of certain circulating peptides/hormones and expresses NPY, agouti-related protein, and pro-opiomelanocortin (POMC); the paraventricular nucleus (PVN), where several key energy regulation pathways from the ARC and lateral hypothalamic area converge and peptides such as the orexins, POMC, galanin, α-melanocyte-stimulating hormone (α-MSH), and neuropeptide Y_1 and Y_5 receptors are expressed; and finally the ventromedial nucleus (VMN) and dorsomedial nucleus (DMN), which have recently been identified as key targets for leptin.[40,41] Studies of lesions within these structures have shown conditions of marked hyperphagia and obesity, probably a result of disruptions in the pathways involving the aforementioned peptides that have been shown to strongly influence energy homeostasis.

During the last couple of decades, our factual knowledge of the hypothalamus has evolved dramatically, as has our understanding of how it controls energy homeostasis. Simple descriptions of the anatomical nuclei, "areas," and fiber tracts have evolved into the characterization of the biochemical neuropeptides, transmitters, and receptors that in concert exert their effects to maintain energy balance. In general, these peptides can be classified as being either anabolic or catabolic in nature; under normal conditions, a dynamic equilibrium exists between these 2 types of peptides that serves to maintain an individual's homeostatic body weight. The anabolic or orexigenic peptides cause decreases in energy expenditure and increases in food intake and fat storage, while the catabolic or anorexigenic peptides cause increases in energy expenditure and decreases in food intake and fat storage. There has been an explosion of findings in recent years regarding the biological pathways of feeding behavior and obesity. Probably some of the most significant findings in this regard have evolved from the characterization of the peptides and pathways that seem to exert the strongest effects on the regulation of feeding, namely the key discoveries of the leptin (catabolic) and NPY (anabolic) pathways (see Table 3).

Leptin and Insulin Afferent Signals

Several lines of evidence have implicated leptin and insulin as afferent peripheral "adipostats," signaling the size of peripheral fat stores to the brain.[40] Leptin is the product of the obesity gene (*ob*) and is secreted primarily by adipocytes in direct proportion to the amount of fat stored within that cell. Although the precise relationship between leptin and weight regulation remains to be fully determined, it is believed to act on the "ObRb" splice variant or "long form" of the leptin receptor at the level of the hypothalamus, where it initiates a cascade of events that lead to the regulation of appetite, energy expenditure, and satiation. More specifically, the ObRb leptin receptor variant is highly expressed in the ARC, VMN, and DMN, which collectively regulate energy homeostasis.[42]

Leptin enters central circulation after being released by adipocytes and then reaches the hypothalamus, where it functions as a feedback mechanism signaling to the CNS the amount of peripheral adipose tissue. This occurs because serum leptin concentrations are positively correlated with BMI and percentage of body fat.[43] Leptin is catabolic in nature because increases in plasma leptin concentrations or direct administration of the hormone to the brain significantly inhibits food intake and fat storage while promoting energy expenditure.[43,44] Both mice and humans deficient in leptin are obese.[45] Leptin inhibits many of the anabolic neuropeptides of the hypothalamus while stimulating several of those that are catabolic.

In 1994, Zhang et al.[46] identified the mouse ob gene and its role in obesity. Mutations in this gene result in the *ob/ob* mouse, which exhibits marked hyperphagia and obesity. Peripheral or central administration of leptin to *ob/ob* mice reverses these phenotypic traits by reducing food intake and increasing metabolic energy expenditure.[43,47] In addition, genetic mutations that alter leptin sensitivity by disrupting the transport of leptin across the blood-brain barrier (via the leptin receptor ObRa or "short-form" variant[48,49]) or leptin functioning at the ObRb receptor also result in hyperphagia and obesity. Additional rodent models of obesity such as the *db/db* mouse[50,51] and the *fa/fa* rat[52] exhibit genetic mutations in the leptin receptor and are thus insensitive to the hormone. This often leads to hyperleptinemia related to an oversecretion of this hormone.

Studies indicate that treatment with clozapine or olanzapine significantly increases plasma leptin levels.[28,53–55] As patients gain weight while being treated with the antipsychotic, they increase their peripheral fat stores; consequently, an increase in leptin secretion from adipocytes is expected. However, some have postulated that antipsychotics may disrupt the central leptin pathways in the hypothalamus, leading to leptin insensitivity and subsequent hyperleptinemia.[56]

Insulin displays similar characteristics to leptin as a peripheral adiposity signal. Insulin circulates at levels that are indicative of peripheral body fat, and its circulating

concentration increases with obesity and decreases during periods of low peripheral body fat.[57] Insulin enters the CNS in direct proportion to the circulating plasma levels[58] via receptor-mediated transport mechanisms expressed in brain microvessels that allow passage across the blood-brain barrier.[40] Although insulin receptors are widely expressed in the CNS, they are more numerous in brain regions involved in energy homeostasis. For example, the hypothalamic ARC displays a high concentration of insulin receptors,[59] as does the hypothalamic DMN,[40] which both contribute to the regulation of food intake behavior.

Further support for insulin as an adiposity signal stems from the findings of Bruning et al.,[60] who demonstrated that mice lacking CNS-specific insulin receptors (NIRKO mice) have increased rates of obesity and percentage of body fat. They further indicate the importance of central insulin pathways in the regulation of both energy expenditure and food intake. Intracerebroventricular infusion of insulin has repeatedly been shown to reduce food intake,[61] and insulin deficiency is known to increase food intake, a behavior that is reversible by insulin injection.[62]

Injection of insulin antibodies into the VMN of some normal animals reveals an increase in food intake[63] and an increase in body weight.[64] Woods et al.[42] point out that unlike leptin-deficient individuals, people with insulin deficiency are not obese, even though they increase their food intake. They indicate that adipocytes cannot store fat in the absence of insulin and that, in insulin deficiency, the brain continuously strives to increase body fat by increasing food intake but the excess calories are eliminated rather than stored as fat. In addition, insulin stimulates leptin production and secretion from adipocytes,[65] while leptin affects insulin secretion from pancreatic β cells.[66]

Atypical antipsychotics such as clozapine and olanzapine have been reported to increase insulin secretion.[54,55,67] Wirshing et al.[68] postulate that some atypical antipsychotics lead to diabetes mellitus via a serotonergic-mediated pathway that in turn leads to insulin insensitivity. The development of atypical antipsychotic–induced diabetes in turn may result in the classical macrovascular and microvascular complications associated with this metabolic anomaly as well as short-term complications such as diabetic ketoacidosis.[69] Furthermore, administration of insulin inhibits ARC expression of another important hypothalamic anabolic peptide, NPY; at low circulating insulin concentrations, NPY peptide and messenger RNA (mRNA) levels are increased in the hypothalamus.[70,71]

Neuropeptide Y Central Signals

NPY is a 36–amino acid neuropeptide that is widely expressed throughout the brain, with relatively high concentrations found in specific nuclei of the hypothalamus. NPY is synthesized within neurons of the ARC that project to the PVN, where it is subsequently released and believed to elicit its effects on feeding behavior.[72] It is the most orexigenic molecule, and its anabolic effects are primarily mediated through neuropeptide Y_1 and Y_5 receptor subtypes.[73] Intracerebroventricular infusion of NPY results in increased food intake, hyperinsulinemia, hypertriglyceridemia, insulin resistance, and increased body weight,[73] conditions that are commonly associated with use of atypical antipsychotics.[74]

NPY is a key peptide in the regulation of energy homeostasis; several links to both anabolic and catabolic pathways have been established. Leptin and insulin receptors are colocalized on ARC neurons; when stimulated in states of positive energy balance (e.g., weight gain), both reduce NPY expression and release, causing decreased food intake. Conversely, in states of negative energy balance (e.g., starvation), when circulating leptin and insulin levels are low, ARC NPY expression is increased, causing a stimulation of the anabolic pathways.[75] Increased NPY release into the PVN, DMN, and VMN stimulates appetite, causes weight gain,[76] and reduces energy expenditure.[77] This feedback mechanism serves to maintain energy balance by influencing both food intake and energy expenditure. The orexigenic effects of increased hypothalamic NPY are abolished by adrenalectomy, while glucocorticoid replacement restores the normal response.[73,78]

Despite the central role of NPY in feeding behavior and weight gain, knockout mice lacking NPY Y_1 or Y_5 receptors have failed to demonstrate the expected decrease in feeding and weight.[79,80] Discrepancies in the effects of NPY are suggestive of the complex nature of energy homeostasis and allude to alternate compensatory mechanisms that maintain the required response pathways through redundancy. The following section briefly discusses components of these putative mechanisms. Both typical and atypical antipsychotics have been shown to modulate NPY expression in the hypothalamus as well as other brain regions. Inconsistencies among several studies regarding the direction of change (increase or decrease) in NPY following antipsychotic treatment have been noted.[81–83] These discrepancies are likely related to the chronicity of antipsychotic administration (acute vs. long term), the type of antipsychotic used (typical vs. atypical), and its receptor-binding profile in addition to the multifactorial nature of energy homeostasis control.

Other Central and Peripheral Signals

The afferent leptin and insulin signals initiate a cascade of events that both activate and inhibit several parallel but overlapping central and peripheral circuits. As mentioned, these afferent signals decrease NPY expression in the ARC to reduce food intake and increase energy expenditure. These same afferent signals stimulate a parallel pathway via increased expression of POMC in the ARC. POMC is subsequently cleaved into a number of smaller melanocortin peptides, of which α-MSH is of particular interest, through its link to the anorexigenic effects of

leptin. α-MSH is an agonist at melanocortin receptors, specifically the MC3R and MC4R subtypes that reduce appetite and augment energy utilization on stimulation[42,84] (see Figure 1). Administration of an MC4R selective antagonist attenuates the anorexigenic action of leptin, suggesting that its functions are mediated in part via the melanocortin pathway. An endogenous MC3R/MC4R antagonist, agouti-related protein, is coexpressed with NPY within the ARC and functions in parallel with NPY to increase food intake and weight gain. This is accomplished via dampening of the POMC/α-MSH anorexigenic pathways.[85] In summary, the relative equilibrium between the POMC/α-MSH anorexigenic circuits and the NPY/agouti-related protein orexigenic circuits serves to maintain homeostasis in an individual's overall energy balance.

Several other endogenous molecules that are expressed in the hypothalamus have been discovered to participate in the regulation of energy homeostasis. These include orexigenic peptides such as the orexins/hypocretins, galanin, and melanin-concentrating hormone as well as the anorexigenic cocaine and amphetamine-regulated transcript and corticotropin-releasing factor peptides (see Figure 1). It is beyond the scope of this report to discuss the mechanisms of the aforementioned peptides (for a comprehensive review, please refer to Jeanrenaud and Rohner-Jeanrenaud[72] and Halford and Blundell[86]). Table 3 summarizes the functional significance of these as well as other molecules and their relation to antipsychotic medications.

DISSECTING THE PATHWAYS AND ANTIPSYCHOTIC DISRUPTION: CNS CANDIDATES (PHARMACODYNAMICS)

The Serotonin System (5-HT_{2C}, 5-HT_{2A}, and 5-HT_{1A} Receptors)

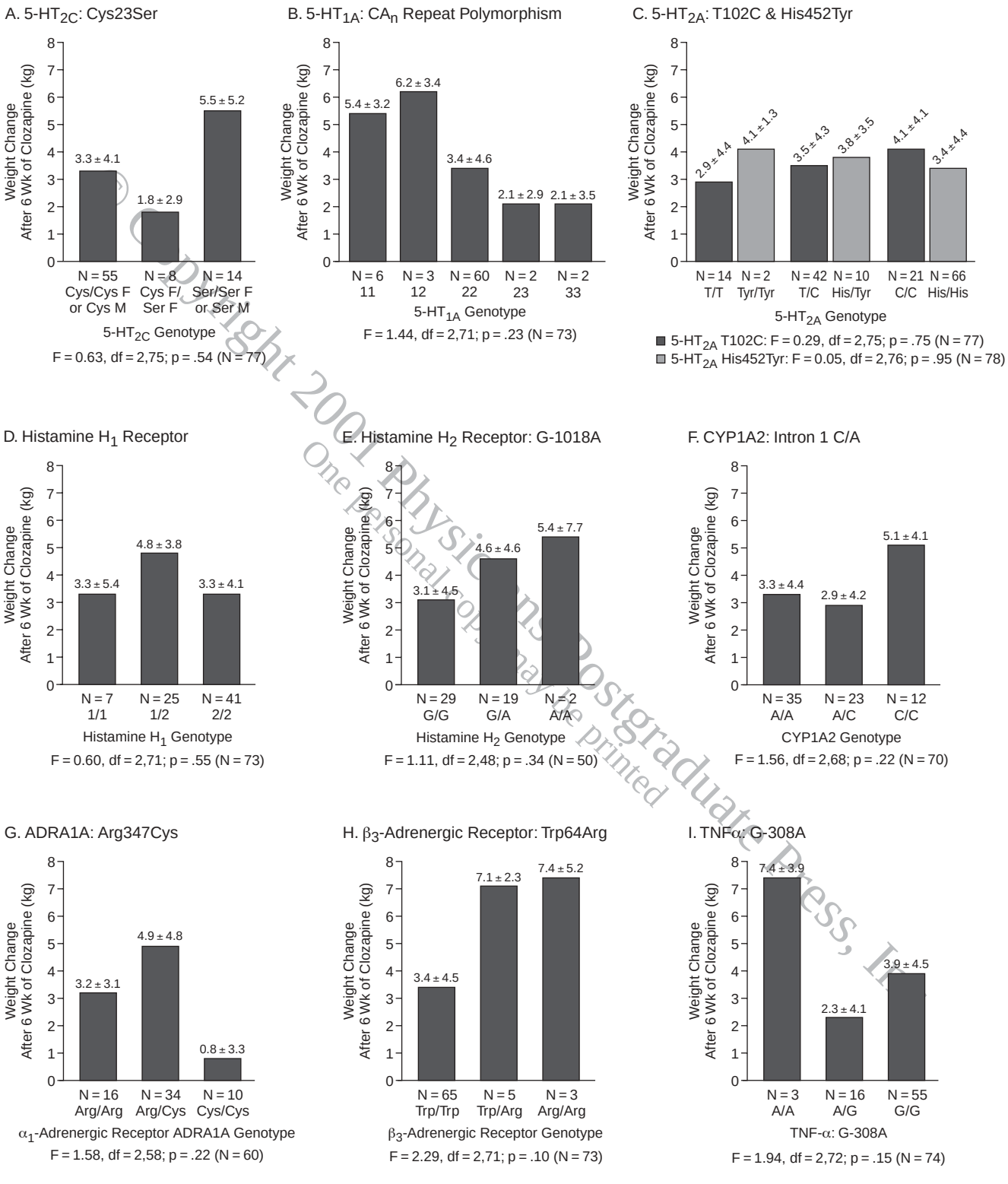
Rationale. A large body of evidence supports a role for the serotonin (5-HT) system in regulating feeding behavior (reviewed in Comuzzie and Allison[31]). Studies in both animals and humans have shown that increasing serotonin results in decreased feeding and decreasing serotonin increases feeding.[87–89] Interestingly, it has been shown that agonists at the 5-HT_1 family of receptors cause hyperphagia; conversely, agonists at the 5-HT_2 family of receptors cause hypophagia (reviewed in Davis and Faulds[90]). *d*-Fenfluramine and other proserotonergic drugs such as the selective serotonin reuptake inhibitors fluoxetine and sertraline cause marked hypophagia and weight loss in both animals and humans.[91] More specifically, rat studies have shown that 5-HT_{1A} agonists as well as 5-$HT_{2C/2A}$ antagonists cause a marked increase in feeding.[92] It is interesting to note that clozapine is a potent 5-$HT_{2C/2A}$ antagonist and a partial 5-HT_{1A} agonist. Autoradiographic studies have shown that both 5-$HT_{2C/2A}$ and 5-HT_{1A} receptors are localized in high density in the VMN and DMN within the hypothalamic satiety control centers.[93,94]

Perhaps the most compelling evidence supporting a role for the 5-HT_{2C} receptor in feeding behavior is from a study of knockout mice lacking 5-HT_{2C} receptors. The knockout mice were overweight compared with wild-type mice; based on paired feeding analysis, this appeared to be due to increased feeding as opposed to metabolic changes in these animals.[95] Further work by this group demonstrated that these 5-HT_{2C} knockout mice continued to be hyperphagic despite administration of leptin, which suggests an overlap between leptin and serotonergic signaling pathways.[96] Leptin has been shown to increase brain serotonin turnover via a nitric oxide synthase–mediated pathway, suggesting that the satiety effects of leptin are at least in part mediated by alterations in central serotonin metabolism.[97] Although the 5-HT_{2C} receptor appears to be involved, there is evidence suggesting that its role may not be as important as once thought. Ziprasidone, an atypical antipsychotic with the least weight gain liability, has very high affinity for the 5-HT_{2C} receptor, evidence that diminishes the relative importance of this receptor. There is also contradictory evidence for the 5-HT_{1A} receptor in that olanzapine has very low affinity for this receptor subtype yet exhibits a similarly high weight gain liability as observed with clozapine.

Several lines of evidence also support a role for the 5-HT_{2A} receptor in antipsychotic-induced weight gain. Currie and Coscina[98] demonstrated that 5-HT_{2A} as opposed to 5-HT_{2C} receptors mediate the effects of NPY. They found that the hyperphagic and hypometabolic effects of central NPY are diminished by previous PVN infusion of a 5-$HT_{2A/2C}$-specific agonist. These effects were not observed following infusion of a 5-$HT_{1B/2C}$-specific agonist, which suggests the increased importance of the 5-HT_{2A} receptor in both feeding and energy substrate utilization. 5-HT_{2A} antagonism by atypical antipsychotics may disrupt this inhibition of NPY, leading to unopposed action of the most potent orexigenic pathway. In addition, genetic variation in the 5-HT_{2A} receptor was found to be associated with increased food and alcohol intake in obese individuals.[99] Although some conflicting evidence exists, for the most part, those antipsychotics that induce significant weight gain share commonalities such as 5-$HT_{2C/2A}$ antagonism, 5-HT_{1A} agonism, and histamine H_1 antagonism (reviewed in Baptista[100]). These receptors therefore become prime candidates in the investigation of genetic determinants of clozapine-induced weight gain.

Methods and Results. In light of these findings, we investigated genetic polymorphisms in the 5-$HT_{2C/1A/2A}$ receptor genes to assess their putative role in clozapine-induced weight gain. We investigated the common cysteine to serine amino acid substitution at position 23 of the 5-HT_{2C} protein (Cys23Ser), which is located in the first exon of the gene. Receptors with the serine variant show a higher in vitro affinity for *m*-chlorophenylpiperazine (*m*-CPP), a 5-HT_{2C} selective agonist.[101] Also, Lappalainen

Figure 3. Our Novel Candidate Gene Genetic Association Results: Comparing Mean ± SD Change in Weight After 6 Weeks of Clozapine Among Candidate Genotypes[a]



[a]Abbreviations: Arg = arginine, CA_n = CA repeat polymorphism, Cys = cysteine, F = female, His = histidine, M = male, Ser = serine, Trp = tryptophan, Tyr = tyrosine, A = adenine, C = cytosine, G = guanine, T = thymine.

et al.[102] have demonstrated an increase in the cerebrospinal fluid concentration of 3-methoxy-4-hydroxyphenylglycol (MHPG), a norepinephrine metabolite in individuals having only the serine variant. We genotyped our patients for this polymorphism using the methods described in our previous study of clozapine response.[103] We found that there was a trend for patients carrying only the serine variant to have higher mean weight gain following treatment with clozapine (Figure 3A).

Regarding the 5-HT_{1A} gene, we investigated the CA_n dinucleotide repeat polymorphism using the methods of Bolos et al.[104] No genetic association could be detected for any of the observed alleles in the 5-HT_{1A} gene (Figure 3B).

We also investigated 2 polymorphisms within the 5-HT_{2A} gene, namely the silent T102C polymorphism in exon 1 that is in strong linkage disequilibrium with the promoter A-1437G polymorphism and the exon 3 histidine to tyrosine amino acid substitution at position 452 (His452Tyr). Ozaki et al.[105] demonstrated that the tyrosine variant receptors show a blunted serotonin-induced response to calcium. These polymorphisms were genotyped using methods similar to our previous studies.[37,103] No associations were observed for any of the 5-HT_{2A} polymorphisms studied (Figure 3C).

The Histaminergic System (Histamine H_1 and H_2 Receptors)

Rationale. It is well established that histamine H_1 receptor antagonism causes increased feeding and weight gain.[68,100,106] Autoradiographic studies have shown that histamine H_1 receptors are localized in high density in the VMN and the PVN within the hypothalamus.[107] Antagonism of the histamine H_1 receptor or depletion of neuronal histamine (hist) in the VMN and PVN was found to increase the level of food intake in normal rats, whereas histamine H_1 agonism reduced feeding.[108] Injection of betahistine, an H_1 receptor agonist, caused hypophagia in pygmy goats.[109] In a recent study by Morimoto et al.,[110] central injection of leptin into mouse brain caused a marked decrease in feeding and weight in wild-type control mice but not in histamine H_1 receptor knockout mice. In addition, when injecting α-fluoromethylhistidine, a specific and irreversible inhibitor of histidine decarboxylase (required for histamine biosynthesis), normal mice did not display the expected hypophagic response to leptin, whereas non–α-fluoromethylhistidine-treated control mice did. Following bolus intracerebroventricular administration of leptin, an elevated hypothalamic neuronal histamine turnover rate was noted; a lower histamine turnover was observed in leptin-deficient mice.[111] These results indicate that leptin may operate directly through interaction with a histamine H_1 receptor–mediated pathway to elicit effects on both satiety control and energy expenditure. Given that neuronal histamine has been shown to increase sympathetic nerve activity with a subsequent increase in basal metabolic rate,[112] the authors speculate that leptin reduces mouse body weight not only through a decrease in food intake but also via an increase in sympathetic neuronal activity that may be mediated by histamine. This suggests a functional interaction between the histaminergic and adrenergic circuits, both of which are involved in energy homeostasis.

In accordance with this hypothesis, recent work by Yoshimatsu et al.[111] found that intracerebroventricular injection of histamine decreased visceral fat content in 2 mouse models of obesity and that this was associated with an up-regulation of both UCP1 in brown adipose tissue (BAT) and UCP3 in white adipose tissue (WAT) compared with pair-fed controls. For a discussion of the mechanism of UCP action on energy expenditure, please refer to the adrenergic section below. This up-regulation was attenuated in histamine H_1 receptor knockout mice, further emphasizing its connection to the adrenergic system, which is known to influence energy efficiency via regulation of peripheral UCP expression.

Wirshing et al.[113] noted an exponential relationship between the maximum amount of weight gained while being treated with an antipsychotic and that particular antipsychotic's affinity for the histamine H_1 receptor. Antipsychotics with the maximum weight gain liabilities (i.e., clozapine and olanzapine) had the greatest affinities for the histamine H_1 receptor, while those with the least amount of weight gain (i.e., haloperidol and sertindole) had the weakest affinity.

Histamine H_2 receptors may also be involved in weight regulation. Although the literature is not as extensive as that of the involvement of histamine H_1 receptors, several studies indicate a possible role.[114–116] A recent case report by Sacchetti et al.[117] suggests that nizatidine, a histamine H_2 receptor antagonist, may control olanzapine-induced weight gain in patients with schizophrenia. Atypical antipsychotics that cause weight gain may interfere with these histaminergic receptors to disrupt both satiety regulation and energy expenditure.

Methods and Results. In the present study, we also investigated the polymorphisims found by Ito et al.[118] in both the histamine H_1 and H_2 genes for their putative association with antipsychotic-induced weight gain. We genotyped the nonfunctional promoter polymorphism of the histamine H_1 gene as well as the G-1018A polymorphism located in the enhancer element of the histamine H_2 promoter region.[118] There were no detectable associations for either H_1 or H_2 polymorphisms and clozapine-induced weight gain (Figure 3D and Figure 3E, respectively).

PERIPHERAL CANDIDATES (PHARMACODYNAMICS AND PHARMACOKINETICS)

Pharmacokinetics (Cytochrome P450 1A2 [CYP1A2])

Rationale. The bioavailability of oral clozapine ranges between 27% and 47% after absorption through the gas-

trointestinal tract and first-pass metabolic inactivation by the liver.[119] Variable absorption and excretion rates and, in particular, variable amounts and activities of liver enzymes may account for the large interindividual variation in plasma levels of the drug.[119] For example, Choc et al.[120] showed that 23 patients with schizophrenia receiving a fixed single dose of clozapine, 75 mg, achieved peak concentrations ranging from 46 to 162 ng/mL. Variability in pharmacokinetic parameters may prevent patients from reaching the recommended therapeutic threshold plasma level of clozapine (between 200 and 420 ng/mL) or can predispose some patients to extremely high plasma clozapine concentrations that may increase patient risk for developing side effects such as weight gain.[121]

Clozapine is largely metabolized in the liver by the CYP1A2 enzyme. This enzyme's activity can be increased by smoking and exposure to polycyclic aromatic hydrocarbons. Conversely, caffeine intake and the antidepressant fluvoxamine may lead to a decrease in CYP1A2 activity in vivo.[122,123] A recent case report of patients who were nonresponsive to clozapine had very high levels of CYP1A2, and the addition of fluvoxamine to the treatment regimen resulted in increased plasma concentrations of clozapine.[124] Jerling et al.[123] reported that patients simultaneously taking clozapine and carbamazepine had a 50% reduction in the plasma concentration/dose ratio of clozapine. Carbamazepine is believed to increase activity of CYP3A4, another CYP enzyme expressed in the liver and the intestines.[119] Thus, therapeutic levels of clozapine are determined in part by the activity of these 2 enzymes, which in turn are affected by other drugs and environmental chemicals. The role of CYP1A2 enzyme polymorphisms may be an important factor affecting interindividual response to clozapine in addition to side effect profiles. (For further discussion, see Ozdemir et al.[121])

Methods and Results. Recently, a (C→A) polymorphism in the first intron of the CYP1A2 gene was found to be associated with variation in CYP1A2 inducibility in healthy volunteers. Sachse et al.[125] have shown that the C/C genotype confers low inducibility for CYP1A2 in smokers. Concurrently, an independent group identified a (G→A) polymorphism in the 5'-flanking region of the CYP1A2 gene at position –2964 from the transcription initiation site in Japanese subjects.[126] This (G→A) single nucleotide polymorphism was associated with a significant decrease of CYP1A2 activity in smokers, as measured by caffeine 3-demethylation rate.

Given the recent discovery of these 2 functional CYP1A2 variants, our group has evaluated the role of the (C→A) polymorphism of CYP1A2 in interindividual variation in clozapine-induced weight gain. Genotyping was conducted according to the methods described in our previous study of this polymorphism as a predictor of typical antipsychotic–induced tardive dyskinesia side effects.[36] Although patients with the C/C genotype exhibited higher mean weight gain, which is consistent with the functionality of this polymorphism, no strong associations were observed (Figure 3F).

The Adrenergic System (β_3-Adrenergic and α_1-Adrenergic Receptors)

Rationale. The sympathetic nervous system promotes responses that prepare the body for strenuous physical activity in the face of emergent/stressful situations (fight or flight response). Apart from the hypercirculatory responses (e.g., increase in heart rate and blood pressure), increases in sympathetic tone prepare the body for increased metabolic demands by providing readily available energy substrates. Energy availability is augmented through increases in glycogenolysis in skeletal muscle and liver, gluconeogenesis in the liver, and lipolysis in adipose tissue. Subsequently, this results in higher levels of plasma glucose, fatty acids, and glycerol, which drive energy expenditure. The adrenergic system (epinephrine, norepinephrine, and adrenergic receptors) is the main efferent pathway mediating these sympathetic responses. It is also involved in the maintenance of basal metabolic rate, thermogenesis, and the efficiency of energy utilization. Interindividual differences in these processes contribute to the variation demonstrated among humans regarding energy homeostasis and body weight.

The recently discovered mitochondrial uncoupling proteins (UCP1–3) provide a link between the adrenergic system and thermogenesis/energy efficiency. UCP1 is exclusively expressed in BAT (involved in thermogenesis),[127] while UCP2 is ubiquitously expressed[128] with a relative preponderance in WAT (involved in fat storage).[129] UCP3 expression is mainly restricted to skeletal muscle[130] but is also present in BAT. Generally, UCPs are present within the inner mitochondrial membrane, where they uncouple oxidative phosphorylation from adenosine triphosphate (ATP) synthesis by allowing H^+ ions back into the inner matrix via UCP-mediated free fatty acid anion proton carriers rather than through the ATP synthase channel. In a highly uncoupled state, fuels are oxidized unrelated to the performance of work and the usable potential energy is lost as heat[131] (see Figure 1). Transgenic mice that overexpress UCP3 are lean when compared with wild-type control mice despite being hyperphagic.[132] Consequently, individuals with higher levels of UCPs are not as efficient at generating ATP and must consume more energy substrates (e.g., glucose, fat) than would an individual with lower levels of UCPs. Individuals with increased UCP levels have greater basal metabolic rates, gain less weight, and are generally more thermogenic. As a result, these differences in UCP levels can contribute to interindividual variations in body weight.

Key first messengers in this UCP pathway are β_3- and α_1-adrenergic receptors. β_3-Adrenergic receptors are expressed on WAT, BAT, skeletal muscle, and pancreatic β cells and centrally in the hypothalamus,[133] while α_1-

adrenergic receptors have an overlapping distribution on WAT and BAT and within the hypothalamic PVN.[134,135] Release of norepinephrine and/or epinephrine or treatment with selective β_3-adrenergic receptor agonists initiates a number of second messenger pathways. β_3-Adrenergic receptor stimulation activates protein kinase A, which through phosphorylation activates perilipin and hormone-sensitive lipase, breaking down fat stores and increasing lipolysis to supply the free fatty acids necessary for mitochondrial UCPs. In a parallel pathway, protein kinase A phosphorylates the CREB transcription factor to increase UCP gene expression. Stimulation of α_1-adrenergic receptors increases intracellular inositol triphosphate (InsP3) and diacylglycerol (DAG). These increases, through yet unknown protein kinase A/protein kinase G targets, also lead to elevated UCP gene expression.[131]

Rodents treated acutely with β_3-selective agonists demonstrate a 2-fold increase in energy expenditure, a 40% to 50% reduction in food intake, and a 10- to 100-fold increase in insulin levels.[136–139] Long-term treatment with β_3-selective agonists decreases fat stores, improves obesity-induced insulin resistance, and increases lipolysis and triglyceride breakdown.[140] β_3-Adrenergic receptor knockout mice are overweight and do not show any of the aforementioned responses to β_3-selective agonists.[138] Furthermore, β_3-adrenergic receptors specifically located on WAT are necessary for a complete rescue of the normal responses to β_3-selective agonists.[140] β_3-Adrenergic stimulation causes an increase in the expression of UCP1–3[141–143] that may mediate some of these responses. β_3-Selective agonists were shown to generate small adipocytes due to increased lipolysis, which was associated with decreased TNF-α and free fatty acid production and has been postulated to be the mechanism of amelioration of insulin resistance[141–143] (see Figure 1). Conversely, human studies using β_3-selective antagonists demonstrate a suppression of lipolysis and fat oxidation.[144]

In several genetic association studies, a polymorphism in the β_3-adrenergic receptor gene that alters a tryptophan amino acid to an arginine amino acid at position 64 of the protein (Trp64Arg) has been associated with insulin resistance,[147] the time of onset of type 2 diabetes mellitus,[146] and an increased capacity for extremely obese individuals to gain weight.[145] Hinney et al.[134] provided a review of these and other genetic association studies investigating this particular genetic polymorphism and how it relates to various weight-related phenotypes, including eating disorders. Additive effects between genetic variation at the β_3-adrenergic receptor gene and the UCP1 gene have been demonstrated to be associated with weight maintenance.[148]

In addition, leptin is known to increase sympathetic tone, and this appears to be mediated by products of POMC cleavage (α-MSH, β-endorphin).[149] Conversely, morbid obesity and a decrease in sympathetic tone were observed in an extended human pedigree that segregates a missense mutation in the leptin gene, resulting in leptin deficiency.[150] Several other mechanistic links connect leptin to the adrenergic system. Recently, it has been shown that leptin transport across the blood-brain barrier is enhanced by epinephrine and other agents that are more selective for α_1-adrenergic receptors.[151] Ephedrine, which is present in the Chinese herbal ma huang, is believed to elicit its anorexigenic responses via stimulation of adrenergic receptors, more specifically at β_3-adrenergic receptors. Atypical antipsychotics such as clozapine are antagonists at β_3- and α_1-adrenergic receptors[24] and may disrupt peripheral as well as central energy homeostasis via these receptors to cause weight gain.

The parasympathetic system generally counters the actions of the sympathetic system; thus, another important novel candidate is the M_3 muscarinic acetylcholine receptor, for which clozapine is a partial agonist.[152] Recently, Yamada et al.[153] have demonstrated that mice lacking muscarinic M_3 receptors are hypophagic, have reduced body weight and peripheral fat stores, and have very low levels of serum leptin and insulin. They further indicate that their results suggest a muscarinic M_3 receptor–mediated facilitation of food intake is present at a site downstream of the hypothalamic leptin/melanocortin system and upstream of the melanin-concentrating hormone system. We are currently screening the muscarinic M_3 receptor for genetic polymorphisms because none have been published to date.

Methods and Results. In light of these findings, we have investigated genetic variations within both the β_3- and α_1-adrenergic receptors to determine if any of these variants are associated with an increased patient propensity to gain weight while being treated with clozapine. We investigated the Trp64Arg polymorphism of the β_3-adrenergic receptor gene as well as the Arg347Cys polymorphism of the α_{1a}-adrenergic receptor gene. We found that individuals homozygous for the cysteine variant of the α_{1a}-adrenergic receptor gene seem to be protected from clozapine-induced weight gain (Figure 3G). Our strongest trend was detected between the Trp64Arg polymorphism of the β_3-adrenergic receptor gene and clozapine-induced weight gain. The presence of the rarer arginine allele appears to be associated with a higher mean change in weight during treatment with clozapine (Figure 3H).

Tumor Necrosis Factor α

Rationale. TNF-α is a pleiotropic cytokine that is a pivotal mediator of cell-to-cell interactions within the immune system and between the immune system and other physiologic systems. It is widely expressed, synthesized mainly by macrophages in response to invasive stimuli, and exerts a myriad of effects influencing immune response, immunomodulation, inflammation, cytotoxicity, angiogenesis, and growth promotion. TNF-α and its soluble and insoluble receptors (TNFR1 and TNFR2) are well known to mediate host responses such as sickness behavior, fever,

sedation, and sleep/wake behavior. More recently, this system has been implicated in the regulation of metabolic processes, weight, and feeding behavior. Although TNF-α is ubiquitously expressed, it has been shown to be expressed in adipose tissue and skeletal muscle, while TNFRs have been discovered in adipocytes.[154–156] An overexpression of TNF-α in adipocytes has been demonstrated in human and rodent obesity.[157] Bullo-Bonet et al.[158] provide a recent review of the TNF system as a key player in obesity and insulin resistance.

It is well established in the literature that clozapine increases serum concentrations of TNF-α.[159–161] Olanzapine produced a rapid increase in soluble TNFR1 and TNFR2 following only 1 week of treatment in patients with schizophrenia.[162] Tricyclic antidepressants, selective serotonin reuptake inhibitors, and some atypical antipsychotics such as clozapine and olanzapine have been shown to activate the TNF-α system by increasing TNF-α or its soluble receptors. Interestingly, this activation precedes the weight gain associated with the use of these psychotropic agents.[28,53,159,161–163]

TNF-α has been shown to inhibit leptin production in subcutaneous and omental adipocytes in humans.[164] Kern et al.[155] described an increased expression of TNF-α in adipose tissue of obese subjects, with a significant positive correlation between levels of TNF-α mRNA and BMI as well as percentage of body fat, which did not exist at BMIs > 45 kg/m^2. It was also shown that TNF-α levels decreased as subjects lost weight. Interestingly, weight loss has been shown to decrease the levels of soluble TNFR1 receptors.[165] More recently, Kern et al.[166] reported that obese subjects had a 7.5-fold increase in TNF-α levels when compared with lean controls and, more importantly, that increased TNF-α levels were inversely correlated with insulin sensitivity.

Several insights have been gained from transgenic mice lacking TNF-α (TNF-α $^{-/-}$), and these TNF-deficient mice show lower fat accumulation and body weight when compared with wild-type control mice (TNF-α $^{+/+}$). Given the link between obesity and insulin resistance, 2 models of obesity (diet-induced and leptin-deficient knockouts) were used to test the involvement of TNF-α in obesity-induced insulin resistance. TNF-α $^{-/-}$ mice, which were made obese as a result of a high-fat diet or leptin deficiency, displayed a significant improvement in insulin sensitivity when compared with their TNF-α $^{+/+}$ counterparts.[167] Contemporarily, Ventre et al.[168] observed similar effects on insulin sensitivity in a hyperphagic model of rodent obesity.

As mentioned, BAT serves more of a thermogenic function than WAT, which functions as a peripheral fat/energy store. In leptin-deficient obese mice lacking both TNFR1 and TNFR2 receptors, there was a decrease in BAT apoptosis (i.e., increased BAT) with an additional increase in both β_3-adrenergic receptor and UCP1 expression,[169] outcomes commonly associated with weight loss. Conversely, a 3-fold increase in TNF-α levels in mice treated with an endotoxin was associated with a corresponding increase in leptin and a decrease in β_3-adrenergic receptor expression.[170] Furthermore, increased TNF-α is known to down-regulate UCP2 expression in WAT via a nitric oxide synthase– and protein kinase G–mediated pathway.[171,172] These effects have been shown to cause weight gain. TNF-α has also been shown to stimulate adipocyte proliferation.[173] Collectively, these studies indicate that TNF-α plays a role in the regulation of peripheral fat storage and insulin resistance.

Genetic association studies provide another line of evidence supporting a role for TNF-α in obesity and insulin resistance. Genetic linkage has been demonstrated between markers at TNF-α and human obesity in Pima Indians[174] and French Canadian Caucasians.[175] A guanine to adenine polymorphism at position –308, near the transcription start site of TNF-α, has been associated with obesity across several populations.[176–179] Several other groups have investigated the role of genetic polymorphisms in TNF-α in the pathogenesis of anorexia and bulimia nervosa as well as in obesity syndromes (reviewed by Holden and Pakula[180]).

Increased levels of TNF-α are also known to cause sedation, hyperinsulinemia, insulin resistance,[154] and hypertriglyceridemia,[157] and these are all well-characterized side effects of atypical antipsychotics such as clozapine and olanzapine. Although the studies presented in this section support a role for increased TNF-α levels in obesity-related syndromes, conflicting evidence also exists given earlier studies connecting TNF-α to catabolic/cachectic states (more thoroughly discussed in Argiles et al.[157]). Two groups have speculated that these discrepancies are a result of differences in the responses to physiologic increases in TNF-α versus "supraphysiologic" increases evident in cachectic states.[157,173] In support of this, Kras et al.[173] present data indicating that, with physiologic increases in TNF-α, anabolic pathways are activated, promoting preadipocyte differentiation and adipocyte growth; at "supraphysiologic" levels, adipocyte dissolution and proteolysis predominate. This is consistent with the aforementioned study by Kern et al.[155] that demonstrated a positive correlation between TNF-α and BMI that plateaus at BMIs > 45 kg/m^2. At these extremely large BMIs, fat content as well as TNF-α levels would be supraphysiologically high, possibly favoring catabolic pathways. Interestingly, Jones et al.[22] recently found that baseline BMI is an important predictor of subsequent weight gain during treatment with atypical antipsychotics. Patients with low baseline BMIs were more likely to exhibit extreme weight gain, whereas those with high baseline BMIs gained much less weight and in some cases lost weight. We postulate that patients with high baseline BMIs may also have increased circulating TNF-α levels at baseline that approach or supersede the supraphysiologic threshold following treatment with atypical antipsychotics. This may explain

the minimal weight gain or weight loss observed in patients with high baseline BMIs. In light of these findings, it is possible that genetic variation in TNF-α may be associated with clozapine-induced weight gain.

Methods and Results. We investigated the G-308A polymorphism located in the 5' untranslated region of the TNF-α gene, using methods similar to previous studies.[181] Allen[182] provided a meta-analysis of studies examining the functional relevance of this polymorphism and concluded that the G/G and G/A genotypes are associated with a 3-fold reduction in TNF-α expression. Consequently, patients with an A/A genotype would have increased expression of TNF-α, and we would expect increased weight gain in these patients. This trend was observed (Figure 3I); our patients who were A/A homozygotes exhibited an approximately 2-fold greater weight gain than those without this genotype.

SUMMARY AND DISCUSSION

A strong genetic component has been determined for the phenotype of obesity. The genetics of antipsychotic-induced weight gain may be unique, although similar pathways are expected to be involved. The focus of this report has been primarily on the genetics of obesity-related pathways that may be disrupted by antipsychotics. Environmental influences were also considered. The importance of environmental factors is demonstrated by a discussion of the influence of "obesigenic" versus "restrictive" environments on genetic components to obesity.[183] In the past decade, particularly in Western society, there has been a shift toward "obesigenic" environments, which include a higher caloric intake, greater food availability, and more sedentary lifestyles. Correlated with this has been the dramatic increase in obesity rates in North America,[5] which has sparked the current health craze toward "restrictive" environments in which people are more conscious of diet and are increasing their physical fitness. An obesigenic environment promotes obesity in those patients predisposed to gaining weight while being treated with antipsychotics, whereas others may be genetically protected from weight gain.

Genetic analysis of the complex trait of antipsychotic-induced weight gain may be additionally complicated by factors such as incomplete penetrance and multiple gene and gene-environment interactions (as reviewed by Nothen et al.[184]). Reviews of the complexity of the obesity pathways have shown that more than 200 genes or markers have been linked to human obesity, and these numbers are continuing to increase.[185,186] Traditionally, 2 main strategies are used for the genetic study of complex illnesses: (1) linkage studies involving families or affected pairs of relatives and (2) association studies using unrelated individuals. Using conventional family-linkage studies, a model is proposed to explain the inheritance pattern of phenotypes and genotypes in the pedigree.[187] Although this is the method of choice for simple Mendelian traits, linkage analysis of complex traits has limited power to identify loci contributing to the phenotype in question (i.e., estimating the many unknown parameters required to model complex traits is extremely difficult).[188] Therefore, to test candidate genes for clozapine-induced weight gain, we utilized a genetic association study design that does not require specification of a genetic model.[188] Use of an association strategy is further supported by the fact that we have strong a priori hypotheses regarding the functional relevance of some of the polymorphisms studied.[189]

Traditional association studies use the nonparametric χ^2 to compare the distributions of genotypes in 2 groups with different phenotypes. If 1 group has a higher occurrence of a particular genotype than the other, this may indicate 1 of 3 things: (1) the alleles making up that genotype are etiologic factors for the phenotype, (2) the alleles predict the phenotype due to their proximity to the actual causative genetic variant, or (3) a false-positive finding has occurred. False-positive findings may result from a multitude of factors, most commonly from the use of ethnically heterogeneous samples (i.e., ethnic groups exhibit differences in the candidate allele frequencies [population stratification]). Although we have used the traditional case-control strategy in the past, we have largely replaced it with newer methods to address the issue of population stratification. We have used an alternate association strategy that uses a more powerful parametric statistic (ANCOVA) that can remedy the problem of population stratification and, to a certain degree, control for environmental factors.[15]

To our knowledge, this article presents the first study to investigate the genetics of both central and peripheral pathways putatively involved in antipsychotic-induced weight gain while providing a synthesis of the obesity literature. In this preliminary/exploratory analysis, we have dissected several obesity pathways and investigated 10 genetic polymorphisms across 9 different candidate genes. Our most interesting results are for the β_3- and α_{1a}-adrenergic receptor genes, the 5-HT_{2C} receptor gene, and the TNF-α gene. Our strongest trend was for the β_3-adrenergic receptor gene, where we found that the presence of the arginine allele predicted higher mean changes in weight while on clozapine (see Figure 3H). Interestingly, the arginine allele has been previously associated with insulin resistance,[147] onset of type 2 diabetes mellitus,[146] and an increased capacity to gain weight.[145] Regarding the α_{1a}-adrenergic receptor gene, individuals homozygous for the cysteine variant seem to be protected from clozapine-induced weight gain (see Figure 3G). Patients with the A/A genotype of the TNF-α gene exhibit an approximate 2-fold greater weight gain than those without this genotype (see Figure 3I). This is in agreement with data investigating the functional relevance of the G-308A promoter polymorphism in that A/A individuals have been shown to have

an increased expression of TNF-α.[181,182] However, these results need to be interpreted cautiously given that only 3 patients were in this A/A genotype category. Female patients who were homozygous or male patients who were hemizygous (5-HT_{2C} is on the X chromosome) for the serine allele of the 5-HT_{2C} receptor gene tended to gain more weight during treatment (see Figure 3A). Although a strong influence appears to be present for the 5-HT_{2C} gene polymorphism, when comparing the mean change in weight during treatment, the contribution of the genetic polymorphism is diminished when analyzing the effect of the covariates incorporated in the analysis. On further scrutiny of the data, it was found that stratification bias secondary to ethnic and response status covariates accounted for most of the differences among genotypic groups. In general, there were no other observable genetic associations for the remaining candidate genes tested (5-HT_{1A}, 5-HT_{2A}, histamine H_1 and H_2 receptor genes, and CYP1A2).

It is important to note that definitive conclusions cannot be drawn from the data reported because the limited sample size may result in both type I (false-positive) and type II (false-negative) errors. It is therefore imperative that readers exercise caution when interpreting the results. The novel data component of this review is intended to provide the reader with an example of some new methods used in genetic association approaches using clozapine-induced weight gain as a model. In addition, this preliminary/exploratory analysis may direct future studies by generating novel hypotheses. It is evident that when studying complex traits, which involve several genes and environmental factors, each with small but significant effects, larger sample sizes are required. We acknowledge that collecting such a sample is difficult and we welcome clinical collaborations. Currently, we are collecting a larger, clinically well-characterized, prospectively assessed sample to address many of the methodological limitations of this current preliminary study.

Another limitation is the rather short assessment period of 6 weeks of clozapine treatment. Studies have shown that maximal weight gain is achieved following approximately 10 to 12 weeks of treatment and plateaus thereafter.[1] Therefore, in our sample, patients with a genetic predisposition to clozapine-induced weight gain may not have expressed the phenotype sufficiently for the statistical test to detect a contribution. We are currently prospectively collecting weight gain phenotypes at multiple timepoints across a 6-month treatment period.

A critical issue in genetic association studies of all types is the limitation associated with multiple testing. It has been a difficult issue to resolve, and others have discussed possible solutions.[13,190] This current study was exploratory in nature and, given limited power due to the relatively small sample size, Bonferroni corrections for multiple testing were not applied. Although our patients had serum concentrations of clozapine assessed to ensure that each achieved the minimum therapeutic threshold (200–420 ng/mL), precise serum levels were not available and thus could not be controlled for as covariates.

An issue of paramount importance in psychiatric pharmacogenetic studies is the definition of the phenotype. The phenotype of antipsychotic-induced weight gain is multifactorial, and, as with all complex phenotypes, several aspects must be considered. Individual measures associated with antipsychotic-induced weight gain include distribution/compartment of adipose tissue, oxygen consumption, waist-to-hip ratio, BMI, waist circumference, fasting blood glucose level, lipid profile (total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides), and serum leptin, insulin, and prolactin levels. Each of these measures may be associated with varying components of the underlying genetic etiologies. The multifaceted nature of weight gain results in phenotypic heterogeneity, which decreases the "signal-to-noise" ratio for the statistical analyses. At a genetic level, different genes or combinations of genes are likely to regulate these individual components of antipsychotic-induced weight gain. Environmental factors can independently or in conjunction with genes contribute to individual components of the phenotype. It is therefore important to select a narrow phenotype that minimizes this heterogeneity, which in turn increases the "signal-to-noise" ratio and the ability to detect genetic associations. In other words, relative to psychiatric behavioral phenotypes, weight gain is easier to assess given that physical and quantifiable measures can be used. As such, the detection of genetic contributors is facilitated. For future studies, we are collecting the aforementioned phenotypic measures.

An unresolved issue in the genetic analysis of complex traits is the identification of the numerous, specific ways in which multiple genes interact to produce a given phenotype. For instance, multiple genes can behave additively, with each gene contributing to a portion of the phenotype exhibited. In addition, genes can behave with heterogeneity, whereby having a risk genotype at one or another or multiple risk loci gives precisely the same phenotype; this would be seen if the risk genes are involved in series along the same pathway. Alternatively, multiple genes can behave epistatically, where one must concurrently have the risk genotype at all of the implicated genes to exhibit the phenotype. This scenario occurs when parallel compensatory pathways exist regulating the phenotype. Disruptions must occur at all of the compensatory pathways to exhibit the phenotype. Further complications arise in that alleles at the various loci can behave in recessive, dominant, codominant, or possibly other modes. These complexities have not been addressed in genetic association studies.

We are currently developing statistical methods that test for these interactions by providing a test statistic indicative of the degree to which a particular data set fits a specifically proposed model of gene-gene interaction. We

have used these methods to detect a recessive-recessive gene-gene interaction between the dopamine D_3 receptor gene and CYP1A2, in which each gene contributes additively to the phenotype of typical antipsychotic–induced tardive dyskinesia.[38,39] These methods are limited in that only small numbers of genes (2–4 genes) can be tested simultaneously for interactions. Arranz et al.[191] have used logistic regression to develop a predictive model of clozapine response that comprised 19 different genetic loci. We envision the development of a full statistical model that incorporates genetic, demographic, clinical, and environmental variables to predict psychiatric pharmacogenetic phenotypes such as antipsychotic-induced weight gain. We are currently developing such a predictive model using principles of computer artificial intelligence that can decipher complex interactions among a large database of input variables to predict a designated output variable. With such a model, complex interactions among large numbers of genetic loci in addition to interactions between these genes and multiple environmental influences may be determined to predict a phenotype, provided that the sample is large and clinically well characterized.

The study of genetic factors that influence a patient's clinical responses to drug treatment and their propensity to develop a side effect—psychiatric pharmacogenetics—is now progressing rapidly. Pharmacogenetic research seeks to uncover genetic factors that will help clinicians identify the best treatment strategies for their patients. Not all patients respond well to all treatment strategies. Some do not improve at all during standard treatments, while others improve but develop debilitating side effects. Our research has focused on pharmacogenetic studies to improve antipsychotic treatment of schizophrenia. We found that genetic variation may account for some of the differences seen among patients both in terms of the efficacy of antipsychotics to alleviate psychosis as well as the propensity to develop antipsychotic-induced side effects. More specifically, we found that by looking at particular genetic variants, one may be able to identify those patients who are most likely to benefit from treatment with clozapine.[13,103,192,193] Furthermore, our team has results indicating that some particular genetic variants may predict those patients who are at greater risk of developing antipsychotic-induced side effects such as tardive dyskinesia.[15,36,37]

Through predictability testing, pharmacogenetic diagnostic kits could break the "trial and error" approach to prescribing antipsychotics. Although in its infancy, pharmacogenetics may in the future lead to individualized pharmacotherapy based on the specific genetic, environmental, and demographic characteristics of each patient. The pharmacogenetic goal of providing treatment based on these client-centered characteristics to maximize efficacy and minimize the risk of adverse events—getting the right medicine to the right patient—will inevitably become common in the not-so-distant future. This may result in increased patient comfort both in terms of higher initial response rates and reduced propensity to developing debilitating side effects. This research may reduce government health care costs by both minimizing wasteful prescribing and the costs associated with side effect care. Furthermore, pharmacogenetic analysis can aid in the elucidation of antipsychotic mechanisms of action, which can lead to the design of newer, more specific therapeutic agents. Pharmacogenetic research can also lead to more efficient clinical drug trials as a result of better characterization of patients. In addition, this work may illuminate some of the underlying causes of schizophrenia.

Note: Should anyone be interested in collaboration, please do not hesitate to contact us, because large multicenter collaborations are necessary to move forward in studying complex traits.

Drug names: carbamazepine (Tegretol and others), clozapine (Clozaril and others), fluoxetine (Prozac), fluvoxamine (Luvox and others), haloperidol (Haldol and others), nizatidine (Axid), olanzapine (Zyprexa), sertraline (Zoloft), ziprasidone (Geodon).

Disclosure of off-label usage: The authors have determined that, to the best of their knowledge, no investigational information about pharmaceutical agents has been presented in this article that is outside U.S. Food and Drug Administration–approved labeling.

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