The Role of Genetic Variation across IL-1β, IL-2, IL-6 and BDNF in Antipsychotic-Induced Weight Gain

by

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A thesis submitted in conformity with the requirements
for the degree of Master of Science

Institute of Medical Science
University of Toronto

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2014

Abstract

Background: Antipsychotics with high weight-inducing propensities influence the expression of immune and neurotrophin genes, which have been independently related to obesity indices. Thus, we investigated whether polymorphisms across interleukin (IL)-1β, IL-2, IL-6, and brain-derived neurotrophic factor (BDNF) genes are associated with antipsychotic-induced weight gain (AIWG).

Methods: Nineteen polymorphisms were genotyped using Taqman® assays in 188 schizophrenia patients on antipsychotic treatment for up to 14 weeks. Mean weight change (%) from baseline was compared across genotypic groups using ANCOVA. A replication sample was used to validate findings.

Results: In Europeans, IL-1β rs16944*GA (p=0.013), IL-1β rs1143634*G (p=0.001), and BDNF Val66Met (Val/Val, p=0.004) were associated with greater AIWG, as were IL-1β rs4849127*A (p=0.049), and IL-1β rs16944*GA (p=0.012) in African Americans. Epistatic effects were observed between BDNF Val66Met and IL-1β rs13032029 (Val/Met+TT, P_{Perm}=0.029), IL-6 rs2069837 (Val/Val+AA, P_{Perm}=0.021), and IL-1β rs16944 (Val/Val+GA, P_{Perm}=0.006).

Conclusions: SNPs across IL-1β and BDNF Val66Met may influence AIWG. Replication of these findings in larger, independent samples is warranted.

Acknowledgments

I would like to extend my deepest gratitude to the many people who contributed to my academic development during my tenure as a Master of Science candidate. Firstly, I would like to thank both of my graduate supervisors, Dr. Daniel Mueller of the Centre for Addiction and Mental Health (CAMH) and Dr. Sidney Kennedy of the University Health Network (UHN), for overseeing my graduate training and thesis development, in addition to providing me with numerous opportunities to participate in research dissemination at the local, national, and international level. I would also like to acknowledge the academic support provided by my committee members, Dr. James Kennedy (CAMH) and Dr. Benjamin Goldstein (Sunnybrook Health Sciences Centre). Collectively, their support has allowed me to harness my academic potential and improve the quality of my graduate thesis. In addition, a special thank you goes to Dr. Arun Tiwari (CAMH) in recognition of his superior training in pharmacogenetic analyses and data interpretation. Dr. Tiwari has been highly involved in this project since its inception, and he has continued to provide invaluable guidance and commitment to my academic success.

The results outlined in this thesis were generated through partnerships with various collaborators. Dr. Jeffrey A. Lieberman of the Columbia University Medical Center and Dr. Herbert Y. Meltzer of the Northwestern University Feinberg School of Medicine are credited for the provision of biological samples that were used for genetic analysis in this study. All genetic analyses were completed within the Tanenbaum Centre for Pharmacogenetics, a division of CAMH. Laboratory support was provided by lab manager Natalie Freeman, in addition to research analyst staff, Sajid Shaikh, Maria Tampakeras, and David Irwin. Specialized laboratory training was provided by post-doctoral fellows, Dr. Vanessa Goncalves and Dr. Clement Zai. I would also like to thank Tamera Arenovich (biostatistical support), Umakajan Umakanthan (IT support), Andrea Smart and Sheraz Cheema (administrative support), and all the CAMH graduate students and trainees who have encouraged me throughout my thesis. Numerous UHN staff and trainees have also contributed to this project including Dr. Susan Rotzinger, Dr. Joseph Geraci (biostatistics), and Joanna Soczynska (immunology, biostatistics). I would also like to acknowledge the research funding received from the Canadian Institutes of Health Research in support of this graduate project.

Furthermore, I am indebted to the Institute of Medical Science (IMS) at the University of Toronto for the opportunity to pursue a Master of Science designation. In conjunction, the academic counselling and assistance I have received from various IMS graduate coordinators and supportive staff have been greatly appreciated.

Finally, I would like to acknowledge the support of my family and friends during this arduous process. In particular, I would like to thank my parents, Ayoma and Trevins Fonseka, as well as my grandmother, Hema Karunaratne for their continuous prayers, support, and faith in me. Thank you for tackling life and all its hardships with me, especially during days where it would have been too difficult to do so alone. I would like to acknowledge Punchi, Uncle, and the kids for their many trips to the airport in support of my conference travels, and my sister Treyomi Fonseka for always encouraging me to see my potential. A special thank you goes to my boyfriend Christopher Brant for his patience, constant support, and numerous attempts to master science alongside me. Thank you for always brightening my days, and strengthening me to push the limits of my success. A very large thank you goes to the many friends who have motivated me to put my best foot forward, and have served as constant reminders that I can accomplish all that I set out to achieve. In particular, thank you Adwoa Afful, Anna Cyriac, Amandeep Sandhu, and Bentley Da Costa. To all these wonderful family and friends, thank you for helping me see the light at the end of the tunnel.

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List of Abbreviations

α7nAch: α7-nicotinic acetylcholine

α-MSH: α-melanocyte-stimulating hormone

AgRP: agouti-related protein

AIWG: antipsychotic-induced weight gain

ANCOVA: analysis of covariance

AP: antipsychotic

APR: acute phase response

ARC: arcuate nucleus

BBB: blood-brain barrier

BDNF: brain-derived neurotrophic factor

BH_4: tetrahydrobiopterin

BMI: body-mass index

BPRS: Brief Psychiatric Rating Scale

BSX: Brain-Specific Homeobox Factor

CAN-BIND: Canadian Biomarker Integration Network in Depression

CART: cocaine- and amphetamine-regulated transcript

CATIE: Clinical Antipsychotic Trials of Intervention Effectiveness

CCK: cholecystokinin

CHI3L1: chitinase-3-like 1

CNS: central nervous system

COX: cyclooxygenase

CRH: corticotropin-releasing hormone

CRP: C-reactive protein

CSF: cerebrospinal fluid

CSWG: clinically significant weight gain

DMH: dorsomedial hypothalamic nucleus

DSM-III-TR: Diagnostic and Statistical Manual of Mental Disorders, Third Edition, Revised

DSM-IV: Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition

ENCODE: Encyclopedia of DNA Elements

EPA: eicosapentaenoic acid

EPS: extrapyramidal symptoms

EPUFAs: essential polyunsaturated fatty acids

FEP: first-episode patient

FGAs: first-generation antipsychotics

GABA: gamma-aminobutyric acid

GI: gastrointestinal

HAMD: Hamilton Rating Scale for Depression

HDL: high density lipoproteins

HIV: human immunodeficiency virus

^{1}H-MRS: proton magnetic resonance spectroscopy

HPA: hypothalamic-pituitary-adrenal

HWE: Hardy-Weinberg equilibrium

IDO: indoleamine 2,3 dioxygenase

IFITM: interferon-induced transmembrane

IFN: interferon

IL: interleukin

IL-1β: interleukin 1-beta

IL-2: interleukin 2

IL-6: interleukin 6

IL-1RA: interleukin-1 receptor antagonist

IL-1RI: interleukin 1 receptor, type I

IL-1RII: interleukin 1 receptor, type II

IL-2R: interleukin 2 receptor

IL-6R: interleukin 6 receptor

JAK: Janus kinase

KBP: kilobase pairs

KYN: kynurenine

KYNA: kynurenic acid

LD: linkage disequilibrium

LHA: lateral/perifornical hypothalamic area

LOD: logarithm of the odds

LPS: lipopolysaccharide

MADRS: Montgomery-Asberg Depression Scale

MAF: minor allele frequency

MAPK: mitogen activated protein kinase

MBMDR: Model-Based Multifactor Dimensionality Reduction

MCH: melanin-concentrating hormone

MCP-1: monocyte chemoattractant protein-1

MDD: major depressive disorder

MetS: metabolic syndrome

MHC: major histocompatibility complex

NF-κB: nuclear factor-κb

NK: natural killer

NMDA: *N*-methyl-D-aspartate

NPY: neuropeptide-Y

NSAIDs: non-steroidal anti-inflammatory drugs

PAMPs: pathogen-associated molecular patterns

PANSS: Positive and Negative Syndrome Scale

PCR: polymerase chain reaction

PET: positron emission tomography

PGE_2: prostaglandin E2

PI-3-kinase: phosphoinositide-3-kinase

Poly I:C: polyinosinic:polycytidylic acid

POMC: proopiomelanocortin

PVN: paraventricular nucleus

QUIN: quinolinic acid

RNS: reactive nitrogen species

ROS: reactive oxygen species

SERPINA3: serpin peptidase inhibitor, clade A, member 3

SGAs: second-generation antipsychotics

sIL-2R: soluble interleukin 2 receptor

sIL-6R: soluble interleukin 6 receptor

SNAP: SNP Annotation and Proxy Search

SNPs: single nucleotide polymorphisms

SNPSpD: Single-Nucleotide Polymorphism Spectral Decomposition

SPECT: single photon emission computed tomography

SPSS: Statistical Package for the Social Sciences

STAT: signal transducer and activator of transcription

sTNFR: soluble tumor necrosis factor receptor

TDO: tryptophan 2,3 dioxygenase

TGF: transforming growth factor

TH_1: type 1 T helper

TH_2: type 2 T helper

TNF-α: tumor necrosis factor-alpha

TRH: thyrotropin-releasing hormone

TrkB: tyrosine kinase receptor

TSPO: translocator protein

UCSC: University of California Santa Cruz

VMN: ventromedial nucleus

VTA: ventral tegmental area

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Chapter 1:

Literature Review

The purpose of this chapter is to review:

- Metabolic disturbances within schizophrenia
- Neuroinflammation within schizophrenia
- Effects of inflammation on metabolic processes

1.1. Introduction

Schizophrenia is a highly debilitating psychiatric condition with a chronic course, and according to diagnostic systems, a global lifetime prevalence rate of approximately 4 per 1000 persons[1]. Clinical conceptualization of schizophrenia is predominantly defined by phenomenological heterogeneity across positive, negative, and cognitive symptom domains[2]. Positive symptoms, which refer to the presence of abnormal mental functions and/or behaviours (e.g. delusions, hallucinations, bizarre or disorganized speech and behaviour), are clinically distinct from negative or deficit symptoms (e.g. blunted affect, amotivation, anhedonia, asociality, poverty of speech)[3]. The severity of cognitive symptoms, which can include disturbances in episodic and working memory, attention, concentration, information processing, learning, and executive function, may be influenced by the presence of affective symptoms[4,5].

Clinical improvement of schizophrenic symptoms is preferentially achieved through treatment with antipsychotic (AP) medication. Beginning with the advent of chlorpromazine in the early 1950s, typical or first-generation antipsychotics (FGAs) were the first class of psychotropic medication that efficaciously treated schizophrenia by primarily reducing positive symptoms and risk for relapse[6]. However, treatment benefits were limited by an adverse side effect profile which commonly included FGA-induced hyperprolactinemia, acute extrapyramidal

symptoms (EPS) (e.g. akathisia, dystonia, parkinsonism), and tardive dyskinesia[7]. A further disadvantage was a high rate of partial and full treatment resistance, particularly with regard to the persistence of negative and cognitive symptoms[6]. To address EPS concerns, atypical or second-generation antipsychotics (SGAs) were introduced with the advent of clozapine in the late 1960s. Clozapine showed considerable promise due to its superior efficacy in treating refractory schizophrenia relative to FGAs[8]. Additional SGAs such as risperidone, olanzapine, sertindole, and ziprasidone were subsequently developed[9]. Collectively, SGAs have been shown to therapeutically outperform FGAs across positive[10], negative[11], cognitive[12,13], and affective[14] symptoms, while maintaining a lower incidence of motor side effects and hyperprolactinemia[15].

In spite of these therapeutic benefits, APs have been shown to cause substantial weight gain in patients. Meta-analyses have reported that weight gain occurs more often with SGAs relative to FGAs, and with greater probability for some SGAs, like clozapine and olanzapine, than others[16,17]. Antipsychotic-induced weight gain (AIWG) is a leading contributor of AP non-compliance[18], and a major risk factor for various medical conditions like obesity, metabolic (e.g. dyslipidemia, hyperglycemia, diabetes mellitus) and vascular (e.g. cardiovascular and cerebrovascular disease, arterial hypertension, ventricular arrhythmias) abnormalities, and premature mortality[19]. Inter-individual variability in the magnitude of weight gain susceptibility has been reported to depend on genetic factors[16,20]. Previous studies have demonstrated an association between genetic variation in genes involved in inflammatory processes, such as tumor necrosis factor-alpha (TNF-α) and leptin, and weight change during AP treatment[21-23].

APs also yield partial treatment benefits by influencing expression of inflammatory cytokines, like interleukin 1-beta (IL-1β), interleukin 2 (IL-2), and interleukin 6 (IL-6), to ultimately reduce the neuroinflammatory signatures observed within schizophrenia[24,25]. Such alterations in cytokine levels can mediate changes in feeding behaviours and disturb metabolic parameters, with reduced inflammation leading to obesity[26-28]. Brain-derived neurotrophic factor (BDNF), a neurotrophin secreted by immune cells as a neuroprotective response to inflammation, is decreased in certain brain regions subsequent to AP treatment[29,30]. At low levels, BDNF can contribute to metabolic disturbances like weight gain[31]. Genetic variation in pro-inflammatory cytokines and BDNF has also been associated with obesity[32-35], and may therefore be implicated in the pharmacogenetics of AIWG.

PART I: METABOLIC DISTURBANCES IN SCHIZOPHRENIA

1.2. Metabolic Pathology and Schizophrenia

Schizophrenia and other severe mental disorders are characterized by a high prevalence of various physical health concerns. In particular, there is a clinically significant co-occurrence of schizophrenia with the metabolic syndrome (MetS) which occurs in approximately 9.8% to 12.9% of untreated schizophrenia patients[36-38]. MetS includes components such as obesity, glucose intolerance, atherogenic dyslipidemia, insulin resistance and hypertension, in addition to an increased risk of type II diabetes mellitus and cardiovascular disease[39,40]. The metabolic pathology of schizophrenia has been documented in multiple case reports prior to the advent of APs (reviewed in Kohen, 2004)[41]. Studies of drug-naive and drug-free schizophrenia patients report central obesity, impaired glucose tolerance, insulin resistance, and elevated fasting plasma levels of glucose, insulin and cortisol relative to healthy controls[42-44]. However, these findings have not been consistently replicated[45-47]. Elevated rates of metabolic disturbances have also been observed in first-degree relatives of schizophrenic patients, suggesting a shared environmental and/or genetic component to metabolic outcomes in schizophrenia[43,48].

1.3. Metabolic Side Effects of Antipsychotic Medication

1.3.1. Antipsychotic-Induced Metabolic Disturbances

Metabolic abnormalities observed within untreated schizophrenia have been shown to worsen subsequent to AP treatment[19]. Zhang *et al.* (2004)[49] identified significant increases in subcutaneous and intra-abdominal fat, in addition to elevated levels of leptin, circulating lipids, and non-fasting glucose after 10 weeks of AP treatment in previously untreated schizophrenia patients. Similarly, a meta-analysis by Mitchell *et al.* (2013)[36] showed that metabolic irregularities like obesity (52.7% vs. 26.6%), elevated triglycerides (41.1% vs. 16.9%), low levels of high density lipoproteins (HDL) (44.7% vs. 20.4%), high blood pressure (39.7% vs.

24.3%), diabetes (12.8% vs. 2.1%), and hyperglycemia (27.8% vs. 6.4%) were elevated in treated as compared to untreated schizophrenic patients.

Weight gain is a common side effect of APs, and affects approximately 15% to 72% of schizophrenic patients receiving acute or maintenance treatment[37]. AIWG has been documented since the introduction of chlorpromazine with reports of steadily increasing weight with treatment that rapidly declines upon drug cessation[50,51]. AIWG often occurs within the first 4 to 12 weeks of treatment and may stabilize over time[52,53]. AIWG has also been observed in other AP-treated psychiatric populations, such as bipolar disorder[54], treatment-resistant major depressive disorder (MDD)[55], mental retardation and autistic disorder[56], and Tourette's syndrome[57]. However, the propensity to induce weight gain varies between APs. A meta-analysis by Lett *et al.* (2012)[16] reported that SGAs carry a greater risk of weight gain compared to FGAs, with clozapine and olanzapine having the highest risk, quetiapine and risperidone having intermediate risk, and aripiprazole and ziprasidone having minimal risk, according to mean weight change values. Among FGAs, low-potency drugs such as chlorpromazine and thioridazine yield greater weight gain effects than high-potency drugs like haloperidol and fluphenazine (refer to Figure 1.1). Some studies also suggest that APs can induce clinically significant weight loss in approximately 10% of patients[58].

FIGURE 1.1: Weight Change Propensities of Antipsychotic Treatments

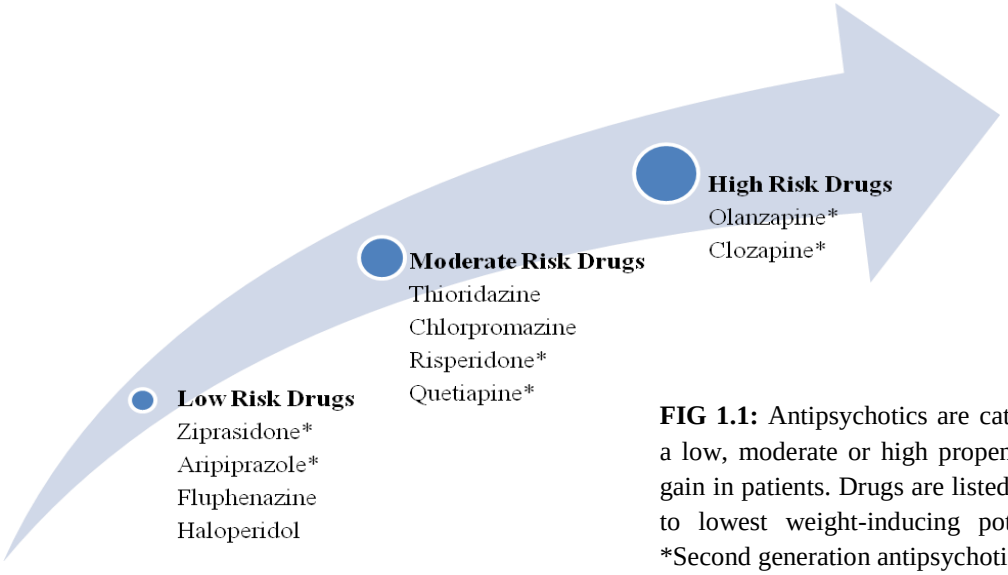


FIG 1.1: Antipsychotics are categorized according to a low, moderate or high propensity to induce weight gain in patients. Drugs are listed in order from highest to lowest weight-inducing potential, per category; *Second generation antipsychotic

1.3.2. Risk Factors for Antipsychotic-Induced Weight Gain

Preliminary data have identified many potential predictors of AIWG which can influence the magnitude of treatment-induced weight change. Such risk factors typically pertain to patient, illness and/or treatment parameters (refer to Figure 1.2). *Patient Parameters.* Pediatric patients are particularly vulnerable to AIWG, as the amount of weight gained with AP treatment negatively correlates with age[59-63]. Elevated risk has been reported for women compared to men[59,61], but the reverse association has also been found[64,65]. Other risk factors include non-white ethnicity[66], increased appetitive behaviours[66,67], and non-smoking status[59]. Cannabis may also influence AIWG by acting on the endogenous endocannabinoid system to increase appetite and weight[68]. An inverse relationship exists between baseline BMI and AIWG, where low pre-treatment BMI correlates with greater weight gain[60,61,69-71]. Low baseline BMI can also predict accelerated weight gain over the course of AP treatment[59]. Patterns of accelerated weight gain, in addition to weight gain early in the course of treatment, predict greater long-term weight gain and a higher plateau for mean weight increase[72]. High parental BMI is predictive of weight gain, and suggests a shared environmental and/or genetic component to AIWG[59,65]. The genetic basis of AIWG has received considerable support from candidate gene studies (refer to Section 1.4.4).

Illness Parameters. Although schizophrenia is an independent risk factor for weight gain (refer to Section 1.2), additional evidence suggests that the magnitude of AIWG differs between schizophrenic subtypes, with the undifferentiated and paranoid subtypes conferring the greatest risk[73-75]. First-episode patient (FEP) status, drug naivety, and high baseline levels of negative symptoms have also been identified as risk factors[52,58,60,62], as has symptomatic improvement which positively correlates with AIWG[61,66,67,74]. *Treatment Parameters.* AIWG increases with the duration of AP treatment[58,74]. Dose effects have been inconsistently reported, with some studies supporting a positive dose-dependent relationship for clozapine, olanzapine[76] and risperidone[77], while others suggest no relationship[60,78]. As shown in Section 1.3.1, different APs confer varying risk of AIWG, with olanzapine and clozapine having the most robust outcomes. Polypharmacy can result in greater weight gain likely due to the combined effects of other weight-inducing drugs[62,79]. For example, Casey *et al.* (2003)[80] observed a two-fold increase in weight during combined risperidone and valproate therapy relative to risperidone monotherapy.

FIGURE 1.2: Risk Factors for Antipsychotic-Induced Weight Gain

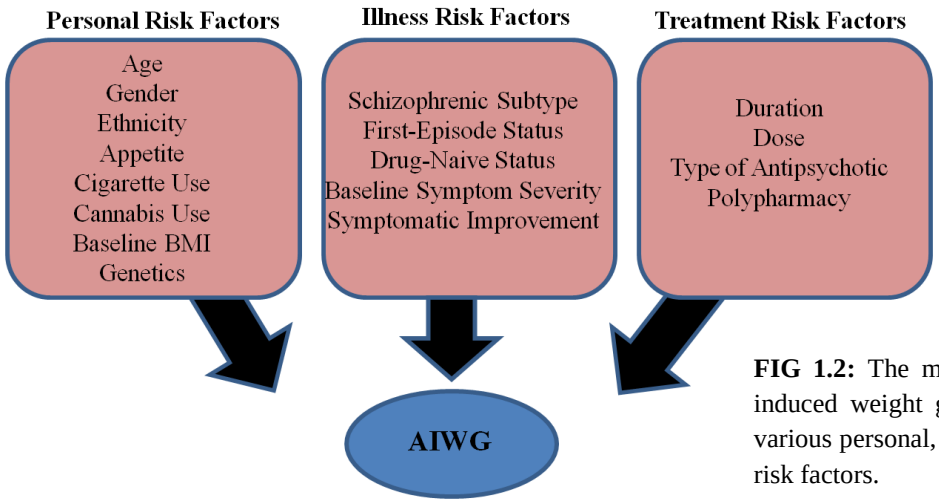


FIG 1.2: The magnitude of antipsychotic-induced weight gain can be influenced by various personal, illness or treatment-related risk factors.

1.4. Mechanisms of Antipsychotic-Induced Weight Gain

1.4.1. Antipsychotic Pharmacodynamics and Weight Gain

The variability in weight gain propensities between APs may be attributed to differences in binding affinities at relevant receptors. FGAs are primarily characterized by pharmacological action at dopamine D_2 receptors but can include effects at muscarinic cholinergic, histamine H_1, and α1-adrenergic receptors[81]. Low potency FGAs like chlorpromazine, loxapine, and thioridazine also interact with serotonin 5-HT_{2A} receptors[82]. Although all SGAs have affinity for D_2 receptors, they dissociate rapidly from these sites, with the majority achieving therapeutic effects through preferential occupancy of 5-HT_{2A} receptors[83]. In addition to D_2 and 5-HT_{2A}, SGAs interact with serotonin and norepinephrine reuptake pumps, and one or more of the following receptors: D_1, D_3, D_4, 5-HT_{1A}, 5-HT_{2C}, 5-HT_3, 5-HT_6, 5-HT_7, α1-adrenergic, α2-adrenergic, H_1, and muscarinic cholinergic receptors[82]. Since these receptors are differentially implicated in weight regulation (refer to Section 1.4.3), diversity in the number and combination of receptor binding sites between APs may contribute to divergent AIWG risk profiles.

1.4.2. Hypothalamic Regulation of Energy Homeostasis

AIWG results from AP-induced disruptions of central and peripheral energy homeostatic pathways, ultimately creating an imbalance favouring energy intake over energy expenditure[84]. Increasing evidence has implicated different genetic, neuronal, and hormonal factors in mediating this association through effects on appetite and satiety signals[85]. Of particular importance are hypothalamic homeostatic circuits which, in controlling food intake and thermoregulation[86], rely on peripherally-released hormones to monitor the body's energy stores and provide appetite-stimulating (orexigenic) or satiation (anorexigenic) signals to the central nervous system (CNS)[87]. Although signals act at various CNS sites, the pathways converge onto the hypothalamus where they are integrated and cue central neurons to secrete relevant neuropeptides[88,89]. Due to the necessity of maintaining adequate energy stores for survival, the most potent pathways signal for increased food intake and reduced energy expenditure[88].

Two primary anorexigenic hormones are leptin and insulin, secreted by adipocytes and pancreatic β-cells, respectively. Both hormones provide information on long-term energy stores, as their circulating concentrations are proportional to the degree of adiposity in the body[90]. The primary CNS interface for these hormones is the arcuate nucleus (ARC) of the hypothalamus which, due to an anatomical position near the base of the brain and poor blood-brain barrier (BBB) insulation, can directly interact with peripheral regulatory factors[91]. The ARC is responsible for reciprocally regulating two types of first-order neurons: (1) anorexigenic neurons co-expressing proopiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART), and (2) orexigenic neurons co-expressing neuropeptide-Y (NPY), and agouti-related protein (AgRP)[92]. These neuropeptides have action at second-order neurons which are localized to three primary hypothalamic sites: lateral/perifornical hypothalamic area (LHA) or 'hypothalamic hunger center', paraventricular nucleus (PVN) or 'hypothalamic satiety center', and the ventromedial nucleus (VMN) which is also involved in mediating satiation via signalling to the PVN in response to blood glucose levels[91]. POMC/CART stimulates the PVN, and inhibits the LHA via α-melanocyte-stimulating hormone (α-MSH). In contrast, NPY activates the LHA and inhibits the VMN, while AgRP inhibits the PVN and antagonizes α-MSH[88,91]. Signals transmitted to the PVN, LHA, and VMN are subsequently translated into autonomic, endocrine

and behavioural responses[89,93]. For example, activated PVN neurons secrete thyrotropin-releasing hormone (TRH) and corticotropin-releasing hormone (CRH) to decrease appetite and increase thermogenesis, whereas LHA neurons secrete orexins A and B and melanin-concentrating hormone (MCH) to increase appetite and decrease thermogenesis[91,94]

In addition to leptin and insulin, inflammatory cytokines such as IL-1, IL-6, and TNF-α also function as adiposity signals[91]. In parallel with these long-term homeostatic controls, various other hormonal and neural signals (e.g. cholecystokinin [CCK], peptide YY, glucagon-like peptide-1, amylin, bombesin) are secreted by the gastrointestinal (GI) tract to provide short-term signals to the CNS and regulate GI function[89,95]. For example, ghrelin is a short-term signalling factor that is secreted by the stomach to yield orexigenic effects by acting on NPY/AgRP neurons[96]. Short-term signalling is important in regulating the size and timing of meals, but has limited effects on adiposity stores[87].

1.4.3. Monoamine Regulation of Energy Homeostasis

To date, a large body of research has investigated the role of monoamines and their AP-targeted receptors in AIWG. Serotonin has been highly studied for its inhibitory effects on feeding behaviours[97], with particular attention paid toward the 5-HT_{2A}, 5-HT_{2C}, and 5-HT_{1A} receptor subtypes. The mechanism of action is based on serotonergic neurons in the mid-brain raphe nucleus projecting onto POMC hypothalamic neurons[85,88,95]. Additional evidence suggests that serotonin yields anorexigenic effects by working in concert with leptin[98,99]. Antagonism of 5-HT_{2A} and 5-HT_{2C} receptors yields increased food intake[100,101], while inconsistent effects of the 5-HT_{1A} receptor have been reported[102,103]. Preferential affinity for 5-HT_{2C} receptors among SGAs relative to FGAs may partially explain their greater propensity to induce AIWG. Dopamine is also a key contributor to food intake, but its effects on weight are dependent on its site of action within the brain. For example, mesolimbic dopamine contributes to the rewarding nature of palatable foods (and therefore, the motivation to eat) while hypothalamic dopamine signals anorexigenic pathways[98]. Evidence suggests that D_2 receptor antagonism by APs increases feeding behaviours, and these effects can be reversed using D_2 receptor agonists[104]. D_2 receptor blockade can also disrupt reward mechanisms of taste[105]. Histamine has been implicated

in energy homeostasis and food intake across many studies. Histamine neurons originate in the tuberomamillary nucleus of the third hypothalamic ventricle and affect weight via dense projections to the hypothalamus[106]. H_1 receptor agonism decreases food intake, and effects are reversible via H_1 receptor antagonism[107,108]. Pharmacological evidence suggests that H_1 receptor affinities are positively correlated with weight gain across FGAs and SGAs[109]. Norepinephrine (noradrenaline) neurons also have projections to the hypothalamus[110]. Chronic administration of norepinephrine into the PVN significantly increases daily food intake in rats[111]. However, α1- and α2-adrenergic receptors yield functionally distinct effects on feeding, where agonism at each site decreases and increases food intake, respectively[112,113]. Since all APs are α1-adrenergic receptor antagonists[114], this may contribute to their effects on AIWG.

1.4.4. Pharmacogenetics of Antipsychotic-Induced Weight Gain

The high degree of inter-individual variability in AIWG has been previously described to be influenced by numerous risk factors (refer to Section 1.3.2). Of particular interest are genetic factors, as monozygotic twin studies suggest AIWG may be under strong genetic control. For example, Theisen *et al.* (2005)[115] found greater similarity in AP-induced body-mass index (BMI) change among monozygotic twins (intrapair difference: 2.78 ± 3.4 kg/m^2) than same-sex sibling pairs (5.55 ± 4.4 kg/m^2). A case report also described similar clozapine-induced weight change between female monozygotic twins (Δweight: 53.1 kg vs. 48.2 kg, final BMI: 38.1 kg/m^2 vs. 33.8 kg/m^2)[116]. To further examine genetic contributions, pharmacogenetic studies have tested the effects of polymorphisms on AIWG. As shown in Sections 1.4.2 and 1.4.3, many hormones and neuropeptides are implicated in energy homeostasis and food intake, and for this reason, are highly investigated within pharmacogenetic research. The serotonin system has been extensively studied, particularly the HTR2C gene, with multiple studies demonstrating protective effects of the -759T promoter variant allele against excessive AIWG[117-121]. Variant sites of the dopamine DRD2 gene, such as rs6277, rs1079598, rs1800497, and rs4436578, have also been implicated in AIWG[122,123]. Other candidates that have been investigated include genes encoding leptin[124], NPY[125], α2-adrenergic receptor[126], H_1 receptor[127], and ghrelin[128]. For a review of the pharmacogenetic literature, refer to Lett *et al. (*2012)[16] and Mueller *et al.* (2013)[16,129].

PART II: NEUROINFLAMMATION IN SCHIZOPHRENIA

1.5. The Inflammatory Response System

1.5.1. The Peripheral Immune Response

The immune response can be broken down into two interconnected branches, (1) innate or non-specific immunity, and (2) adaptive or specific immunity. Innate immunity is the first line of host defence that rapidly mounts an inflammatory response upon detection of invading pathogens and physiological damage[130]. This response is considered non-specific in that it broadly targets conserved microbial features, or pathogen-associated molecular patterns (PAMPs) (e.g. lipopolysaccharide [LPS], unmethylated DNA, peptidoglycan, flagellin), which are common to many pathogens but not present in the host[130-132]. PAMPs bind to pattern recognition receptors expressed on the surface of phagocytic cells (e.g. toll-like receptors on macrophages and neutrophils) or as soluble receptors[131,133]. The binding stimulates phagocytes to (1) engulf and degrade the pathogen, (2) initiate an inflammatory cascade, primarily via the nuclear factor-κb (NF-κB) pathway, to release immune factors (e.g. cytokines, prostaglandins, chemokines, leukotrienes), and (3) initiate an adaptive immune response by stimulating antigen-specific T and B lymphocytes[130,132,134,135]. The adaptive immune response is a second line of defence which confers long-term protection against re-exposure to specific antigens by building immunological memory[136]. Once the pathogen is contained, immune signalling shifts toward anti-inflammatory action to resolve inflammation and restore homeostatic balance[131].

1.5.2. The Central Immune Response

In the CNS, there are two primary immune cells, microglia and astrocytes, which regulate the initiation and termination of the inflammatory response[137]. Microglia are resident macrophages of the CNS that continuously survey the brain for neuronal damage, plaques, and microbes, and provide an immediate response to even minor central pathology[135]. Once

activated, microglia engage in a variety of functions including phagocytosis and antigen-presentation of invading microbes, secretion of oxidative stress markers like reactive oxygen species (ROS) and reactive nitrogen species (RNS), and production of cyclooxygenase (COX)-2, prostaglandin E2 (PGE_2), and inflammatory cytokines (e.g. IL-1, IL-6, TNF-α, interferon [IFN]-γ)[138-142]. Microglia can also confer neuroprotective benefits like enhanced neuronal survival through cellular maintenance and secretion of neurotrophic (e.g. BDNF) and anti-inflammatory factors, synaptic formation and pruning, developmental cell apoptosis, and axon remodelling [141,143,144]. Astrocytes have similar CNS functions including neuronal support, neurotransmission, synaptic formation, and BBB maintenance[142]. Although astrocytes can secrete pro-inflammatory mediators, they primarily produce anti-inflammatory factors[142,145]. Astrocytes also have inhibitory and stimulatory effects on microglia depending on the internal immune state[135].

1.5.3. Neuroimmune Communication

There is evidence for the involvement of immune dysfunction in the etiopathogenesis of schizophrenia, particularly an up-regulation of pro-inflammatory signalling[146]. This relationship is mediated though crosstalk between the immune system and CNS which is bidirectional under normal and pathological conditions[147,148,149]. For example, cytokines and cytokine receptors, such as for IL-1, IL-2, and IL-6, are expressed within the brain and have action at central neurons, with particularly high densities localized to the hippocampus and hypothalamus[149,150]. In addition to interleukins, other cytokines are present in the CNS such as IFNs and the tumor necrosis family[151]. Cytokines are reciprocally regulated by various centrally-released neurotransmitters and neuropeptides[149], and are produced in the CNS by neurons and glial cells[149,151].

Peripheral cytokines can also infiltrate the otherwise immune-privileged CNS through multiple humoral pathways that act in parallel, including: (1) passive transport at disrupted regions of the BBB[152] or through the choroid plexuses and circumventricular organs which are BBB-deficient[153-155], (2) active transport via saturable transport molecules, as observed for IL-1α, IL-1β, IL-1 receptor antagonist (IL-1RA), IL-6, and TNF-α[156,157], and (3) binding to cerebral endothelial cells to stimulate release of secondary inflammatory messengers such as additional cytokines (e.g. IL-1α, IL-1β, IL-6), and PGE_2 via COX-2[158-160]. Immune information is also

directly transmitted to the brain using rapid neural pathways. Neural transmission involves activation of primary afferent nerve fibres in response to local cytokine release in the periphery, like the glossopharyngeal nerve during oral infections[161] and vagal sensory ganglia during abdominal, visceral, and GI infections[162-164]. Once immune information from the periphery reaches the brain, it is reconstituted in the CNS via central cytokine release[165].

1.5.4. Neurotrophin-Immune Crosstalk

Neurotrophins, like BDNF, are involved in numerous processes that are critical for neuronal development and synaptic plasticity throughout development and in response to injury (refer to Table 1.1)[166-169]. Within this role, BDNF interacts with immune cells to mediate neuroimmune cross-talk[168,170,171]. Although primarily secreted by neurons and astrocytes, BDNF and its tyrosine kinase receptor (TrkB) are produced by major peripheral immune cells (e.g. $CD4^+$/$CD8^+$ T lymphocytes, B lymphocytes, monocytes, macrophages)[172,173], and activated microglia[142]. BDNF is proposed to exert a neuroprotective role within the central immune response, as it is secreted by immune cells in response to inflammation to protect the brain from possible damage[174-176]. BDNF and TrkB, therefore, show inverse expression trends relative to inflammatory cytokines like IL-1β, IL-6 and TNF-α[177-179]. In contrast, proBDNF, an immature isoform of BDNF, negatively regulates neuronal remodelling, synaptic transmission, and synaptic transmission via activation of the p75 neurotrophin receptor ($p75^{NTR}$)[180].

1.5.5. Biological Properties of Cytokines

Cytokines are pleiotropic proteins involved in host regulation of both immunologic and non-immunologic processes (e.g. cell growth, migration, development, differentiation)[181-183]. Cytokines are produced by a variety of cell types within the periphery (e.g. endothelial cells, monocytes, macrophages, dendritic cells, natural killer cells, and T cells) and CNS (e.g. astrocytes, microglia), and function as chemical messengers[135,144,184]. The immune capabilities of different cytokines are highly redundant, and ultimately lead to the stimulation of multiple cell

types and the downstream production of inflammatory mediators[37,185]. For example, IL-1 signals for the release of IL-2, IL-6 and TNF-α[186]. Cytokines yield their effects by binding to specific cytokine receptors that are expressed on a variety of peripheral and central cells, and also exist in soluble form[187]. Some receptor subtypes serve as non-functional decoys (e.g. interleukin 1 receptor, type II [IL-1RII], soluble interleukin 2 receptor [sIL-2R])[184,188,189] while others enhance cytokine activity (e.g. soluble interleukin 6 receptor [sIL-6R])[184]. Inhibitory effects are also achieved by non-functional receptor antagonists (e.g. IL-1RA) which compete with cytokines for receptor binding sites[188]. Cytokine-receptor complexes are phosphorylated by Janus kinase (JAK) and Src kinases, and signal through pathways like (1) JAK-STAT (signal transducer and activator of transcription), (2) Ras/MAPK (mitogen activated protein kinase), and (3) phosphoinositide-3-kinase (PI-3-kinase) to activate gene transcription and cellular activity[190].

Cytokines are classified as either pro-inflammatory or anti-inflammatory in function. Pro-inflammatory cytokines, such as IL-1β, IL-2, and IL-6, augment the inflammatory cascade by recruiting leukocytes to sites of infection/injury, activating inflammatory cells, and assisting with the elimination of invading pathogens (refer to Table 1.1)[135,184]. Anti-inflammatory cytokines, like IL-1RA, sIL-2R, IL-4, IL-5 IL-10, are reciprocally designed to down-regulate inflammation via immunosuppressive functions[142,191]. Under certain conditions, cytokines can exert a dual role as both an inflammatory and anti-inflammatory signalling molecule. For example, IL-8 can function to either increase or decrease neutrophil recruitment[186]. Cytokines can also be classified based on T cell origin sources. Type 1 T helper (TH_1) cells produce activators of cell-mediated immunity against intracellular antigens, and thus, typically secrete pro-inflammatory cytokines. Conversely, Type 2 T helper (TH_2) cells enhance humoral or antibody-based reactions against extracellular antigens and allergens, and thus, typically produce anti-inflammatory cytokines [134,142,184,190]. In addition to the traditional TH_1 and TH_2 branches, the more recently identified TH_{17} cells (e.g. IL-17A, IL-17F, IL-21, IL-22) have been implicated in pro-inflammatory functions, particularly defence against extracellular bacterial infections, autoimmune diseases, and tissue immunity[192]. Disruptions of cytokine signalling can result in chronic inflammation that has been implicated in the onset of schizophrenia[193], and other psychiatric conditions, like panic disorder, obsessive-compulsive disorder, bipolar disorder, autism[186,194], and especially MDD[177,195-197]. These inflammatory signatures are generally considered low-grade because they are modest in severity compared to primary inflammatory conditions like rheumatoid arthritis[135].

TABLE 1.1: Biological Properties of Cytokines and BDNF

Cytokine	**Receptor***	**Cell Source**	**Biological Activity**
IL-1β	IL-1RI	Monocytes, macrophages, fibroblasts, endothelial cells, dendritic cells, B lymphocytes, microglia, astrocytes	Stimulation of T, B and endothelial cells; induction of acute phase reaction; hematopoiesis; up-regulation of cytokines and cell adhesion molecules; neuronal depolarization/excitation; increase of NMDA receptor phosphorylation
IL-2	IL-2R	T cells, NK cells, microglia	Stimulation of T, B, and NK cells; generation of cytotoxic T cells; up-regulation of T-cell derived cytokines; facilitation of immunoglobulin synthesis; elimination of self-reactive T cells; T_{reg} cell maintenance
IL-6	IL-6R	Monocytes, macrophages, T cells, hepatocytes, osteoclasts, fibroblasts, microglia, astrocytes	Stimulation of T and B cells; induction of acute phase reaction; hematopoiesis; modulation of cytokine release; production of immunoglobulins
BDNF	TrkB	Neurons, astrocytes, microglia, T cells, B cells, monocytes, macrophages	Neuronal and glial survival, development, differentiation; synaptic/dendritic plasticity; influence production of T-cell derived cytokines; axonal/dendritic growth; neurotransmitter release; neuroprotection; increase survival of thermocyte precursors

*functional receptor; IL-1β = interleukin 1-beta; IL-1RI = interleukin 1 receptor, type I; IL-2 = interleukin 2; IL-2R = interleukin 2 receptor; IL-6 = interleukin 6; IL-6R = interleukin 6 receptor; BDNF = brain-derived neurotrophic factor; TrkB = tyrosine kinase receptor; NK = natural killer cells; NMDA = *N*-methyl-D-aspartate [37,135,151,169,172,185,186,188,198]

1.6. Evidence of Immune Alterations in Schizophrenia

1.6.1. Prenatal Immune Activation Model of Schizophrenia

Maternal exposure to infection during a critical developmental (i.e. prenatal) period is proposed to increase the risk of schizophrenia in the offspring. This occurs when the maternal innate immune response leads to immune alterations in the fetal environment that disrupt early structural and functional neurodevelopment, with downstream implications for post-natal brain dysfunction that typically emerges after adolescence[199,200]. Pre-natal immune challenges may also prime the fetus for post-natal immune hyperactivity toward both immunological and non-immunological stimuli[135], and permanently alter the fetal peripheral immune system[201]. These consequences may be the result of an influx of maternally-generated inflammatory mediators across the placenta and fetal BBB[202], in addition to stimulated expression of pro-inflammatory cytokines within the fetal brain[200]. In particular, there is strong support for increased IL-6, and possibly IL-1, IL-2, IFN-γ, and TNF-α, in mediating these effects[200,203,204]. In addition to cytokine changes, maternal infection can alter neurogenesis and BDNF levels in offspring[205-207].

Preclinical models of maternal exposure to immune-stimulating agents such as influenza[208,209], polyinosinic:polycytidylic acid (poly I:C)[210], LPS[211], and turpentine[211] have demonstrated the emergence of schizophrenia-like outcomes in behavioural, neurostructural and neurochemical domains. For example, immune models have shown alterations of dopamine, and dopamine-related metabolites, neurons, receptors and genes within offspring[201,212,213]. Epidemiological studies also report higher rates of schizophrenia following prenatal exposure to influenza, rubella, measles, polio, herpes simplex, genital and/or reproductive infections, sinusitis, tonsillitis, pneumonia, and Toxoplasma gondii[199]. Additional research suggests synergistic effects of CNS vulnerability from prenatal infection and pre-pubertal stress or environmental interactions (e.g. physical or psychological stress or exposure to pathogens) in the development of schizophrenia (i.e. ‘two hit’ model of schizophrenia) (refer to Figure 1.3)[135,214].

1.6.2. Peripheral Cytokine Alterations in Schizophrenia

Several studies have found altered peripheral cytokine levels in schizophrenia (refer to Figure 1.3), with some discrepancy over which cytokines are elevated and which are lowered. Schizophrenia was initially characterized as an imbalance in TH_1 and TH_2 immune responses, observed as a shift toward exaggerated TH_2 cytokine production[215]. The TH_1/TH_2 imbalance hypothesis received support from studies that found decreased levels of TH_1 cytokines (e.g. IL-2, Il-6, IFN-γ)[216,217], and increased levels of TH_2 cytokines (e.g.IL-10) in schizophrenic patients[218]. However, findings were later challenged by reports of increased pro-inflammatory mediators like IL-1, IL-2, IL-6, IL-8, IL-18, and TNF-α[219-226], elevated levels of circulating monocytes[227], and increased release of PGE_2 and COX-2[228] in schizophrenia. This up-regulation of pro-inflammatory markers occurs within drug-naive, drug-free, and FEP, suggesting that these findings are independent of AP effects[223,229-231]. Similar immune disturbances are observed in unaffected first-degree relatives of schizophrenic patients[232]. A meta-analysis by Potvin *et al.* (2008)[184] confirmed that there is insufficient evidence to support a TH_2 cytokine shift. In addition to immune changes, reductions in serum levels of BDNF have been observed in drug-naive FEP, with levels negatively correlating with symptom severity (refer to Figure 1.3)[233,234].

This discrepant pattern of findings may have resulted from methodological differences between studies involving (1) biological sample type, (2) method of sample preparation and storage, (3) method of in vitro immune stimulation, and (4) method of cytokine quantification. Reliable quantification in serum can also be challenged by short cytokine half-lives and rapid turnover[142]. Other study parameters such as small sample size and variable diagnostic criteria should also be considered[235]. Many additional factors can affect cytokines levels but are not routinely controlled for across studies. Such factors include age[236], ethnicity[237], gender and menstrual cycle phase[238], sleep[239], BMI[240], smoking status[241], alcohol use[242], medical comorbidities such as coronary artery disease, obesity, diabetes, osteoporosis, pain, and autoimmune diseases[194,243,244], exercise[245], food consumption[246], and stress[247]. This is in addition to the effects of clinical variables like duration and severity of schizophrenic illness, and use of medication[248]. Stage of schizophrenic illness is an additional factor to consider, as some cytokines have been identified as state markers of acute symptom exacerbations (e.g. IL-1β, IL-

6, transforming growth factor (TGF)-β) while others may be trait markers (e.g. IL-12, IFN-γ, TNF-α, sIL-2R)[249].

The immunological profile of the periphery may also suggest that schizophrenia is associated with a concurrent increase in pro- and anti-inflammatory activity, evidenced by hyper-production of both types of factors and immune cells[250,251]. For example, Drexhage *et al.* (2011)[252] found higher percentages of pro-inflammatory monocytes, activated $CD3^{+}CD25^{+}$ T cells, and pro-inflammatory TH_{17} cells in schizophrenic patients, in addition to elevated anti-inflammatory $CD4^{+}CD25^{high}FoxP3^{+}$ regulatory T cells, and $IL\text{-}4^{+}$ lymphocytes. It has been proposed that increased anti-inflammatory signalling may be a protective response which develops subsequent to increased pro-inflammatory activity to limit the potentially damaging effects of chronic inflammation[135]. Although the direction of these immune findings has not yet been clearly defined, peripheral cytokines and BDNF may be meaningful proxies of brain activity due to a high degree of correlation between circulating and central levels[253,254].

1.6.3. Central Cytokine Alterations in Schizophrenia

Numerous studies have provided evidence of central inflammation within schizophrenia (refer to Figure 1.3) by quantifying immune markers in cerebrospinal fluid (CSF). CSF investigations have shown elevations of pro-inflammatory mediators (e.g. IL-1β, IL-2, IL-6) and their receptors (e.g. sIL-6R)[135,255,256], reductions of anti-inflammatory factors (e.g. sIL-2R, sIL-1RA, TGF-β receptor)[135,257], and increased numbers of monocytes and macrophages[258]. According to Meyer *et al.* (2011)[135], these patterns of CNS activity may reflect an enhanced inflammatory profile occurring in tandem with central immunosuppression, which differs from peripheral immune patterns in schizophrenia. CSF analyses have also suggested that neuroinflammation may only be present in a subgroup of schizophrenic patients[259]. Similar to in the periphery, CSF reductions in BDNF have been observed in drug-naive FEP, with levels negatively correlating with the severity of symptoms (refer to Figure 1.3)[254].

S100B, a calcium- and zinc-binding protein, is a proxy for brain tissue damage and glial dysfunction, particularly astrocyte over-activation[260]. S100B can have harmful effects like

neuronal apoptosis, production of inflammatory factors (e.g. COX-2, PGE_2, IL-1β) and NOS, and up-regulation of TNF-α secretion from monocytes and microglia[142]. CSF and serum levels of S100B are elevated within schizophrenia[261-263], with findings confirmed in drug-free patients[264]. Post-mortem investigations have observed S100B-imunoreactive astroglia in brain regions implicated in the pathophysiology of schizophrenia like the anterior cingulate cortex, dorsolateral prefrontal cortex, orbitofrontal cortex and hippocampus[142]. Reports of whether increased S100B occurs in the presence of astrogliosis have been inconsistent[265,266].

Microglia activation and enhanced densities, particularly in the temporal and frontal cortex, have also been implicated in schizophrenia according to post-mortem studies[267,268]. Zhu *et al.* (2014)[269] observed that intrahippocampal LPS injections into rats led to behavioural changes that were comparable to the schizophrenic phenotype (e.g. social and pre-pulse inhibition deficits) and to an elevated number of microglia in the hippocampus, cerebral cortex and thalamus. Positron emission tomography (PET) has confirmed enhanced microglia in schizophrenia, in real time, by quantifying translocator protein (TSPO) binding density activation[270]. TSPO is typically expressed at low levels in the brain but, in the presence of activated microglia, can be detected in the outer mitochondrial membrane[271]. Such elevations of activated microglia are observable in the first 5 years of schizophrenia[272]. Studies have also shown decreased BDNF concentrations within the cortex and hippocampus of schizophrenic patients, in addition to reduced TrkB and BDNF-positive neurons in the CNS[273].

1.6.4. Immunogenetics and Schizophrenia

Many genes that have been implicated in conferring risk of schizophrenia are also involved in the inflammatory response (refer to Figure 1.3). Most significantly, genetic variants within the extended region of the major histocompatibility complex (MHC), which is an area of high immune relevance on chromosome 6p22.1, are the most likely susceptibility genes for schizophrenia[274,275]. Similar findings have emerged for genes encoding the IL-1 cytokine family on chromosome 2q13[191]. Numerous cytokine polymorphisms are also associated with schizophrenia and its clinical features, such as variants localized to genes encoding IL-1β (rs1143633, rs16944, rs4848306), IL-2 (rs2069762), IL-6 (rs1800795), IL-6R (rs8192284),

monocyte chemoattractant protein-1 (MCP-1, rs1024611), TNF-α (rs1800629, rs1799724, rs361525), IL-3RA (rs6603272), IL-4 (rs2243250), IL-8 (rs2227307), IL-10 (rs1800896), IL-18 (rs187238, rs1946518), and IL-28B (rs8099917) (for review, see Zakharyan & Boyajyan, 2014)[193]. Adjunct imaging data have shown that the T, or high transcriptional, allele for IL-1β rs16944 is associated with brain volume reductions[276] and differential brain activation[277] in schizophrenia. Expression analyses have shown that several genes involved in immune response pathways, such as serpin peptidase inhibitor, clade A, member 3 (SERPINA3), interferon-induced transmembrane (IFITM) proteins, Chitinase-3-like 1 (CHI3L1), heat shock proteins, and CD14 antigen are up-regulated in schizophrenia, particularly within the prefrontal cortex[278,279]. Expression of inflammatory cytokines (e.g. IL-1β, IL6, TNF-α) is also enhanced in circulating monocytes[144] and leukocytes [280] in schizophrenia.

Genetic variance in BDNF has also been associated with schizophrenia, particularly the Val66Met and C270T polymorphisms[281,282]. However, findings have not been consistently replicated[283,284]. A meta-analysis by Gratacos *et al.* (2007)[285] confirmed the association between Val66Met and schizophrenia, reporting that homozygous carriers of the Met/Met genotype were at a 19% elevated risk compared to heterozygotes. Val66Met has also been associated with clinical features of schizophrenia, such as age of onset[286], symptom severity[286], cognitive difficulties[287,288], and alterations in brain morphology[289,290]. Epigenetic alterations of BDNF, particularly DNA methylation signatures, may also contribute to schizophrenia[291,292]

FIGURE 1.3: Immune and Neurotrophin Alterations in Schizophrenia

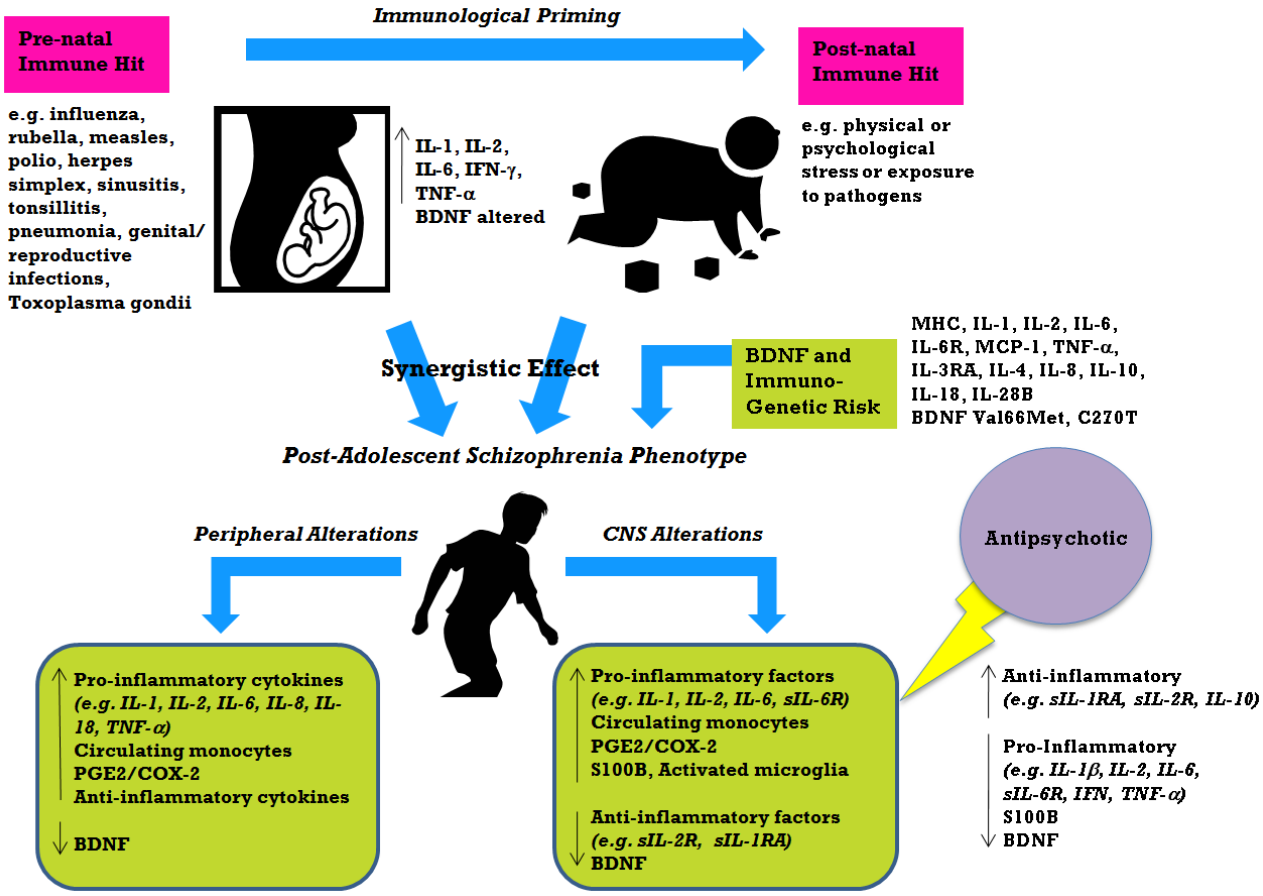


FIG 1.3: Maternal exposure to infection during prenatal development, in synergy with post-natal stress and schizophrenia susceptibility genes, lead to the development of the schizophrenic phenotype after adolescence. This phenotype is characterized by alterations in inflammatory factors, immune cells and BDNF within the periphery and CNS. Immune dysfunction is often normalized with antipsychotic treatment, while BDNF deficiency remains unchanged or may even undergo further reductions.
IL = interleukin, IFN = interferon, TNF-α = tumor necrosis factor alpha, BDNF = brain-derived neurotrophic factor, MHC = major histocompatibility complex, MCP-1 = monocyte chemoattractant protein-1, PGE2 = prostaglandin E2, COX-2 = cyclooxygenase-2, CNS = central nervous system

1.6.5. Mechanisms of Cytokine-Induced Schizophrenia

Schizophrenia has been predominantly characterized by a highly interconnected disturbance of dopamine and glutamate neurotransmission. Dopamine dysregulation has been described by hyperactivity in the striatum (i.e. mesolimbic dopamine pathway) and hypoactivity

in the frontal cortex (i.e. mesocortical dopamine pathway), with the former contributing to positive symptoms and the latter to cognitive, negative and affective symptoms[81,293]. Dopaminergic alterations are a possible consequence of glutamatergic hypofunction due to impaired synaptic signalling at the glutamate receptor, *N*-methyl-D-aspartate (NMDA)[293,294]. Neurotransmitter disruptions can also occur for gamma-aminobutyric acid (GABA), serotonin, opioids, and acetylcholine[293].

Inflammatory cytokines, like IL-1β, IL-6, and TNF-α, may contribute to these disturbances, as they have been shown to affect glutamate release via activation of the enzyme, indoleamine 2,3 dioxygenase (IDO)[295]. IDO is found in immune cells (e.g. macrophages, dendritic cells), and in all organs including the brain[165]. IDO degrades tryptophan, a serotonin precursor, into kynurenine (KYN) which is subsequently converted to kynurenic acid (KYNA) in astrocytes and quinolinic acid (QUIN) in microglia[177]. Cortisol can also initiate this pathway via tryptophan 2,3 dioxygenase (TDO)[165]. Increased KYNA inhibits signaling at NMDA and α7-nicotinic acetylcholine (α7nAch) receptors which leads to blockade of glutamate release[135,142,296]. Studies confirm that KYN and KYNA are both elevated in the CSF [297-299] and post-mortem brain tissue[300] of schizophrenic patients. Post-mortem analyses and studies employing various methodologies like PET/single photon emission computed tomography (SPECT), and proton magnetic resonance spectroscopy (^{1}H-MRS) have confirmed abnormalities in glutamatergic indices in both medicated and unmedicated patients[105,301].

Glutamate is the major excitatory neurotransmitter in the CNS, and its dysfunction has been implicated in positive, negative and cognitive symptoms, and changes in brain morphology in schizophrenia[57,203,228,294,302]. *Positive Symptoms.* Corticobrainstem glutamate neurons inhibit dopamine release from mesolimbic neurons by signaling through an inhibitory GABA interneuron in the ventral tegmental area (VTA). NMDA hypoactivity within this projection would thus limit these capabilities, and lead to mesolimbic dopamine hyperactivity[81,294]. Indeed, studies show that increased KYNA in schizophrenia leads to hyperactivity of dopamine neurons in the VTA[303], and administration of NMDA receptor antagonists increases dopamine in the rat nucleus accumbens and striatum[304]. *Negative/Cognitive/Affective Symptoms.* Glutamate projections to the mesocortical dopamine pathway do not signal through GABA, and thus, can normally function to excite dopamine neurons. Thus, in the presence of NMDA hypoactivity,

mesocortical dopamine neurons are also hypoactive[81]. Similar dopaminergic effects may be achieved through cytokine-induced reductions in tetrahydrobiopterin (BH_4) bioavailability, an enzyme cofactor required for the dopamine synthetic pathway, through production of ROS and NOS[305]. Taken together, cytokines yield the dopaminergic profile found within schizophrenia by altering striatal and prefrontal dopamine levels[201,212,213,227,306,307]. Negative symptoms may also be mediated by IDO-based reduction in tryptophan bioavailability leading to central serotonin deficiency[135]. Cytokine-induced glutamatergic hypofunction may also mediate reductions in BDNF expression, as activation of NDMA receptors increases levels of BDNF mRNA, whereas NMDA antagonism has opposite effects[308,309]. Another potential contributing factor is monoamine alterations, particularly serotonin, as 5-$HT_{2A/2C}$ receptor agonists significantly elevate BDNF mRNA expression[166]. Dopamine has also been shown to modulate levels of BDNF mRNA and protein through D_1 receptor activity[310].

1.6.6. Anti-inflammatory Properties of Antipsychotics

Several studies suggest that APs have immunomodulatory properties, particularly anti-inflammatory effects. Long-term treatment with APs has been shown to increase expression of anti-inflammatory mediators (e.g. sIL-1RA, sIL-2R, and IL-10) while simultaneous reducing pro-inflammatory markers (e.g. IL-1β, IL-2, IL-6, sIL-6R, IFN, and TNF-α) in the periphery and CNS[25,135,186]. APs have also been shown to reduce levels of S100B[311]. Additional support is derived from reports of AP efficacy in treating tuberculosis/mycobacterial infections[312], and rheumatoid arthritis[313]. APs may mediate these effects by suppressing production of pro-inflammatory cytokines and nitric oxide from activated microglia[134,138]. Among APs, SGAs have more potent anti-inflammatory properties relative to FGAs[135,314]. The capacity of APs to normalize the inflammatory signatures of schizophrenia contributes to their clinical efficacy. For example, reduction of serum IL-2 subsequent to AP therapy correlates with greater improvement in symptom severity[314]. Baseline levels of pro-inflammatory markers can serve as a predictive biomarker of treatment response, as it is shown to negatively correlate with clinical improvement[314]. Genetic variation across immune genes also affects clinical response to APs, as observed for IL-1RA[315], MCP-1[316], and TNF-α[317].

Augmentation of APs with adjunctive anti-inflammatory compounds has shown superior treatment benefits in reducing positive and negative symptoms compared to AP monotherapy[318]. Anti-inflammatory add-on therapy is also advantageous among AP-resistant patients[319,320]. Various agents have been studied in schizophrenia including minocycline[321], and non-steroidal anti-inflammatory drugs (NSAIDs) like the COX-2 inhibitor celecoxib[322] and COX-1/2 inhibitor acetylsalicylic acid[323]. Essential polyunsaturated fatty acids (EPUFAs) like omega-3 eicosapentaenoic acid (EPA) and estrogens like 17β-estradiol have mild anti-inflammatory properties and show therapeutic benefits for schizophrenia[324,325]. Similar findings have been reported for *N*-acetylcysteine which has both anti-inflammatory and antioxidant properties[324].

Conversely, BDNF is not typically normalized with AP therapy. Instead, APs have been shown to cause a further reduction in BDNF, likely mediated through 5HT receptor inhibition[176]. Numerous studies report decreased serum BDNF levels under FGA and SGA treatment relative to controls[326-328]. Pirildar *et al.* (2004)[329] showed that untreated schizophrenic patients had lower baseline BDNF levels compared to controls, and levels were unchanged after 6 weeks of AP treatment. Rizos *et al.* (2010)[330] reported similar findings after measuring serum BDNF in schizophrenic patients during relapse and again after 6 weeks of AP treatment. Pillai *et al.* (2006)[331] found that central levels of BDNF protein in the rat brain were unchanged by olanzapine, largely decreased by haloperidol and chlorpromazine, and moderately decreased by risperidone after 90 days of treatment, with levels continuing to decline for up to 180 days. However, findings are not consistently replicated, as some trials report AP-mediated improvements in BDNF levels[332].

PART III: INFLAMMATION AND METABOLIC PROCESSES

1.7. Cytokines and Anorexia of Infection

1.7.1. Anorexia of Infection and the Acute Phase Response

Inflammatory cytokines have been closely implicated in metabolic regulation, particularly a reduction in food-motivated behaviours, food intake, and body weight[333,334]. Cytokines mediate these effects as part of the acute phase response (APR) of innate immunity[335]. The APR is an integrated set of immune, endocrine, metabolic, behavioural, and neural alterations that are mounted by the innate immune system upon immediate recognition of an immunological insult[336,337]. In addition to anorexia, the APR can include reactions like fever, lethargy, altered plasma protein concentrations (e.g. C-reactive protein, CRP), and increased leukocyte counts[338]. APR reactions, including anorexia, form a critically adaptive defence strategy that takes priority over regular homeostatic controls to provide short-term survival benefits to the host[335,339]. The survival benefits of anorexia can include: (1) reducing food scavenging behaviours to conserve energy and heat, (2) reducing access to food-derived micronutrients, such as iron and zinc, that can be used by pathogens to flourish, (3) limiting potentially harmful metabolic APR effects, and (4) promoting apoptosis of infected cells[336,337,339]. In support of the adaptive effects of anorexia, Murray & Murray (1979)[340] showed that force-feeding infected mice to a normal energy intake resulted in increased mortality and reduced survival time compared to infected mice fed ad libitum. Similarly, Wing & Young (1980)[341] found that acutely starved mice infected with Listeria monocytogenes showed significantly less mortality than fed mice.

1.7.2. Cytokines as Mediators of Anorexia

Inflammatory cytokines, such as IL-1α, IL-1β, IL-2, IL-6, IL-8, TNF-α, and IFNs have been shown to suppress food intake, both individually and synergistically, after peripheral and central administration[342-350]. Alterations in feeding behaviours are typically observed as

reductions in meal size, duration, and frequency, and longer inter-meal intervals[344,349,351]. Aubert *et al.* (1995)[352] showed that rats treated with IL-1β had a reduced total caloric intake, and consumed relatively more carbohydrates and less protein. Taste aversion can also result from cytokine administration but this does not appear to be a major component of anorexic action[336,353]. Studies have shown an attenuated anorexic response within cytokine and/or cytokine receptor gene knock-out models[354,355], and upon antagonism of cytokine action[351,356-358]. However, chronic cytokine administration can eventually lead to tolerance toward their hypophagic effects, and thus, recovery of food intake[359,360]. In addition to altering feeding behaviours, cytokines influence body weight through effects on energy expenditure (e.g. change in body temperature)[361,362].

1.8. Mechanisms of Cytokine-Induced Anorexia

1.8.1. Cytokines and Peripheral Satiety Signals

As shown in Section 1.5.3, peripheral cytokines have the ability to stimulate afferent nerve fibres, like the vagus nerve of the abdomen and GI tract which relay signals through the nucleus of the solitary tract to the PVN of the hypothalamus[91]. This neural route may be a potential mechanism through which cytokines mediate their anorexic effects, as subdiaphragmatic vagotomy can attenuate suppression of both food intake[363] and food-motivated behaviour[363] in some but not all cases[364]. Cytokines may work in concert with the peripheral satiety hormone CCK to stimulate the vagus nerve. Cytokines induce release of CCK, and activation of CCK_A receptors partially contributes to vagal-mediated hypophagic outcomes[336]. In addition, cytokines, like IL-1 and TNF-α, elevate plasma leptin levels to suppress food intake, and can act directly on adipocytes to stimulate leptin secretion[309,365-368]. Cytokines can also influence levels of insulin and glucagon[369]. In further, IL-1α, IL-1β, IL-6, and TNF-α reduce stomach muscle contractions to yield gastric stasis (i.e. delayed gastric emptying) which contributes to anorexia by influencing meal size and length of inter-meal intervals[370].

1.8.2. Cytokines and Central Satiety Controls

In the CNS, cytokines have direct action at hypothalamic neurons in the LHA, PVN, and VMN in mediating feeding behaviours[369]. In support, cytokine mRNA and protein (e.g. IL-1β, TNF-α), and cytokine receptors have been found in multiple brain regions including the hypothalamus[371-373], with greatest concentrations in the VMH[333]. In anorexic tumor-bearing rats, IL-1β and IL-1R mRNA are up-regulated in the hypothalamus, and CSF levels of IL-1 negatively correlate with food intake[333]. Kent *et al.* (1994)[374] demonstrated that direct administration of recombinant IL-1β into the VMN both time- and dose-dependently induced anorexia and weight loss, with food and water consumption reduced by 45% and 30%, respectively. VMN-injected cytokine antagonists have been shown to attenuate anorexic outcomes[375,376]. For example, Laviano *et al.* (1995)[375] showed that central injections of IL-1RA into the VMN improved food intake in anorexic tumor-bearing rats.

Cytokines can also mediate anorexic effects by influencing hypothalamic production of various neurotransmitters and neuropeptides that are relevant to the regulation of food intake. For example, central cytokine administration can reduce levels of NPY[377]. Sonti *et al.* (1996)[378] observed that NPY administration blocks the anorexic effects of IL-1β when both are administered together, and induces feeding in anorexic rats pre-treated with IL-1β . A similar relationship exists between NPY and IFN-α[346]. LPS and IL-1β have been shown to decrease plasma ghrelin levels, an effect which can be blocked by administration of exogenous ghrelin, IL-1RA and the NSAID indomethacin[379]. Cytokines also interact with other neuropeptides like POMC-derived peptides (e.g. α-MSH), hypocretins/orexins, CART, MCH, and AgRP[369,373]. Anorexic outcomes may also result from the ability of cytokines to influence the expression of hypothalamic neurotransmitters like dopamine, serotonin, histamine, and norepinephrine which, as shown in Section 1.4.3, are involved in regulating food intake[369,380].

In addition, prostaglandins, especially PGE_2, and their precursors have been shown to reduce food intake and influence GI motility[381-386]. C-*fos* mRNA studies show that central injection of PGE_2 activates neurons within the PVN[387]. These anorexic effects are typically reversible by COX inhibitors like aspirin, flurbiprofen, indomethacin and paracetamol[381,383]. In support, pre-treatment with COX-inhibitors can partially block the anorexic and gastric emptying

effects of IL-1α, IL-1β, and TNF[370]. COX gene knockout models suggest a greater involvement of COX-2 in cytokine-induced hypophagia than COX-1, although COX-1 may contribute to early-stage anorexic responses[388-390]. Furthermore, cytokines activate the hypothalamic-pituitary-adrenal (HPA) axis[391,392], and simulate release of CRH[393-396] which then acts within the brain to reduce food intake[397]. Uehara *et al.* (1989)[398] demonstrated that pre-treatment with CRH-antiserum blocks reductions in food intake subsequent to IL-1β administration.

1.9. Cytokine Involvement in Weight Gain and Obesity

1.9.1. Inflammatory Feedback Response in Obesity

In Section 1.4.2, inflammatory cytokines were identified as a type of adiposity signal that, separate from their role in illness anorexia, convey information on long-term energy stores to the hypothalamus under normal conditions[91]. During fat accumulation and adipocyte enlargement, adipose tissue (particularly within visceral fat) becomes a site of active inflammation as it undergoes molecular and cellular changes, accumulates macrophages, and secretes various immune factors like inflammatory cytokines[399,400]. These cytokines subsequently suppress feeding and induce energy expenditure (refer to Section 1.7.2) via a feedback loop to prevent obesity, and thus, maintain homeostatic balance (refer to Figure 1.4)[401]. Therefore, once caloric restriction is achieved, it is accompanied by a potent anti-inflammatory effect which can include reduced production of inflammatory cytokines and prostaglandins, lowered blood lymphocyte counts, and reduced macrophage activation and infiltration into adipocytes[400,402,403]. Perturbations of adiposity signalling may cause a shift in weight gain outcomes. For example, a developed resistance to inflammatory signalling leads to continued fat accumulation, and ultimately, obesity[401]. In support, obesity (and MetS) is a condition associated with low-grade chronic inflammation[404]. Inflammation within obesity, particularly increased levels of TNF-α and IL-6, has also been shown to influence the insulin signalling pathway, thereby leading to insulin resistance[405]. This can, in turn, lead to hyperglycemia and type II diabetes, which may, through glucose excretion in the urine, be an extreme method to rid the body of surplus

energy[401]. Similar to inflammation resistance, a deficit of inflammatory signalling in the face of increased adiposity would likely yield similar obesity outcomes. Supportive evidence may be derived from O'Rourke *et al.* (2006)[406] who found decreased serum cytokine protein and mRNA levels in peripheral blood mononuclear cells of obese compared to lean patients, which occurred in spite of an intact capacity to up-regulate cytokine expression in response to leptin.

FIGURE 1.4: Immune and Neurotrophin Involvement in Obesity Signalling

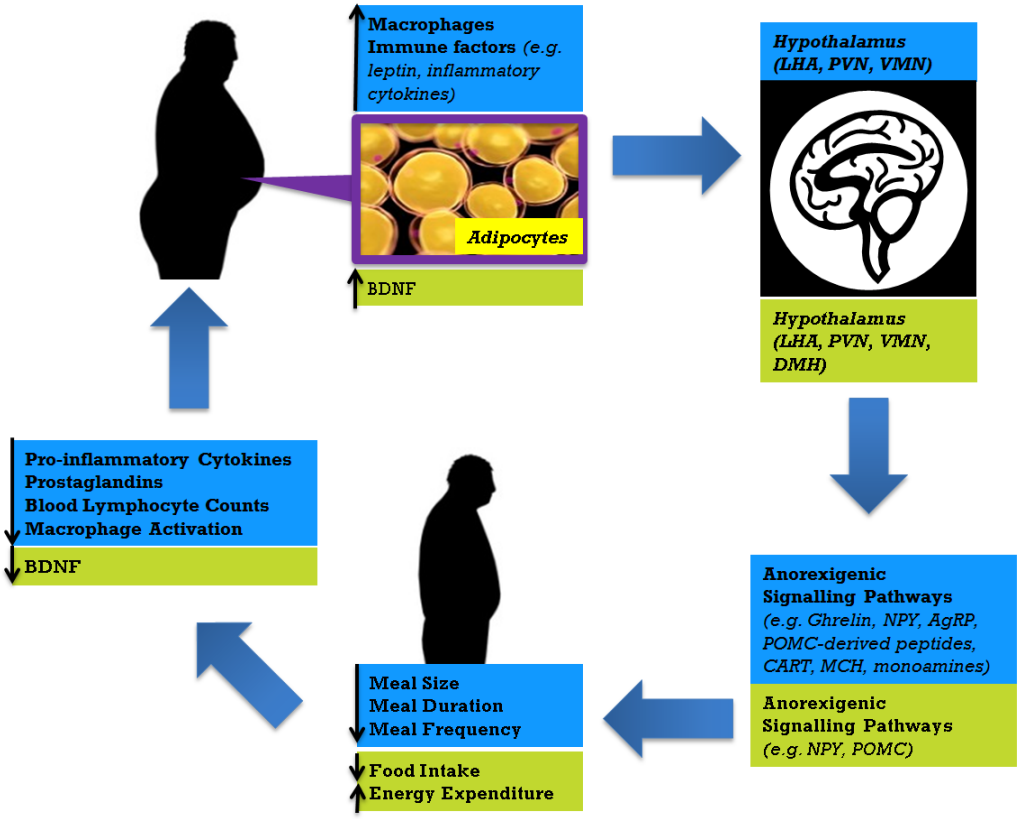


FIG 1.4: The adiposity signalling pathways of inflammatory cytokines (in blue) and BDNF (in green) are shown. Both markers, which are elevated during fat accumulation, act within the hypothalamus to initiate anorexigenic pathways, to ultimately decrease food intake and increase energy expenditure. Once caloric restriction is achieved, cytokine and BDNF levels are reduced. Perturbations of adiposity signalling by altering cytokine or BDNF levels may shift weight gain outcomes.

1.9.2. Genetic Knockout Models of Cytokine-Induced Obesity

Various models have tested the association between deficit cytokine production and obesity outcomes using genetic knockout models. Wallenius *et al.* (2002)[27] observed that IL-6 deficient mice (IL-$6^{-/-}$) developed mature-onset obesity accompanied by increased food intake, altered carbohydrate and lipid metabolism, increased leptin levels, and reduced responsiveness to leptin treatment. IL-6 administration was able to partially reverse these effects by lowering body weight and leptin levels, and increasing energy expenditure. Investigations of IL-1 involvement have yielded similar findings. Garcia *et al.* (2006)[407] found that IL-1RI deficient mice (IL-$1RI^{-/-}$) developed mature-onset obesity relative to wild-type mice by 5 to 6 months of age, with a 20% weight difference emerging by 9 months. IL-$1RI^{-/-}$ mice also developed a 1.5 to 2.5-fold increase in visceral and subcutaneous fat mass, insulin resistance, increased leptin levels and, prior to obesity onset, reduced locomotor activity and reduced suppression of body weight and food intake in response to leptin. Similar outcomes were observed by McGillicuddy *et al.* (2013)[408] in IL-$1RI^{-/-}$ mice which developed mature-onset obesity after 6 months despite being fed a low-fat diet. Netea *et al.* (2006)[409] showed that deficiency of IL-18 (IL-$18^{-/-}$) in mice leads to higher leptin levels, hyperphagia, increased cholesterol and triglyceride concentrations, obesity, and insulin resistance, which can all be partly reversed with central administration of recombinant IL-18. Obesity differences, relative to control mice, first appeared at 6 months of age, with the greatest difference of 38.1% occurring at 12 months. Mice deficient for the IL-18 receptor (IL-$18R^{-/-}$) also displayed similar obesity and insulin resistance outcomes.

There is an observed synergy between cytokines in yielding weight change effects. Chida *et al.* (2006)[26] found that mice with a combined IL-1 and IL-6 deficiency (IL-$1^{-/-}$/IL-$6^{-/-}$) developed obesity by 10 weeks while mice with either a IL-1 or IL-6 knockout remained normal at this age. IL-$1^{-/-}$/IL-$6^{-/-}$ mice also had significantly higher daily food intake and greater suppression of anorexic effects after peripheral IL-1 administration. Investigations of the effects of IL-1RA have also been conducted. Matsuki *et al.* (2003)[410] showed that IL-$1RA^{-/-}$ mice, in which excess IL-1 signaling may occur, have a lean phenotype through impaired body fat accumulation and lipid storage, and resistance against the obesity-inducing effects of both monosodium glutamate and a high-fat diet. Similar results were obtained by Somm *et al.* (2005)[411] after studies of IL-$1RA^{-/-}$ mice revealed a lean phenotype based on reduced fat mass,

dysfunctional adipogenesis, and increased energy expenditure. Horai *et al*. (1998)[412] also observed reduced body weight in IL-1RA$^{-/-}$ mice relative to heterozygote IL-1RA$^{+/-}$ littermates.

1.9.3. Cytokine Genetic Variants and Obesity

Polymorphisms in cytokine genes show an association with human obesity. Andersson *et al.* (2009)[413] found that the 3' untranslated region variant, rs4252041 (C>T), of the IL-1RA gene was associated with lower total and regional fat mass, total fat percent (%) and BMI in 1,068 young men. Um *et al.* (2011)[414] found two IL-1α polymorphisms, C-889T (rs1800587) and G+4845T (rs17561), that were associated with an increase in BMI in obese healthy women. Lee *et al.* (2008)[415] investigated the IL-1β +3953 (rs1143634) variant site, finding a higher frequency of the T (CT/TT), or high transcriptional, allele among lean BMI (<25 kg/m^2) versus overweight BMI (25-29 kg/m^2) females. The same variant site was tested by Manica-Cattani *et al*. (2010)[32] in 880 Caucasian subjects, similarly finding a higher T allele frequency in non-overweight than overweight and obese groups. Strandberg *et al.* (2006)[416] also tested the +3953 variant but against body fat mass in 1,068 young men. Results showed that carriers of the T allele had significantly lower total fat mass, in addition to reduced arm, leg, and trunk fat. Additional investigations by Strandberg *et al.* (2008)[417] found an association for both the IL-1β -31T>C (rs1143627) and IL-6 -174 G>C (rs1800795) polymorphisms with total and regional fat mass in 3,014 elderly men. The IL-6 -174C variant has also been associated with higher BMI and risk of obesity-related metabolic indices like insulin resistance and high systolic blood pressure[418,419], while the -174G variant has been associated with a lean phenotype, low waist circumference, and low concentrations of insulin or glucose[33]. Andersson *et al.* (2010)[420] found the IL-6 polymorphism rs10242595 G>A to be associated with low BMI and total body fat mass, and smaller regional fat masses. In addition, the IL-6 promoter polymorphism rs2069827 G>T is associated with elevated early-adulthood BMI, baseline BMI, and waist circumference in men and women, separately[421]. Wolford *et al.* (2003)[422] studied multiple polymorphisms across the IL-6R gene (rs4845623 T>C, c_1981308_10 T>C, rs2228145 T>G, c_1158918_10 G>C, rs2229328 T>C) finding that carriers of the variant allele for these sites had a higher mean BMI compared to those with the wild-type allele when tested among 700 non-diabetic Pima Indians.

1.9.4. Neurotrophin Involvement in Obesity

BDNF has been implicated in the regulation of feeding and energy metabolism (refer to Figure 1.4). The highest levels of BDNF protein are within the hippocampus and hypothalamus[423], and BDNF mRNA has been isolated within these brain regions[166]. In particular, although both BDNF and TrkB are expressed in the VMN, LHA, PVN, and dorsomedial hypothalamic nucleus (DMH), BDNF is greatest in the VMN[424]. BDNF has also been shown to affect NPY and POMC expression[425-427], and the serotonin system within the hypothalamus[428,429]. The suggested role of BDNF within feeding is anorexigenic, with reports of central BDNF administration suppressing food intake[428,430,431] and increasing energy expenditure[431] leading to weight loss. Central BDNF protein has been shown to decrease subsequent to food deprivation, and increase with re-feeding[430]. Serum BDNF levels are positively correlated with body weight and BMI[432]. Investigations of BDNF gene mutations provide further support for this association. Kernie *et al.* (2000)[433] found that approximately 50% of $BDNF^{+/-}$ heterozygous mice develop obesity, evidenced by a 300% increase in fat by 9 months, in addition to leptin resistance and hyperinsulinemia. Lyons *et al*. (1999)[429] observed that $BDNF^{+/-}$ mice develop chronic hyperphagia and obesity by 2 to 4 months of age, with a 34% higher body weight than wild-type littermates by 12 to 18 months. The literature further suggests that polymorphisms within the BDNF gene, particularly Val66Met, are associated with obesity, with variable reports on the causal allele. Gunstad *et al.* (2006)[35] first identified that the Met/Met genotype was associated with a lower BMI than Val allele carriers in 481 healthy adults. This finding was subsequently replicated in other studies[434-436]. However, some studies report the reverse association linking the 66Met allele to obesity[437,438], while others report no association[439]. Reduced BDNF levels have also been noted within eating disordered patients[440,441], while elevated BDNF is observable in obese individuals[432].

1.10. Conclusions

There is a growing body of evidence that supports the role of cytokine and neurotrophin (BDNF) dysfunction in both schizophrenia and metabolic disturbances like weight gain. In considering the proposed immunomodulatory properties of APs, in addition to their effects on BDNF, it is plausible that these factors contribute to AIWG. This theory has received some attention in recent years, starting with Klunge *et al.* (2009)[442] who proposed that the effects of clozapine and olanzapine on weight gain may be closely related to their effects on cytokine networks after finding that levels of TNF-α, soluble tumor necrosis factor receptor (sTNFR)-1, sTNFR-2, and sIL-2R correlated with BMI after 6 weeks of treatment. Similarly, Song *et al.* (2014)[443] observed significant alterations in serum levels of IL-1β, IL-6, and TNF-α, in addition to body weight, over 6 months of risperidone treatment in drug-naïve FEPs. In fact, patients who experienced clinically significant (>7%) weight gain by the end of treatment had higher levels of all cytokine markers compared to patients who gained <7% of weight. Klemettila *et al.* (2014)[444] tested similar associations in 190 treatment-resistant schizophrenia patients on clozapine and found that levels of IL-1RA and CRP were associated with obesity, as was IL-6 within females. However, no studies, to the author's knowledge, have investigated the effects of cytokine polymorphisms on AIWG. BDNF has also been tested within AIWG with various lines of evidence suggesting involvement of reduced serum levels, and polymorphisms such as rs11030101, rs1519480, and Val66Met in mediating weight gain[445-448]. In spite of this work, there are no studies, to the author's knowledge, that have tested the epistatic effects between cytokine and BDNF polymorphisms on AIWG. At this stage, details of these associations have yet to be clearly elucidated and require further research.

Chapter 2:

Research Aims and Hypotheses

The purpose of this chapter is to outline:

- ❖ Research aims of the proposed study
- ❖ Research hypotheses of the proposed study

2.1. Research Aims

- Aim 1: To investigate whether variants in the genes encoding IL-1β, IL-2, and IL-6, in addition to BDNF Val66Met, are associated with:
    A. AIWG
    B. Clinically significant (≥7% weight change from baseline) AIWG (herein referred to as *clinically significant weight gain*, *CSWG*)

- Aim 2: To investigate whether variants in the genes encoding IL-1β, IL-2, and IL-6 have epistatic (or synergistic) effects with BDNF Val66Met in their association with AIWG.

- Aim 3: To determine whether variants from *Aim 1* that are significantly associated with AIWG replicate in an independent sample (CATIE sample).

2.2. Research Hypotheses

Based on the aforementioned research aims, the following hypotheses were formulated:

- Hypothesis 1: If antipsychotics decrease expression of IL-1β, IL-2, and IL-6, and either further antagonize or do not improve already reduced BDNF levels, and low levels of inflammatory cytokines and BDNF are associated with hyperphagia and weight gain, then genetic variation across IL-1β, IL-2, and IL-6, in addition to BDNF Val66Met, will be associated with:
    A. AIWG
    B. CSWG

- Hypothesis 2: If pro-inflammatory cytokines and BDNF show inverse expression trends within schizophrenia, and cytokines and neurotrophins interact as part of neuroimmune crosstalk, then variants across IL-1β, IL-2, and IL-6 will interact with BDNF Val66Met to yield AIWG.

- Hypothesis 3: If variants that were identified as being significantly associated with AWIG from *Aim 1* are informative of a true causal relationship, then these associations will replicate in an independent sample (CATIE sample).

Chapter 3:

Methodology

The purpose of this chapter is to:

- ❖ Provide an overview of data collection parameters
- ❖ Describe SNP selection and genotyping procedures
- ❖ Describe statistical analyses

3.1. Study Design

To determine the genetic basis of AIWG, study participants were administered AP medication and monitored for weight change outcomes. Participants had the following information collected:

- Demographic information including age, sex, and ethnicity
- Clinical information including baseline symptom severity, type of AP medication received, and duration of AP treatment
- Weight change information including baseline weight (in kilograms, kg), and weight (in kg) at the end of AP treatment. Information was used to determine the amount of weight gained over the course of AP treatment, as a percentage (%) of baseline weight (herein referred to as *weight change (%) from baseline*).
- 10-20mL of venous blood for genetic analysis

The effect of genetic variance on AIWG was determined by comparing weight change (%) from baseline across genotypic groups for each SNP of interest. Study parameters are described in further detail in the below-listed sections.

3.2. Study Participants

Participants were 188 English-speaking males (n=126) and females (n=62), 18 to 60 years of age, with a Diagnostic and Statistical Manual of Mental Disorders, Third Edition, Revised (DSM-III-TR) or Fourth Edition (DSM-IV) diagnosis of chronic schizophrenia or schizoaffective disorder. Analyses were not restricted to schizophrenia participants because these cases could not be isolated from schizoaffective disorder participants due to lack of available information. Participants with a diagnosis of schizoaffective disorder comprised approximately 10% of the total sample. Participants were primarily of European (n=128) or African descent (n=50), with ethnicity ascertained using self-reported ancestry over three generations. Additional demographic and clinical characteristics are presented in Table 3.1. All participants provided written informed consent. The study was approved by all participating institutional ethics review boards. Participants were recruited across three primary sites:

Sample A. Charite University Medicine in Berlin, Germany (DJ Mueller, I Puls, n=70). Inclusion criteria included a Positive and Negative Syndrome Scale (PANSS)[a] score of $\geq$60, and either first manifestation or relapse of schizophrenic symptoms with significant deterioration. Participants were treated with one of nine AP medications, which primarily included clozapine, haloperidol, olanzapine or risperidone, for up to six weeks.

Sample B. Case Western Reserve University in Cleveland, Ohio (HY Meltzer, JA Lieberman, n=69). Eligible participants were treatment refractory or intolerant to FGAs. Participants did not have prior exposure to SGAs and, where clinically possible, were drug-free for two to four weeks prior to the start of the study. Participants were treated with clozapine for six weeks. Clozapine dosing was variable and serum levels were monitored to confirm compliance.

[a]The PANSS is a 30-item, clinician-rated instrument which assesses the severity of schizophrenic illness using a 7-point rating scale (from 1=absent to 7=extreme) across the following domains: positive symptoms, negative symptoms, and global psychopathology. The PANSS is an adaptation of 18 items from the Brief Psychiatric Rating Scale (BPRS) and 12 items from the Psychopathology Rating Schedule[449].

Samples A and B had additional exclusionary criteria. Participants were excluded from the study if any of the following criteria were endorsed:

- Pregnancy or breast feeding
- Organic brain disorder or severe head injury
- Severe or unstable medical condition requiring treatment including hepatitis C, human immunodeficiency virus (HIV), thyroid disorder or diabetes mellitus
- Substance dependence
- Mental retardation
- Severe personality disorder

Sample C. Hillside Hospital in Glen Oaks, New York (JA Lieberman, n=49). Eligible participants had a PANSS score of $\geq$60, and showed poor response to FGAs as evidenced by persistent positive symptoms and reduced functioning over the past two years. Participants did not have prior exposure to SGAs. Participants were randomly assigned to clozapine (500mg/day), haloperidol (20mg/day), olanzapine (20mg/day), or risperidone (8mg/day) for up to 14 weeks. Dosing was adjusted in response to clinical status. Exclusionary criteria were an intolerance or failure to attain clinical improvement during past trials of the study medications, and/or receipt of depot antipsychotics $\leq$1 month prior to the start of the study (for more details, see Volavka *et al.* 2002)[450].

TABLE 3.1: Demographic and Clinical Sample Characteristics

Characteristics	**Sample A n=70**	**Sample B n=69**	**Sample C n=49**	**Total Sample n=188**
Sex				
Female	32 (45.7%)	24 (34.8%)	6 (12.2%)	62 (33.0%)
Male	38 (54.3%)	45 (65.2%)	43 (87.8%)	126 (67.0%)
Age (years)	35.04 ± 11.9	33.04 ± 8.0	40.74 ±7.5	35.80 ± 10.0
Baseline Weight (kg)	82.31 ± 16.4	74.54 ± 13.1	84.86 ± 18.3	80.07 ± 16.3
Weight Change (kg)	3.48 ± 3.8	3.60 ± 4.4	4.76 ± 6.6	3.85 ± 4.9
Weight Change (%)	4.09 ± 4.3	5.11 ± 6.3	6.18 ± 8.6	5.01 ± 6.4
CSWG				
<7%	52 (74.3%)	44 (63.8%)	32 (65.3%)	128 (68.1%)
≥7%	18 (25.7%)	25 (36.2%)	17 (34.7%)	60 (31.9%)
Baseline BPRS †	50.81 ± 15.7	50.98 ± 14.5	53.74 ± 7.5	51.89 ± 12.9
Study Duration (weeks)	5.23 ± 1.4	6.00 ± 0.0	12.02 ± 3.6	7.26 ± 3.5
Ethnicity				
European	69 (98.6%)	49 (71.0%)	10 (20.4%)	128 (68.1%)
African	1 (1.4%)	20 (29.0%)	29 (59.2%)	50 (26.6%)
Other ǂ	-----	-----	10 (20.4%)	10 (5.4%)
Study Medications				
Clozapine	8 (11.4%)	69 (100%)	10 (20.4%)	87 (46.3%)
Haloperidol	6 (8.6%)	-----	9 (18.4%)	15 (8.0%)
Olanzapine	14 (20.0%)	-----	18 (36.7%)	32 (17.0%)
Risperidone	19 (27.1%)	-----	12 (24.5%)	31 (16.5%)
Others ‖	22 (31.4%)	-----	-----	22 (11.7%)

Values represent number (percent) or mean ± standard deviation; †For comparability across samples, BPRS information was used to assess severity of schizophrenic illness. BPRS total scores were extracted from the PANSS for Samples A and C; ǂIncludes Hispanic, Asia Pacific, American Indian; ‖Includes quetiapine, fluphenazine, aripiprazole, ziprasidone, amisulpride; CSWG = clinically significant weight gain, BPRS = Brief Psychotic Rating Scale

3.3. SNP Selection

A total of 19 putative functional (13) and tag (6) single nucleotide polymorphisms (SNPs)[b] were selected for analysis across IL-1β, IL-2, IL-6 and BDNF (listed below). For the purpose of this study, functional properties (denoted by *) refer to SNPs that have been shown to either (i) affect gene expression directly, (ii) be in high linkage disequilibrium (LD, $r^2 \geq 0.8$)[c] with a secondary SNP known to affect gene expression, or (iii) reside within or in close proximity to a regulatory region of the gene (e.g. promoter region, transcription factor binding site, insulator region). Tagger properties refer to SNPs that capture variation across the gene from being in high LD with other SNPs in the gene region. The following SNPs were selected for genetic analysis:

- **Interleukin 1β** (IL-1β) (located on chromosome 2, refer to Figure 3.1):

  rs4849127, rs13032029 (*), rs16944 (*), rs3136558, rs1143634 (*), rs1143643 (*)

- **Interleukin 2** (IL-2) (located on chromosome 4, refer to Figure 3.2):

  rs2069762 (*), rs2069778, rs2069779, rs2069772, rs2069776 (*)

- **Interleukin 6** (IL-6) (located on chromosome 7, refer to Figure 3.3):

  rs2069827 (*), rs1800795 (*), rs2069837 (*), rs2066992 (*), rs2069840 (*), rs2069861, rs10242595 (*)

- **Brain-Derived Neurotrophic Factor** (BDNF) (located on chromosome 11, refer to Figure 3.4):

  Val66Met (rs6265*)

[b] A SNP refers to a type of genetic variation which occurs when a nucleotide (A, T, C, G) at a given locus in the DNA sequence differs between individuals of a population[451].

[c] Linkage disequilibrium (LD) refers to the non-random association of alleles at different loci. The r^2 statistic, which is a measure of LD, is the squared correlation between allele frequencies at two loci. The value of the r^2 statistic ranges from 0 (loci are randomly associated, and thus, in perfect equilibrium) to 1 (loci provide identical genetic information, and thus, are in perfect disequilibrium)[451]. An r^2 cut-off of 0.8 between SNPs implies that the genotype of SNP1 can be reliably predicted 80% of the time if the genotype of SNP2 is known. The use of $r^2 \geq 0.8$ between loci is a general cut-off to identify SNPs that do not need to be investigated separately.

Putative functional SNPs were selected based on functional evidence in the literature and through use of Encyclopedia of DNA Elements (ENCODE)[452] data which were visualized via University of California Santa Cruz (UCSC) Human Genome Browser[453], and further explored using HaploReg[454] version 1.0, and RegulomeDB[455].

The International HapMap Project[456] database of human genetic variation was used in conjunction with Haploview[457] version 4.2 to identify tag SNPs ($r^2 \geq 0.8$, minor allele frequency (MAF)[d] ≥ 0.05) that were 10 kilobase pairs (kbp) upstream and 2kbp downstream of each interleukin gene. No tag SNPs were selected for BDNF. Haploview[457] version 4.2 was also used to assess Hardy-Weinberg equilibrium (HWE)[e] and MAFs for all SNPs. The selected SNPs covered $\geq$90% of the common genetic variation of IL-1β, IL-2 and IL-6.

[d]A minor allele frequency (MAF) is the lowest allele frequency at a given variant site within a specified population. In this study, MAFs for tag SNP selection were based on subjects of European ancestry from the International HapMap Project[456]. The MAF was set at 5% to ensure that SNPs would be adequately polymorphic.

[e]The Hardy-Weinberg equilibrium (HWE) law states that allele and genotype frequencies will remain constant across generations in a given population in the absence of evolutionary influences like non-random mating, mutation, migration, and natural selection[458.] A departure from HWE may indicate selection pressure effects or, more likely, a genotyping error[451].

FIGURE 3.1: IL-1β Gene Diagram of Polymorphisms

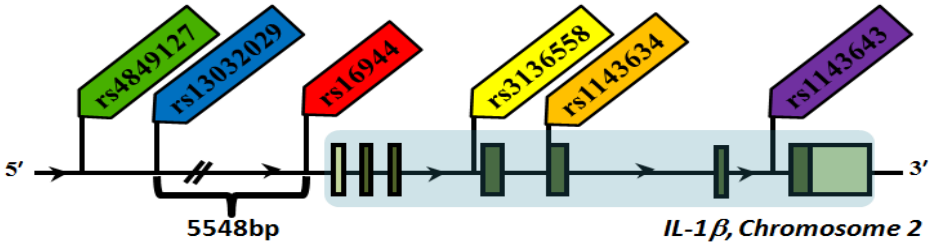


FIGURE 3.2: IL-2 Gene Diagram of Polymorphisms

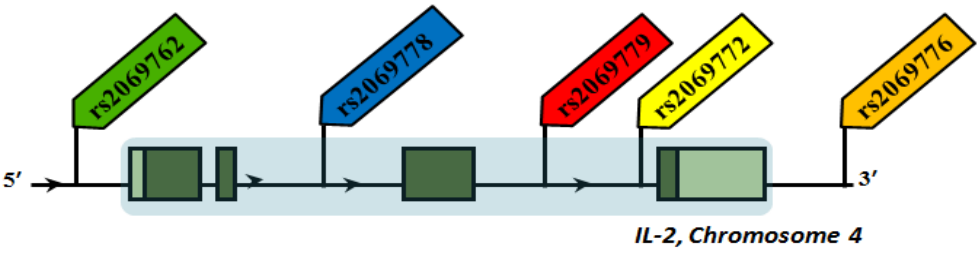


FIGURE 3.3: IL-6 Gene Diagram of Polymorphisms

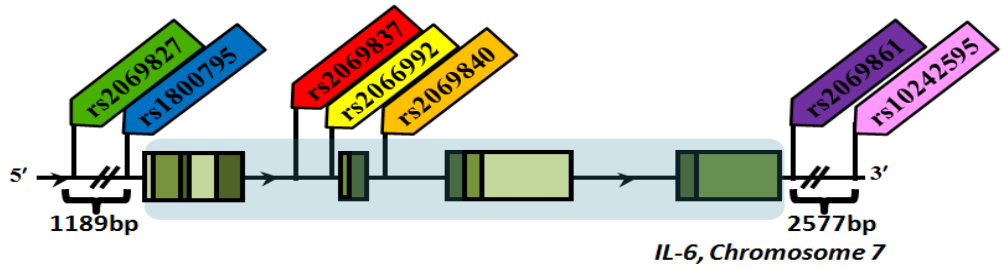


FIGURE 3.4: BDNF Gene Diagram of Polymorphisms

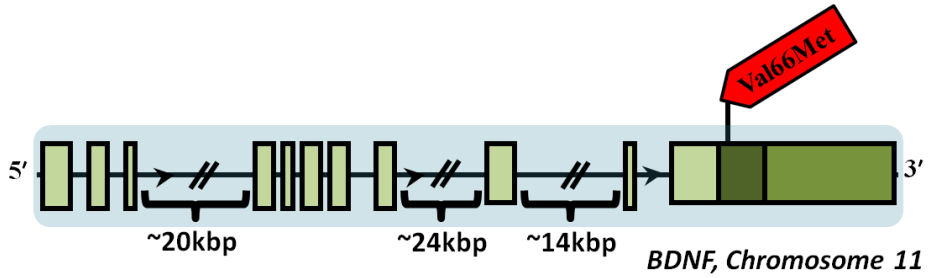


FIG 3.1 to 3.4: The structure of each gene from the present study is depicted, with the full gene region highlighted. Green boxes represent coding exon regions, light green boxes represent non-coding exon regions, and intermediate green boxes represent alternate splicing regions. Study SNPs are tagged based on their location within their respective gene region.

3.4. Genotyping Procedures

Genotype information was determined using polymerase chain reaction (PCR). Venous blood (10-20mL) was obtained from all study participants. Genomic DNA was extracted at the Centre for Addiction and Mental Health using the high-salt method[460]. DNA samples were stored between 2°C to 8°C. All samples were centrifuged using the Allegra™ X-22 Centrifuge (Beckman Coulter™ Inc, Indianapolis, IN) at 2000rpm for 1 minute prior to PCR preparations. Genotyping was performed blind to weight change status. Of the 19 study SNPs, 16 were genotyped using Taqman® OpenArray® technology, while the remaining three were genotyped using Taqman® SNP genotyping assays.

3.4.1. Taqman® OpenArray® Genotyping

SNPs were genotyped using the format-16 Taqman® OpenArray® Genotyping Plate (Applied Biosystems Inc, Foster City, CA). Taqman® assays for 16 SNPs were pre-loaded onto the arrays. Arrays were stored at -20°C to 5°C, and thawed at room temperature prior to use. The quantity of DNA per sample was measured using NanoDrop 8000 spectrophotometer, in conjunction with NanoDrop 8000 Software version 2.0.0 (Thermo Scientific Inc, Wilmington, DE). Spectrophotometer readings were performed according to the manufacturer's protocol. Samples had nucleic acid concentrations in the range of 20-100ng/μL.

PCR was performed using the following reagents: 1.5μL of DNA, and 1.5μL Taqman® OpenArray® Genotyping Master Mix, with a total volume of 3μL per subject. Reagent mixtures were centrifuged at 2000rpm for 1 minute prior to being loaded onto the array using the OpenArray® AccuFill™ System in conjunction with the OpenArray® AccuFill™ System Software version 1.1 (Applied Biosystems Inc). Each array held 144 samples and four negative controls[f]. DNA target sequences were immediately amplified using the QuantStudio™ 12K Flex Real-Time PCR system in conjunction with the QuantStudio™ 12K Flex Software version 1.2.1 (Applied Biosystems Inc), according to the following conditions: (Stage 1) 93°C for 10 minutes,

[f] Negative controls are included to ensure that samples are free from contamination. In the absence of contamination, negative controls should not amplify.

(Stage 2) 50 cycles of 95°C for 45 seconds, 94°C for 13 seconds, and 53°C for 2 minutes and 14 seconds, and (Stage 3) 25°C for 2 minutes.

SNPs IL-1β rs3136558, IL-2 rs2069779, and IL-6 rs2069861 did not successfully plate onto OpenArray® technology and were re-genotyped using Taqman® SNP assays (Applied Biosystems Inc) according to the protocol described in Section 3.4.2.

3.4.2. Taqman® SNP Genotyping

IL-2 rs2069776, IL-6 rs1800795, and BDNF Val66Met (rs6265) were genotyped using Taqman® SNP genotyping assays (Applied Biosystems Inc). Assays were stored between -15°C to -25°C. Each assay was separately mixed using the Vortex Genie® 2 (Thermo Scientific Inc) prior to use in the PCR experiments. Taqman® assays included forward and reverse primers specific to the DNA target sequence of interest, and two fluorescent-dye-labelled probes to differentiate each allele of the target SNP. PCR was performed using the following reagents: 1μL of DNA, 5μL of Taqman® Genotyping Master Mix, 0.25μL of Taqman® SNP assay, and 3.75μL of Qiagen nuclease-free water, with a total volume of 10μL per subject. MicroAmp® Optical 96-Well Reaction Plates (Applied Biosystems Inc) were used in the PCR experiments. Plates included a minimum of four negative controls. All genotyping procedures were carried out according to the manufacturer's protocol. Once added to the plate, reagent mixtures were centrifuged at 2000rpm for 1 minute prior to amplification. The DNA target sequence was immediately amplified using 2720 thermal cyclers (Applied Biosystems Inc), according to the following conditions: (Stage 1) 95°C for 10 minutes, and (Stage 2) 50 cycles of 92°C for 15 seconds and 60°C for 1 minute.

3.4.3. Genotype Calls

SNP genotyping calls were based on allelic discrimination and genotype cluster plots. For Taqman® SNP genotyping, allele-specific fluorescence was measured on the ViiA™ 7 using the ViiA™ 7 RUO Software version 1.2.2 (Applied Biosystems Inc). For Taqman® OpenArray®

genotyping, allele-specific fluorescence was measured directly on the QuantStudio™ 12K Flex Real-Time PCR system using the Taqman® Genotyper Software version 1.3. Genotyping calls were confirmed by two independent researchers after visual inspection of allelic discrimination plots (refer to Figure 3.5). To verify genotyping accuracy, 10% of the total sample was re-genotyped. The concordance rate was 99.9%. Discordant genotypes were removed, and entered as missing values for statistical analysis. The SNP genotyping rate was >97% and subject genotyping rate was >89% in this study.

FIGURE 3.5: Genotype Clusters of an Allelic Discrimination Plot

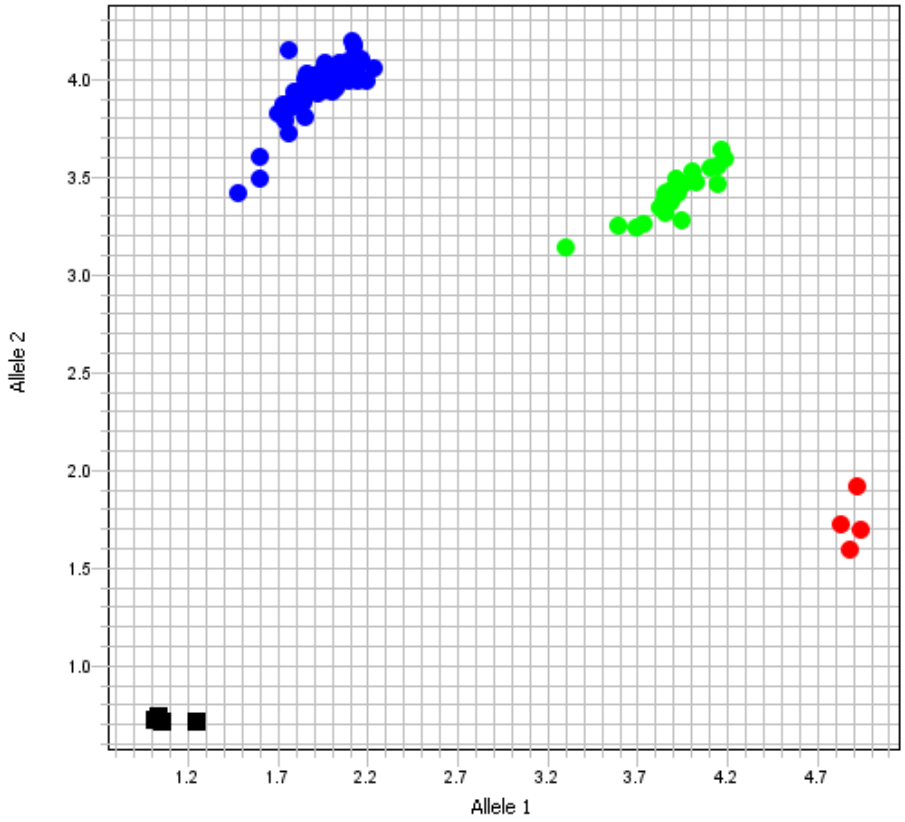


FIG 3.5: An allelic discrimination plot is a visual representation of genotype clusters for a given SNP, as determined by allele-specific fluorescence. Red dots represent individuals homozygous for Allele 1. Blue dots represent individuals homozygous for Allele 2. Green dots represent individuals heterozygous for Allele 1 and Allele 2. A high quality PCR reading presents clearly defined, well-separated clusters. Negative controls (black squares) show zero or minimal fluorescence in the absence of PCR contamination. Individuals with undetermined genotypes are represented by an 'X' (not shown).

3.5. Statistical Analysis

In association testing, categorical variables (CSWG, ≥7%) were compared using Pearson χ^2, and continuous variables (weight change, %) were analyzed using analysis of covariance (ANCOVA). Pearson's correlation coefficients, Pearson's χ^2, and Student t-tests were used to determine any confounding associations between each weight change outcome variable and age, sex, duration of treatment, baseline weight, and baseline Brief Psychotic Rating Scale (BPRS)[g] score. To determine the association between genetic variation and AIWG, two major association analyses were conducted (described below in Sections 3.5.1 and 3.5.2). Within each major analysis, we tested the association of SNPS with AIWG in (i) total, (ii) race-stratified, and (iii) race- and treatment-stratified samples. Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS; SPSS Inc, Chicago, Ill) version 20.0.

3.5.1. Association Analysis I: Weight Change (%) from Baseline

The dependent variable of weight change (%) from baseline was compared across genotypic groups using ANCOVA. Baseline weight and duration of treatment were identified as confounding variables (described in further detail in Section 4.1.2) and entered into the model as covariates. Post-hoc analyses were carried out for all significant SNPs. The effect of each genotype was investigated separately, where possible. However, where there were fewer than five individuals homozygous for the minor allele of a given SNP, these participants were merged with heterozygotes and assessed according to a dominant model of genetic inheritance. If $n<5$ in the merged group, the SNP analysis was not tested for an association with weight change (%). Effect size estimates were reported using eta squared values. Despite the fact that 67% of the total sample was male, gender sub-analyses were not conducted as this variable was not associated with weight change (refer to Section 4.1.2).

[g]The Brief Psychotic Rating Scale is an 18-item, clinician-rated instrument which assesses the severity of both psychotic and non-psychotic symptom dimensions associated with schizophrenia or other psychotic illnesses using a 7-point rating scale (from 1=not present to 7=extremely severe)[461].

The dependent variable was assessed for normality using the Shapiro-Wilk test. Where $p<0.05$, data were visually inspected using normality plots. Visual calls were confirmed by two independent researchers. Homogeneity of variances between genotypic groups was determined using Levene's F statistic. Where testing assumptions were violated, the non-parametric Kruskal-Wallis test was performed. Statistical tests were considered significant at the $p<0.05$ level.

3.5.2. Association Analysis II: Clinically Significant Weight Gain

The variable of weight change (%) from baseline was dichotomized to reflect clinically significant weight change, where patients at or above 7% weight change ('clinically significant weight gain' or CSWG, n=60) were compared against the remainder of the sample ('non-clinically significant weight gain' or non-CSWG, n=128). Weight gain of 7% or more was considered clinically significant, as defined according to the Food and Drug Administration[462]. Pearson χ^2 was used to determine the effect of genotype on CSWG status. No statistical covariates were entered. To ensure an adequate number of individuals across factor space, all investigations were carried out according to a dominant model of inheritance. If any of the *expected cell counts* were $5>n\geq1$, the Fisher Exact test was performed. If n=0 for any cells of factor space, the SNP analysis was not tested for an association with CSWG. Statistical tests were considered significant at the $p<0.05$ level.

3.5.3. Haplotype and Allelic Analyses

Unphased[463] version 3.1.5 was used to investigate haplotype and allelic effects of IL-1β, IL-2, IL-6, and BDNF[h]. Three-marker haplotypes were constructed and analyses were carried out to identify any associations with weight change (%) from baseline. Investigations were restricted to the subset of patients treated with either clozapine or olanzapine. Analyses were performed separately for patients of European and African ancestry. Haplotypes with frequencies less than 5% were excluded from analysis. Where tests of overall association were significant at the

[h]Haplotypes were not tested for BDNF

$p<0.05$ level, post-hoc comparisons were reviewed. Allelic analyses were performed under the same conditions and examined individual allele effects at each locus.

3.5.4. Gene-Gene Interaction Analyses

The association between bi-locus genotypes and weight change (%) from baseline was investigated using Model-Based Multifactor Dimensionality Reduction (MBMDR)[464] operated through R version 3.0.2 (R Foundation for Statistical Computing, Vienna, Austria). Epistatic effects were modelled between BDNF Val66Met and variant sites across IL-1β, IL-2, and IL-6. Significance was calculated using a permutation test based on 1000 permutations. Investigations were restricted to the subset of patients treated with either clozapine or olanzapine. Analyses were performed separately for patients of European and African ancestry. Epistatic effects were considered significant at the $p<0.05$ level.

3.5.5. Multiple Testing Corrections

Single-Nucleotide Polymorphism Spectral Decomposition (SNPSpD)[465] was used to correct for multiple comparisons across association analyses. Adjusted p-values were derived from a variation of the Nyholt correction using the Li and Ji method[466]. The Li and Ji method more accurately estimates the effective number of independent tests (M_{eff}) to control the type I error rate without lowering statistical power. To avoid excessively conservative results, the Li and Ji method was applied wherever its proposed M_{eff} (i.e. $M_{eff\ Li}$) was lower than the standard M_{eff}, as recommended[465]. Gene-wide corrections were calculated for each of IL-1β ($p_{IL\text{-}1\beta}$), IL-2 ($p_{IL\text{-}2}$), and IL-6 ($p_{IL\text{-}6}$); a gene-wide correction was not applicable for BDNF. A hypothesis-wide correction (p_{corr}) was applied by multiplying unadjusted p-values by a summation of $M_{eff\ Li}$ values for each of the four genes.

For haplotype and gene-gene interaction analyses, Bonferroni corrections were applied. For haplotype investigations, gene-wide corrections were applied to tests of overall association, based on the number of three-marker haplotypes examined across each gene. Hypothesis-wide

corrections were not applied. Allelic analyses were corrected for the number of SNPs tested. For gene-gene interaction effects, corrections were based on the number of pair-wise interactions investigated. Since corrections were applied to statistics of overall association, none were applied to post-hoc comparisons.

3.5.6. Power Calculations

Power calculations were performed using Quanto[467] version 1.2.4. Assuming a MAF of 0.05 and a sample size of n=188, we had more than 80% power to detect a mean difference of 4.2% between carriers and non-carriers of the risk genotype (explains 4.1% of the variance) in an additive model of genetic inheritance or a mean difference of 4.4% between carriers and non-carriers of the risk genotype (explains 4.2% of the variance) in a dominant model of genetic inheritance.

Chapter 4:

Results

The purpose of this chapter is to review results from:

- Descriptive Statistics
- Association Analysis I – Weight Change (%) from Baseline
- Association Analysis II – Clinically Significant Weight Gain
- Haplotype and Allelic Analyses
- Gene-Gene Interaction Analyses

4.1. Descriptive Statistics

4.1.1. Clinical and Demographic Sample Characteristics

One hundred and eighty-eight individuals, consisting of 126 males and 62 females, were included in the study. All participants had a DSM-III-TR or DSM-IV diagnosis of chronic schizophrenia or schizoaffective disorder. Participants were 18 to 60 years of age, with a mean age of 35.8 ± 10 years. Participants were primarily of European (n=128) or African descent (n=50), with ten participants reporting Hispanic, Asian Pacific or American Indian ancestry.

Participants were primarily treated with one of four APs including clozapine (n=87), olanzapine (n=32), risperidone (n=31) or haloperidol (n=15). All remaining participants (n=22) were treated with quetiapine, fluphenazine, aripiprazole, ziprasidone or amisulpride. The average length of AP treatment was 7.26 ± 3.5 weeks. Mean baseline weight was 80.07 ± 16.3kg, and mean weight change subsequent to AP treatment was 3.85 ± 4.9kg. Refer to Table 3.1 for further demographic and clinical characteristics.

4.1.2. Statistical Covariates of AIWG

Weight change (%) from baseline did not significantly differ between recruitment sites (p=0.214). However, significant site-specific differences were observed for baseline weight (p=0.001) and duration of treatment (p<0.001). Among the total sample, confounding associations were observed between weight change (%) and duration of treatment (p=0.006) but not participant age (p=0.953), gender (p=0.557), baseline weight (p=0.069) or baseline BPRS score (p=0.819). In the subset of patients of European ancestry treated with clozapine or olanzapine, no associations were found between any of the aforementioned variables (p>0.3). In the subset of patients of African ancestry treated with clozapine or olanzapine, an association was observed between weight change (%) and baseline weight (p=0.047), while all other associations were non-significant (p>0.06). Since baseline weight and treatment length influenced the amount of weight gained with AP treatment, they were entered as statistical covariates for Association Analysis I.

The same variables were re-assessed to determine confounding associations with CSWG, as variable dichotomization may yield different covariates. None were found to be associated with CSWG status for the total sample or among patients of European or African ancestry treated with clozapine or olanzapine (p>0.09). We elected not to force include covariates identified for Association Analysis I (e.g. baseline weight, duration of treatment) because controlling for variables that are not significantly associated with the outcome measure can increase statistical noise. Thus, no variables were entered as statistical covariates for Association Analysis II.

4.1.3. Linkage Disequilibrium, Hardy Weinberg Equilibrium and Minor Allele Frequencies

None of the 19 SNPs analyzed in this study were in high LD with each other (r^2<0.8, refer to Figure 4.1 to 4.3). All SNPs maintained HWE (p>0.05) (refer to Table 4.2 and 4.3) except IL-2 rs2069779 (p=0.026, European sub-sample). IL-2 rs2069779 was also monomorphic in patients of African ancestry and was, therefore, excluded from further analyses. All remaining SNPs had a MAF greater than 5% in patients of European ancestry (refer to Table 4.2). In

patients of African ancestry, IL-2 rs2069778, IL-6 rs2069827, and IL-6 rs2069861 had a MAF less than 5% (refer to Table 4.3) but were still analyzed to maintain procedural consistency.

FIGURE 4.1: IL-1β Linkage Disequilibrium Plots

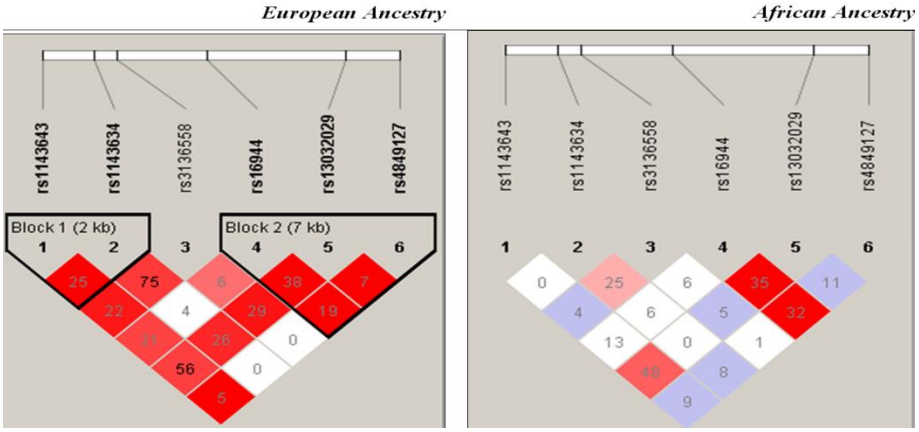


FIGURE 4.2: IL-2 Linkage Disequilibrium Plots

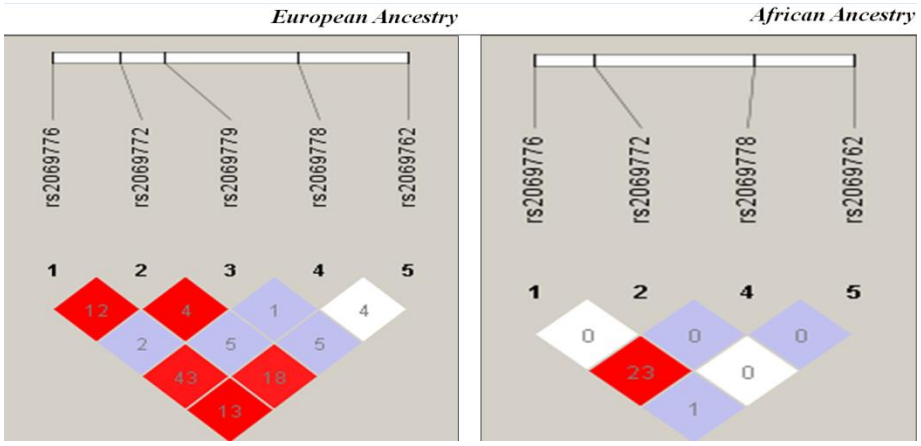


FIGURE 4.3: IL-6 Linkage Disequilibrium Plots

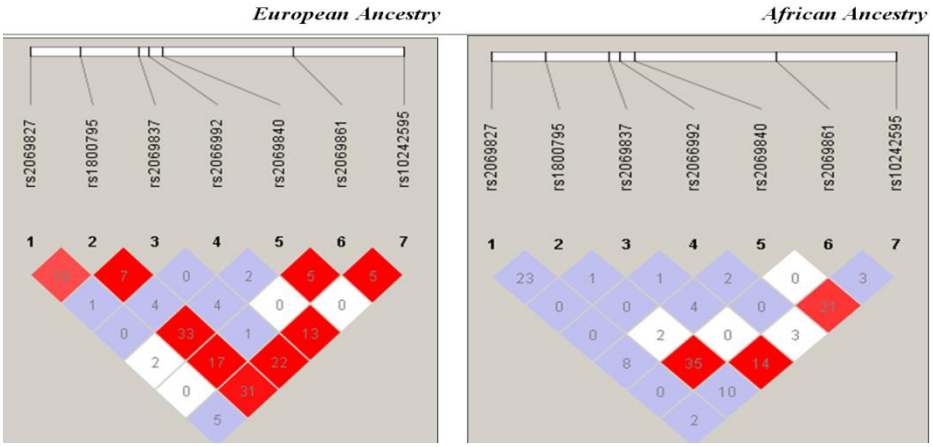


FIG 4.1 to 4.3: Linkage disequilibrium (LD) plots are based on data obtained from study participants of European ancestry (left) and African ancestry (right) separately (Haploview[457] v4.2). LD plot values represent r^2 statistics. LD plots are formatted according to the following color scheme: 'bright red' represents D'=1 and logarithm of the odds (LOD)[i] ≥2, 'shades of pink/red' represents D'<1 and LOD ≥2, 'blue' represents D'=1 and LOD <2, and 'white' represents D'<1 and LOD <2.

[i]D' is a second measure of LD between loci with statistics ranging from 0 (indicative of no LD) to 1 (indicative of complete LD). Logarithm of the odds (LOD) is a measure of confidence in the value of D'[451].

4.2.1. Total Sample Analyses

Weight change (%) from baseline was compared across genotypic groups, with baseline weight and duration of treatment entered as statistical covariates. In the total sample, four genotypic associations involving IL-1β and BDNF with weight change (%) were observed. No associations were observed for variants across IL-2 or IL-6 (refer to Table 4.1).

Finding #1*:* A significant genotypic association was found for IL-1β rs4849127 where carriers of the A-allele gained approximately 4% more weight than individuals homozygous for the G-allele (p=0.001, refer to Figure 4.4), according to a dominant model of genetic inheritance. Under this model, IL-1β rs4849127 explains approximately 5.9% of the variance in weight change (%) from baseline. This finding remained significant after a gene-wide ($p_{IL\text{-}1\beta}$=0.004) and hypothesis-wide (p_{corr}=0.014) correction. However, with approximately 93% of A-allele carriers having the heterozygous GA genotype, results may be driven by an over-dominance model.

Finding #2: A significant genotypic association was found for IL-1β rs16944 where individuals with the heterozygous GA genotype gained approximately 4% more weight than individuals with either homozygous genotype (p=0.001, refer to Figure 4.5). Under this model, IL-1β rs16944 explains approximately 7.3% of the variance in weight change (%) from baseline. This finding remained significant after a gene-wide ($p_{IL\text{-}1\beta}$=0.004) and hypothesis-wide (p_{corr}=0.014) correction. Post-hoc comparisons confirmed that individuals with the GA genotype significantly differed from both the AA (p=0.008) and GG (p=0.001) genotype groups.

FIGURE 4.4: Weight Change (%) Distribution for IL-1β rs4849127 in the Total Sample

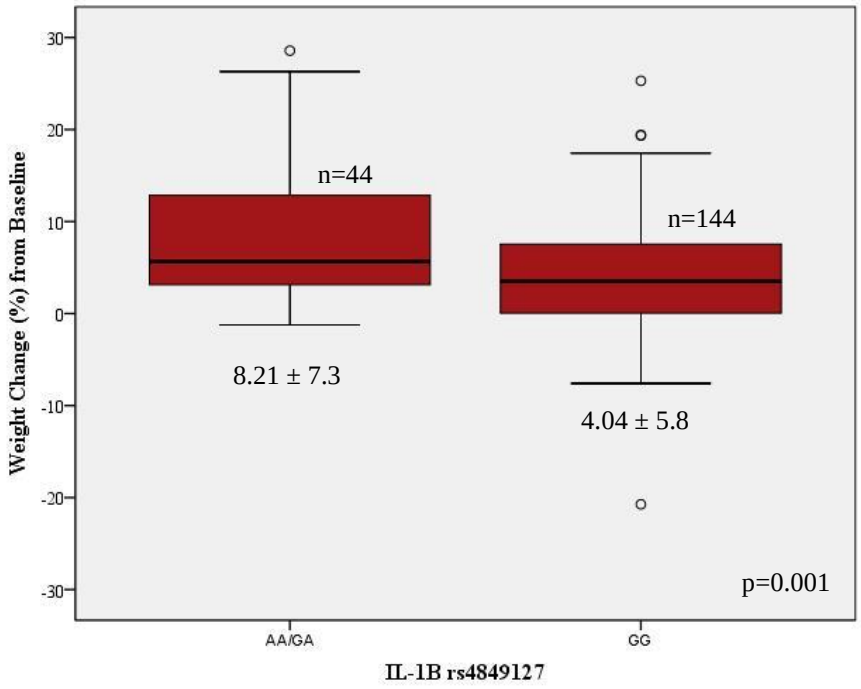
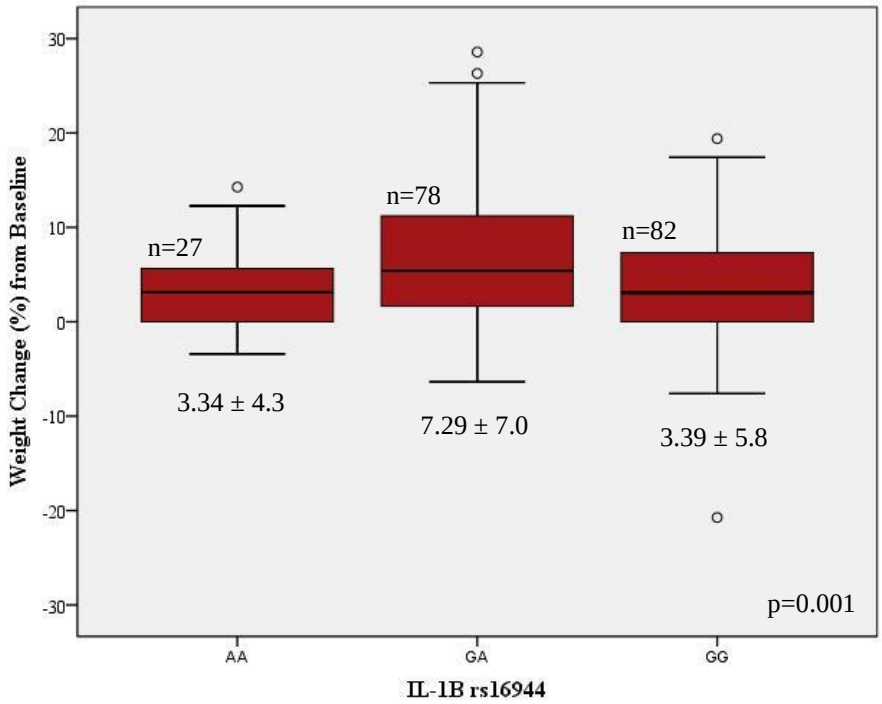
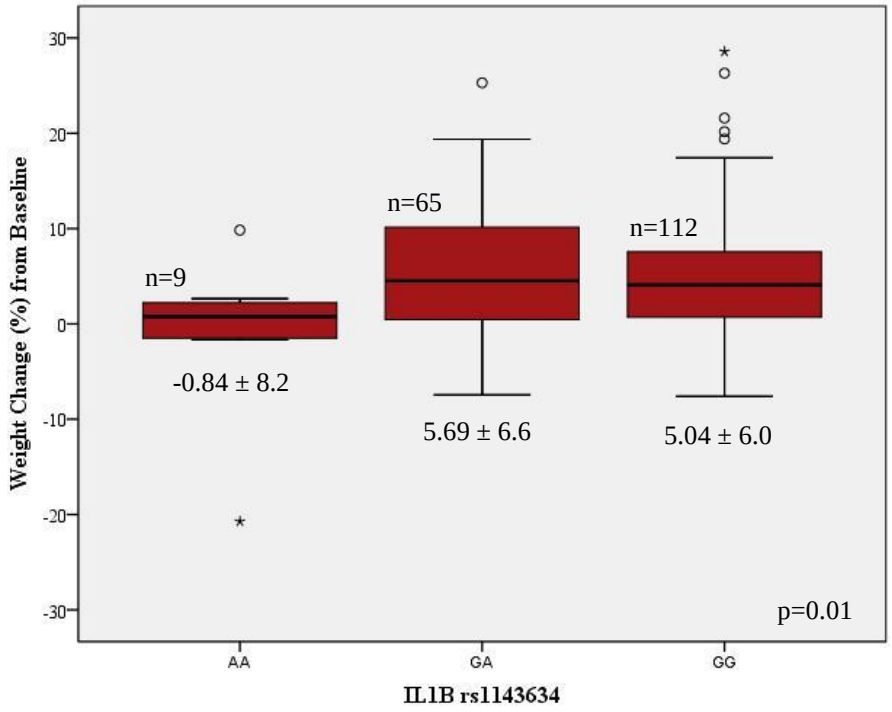


FIGURE 4.5: Weight Change (%) Distribution for IL-1β rs16944 in the Total Sample



Finding #3: A significant genotypic association was found for IL-1β rs1143634 where individuals with the heterozygous GA genotype gained more weight than individuals with either homozygous genotype (p=0.010, refer to Figure 4.6). Under this model, IL-1β rs1143634 explains around 5.0% of the variance in weight change (%) from baseline. This finding remained significant after a gene-wide ($p_{IL\text{-}1\beta}$=0.04) but not a hypothesis-wide (p_{corr}=0.14) correction.

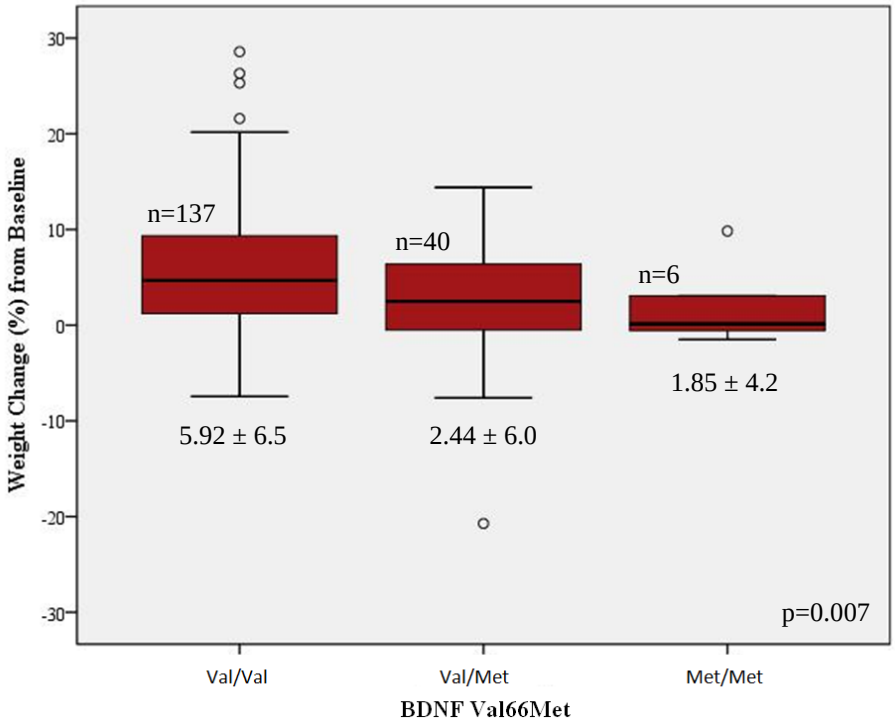
FIGURE 4.6: Weight Change (%) Distribution for IL-1β rs1143634 in the Total Sample



Post-hoc comparisons revealed that individuals with the GA genotype only statistically differed from the AA (p=0.003) but not the GG (p=0.226) genotype group. A re-analysis of the data was performed with G-allele carriers merged. Results showed that individuals homozygous for the A-allele gained approximately 6% less weight than G-allele carriers (AA vs. GA + GG; -0.84 ± 8.2 vs. 5.28 ± 6.2; p=0.005). Under this model, IL-1β rs1143634 explains approximately 4.3% of the variance in weight change (%) from baseline. This finding remained significant after a gene-wide ($p_{IL\text{-}1\beta}$=0.02) but not a hypothesis-wide (p_{corr}=0.07) correction.

Finding #4: A significant genotypic association was found for BDNF Val66Met where individuals with the Val/Val genotype gained more weight than individuals with the Val/Met or Met/Met genotype (p=0.007, refer to Figure 4.7). Under this model, BDNF Val66Met explains about 5.4% of the variance in weight change (%). This finding did not remain significant after a hypothesis-wide correction (p_{corr}=0.098).

FIGURE 4.7: Weight Change (%) Distribution for BDNF Val66Met in the Total Sample



Post-hoc comparisons revealed that the Val/Val genotype group did not significantly differ from the Met/Met group (p=0.184). In fact, the Met/Met group did not differ from the Val/Met genotype group either (p=0.968). This may have resulted from the genetic model being underpowered due to low numbers in the Met/Met group (n=6). A re-analysis of the data using a dominant model of inheritance was performed with Met allele carriers merged. Results showed that carriers of the Met allele gained approximately 4% less weight than individuals homozygous for the Val allele (Met/Met + Val/Met vs. Val/Val; 2.36 ± 5.8 vs. 5.92 ± 6.5, p=0.002). Under this model, BDNF Val66Met still explains about 5.4% of the variance in weight change (%). This finding remained significant after a hypothesis-wide correction (p_{corr}=0.028).

TABLE 4.1: Results from Association Analysis of SNPs with AIWG in the Total Sample

SNP	Genotype	Frequency[a]	ΔWeight (%)[a]	χ^2/ F Value	P Value[b]	p_{corr}[c]
IL-1β rs4849127	AA	3 (1.6%)	4.18 ± 4.8	11.1	ǂ ‖***0.001**	***0.014**
	GA	41 (21.8%)	8.50 ± 7.4			
	GG	144 (76.6%)	4.04 ± 5.8			
IL-1β rs13032029	CC	63 (34.2%)	5.40 ± 6.2	0.61	0.545	1.00
	CT	86 (46.7%)	5.20 ± 6.9			
	TT	35 (19.0%)	3.22 ± 5.1			
IL-1β rs16944	AA	27 (14.4%)	3.34 ± 4.3	13.6	‖***0.001**	***0.014**
	GA	78 (41.5%)	7.29 ± 7.0			
	GG	82 (43.6%)	3.39 ± 5.8			
IL-1β rs3136558	AA	116 (62.7%)	5.45 ± 6.4	0.98	0.376	1.00
	GA	57 (30.8%)	4.60 ± 6.7			
	GG	12 (6.5%)	2.39 ± 4.8			
IL-1β rs1143634	AA	9 (4.8%)	-0.84 ± 8.2	4.73	***0.010**	0.140
	GA	65 (34.9%)	5.69 ± 6.6			
	GG	112 (60.2%)	5.04 ± 6.0			
IL-1β rs1143643	CC	75 (40.8%)	5.46 ± 7.2	0.09	0.918	1.00
	CT	84 (45.7%)	4.70 ± 6.0			
	TT	25 (13.6%)	4.45 ± 5.6			
IL-2 rs2069762	AA	98 (53.0%)	5.60 ± 6.3	1.75	0.177	1.00
	CA	74 (40.0%)	4.39 ± 5.7			
	CC	13 (7.0%)	2.61 ± 9.4			
IL-2 rs2069778	AA	1 (0.5%)	1.66	0.16	ǂ0.686	1.00
	GA	34 (18.6%)	4.64 ± 5.3			
	GG	148 (80.9%)	5.11 ± 6.7			
IL-2 rs2069772	CC	12 (6.5%)	4.27 ± 4.0	0.32	0.723	1.00
	CT	62 (33.7%)	4.35 ± 5.8			
	TT	110 (59.8%)	5.36 ±7.0			
IL-2 rs2069776	CC	6 (3.3%)	7.32 ± 6.2	0.74	0.478	1.00
	CT	61 (33.3%)	5.39 ± 6.8			
	TT	116 (63.4%)	4.72 ± 6.3			

IL-6 rs2069827	GG	156 (83.9%)	5.18 ± 6.7	0.22	ǂ0.638	1.00
	GT	28 (15.1%)	4.14 ± 5.0			
	TT	2 (1.1%)	1.85 ± 2.6			
IL-6 rs1800795	CC	87 (47.5%)	6.15 ± 6.4	2.35	0.099	1.00
	CG	70 (38.3%)	4.49 ± 6.3			
	GG	26 (14.2%)	2.71 ± 6.6			
IL-6 rs2069837	AA	153 (82.7%)	4.83 ± 6.6	0.19	ǂ0.664	1.00
	GA	29 (15.7%)	5.05 ± 5.6			
	GG	3 (1.6%)	7.23 ± 1.3			
IL-6 rs2066992	GG	157 (86.7%)	4.93 ± 6.5	0.10	ǂ0.753	1.00
	GT	23 (12.7%)	5.42 ± 6.6			
	TT	1 (0.5%)	5.10			
IL-6 rs2069840	CC	101 (54.6%)	4.96 ± 6.6	0.20	0.821	1.00
	CG	66 (35.7%)	4.82 ± 6.1			
	GG	18 (9.7%)	5.77 ± 7.1			
IL-6 rs2069861	CC	155 (83.8%)	5.31 ± 6.3	1.46	ǂ0.229	1.00
	CT	29 (15.7%)	3.17 ± 7.0			
	TT	1 (0.5%)	4.37			
IL-6 rs10242595	AA	31 (16.8%)	6.58 ± 6.8	0.70	0.499	1.00
	GA	74 (40.0%)	4.73 ± 5.8			
	GG	80 (43.2%)	4.66 ± 6.9			
BDNF Val66Met	Val/Val	137 (74.9%)	5.92 ± 6.5	5.04	***0.007**	0.098
	Val/Met	40 (21.9%)	2.44 ± 6.0			
	Met/Met	6 (3.3%)	1.85 ± 4.2			

Covariate information was missing for two participants; [a]Values represent number (percent) or mean ± standard deviation; [b]Uncorrected p-values; [c]P-value after a hypothesis-wide correction; *Value significant at $p<0.05$ level; ǂP-value calculated under a dominant model of genetic inheritance; ‖Value calculated using Kruskal-Wallis; AIWG = antipsychotic-induced weight gain, SNP = single nucleotide polymorphism, IL-1β = interleukin 1 beta, IL-2 = interleukin 2, IL-6 = interleukin 6, BDNF = brain-derived neurotrophic factor

4.2.2. Race-Stratified Analyses

Participants were mainly of European (n=128) or African ancestry (n=50), and SNP allele frequencies differed between ethnic groups (refer to Table 4.2 and 4.3). To avoid a population stratification bias, a race-stratified sub-analysis was tested using parameters from Section 4.2.1.

4.2.2.1. Sub-Analysis of Patients of European Ancestry

In patients of European ancestry, three genotypic associations involving IL-1β and BDNF with weight change (%) from baseline were observed. No associations were observed for variants across IL-2 or IL-6 with weight change (%) (Refer to Table 4.2).

Finding #1: A significant genotypic association was found for IL-1β rs16944 where individuals with the heterozygous GA genotype gained more weight than individuals with either homozygous genotype (p=0.013, refer to Figure 4.8). Post-hoc comparisons confirmed that heterozygote individuals significantly differed from the AA (p=0.017) and GG (p=0.019) genotype groups. Under this model, IL-1β rs16944 explains about 6.9% of the variance in weight change (%). However, this finding did not remain significant after a gene-wide ($p_{IL\text{-}1\beta}$=0.052) or hypothesis-wide (p_{corr}=0.182) correction.

Finding #2: A significant genotypic association was found for IL-1β rs1143634 where individuals with the heterozygous GA genotype gained more weight than individuals with either homozygous genotype (p=0.004, refer to Figure 4.9). Under this model, IL-1β rs1143634 explains about 8.7% of the variance in weight change (%). This finding remained significant after a gene-wide ($p_{IL\text{-}1\beta}$=0.016) but not a hypothesis-wide (p_{corr}=0.056) correction. Post-hoc comparisons for IL-1β rs1143634 revealed that individuals with the GA genotype only statistically differed from the AA (p=0.001) but not the GG (p=0.441) genotype group. A re-analysis of the data was performed with G-allele carriers merged. Results showed that individuals homozygous for the A-allele gained about 7% less weight than G-allele carriers (AA vs. GA + GG; -2.17 ± 7.7 vs. 4.42 ± 5.2, p=0.001). Under this model, IL-1β rs1143634 explains around 8.3% of the variance in weight change (%). This finding remained significant after a gene-wide ($p_{IL\text{-}1\beta}$=0.004) and hypothesis-wide (p_{corr}=0.014) correction.

FIGURE 4.8: Weight Change (%) Distribution for IL-1β rs16944 in Europeans

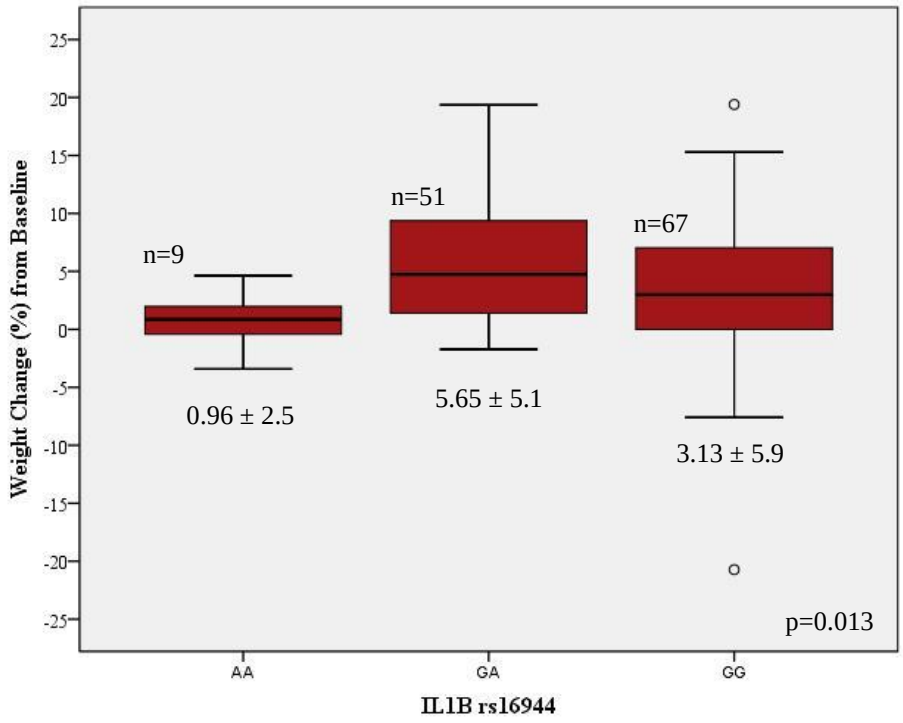
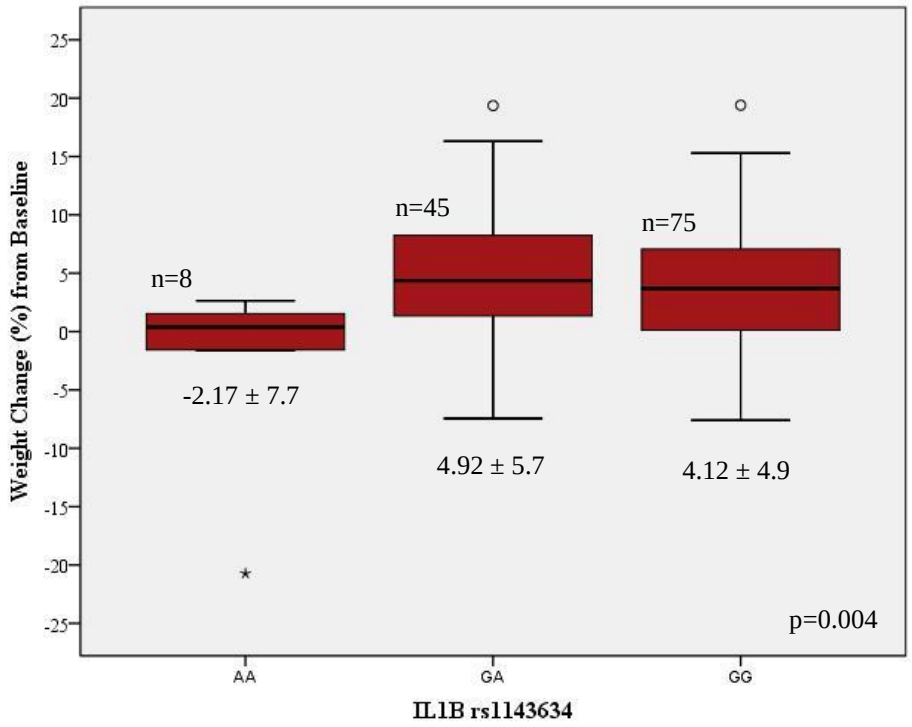


FIGURE 4.9: Weight Change (%) Distribution for IL-1β rs1143634 in Europeans



Finding #3: A significant genotypic association was found for BDNF Val66Met where individuals with the Val/Val genotype gained more weight than individuals with the Val/Met or Met/Met genotype (p=0.014, refer to Figure 4.10). Under this model, BDNF Val66Met explains about 6.8% of the variance in weight change (%). This finding did not remain significant after a hypothesis-wide correction (p_{corr}=0.196). Post-hoc comparisons revealed that the Val/Val genotype group did not significantly differ from the Met/Met group (p=0.073). In fact, the Met/Met group did not differ from the Val/Met genotype group either (p=0.508). This may have resulted from the model being underpowered due to low numbers in the Met/Met group (n=5). A re-analysis of the data was performed with Met allele carriers merged. Results showed that carriers of the Met allele gained approximately 4% less weight than individuals homozygous for the Val allele (Met/Met + Val/Met vs. Val/Val; 1.97 ± 5.9 vs. 5.01 ± 5.2, p=0.004). Under this model, BDNF Val66Met explains approximately 6.5% of the variance in weight change (%). This finding did not remain significant after a hypothesis-wide correction (p_{corr}=0.056).

FIGURE 4.10: Weight Change (%) Distribution for BDNF Val66Met in Europeans

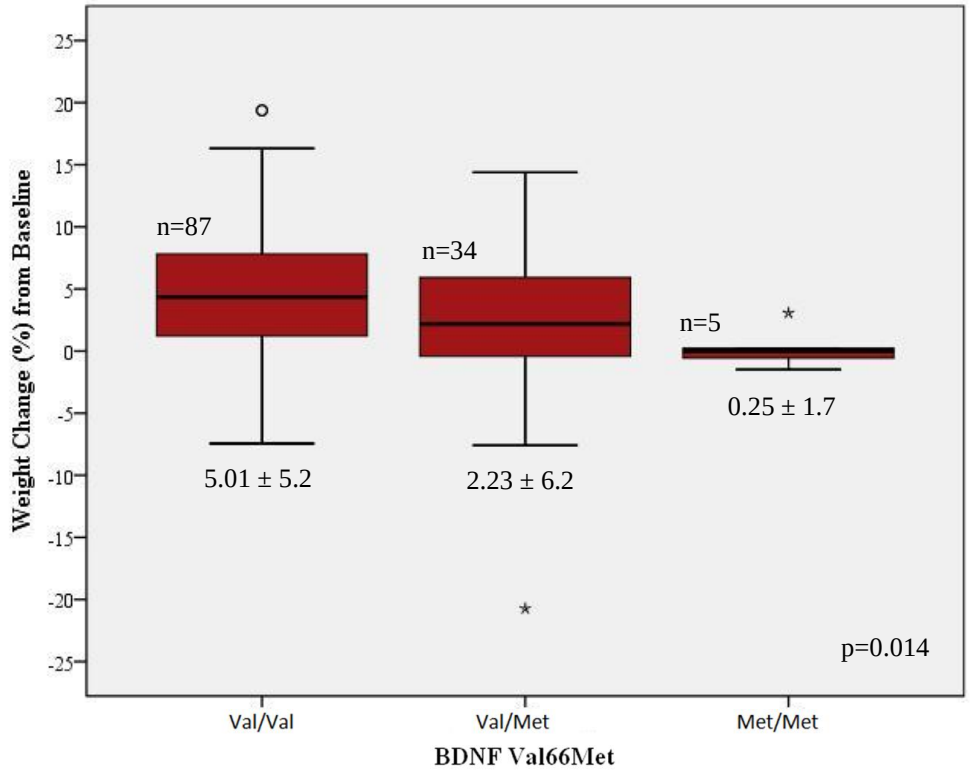


TABLE 4.2: Results from Association Analysis of SNPs with AIWG in Europeans

SNP	Genotype	ΔWeight (%)[a] All Drugs (n= 128)	P Value[b]	p_{corr}[c]	ΔWeight (%)[a] CLZ or OLZ (n= 74)	P -Value[b]	p_{corr}[c]	MAF[d]	HWE P-Value[d]
IL-1β rs4849127	AA	-----	0.141	1.00	-----	0.282	1.00	0.07	1.00
	GA	5.92 ± 4.6 (17)			6.64 ± 5.1 (12)				
	GG	3.71 ± 5.6 (111)			4.39 ± 5.9 (62)				
IL-1β rs13032029	CC	4.09 ± 5.3 (31)	0.828	1.00	4.66 ± 5.8 (21)	0.654	1.00	0.49	0.967
	CT	4.00 ± 5.6 (62)			5.01 ± 5.4 (32)				
	TT	3.35 ± 5.2 (33)			3.50 ± 5.9 (19)				
IL-1β rs16944	AA	0.96 ± 2.5 (9)	***0.013**	0.182	1.12 ± 3.0 (6)	0.056	0.784	0.27	1.00
	GA	5.65 ± 5.1 (51)			6.52 ± 5.6 (33)				
	GG	3.13 ± 5.9 (67)			3.72 ± 5.8 (35)				
IL-1β rs3136558	AA	3.94 ± 5.2 (76)	0.616	1.00	4.64 ± 6.1 (45)	0.850	1.00	0.24	0.509
	GA	4.46 ± 6.3 (43)			5.31 ± 5.7 (23)				
	GG	2.34 ± 3.9 (9)			3.51 ± 4.3 (6)				
IL-1β rs1143634	AA	-2.17 ± 7.7 (8)	***0.004**	0.056	0.84 ± 1.9 (4)	ǂ0.757	1.00	0.24	0.857
	GA	4.92 ± 5.7 (45)			5.59 ± 6.0 (27)				
	GG	4.12 ± 4.9 (75)			4.60 ± 5.8 (43)				
IL-1β rs1143643	CC	3.86 ± 6.6 (37)	0.859	1.00	5.09 ± 5.7 (27)	0.903	1.00	0.44	0.648
	CT	3.86 ± 4.9 (66)			4.35 ± 5.6 (32)				
	TT	4.50 ± 5.8 (23)			5.10 ± 7.0 (14)				

IL-2 rs2069762	AA	3.91 ± 4.8 (54)	0.321	1.00	3.85 ± 4.8 (34)	0.377	1.00	0.33	0.200
	CA	4.43 ± 5.1 (64)			5.66 ± 6.2 (33)				
	CC	1.83 ± 10.5 (10)			4.92 ± 8.3 (7)				
IL-2 rs2069778	AA	1.66 (1)	ǂ0.649	1.00	1.66 (1)	ǂ0.773	1.00	0.12	0.984
	GA	4.52 ± 5.1 (27)			4.57 ± 5.4 (18)				
	GG	3.90 ± 5.8 (97)			4.96 ± 6.1 (53)				
IL-2 rs2069772	CC	4.27 ± 4.0 (12)	0.986	1.00	3.15 ± 3.5 (8)	0.530	1.00	0.30	0.911
	CT	3.91 ± 5.6 (52)			4.66 ± 6.4 (31)				
	TT	3.97 ± 5.8 (63)			5.14 ± 5.8 (34)				
IL-2 rs2069776	CC	4.34 ± 4.6 (4)	ǂ0.582	1.00	2.15 ± 1.9 (3)	ǂ0.940	1.00	0.22	0.466
	CT	4.37 ± 5.2 (47)			5.10 ± 5.9 (29)				
	TT	3.86 ± 5.9 (75)			4.93 ± 6.0 (40)				
IL-6 rs2069827	GG	3.92 ± 5.7 (103)	ǂ0.683	1.00	4.70 ± 5.8 (61)	ǂ0.746	1.00	0.11	0.825
	GT	4.58 ± 4.9 (23)			5.45 ± 5.7 (12)				
	TT	1.85 ± 2.6 (2)			0E-14 (1)				
IL-6 rs1800795	CC	4.67 ± 4.3 (41)	0.524	1.00	4.44 ± 4.8 (21)	0.967	1.00	0.44	0.823
	CG	4.07 ± 6.0 (60)			5.22 ± 6.5 (39)				
	GG	3.08 ± 6.4 (25)			4.56 ± 5.4 (12)				
IL-6 rs2069837	AA	4.10 ± 5.9 (107)	ǂ0.719	1.00	5.06 ± 6.1 (63)	ǁ0.479	1.00	0.09	1.00
	GA	3.32 ± 3.5 (20)			3.00 ± 2.9 (11)				
	GG	7.92 (1)			-----				
IL-6 rs2066992	GG	3.96 ± 5.7 (111)	0.624	1.00	4.85 ± 6.0 (62)	0.941	1.00	0.05	1.00
	GT	4.88 ± 5.2 (13)			5.01 ± 5.5 (9)				
	TT	-----			-----				

IL-6 rs2069840	CC	3.70 ± 5.7 (64)	0.835	1.00	4.15 ± 5.6 (37)	0.299	1.00	0.31	0.315
	CG	4.32 ± 5.7 (49)			5.92 ± 6.1 (31)				
	GG	4.29 ± 4.4 (15)			2.44 ± 4.3 (6)				
IL-6 rs2069861	CC	4.29 ± 5.2 (99)	ǂ0.257	1.00	4.82 ± 5.9 (56)	ǂ0.933	1.00	0.11	1.00
	CT	2.83 ± 6.7 (27)			4.58 ± 5.7 (17)				
	TT	4.37 (1)			4.37 (1)				
IL-6 rs10242595	AA	4.48 ± 4.0 (14)	0.883	1.00	4.88 ± 3.4 (8)	0.691	1.00	0.30	0.342
	GA	3.74 ± 5.5 (48)			4.01 ± 6.1 (29)				
	GG	4.16 ± 5.9 (65)			5.32 ± 6.0 (37)				
BDNF Val66Met	Val/Val	5.01 ± 5.2 (87)	***0.014**	0.196	5.95 ± 5.7(50)	ǂ***0.025**	0.350	0.18	0.624
	Val/Met	2.23 ± 6.2 (34)			2.82 ± 5.5 (20)				
	Met/Met	0.25 ± 1.7 (5)			-0.74 ± 1.1 (2)				

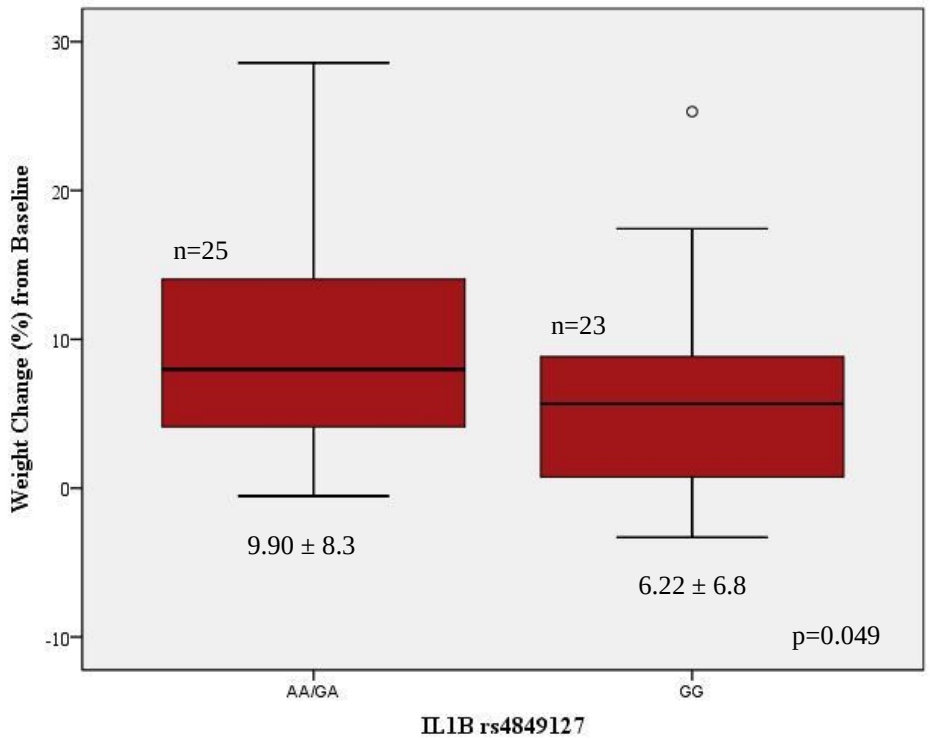
[a]Values represent mean ± standard deviation (number); [b]Uncorrected p-values; [c]P-value after a hypothesis-wide correction; [d]Values calculated among all patients of European ancestry; ǂP-value calculated under a dominant model of genetic inheritance; ǁValue calculated using Kruskal-Wallis; *****Value significant at $p<0.05$ level; SNP = single nucleotide polymorphism, CLZ = clozapine, OLZ = olanzapine, MAF = minor allele frequency, HWE = Hardy-Weinberg equilibrium, IL-1β = interleukin 1 beta, IL-2 = interleukin 2, IL-6 = interleukin 6, BDNF = brain-derived neurotrophic factor

4.2.2.2. Sub-Analysis of Patients of African Ancestry

In the sub-sample of patients of Africa ancestry, two genotypic associations involving IL-1β with weight change (%) were observed. No genotypic associations were observed for variants across IL-2, IL-6 or BDNF with weight change (%) (refer to Table 4.3).

Finding #1: A significant genotypic association was found for IL-1β rs4849127 where carriers of the A-allele gained approximately 4% more weight than individuals homozygous for the G-allele (p=0.049, refer to Figure 4.11), according to a dominant model of inheritance. However, with around 92% of A-allele carriers having the heterozygous GA genotype, results may be driven by an over-dominance model. Under this model, IL-1β rs4849127 explains approximately 8.5% of the variance in weight change (%). This finding did not remain significant after a gene-wide ($p_{IL\text{-}1\beta}$=0.245) or hypothesis-wide (p_{corr}=0.784) correction.

FIGURE 4.11: Weight Change (%) Distribution for IL-1β rs4849127 in Africans



Finding #2: A significant genotypic association was found for IL-1β rs16944 where individuals with the heterozygous GA genotype gained more weight than individuals with either homozygous genotype (p=0.012, refer to Figure 4.12). Post-hoc comparisons confirmed that individuals with the GA genotype significantly differed from both the AA (p=0.006) and GG (p=0.037) genotype groups. Under this model, IL-1β rs16944 explains approximately 18.1% of the variance in weight change (%). This finding did not remain significant after a gene-wide ($p_{IL-1\beta}$=0.06) or hypothesis-wide (p_{corr}=0.192) correction.

FIGURE 4.12: Weight Change (%) Distribution for IL-1β rs16944 in Africans

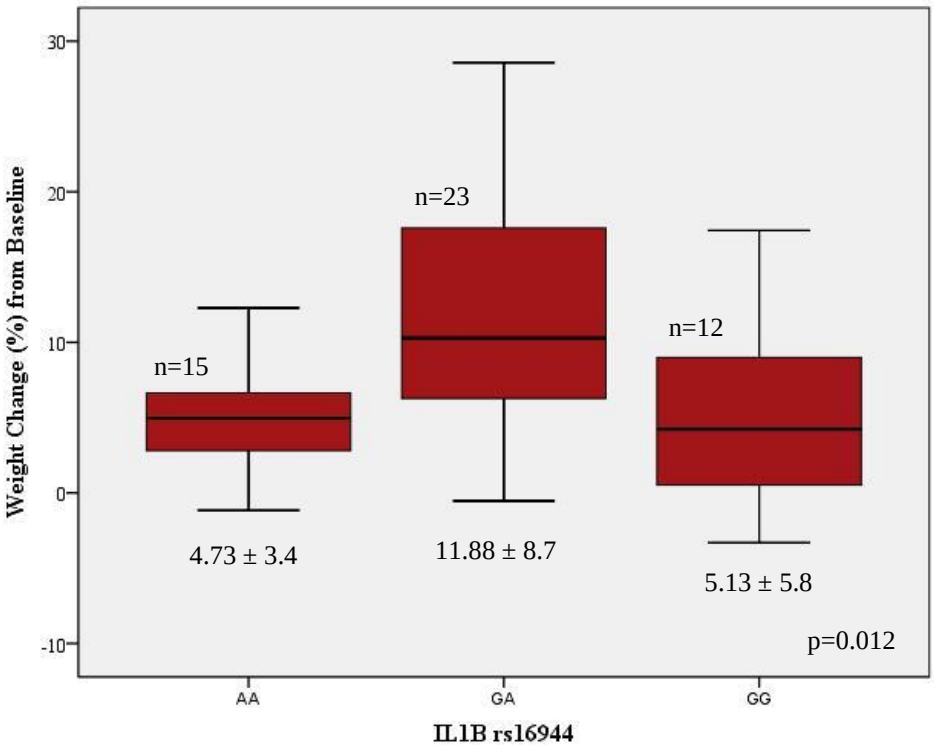


TABLE 4.3: Results from Association Analysis of SNPs with AIWG in Africans

SNP	Genotype	ΔWeight (%)[a] All Drugs (n=50)	P Value[b]	p_{corr}[c]	ΔWeight (%)[a] CLZ or OLZ (n=37)	P -Value[b]	p_{corr}[c]	MAF[d]	HWE P-Value[d]
IL-1β rs4849127	AA	6.89 ± 1.6 (2)	ǂ***0.049**	0.784	6.89 ± 1.6 (2)	ǂ***0.027**	0.432	0.27	0.458
	GA	10.16 ± 8.6 (23)			11.79 ± 8.9 (18)				
	GG	6.22 ± 6.8 (23)			7.19 ± 7.5 (16)				
IL-1β rs13032029	CC	7.60 ± 6.4 (27)	ǂ0.415	1.00	8.07 ± 7.3 (20)	0.525	1.00	0.24	0.312
	CT	9.23 ± 9.3 (20)			11.22 ± 9.3 (16)				
	TT	0.84 (1)			-----				
IL-1β rs16944	AA	4.73 ± 3.4 (15)	ǁ***0.012**	0.192	4.68 ± 3.9 (11)	ǁ***0.008**	0.128	0.47	0.741
	GA	11.88 ± 8.7 (23)			13.46 ± 8.7 (19)				
	GG	5.13 ± 5.8 (12)			5.98 ± 6.4 (7)				
IL-1β rs3136558	AA	9.07 ± 7.4 (35)	ǂ0.409	1.00	10.49 ± 7.6 (28)	0.655	1.00	0.14	1.00
	GA	5.28 ± 8.6 (12)			5.89 ± 10.1 (8)				
	GG	9.83 (1)			-----				
IL-1β rs1143634	AA	9.83 (1)	ǂ0.272	1.00	-----	0.252	1.00	0.19	0.885
	GA	8.18 ± 7.9 (17)			8.99 ± 8.1 (14)				
	GG	8.05 ± 7.9 (30)			9.78 ± 8.5 (22)				
IL-1β rs1143643	CC	8.18 ± 7.4 (31)	ǂ0.917	1.00	9.26 ± 8.0 (23)	ǂ0.773	1.00	0.20	1.00
	CT	8.62 ± 8.9 (15)			10.09 ± 9.4 (12)				
	TT	3.85 ± 4.3 (2)			6.86 (1)				

IL-2 rs2069762	AA	8.08 ± 7.46 (39)	ǂ0.784	1.00	9.13 ± 8.1 (30)	0.727	1.00	0.09	0.663
	CA	6.56 ± 9.5 (7)			9.35 ± 9.8 (5)				
	CC	9.29 (1)			-----				
IL-2 rs2069778	AA	-----	-----	-----	-----	-----	-----	0.04	1.00
	GA	6.18 ± 2.9 (4)			6.86 (1)				
	GG	8.31 ± 8.0 (44)			9.54 ± 8.4 (35)				
IL-2 rs2069772	CC	-----	0.664	1.00	-----	0.555	1.00	0.08	1.000
	CT	8.09 ± 6.1 (8)			10.24 ± 5.4 (6)				
	TT	8.13 ± 8.2 (39)			9.34 ± 8.9 (29)				
IL-2 rs2069776	CC	13.29 ± 4.9 (2)	ǂǂ0.341	1.00	16.75 (1)	ǂ0.079	1.00	0.15	0.587
	CT	10.20 ± 10.2 (11)			13.09 ± 10.6 (8)				
	TT	7.23 ± 6.8 (36)			8.18 ± 7.5 (26)				
IL-6 rs2069827	GG	8.22 ± 7.9 (46)	-----	-----	9.47 ± 8.3 (36)	-----	-----	0.02	1.00
	GT	6.19 ± 4.4 (2)			-----				
	TT	----			-----				
IL-6 rs1800795	CC	8.06 ± 7.7 (40)	0.545	1.00	9.17 ± 8.0 (32)	-----	-----	0.08	1.00
	CG	8.78 ± 9.3 (7)			13.63 ± 13.5 (3)				
	GG	----			-----				
IL-6 rs2069837	AA	7.68 ± 7.9 (36)	ǂ0.863	1.00	8.91 ± 8.5 (27)	ǂ0.847	1.00	0.14	0.465
	GA	8.89 ± 7.5 (9)			11.09 ± 8.2 (6)				
	GG	6.89 ± 1.6 (2)			6.89 ± 1.6 (2)				

IL-6 rs2066992	GG	8.23 ± 7.5 (39)	0.500	1.00	9.52 ± 7.9 (30)	0.956	1.00	0.08	1.00
	GT	6.93 ± 9.3 (8)			8.23 ± 11.6 (5)				
	TT	-----			-----				
IL-6 rs2069840	CC	8.17 ± 7.3 (32)	ǂ0.917	1.00	9.33 ± 7.6 (26)	ǂ0.925	1.00	0.19	0.452
	CG	6.90 ± 7.4 (13)			8.39 ± 9.1 (7)				
	GG	13.17 ± 14.2 (3)			13.17 ± 14.2 (3)				
IL-6 rs2069861	CC	8.15 ± 7.7 (46)	-----	-----	9.57 ± 8.2 (34)	-----	-----	0.03	1.00
	CT	7.80 ± 12.7 (2)			7.80 ± 12.7 (2)				
	TT	-----			-----				
IL-6 rs10242595	AA	9.52 ± 7.9 (15)	ǁ0.717	1.00	11.09 ± 8.0 (12)	ǁ0.658	1.00	0.46	0.979
	GA	6.79 ± 5.6 (24)			8.05 ± 6.0 (16)				
	GG	9.09 ± 10.6 (11)			9.68 ± 11.6 (9)				
BDNF Val66Met	Val/Val	8.42 ± 8.1 (42)	ǂ0.518	1.00	9.81 ± 8.3 (34)	-----	-----	0.06	0.300
	Val/Met	4.78 ± 4.4 (4)			-0.61 (1)				
	Met/Met	9.83 (1)			-----				

Covariate information was missing for two participants. Where p-value not shown, analysis not performed due to <2 genotypic groups for comparison or <5 subjects in merged comparison group. [a]Values represent mean ± standard deviation (number); [b]Uncorrected p-values; [c]P-value after a hypothesis-wide correction; [d]Values calculated among all patients of African ancestry; ǂP-value calculated under a dominant model of genetic inheritance; ǁValue calculated using Kruskal-Wallis; *Value significant at p<0.05 level; SNP = single nucleotide polymorphism, CLZ = clozapine, OLZ = olanzapine, MAF = minor allele frequency, HWE = Hardy-Weinberg equilibrium, IL-1β = interleukin 1 beta, IL-2 = interleukin 2, IL-6 = interleukin 6, BDNF = brain-derived neurotrophic factor

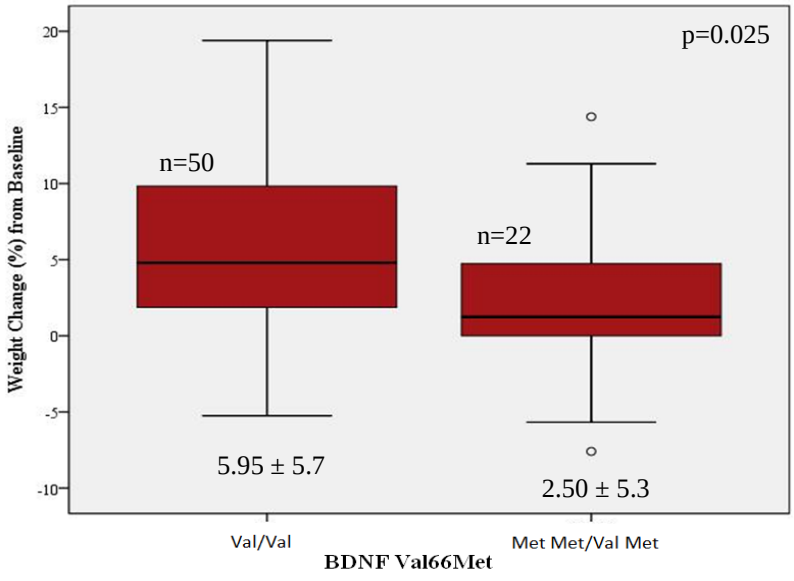
4.2.3. Race-and Treatment-Stratified Analyses

Participants were administered AP treatment that primarily included clozapine (46.3%), haloperidol (8.0%), olanzapine (17.0%) or risperidone (16.5%) (refer to Table 3.1). Of these APs, the greatest weight change (%) from baseline was observed for clozapine (5.55 ± 6.3) and olanzapine (8.01 ± 8.1) compared with haloperidol (0.70 ± 7.0) or risperidone (3.87 ± 3.8). This is consistent with the literature which has identified olanzapine and clozapine as high-risk drugs for AIWG[16]. Thus, a sub-analysis restricted to individuals on clozapine or olanzapine was performed. To maintain sample homogeneity, race stratification was applied.

4.2.3.1. Sub-Analysis of Patients of European Ancestry on Clozapine or Olanzapine

In the sub-sample of patients of European ancestry on clozapine or olanzapine, only BDNF Val66Met was associated with weight change (%) from baseline. Results showed that Met allele carriers gained approximately 3% less weight than individuals homozygous for the Val allele (p=0.025, refer to Figure 4.13). Under this model, BDNF Val66Met explains about 7.2% of the variance in weight change (%). However, this finding did not remain significant after a hypothesis-wide correction (p_{corr}=0.35). No genotypic associations were observed for variants across IL-1β, IL-2 or IL-6 with weight change (%) (refer to Table 4.2).

FIGURE 4.13: Weight Change (%) Distribution for BDNF Val66Met in Europeans on CLZ/OLZ

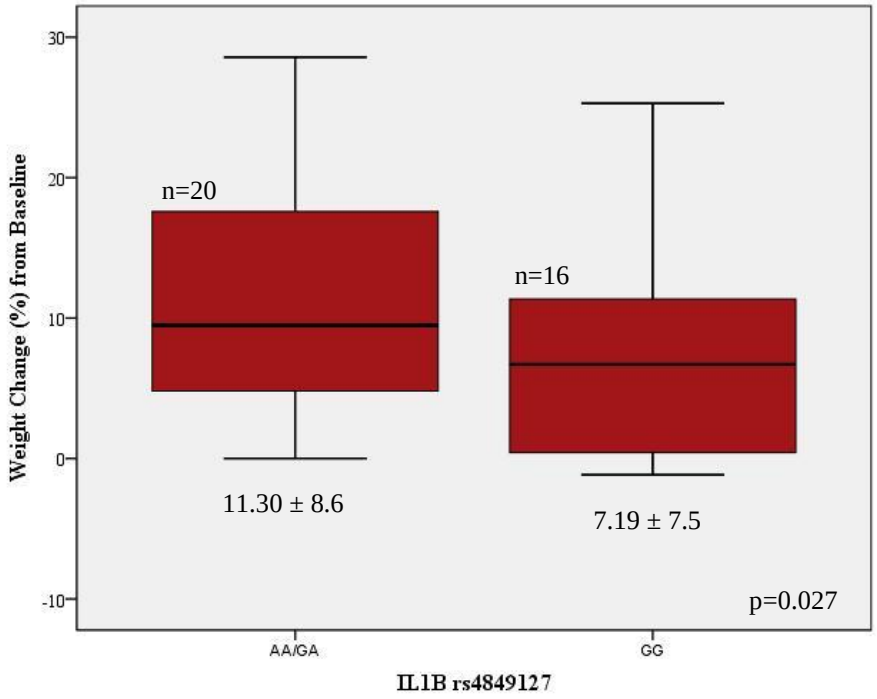


4.2.3.2. Sub-Analysis of Patients of African Ancestry on Clozapine or Olanzapine

In the sub-sample of patients of Africa ancestry on clozapine or olanzapine, two genotypic associations involving IL-1β with weight change (%) were observed. No associations were observed for IL-2, IL-6 or BDNF with weight change (%) (refer to Table 4.3).

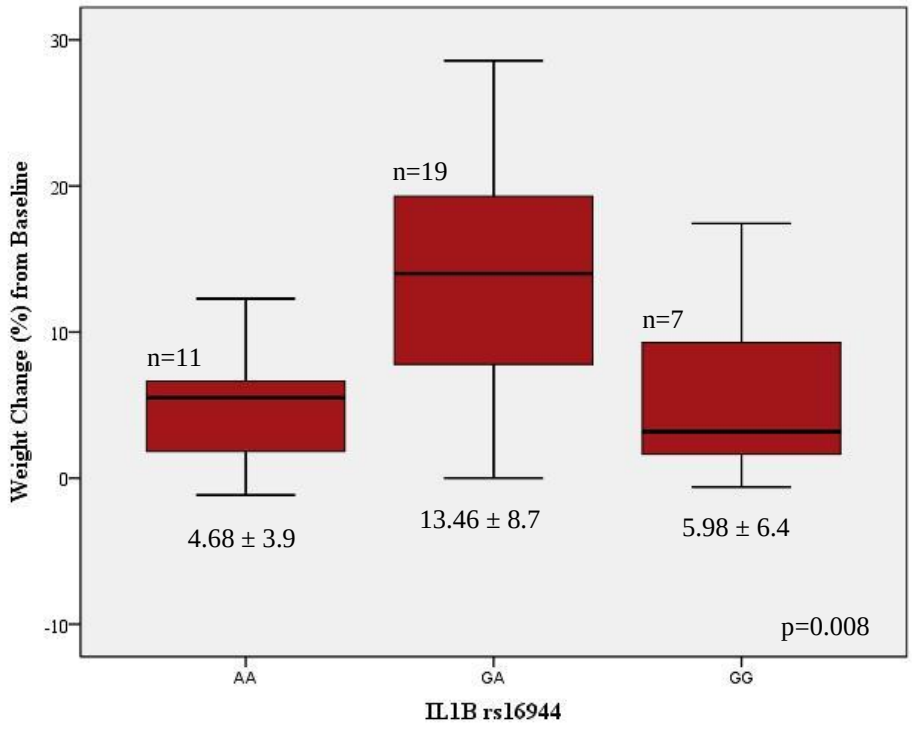
Finding #1: A significant genotypic association was found for IL-1β rs4849127 where carriers of the A-allele gained approximately 4% more weight than individuals homozygous for the G-allele (p=0.027, refer to Figure 4.14), as assessed according to a dominant model of genetic inheritance. However, with approximately 90% of A-allele carriers having the heterozygous GA genotype, results may be driven by an over-dominance model. Under this model, IL-1β rs4849127 explains around 14.3% of the variance in weight change (%). This finding did not remain significant after a gene-wide ($p_{IL\text{-}1\beta}$=0.135) or hypothesis-wide (p_{corr}=0.432) correction.

FIGURE 4.14: Weight Change (%) Distribution for IL-1β rs4849127 in Africans on CLZ/OLZ



Finding #2: A significant genotypic association was found for IL-1β rs16944 where individuals with the heterozygous GA genotype gained more weight than individuals with either homozygous genotype (p=0.008, refer to Figure 4.15). Under this model, IL-1β rs16944 explains approximately 26.8% of the variance in weight change (%). Post-hoc comparisons confirmed that individuals with the GA genotype significantly differed from the AA group (p=0.003) but, unlike in previous analyses, not the GG genotype group (p=0.069). In fact, the GG group did not differ from the AA group either (p=0.821). This may have resulted from the genetic model being underpowered due to low numbers in the GG group (n=7), as merging G allele carriers weakened the genotypic association (p=0.061). This finding remained significant after a gene-wide ($p_{IL\text{-}1\beta}$=0.04) but not a hypothesis-wide (p_{corr}=0.128) correction.

FIGURE 4.15: Weight Change (%) Distribution for IL-1β rs16944 in Africans on CLZ/OLZ



4.3. Haplotype and Allelic Analyses

Three-marker haplotypes were tested for an association with weight change (%) from baseline in patients treated with clozapine or olanzapine. Analyses were performed separately for patients of European and African ancestry. Among all analyses performed, only one significant haplotype on the IL-1β gene was identified in the subset of patients of African ancestry between the following variant sites: rs1143643, rs1143634, rs3136558 ($p<0.001$, refer to Table 4.4**)**. The overall test of association remained significant after a gene-wide correction ($p_{IL\text{-}1\beta}=0.002$). Post-hoc comparisons revealed that the T-A-G haplotype was responsible for this association ($\chi^2=6.06$, $p=0.014$). However, an examination of the LD plot for this haplotype (refer to Figure 4.1) revealed that the rs1143643 and rs1143634 loci are not in high LD with each other (D'=0.37, LOD=0.04). LD information for the remaining pairwise combinations includes: rs1143643 and rs3136558 (D'=1, LOD=0.27), and rs1143634 and rs3136558 (D'=0.61, LOD=2.73). This indicates that this haplotypic combination has a low likelihood of existing in nature. No haplotypes were identified for IL-2 or IL-6 in this sub-sample (refer to Table 4.4). No haplotypes were identified across IL-1β, IL-2 or IL-6 in patients of European ancestry on clozapine or olanzapine (refer to Appendix A).

Allelic investigations were performed for all SNPs under the same testing conditions. Among all analyses, an allelic association was observed for BDNF Val66Met in patients of European ancestry on clozapine or olanzapine, where the Met allele was associated with lower weight gain (%) from baseline as compared to the alternate Val allele ($\chi^2=6.76$, $p=0.009$). This result did not remain significant after a hypothesis-wide correction ($p_{corr}=0.162$). This finding is supported by results from Association Analysis I (refer to Section 4.2.3.1, Figure 4.13). BDNF Val66Met could not be tested in patients of African ancestry on clozapine or olanzapine due to less than five patients in the minor allele group. No allelic associations were observed for variants across IL-1β, IL-2 or IL-6 in patients of European or African ancestry on clozapine or olanzapine (refer to Table 4.5).

TABLE 4.4: Haplotype Analysis in Patients of African Ancestry on CLZ/OLZ

Test Markers	**Overall P-Value**[b]	**Haplotype**	**Frequency**[a]	χ^2	**Haplotype P-Value**[b]
IL-1β rs1143643 IL-1β rs1143634 IL-1β rs3136558	***<0.001**	C-A-A	8 (12.1%)	2.72	0.099
		C-G-A	44 (66.7%)	1.88	0.171
		T-A-G	4 (6.1%)	6.06	***0.014**
		T-G-A	10 (15.2%)	3.76	0.053
IL-1β rs1143634 IL-1β rs3136558 IL-1β rs16944	0.054	A-A-G	7 (10.6%)	3.63	0.057
		A-G-G	5 (7.6%)	3.29	0.070
		G-A-A	36 (54.6%)	0.64	0.424
		G-A-G	18 (27.3%)	0.43	0.513
IL-1β rs3136558 IL-1β rs16944 IL-1β rs13032029	0.673	A-A-C	37 (54.4%)	0.43	0.512
		A-G-C	11 (16.2%)	0.12	0.726
		A-G-T	15 (22.1%)	1.57	0.210
		G-G-C	5 (7.4%)	0.06	0.805
IL-1β rs16944 IL-1β rs13032029 IL-1β rs4849127	0.191	A-C-A	22 (29.7%)	1.04	0.309
		A-C-G	19 (25.7%)	3.98	0.046
		G-C-G	16 (21.6%)	<0.01	0.978
		G-T-G	17 (23.0%)	0.88	0.347
IL-2 rs2069776 IL-2 rs2069772 IL-2 rs2069778	0.159	C-T-G	9 (13.2%)	3.68	0.055
		T-C-G	6 (8.8%)	0.04	0.848
		T-T-G	53 (77.9%)	2.89	0.089
IL-2 rs2069772 IL-2 rs2069778 IL-2 rs2069762	0.949	C-G-A	6 (8.8%)	0.10	0.748
		T-G-A	57 (83.8%)	0.08	0.784
		T-G-C	5 (7.4%)	<0.01	0.969
IL-6 rs2069827 IL-6 rs1800795 IL-6 rs2069837	0.781	G-C-A	54 (84.4%)	0.08	0.780
		G-C-G	10 (15.6%)	0.08	0.780

IL-6 rs1800795 IL-6 rs2069837 IL-6 rs2066992	0.611	C-A-G	49 (79.0%)	0.20	0.652
		C-A-T	4 (6.5%)	0.83	0.361
		C-G-G	9 (14.5%)	0.01	0.908
IL-6 rs2069837 IL-6 rs2066992 IL-6 rs2069840	0.672	A-G-C	44 (62.9%)	0.02	0.881
		A-G-G	13 (18.6%)	0.63	0.426
		A-T-C	4 (5.7%)	0.93	0.335
		G-G-C	9 (12.9%)	<0.01	0.967
IL-6 rs2066992 IL-6 rs2069840 IL-6 rs2069861	0.587	G-C-C	51 (75.0%)	0.50	0.480
		G-G-C	12 (17.7%)	1.07	0.302
		T-C-C	5 (7.4%)	0.11	0.737
IL-6 rs2069840 IL-6 rs2069861 IL-6 rs10242595	0.354	C-C-A	39 (57.4%)	0.18	0.670
		C-C-G	19 (27.9%)	1.56	0.212
		G-C-G	10 (14.7%)	0.97	0.325

[a]Values refer to frequency among haplotype options for the specified test markers; [b]Uncorrected p-values; *Value significant at $p<0.05$ level; IL-1β = interleukin 1 beta, IL-2 = interleukin 2, IL-6 = interleukin 6

TABLE 4.5: Allelic Analysis in Patients of European and African Ancestry on CLZ/OLZ

		European Ancestry (n=74)			**African Ancestry (n=37)**		
Test Marker	**Allele**	**Frequency**[a]	**χ^2**	**P-Value**[b]	**Frequency**[a]	**χ^2**	**P-Value**[b]
IL-1β rs4849127	A	12 (8.1%)	1.40	0.237	22 (29.7%)	1.04	0.309
	G	136 (91.9%)			52 (70.3%)		
IL-1β rs13032029	C	74 (51.4%)	0.45	0.502	57 (77.0%)	0.88	0.347
	T	70 (48.6%)			17 (23.0%)		
IL-1β rs16944	A	45 (30.4%)	0.21	0.650	41 (55.4%)	0.67	0.413
	G	103 (69.6%)			33 (44.6%)		
IL-1β rs3136558	A	113 (76.4%)	0.01	0.939	66 (89.2%)	1.74	0.187
	G	35 (23.7%)			8 (10.8%)		
IL-1β rs1143634	A	35 (23.7%)	0.09	0.765	14 (18.9%)	0.05	0.817
	G	113 (76.4%)			60 (81.1%)		

IL-1β rs1143643	C	86 (58.9%)	0.01	0.907	60 (81.1%)	0.01	0.919
	T	60 (41.1%)			14 (18.9%)		
IL-2 rs2069762	A	101 (68.2%)	0.98	0.323	67 (93.1%)	<0.01	0.950
	C	47 (31.8%)			5 (6.9%)		
IL-2 rs2069778	A	20 (13.9%)	0.20	0.654	-----	-----	-----
	G	124 (86.1%)			72 (100%)		
IL-2 rs2069772	C	47 (32.2%)	0.68	0.411	6 (8.3%)	0.06	0.806
	T	99 (67.8%)			66 (91.7%)		
IL-2 rs2069776	C	35 (24.3%)	0.12	0.733	10 (13.9%)	3.25	0.071
	T	109 (75.7%)			62 (86.1%)		
IL-6 rs2069827	G	134 (90.5%)	<0.01	0.953	74 (100%)	-----	-----
	T	14 (9.5%)			-----		
IL-6 rs1800795	C	81 (56.3%)	0.03	0.873	66 (100%)	-----	-----
	G	63 (43.8%)			-----		
IL-6 rs2069837	A	137 (92.6%)	1.12	0.291	62 (86.1%)	0.01	0.908
	G	11 (7.4%)			10 (13.9%)		
IL-6 rs2066992	G	133 (93.7%)	0.01	0.941	67 (93.1%)	0.09	0.758
	T	9 (6.3%)			5 (6.9%)		
IL-6 rs2069840	C	105 (71.0%)	0.07	0.790	61 (82.4%)	0.33	0.567
	G	43 (29.1%)			13 (17.6%)		
IL-6 rs2069861	C	129 (87.2%)	0.03	0.869	70 (100%)	-----	-----
	T	19 (12.8%)			-----		
IL-6 rs10242595	A	45 (30.4%)	0.38	0.539	40 (54.1%)	0.26	0.608
	G	103 (69.6%)			34 (46.0%)		
BDNF Val66Met	Met	24 (16.7%)	6.76	***0.009**	1 (1.4%)	-----	-----
	Val	120 (83.3%)			71 (98.6%)		

Where p value is not shown, analysis could not be performed due to n<5 in minor allele group. [a]Values refer to frequency among allelic options for the specified test marker; [b]Uncorrected p-values; *Value significant at p<0.05 level; IL-1β = interleukin 1 beta, IL-2 = interleukin 2, IL-6 = interleukin 6, BDNF = brain-derived neurotrophic factor

4.4. Association Analysis II: Clinically Significant Weight Gain

SNPs were tested using Pearson χ^2 for an association with CSWG status, defined by a weight change (%) from baseline equal to or greater than 7%. No covariates were entered into the statistical model. Among the total sample, two genotypic associations were observed involving IL-1β and IL-6 with CSWG status. No associations were observed for variants across IL-2 or BDNF (refer to Table 4.6)

Finding #1: For IL-1β rs4849127, an association was observed between CSWG and carriers of the A-allele (OR=2.17, CI: 1.08 – 4.35, p=0.028), where the A-allele conferred risk for CSWG. This finding did not remain significant after a gene-wide ($p_{IL\text{-}1\beta}$=0.112) or hypothesis-wide (p_{corr}=0.392) correction. However, with about 93% of A-allele carriers having the heterozygous GA genotype, results may be driven by an over-dominance model, as observed in Section 4.2.1.

Finding #2: For IL-6 rs1800795, an association was observed between CSWG and carriers of the G-allele (OR=0.53, CI: 0.28 – 0.98, p=0.042), where the G-allele was protective against CSWG. This finding did not remain significant after a gene-wide ($p_{IL\text{-}6}$=0.252) or hypothesis-wide (p_{corr}=0.588) correction.

No genotypic associations were observed for IL-1β, IL-2, IL-6 or BDNF with CSWG among patients of European ancestry (refer to Appendix B), patients of African ancestry (refer to Appendix C), patients of European ancestry on clozapine or olanzapine (refer to Appendix D), or patients of African ancestry on clozapine or olanzapine (refer to Appendix E).

However, since heterozygosity at IL-1β rs16944 has consistently shown an association with AIWG, the GA group was tested for an association with CSWG. An examination across genotypes revealed a significant association in the total sample (χ^2 =9.47, p=0.009, $p_{IL\text{-}1\beta}$=0.036, p_{corr}=0.126) and in patients of African ancestry (χ^2 =9.19, p=0.010, $p_{IL\text{-}1\beta}$=0.05, p_{corr}=0.16). Analyses could not be computed for patients of European ancestry or among Europeans or Africans on clozapine or olanzapine due to expected cell counts less than five.

TABLE 4.6: Genetic Predictors of Antipsychotic-Induced CSWG in the Total Sample

SNP	Genotype	CSWG (n= 60)[a]	Non-CSWG (n= 128)[a]	OR	95% CI	χ^2	P-Value[b]	p_{corr}[c]
IL-1β rs4849127	AA/GA	20 (33.3%)	24 (18.8%)	2.17	1.08 – 4.35	4.85	***0.028**	0.392
	GG	40 (66.7%)	104 (81.2%)					
IL-1β rs13032029	TT/CT	37 (63.8%)	86 (67.2%)	0.86	0.45 – 1.65	0.21	0.650	1.00
	CC	21 (36.2%)	42 (32.8%)					
IL-1β rs16944	AA/GA	38 (63.3%)	67 (52.8%)	1.55	0.82 – 2.91	1.85	0.174	1.00
	GG	22 (36.7%)	60 (47.2%)					
IL-1β rs3136558	GG/GA	21 (35.0%)	48 (37.8%)	0.89	0.47 – 1.68	0.14	0.712	1.00
	AA	39 (65.0%)	79 (62.2%)					
IL-1β rs1143634	AA/GA	28 (46.7%)	46 (35.9%)	1.56	0.84 – 2.91	1.97	0.160	1.00
	GG	32 (53.3%)	82 (64.1%)					
IL-1β rs1143643	TT/CT	31 (52.5%)	79 (62.2%)	0.67	0.36 - 1.26	1.56	0.212	1.00
	CC	28 (47.5%)	48 (37.8%)					
IL-2 rs2069762	CC/CA	23 (39.0%)	64 (50.0%)	0.64	0.34 – 1.20	1.97	0.160	1.00
	AA	36 (61.0%)	64 (50.0%)					
IL-2 rs2069778	AA/GA	11 (18.3%)	24 (19.2%)	0.95	0.43 – 2.08	0.02	0.888	1.00
	GG	49 (81.7%)	101 (80.8%)					
IL-2 rs2069772	CC/CT	22 (37.9%)	52 (40.6%)	0.89	0.47 – 1.69	0.12	0.728	1.00
	TT	36 (62.1%)	76 (59.4%)					

IL-2 rs2069776	CC/CT	24 (40.0%)	43 (34.4%)	1.27	0.67 – 2.40	0.55	0.458	1.00
	TT	36 (60.0%)	82 (65.6%)					
IL-6 rs2069827	TT/GT	8 (13.3%)	22 (17.2%)	0.74	0.31 – 1.78	0.45	0.501	1.00
	GG	52 (86.7%)	106 (82.8%)					
IL-6 rs1800795	GG/CG	25 (41.7%)	72 (57.6%)	0.53	0.28 – 0.98	4.13	***0.042**	0.588
	CC	35 (58.3%)	53 (42.4%)					
IL-6 rs2069837	GG/GA	13 (22.0%)	20 (15.6%)	1.53	0.70 – 3.33	1.14	0.285	1.00
	AA	46 (78.0%)	108 (84.4%)					
IL-6 rs2066992	TT/GT	7 (11.9%)	17 (13.7%)	0.85	0.33 – 2.17	0.12	0.730	1.00
	GG	52 (88.1%)	107 (86.3%)					
IL-6 rs2069840	GG/CG	26 (43.3%)	58 (45.7%)	0.91	0.49 – 1.69	0.09	0.764	1.00
	CC	34 (56.7%)	69 (54.3%)					
IL-6 rs2069861	TT/CT	8 (13.6%)	23 (18.0%)	0.72	0.30 – 1.71	0.57	0.451	1.00
	CC	51 (86.4%)	105 (82.0%)					
IL-6 rs10242595	AA/GA	38 (63.3%)	69 (54.3%)	1.45	0.77 – 2.73	1.35	0.245	1.00
	GG	22 (36.7%)	58 (45.7%)					
BDNF Val66Met	Met/Met + Val/Met	10 (16.9%)	36 (28.6%)	0.51	0.23 – 1.12	2.91	0.088	1.00
	Val/Val	49 (83.1%)	90 (71.4%)					

[a]Values represent number (percent) or mean ± standard deviation; [b]Uncorrected p-values; [c]P-value after a hypothesis-wide correction; *Value significant at $p<0.05$ level; SNP = single nucleotide polymorphism; CSWG = clinically significant weight gain, $\geq$7% weight change from baseline, OR = odds ratio, CI = confidence interval, IL-1β = interleukin 1 beta, IL-2 = interleukin 2, IL-6 = interleukin 6, BDNF = brain-derived neurotrophic factor

4.5. Gene-Gene Interaction Analyses

Epistatic effects were modelled between variant sites across each of IL-1β, IL-2, and IL-6 and the BDNF Val66Met locus for an association with weight change (%) from baseline. Analyses were restricted to patients treated with either clozapine or olanzapine, and performed separately for patients of European and African ancestry. Among these sub-samples, three interactions were observed involving IL-1β and IL-6 with BDNF Val66Met.

Interaction #1: In patients of European ancestry, an interaction was observed between IL-1β rs13032029 and BDNF Val66Met (β=-7.63, Wald=9.70, Permutation P=0.029, refer to Table 4.7). This finding did not remain significant after a gene-wide ($p_{IL\text{-}1\beta}$=0.174) or hypothesis-wide (p_{corr}=0.493) correction. Carriers of the TT genotype at IL-1β rs13032029 and the Val/Met genotype at BDNF Val66Met were identified as low risk (least weight gain) for AIWG compared to individuals with any other genotypic combination at these two loci. A re-analysis of the data using ANCOVA to compare patients with the combined TT + Val/Met genotype against all other patients confirmed this finding (TT + Val/Met vs. CC or CT + Val/Val or Met/Met; -2.44 ± 3.9 vs. 5.19 ± 5.4; p=0.004, refer to Table 4.9 and Figure 4.16).

Interaction #2: In patients of European ancestry, an interaction was observed between IL-6 rs2069837 and BDNF Val66Met (β=3.95, Wald=9.12, Permutation P=0.021, refer to Table 4.7). This finding did not remain significant after a gene-wide ($p_{IL\text{-}6}$=0.147) or hypothesis-wide (p_{corr}=0.357) correction. Carriers of the AA genotype at IL-6 rs2069837 and the Val/Val genotype at BDNF Val66Met were identified as high risk (greatest weight gain) for AIWG compared to individuals with any other genotypic combination at these two loci. A re-analysis of the data using ANCOVA to compare patients with the combined AA + Val/Val genotype against all other patients confirmed this finding (AA+ Val/Val vs. GG or GA + Val/Met or Met/Met; 6.60 ± 6.0 vs. 2.65 ± 4.7; p=0.006, refer to Table 4.9 and Figure 4.17).

Interaction #3: In patients of African ancestry, an interaction was observed between IL-1β rs16944 and BDNF Val66Met (β=8.59, Wald=13.06, Permutation P=0.006, refer to Table 4.8). This finding remained significant after a gene-wide ($p_{IL\text{-}1\beta}$=0.036) but not a hypothesis-wide (p_{corr}=0.102) correction. Carriers of the GA genotype at IL-1β rs16944 and the Val/Val genotype

at BDNF Val66Met were identified as high risk (greatest weight gain) for AIWG compared to individuals with any other genotypic combination at these two loci. A re-analysis of the data using ANCOVA to compare patients with the combined GA + Val/Val genotype against all other patients confirmed this finding (GA + Val/Val vs. GG or AA + Val/Met or Met/Met; 13.77 ± 8.8vs. 5.01 ± 5.0; p=0.002, refer to Table 4.9 and Figure 4.18).

TABLE 4.7: Gene-Gene Interaction against BDNF Val66Met in Europeans on CLZ/OLZ

SNP	Genotype[a]	Risk Group[b]	Beta	Wald	P-Value	Perm P-Value[c]
IL-1β rs4849127	GG + Val/Met	Low	-3.40	4.49	***0.038**	0.156
IL-1β rs13032029	TT + Val/Met	Low	-7.63	9.70	***0.003**	***0.029**
IL-1β rs16944	GA + Val/Val	High	3.90	7.94	***0.006**	0.063
IL-1β rs3136558	AA + Val/Met	Low	-3.93	4.50	***0.038**	0.310
IL-1β rs1143634	GG + Val/Met	Low	-3.93	4.50	***0.038**	0.322
IL-2 rs2069762	CC + Val/Met	Low	-8.39	4.25	***0.043**	0.273
IL-2 rs2069778	GG + Val/Val	High	2.80	4.13	***0.046**	0.305
IL-2 rs2069772	TT + Val/Val	High	2.76	3.78	0.056	0.394
IL-6 rs2069827	GT + Val/Val	High	4.65	4.24	***0.043**	0.28
IL-6 rs1800795	CG + Val/Val	High	3.06	4.75	***0.033**	0.28
IL-6 rs2069837	AA + Val/Val	High	3.95	9.12	***0.004**	***0.021**
IL-6 rs2066992	GG + Val/Val	High	2.88	4.17	***0.045**	0.193
IL-6 rs2069840	CG + Val/Val	High	2.87	3.88	0.053	0.316
IL-6 rs10242595	GG + Val/Val	High	3.28	5.42	***0.023**	0.175

All interactions were performed against BDNF Val66Met. An interaction model was not found between BDNF Val66Met and IL-1β rs1143643, IL-2 rs2069776 or IL-6 rs2069861. [a]Values represent the respective interleukin + BDNF genotype; [b]High risk represents greatest change in weight (%) from baseline, and low risk represents least change in weight (%) from baseline; [c]Uncorrected p-values based on 1000 permutations; *Value significant at $p<0.05$ level; SNP = single nucleotide polymorphism; IL-1β = interleukin 1 beta, IL-2 = interleukin 2, IL-6 = interleukin 6, BDNF = brain-derived neurotrophic factor

TABLE 4.8: Gene-Gene Interaction against BDNF Val66Met in Africans on CLZ/OLZ

SNP	Genotype[a]	Risk Group[b]	Beta	Wald	P-Value	Perm P-Value[c]
IL-1β rs4849127	AG+ Val/Val	High	4.62	2.97	0.094	0.274
IL-1β rs16944	GA+ Val/Val	High	8.59	13.06	***0.00097**	***0.006**

All interactions were performed against BDNF Val66Met. An interaction model was not found between BDNF Val66Met and all remaining SNPs. [a]Values represent the respective interleukin + BDNF genotype; [b]High risk represents greatest change in weight (%) from baseline, and low risk represents least change in weight (%) from baseline; [c]Uncorrected p-values based on 1000 permutations; *Value significant at $p<0.05$ level; SNP = single nucleotide polymorphism, IL-1β = interleukin 1 beta, IL-2 = interleukin 2, IL-6 = interleukin 6, BDNF = brain-derived neurotrophic factor

TABLE 4.9: ANCOVA Analysis of Gene-Gene Interaction Effects against BDNF Val66Met in Patients of European and African Ancestry on CLZ/OLZ

Gene	Genotype[a]	Ancestry	Frequency[b]	ΔWeight (%)[b]	P-Value
IL-1β rs16944	GG or AA + Val/Met or Met/Met	African	17 (48.6%)	5.01 ± 5.0	**‖*0.002**
	GA + Val/Val		18 (51.4%)	13.77 ± 8.8	
IL-1β rs13032029	CC or CT + Val/Val or Met/Met	European	65 (92.9%)	5.19 ± 5.4	***0.004**
	TT + Val/Met		5 (7.1%)	-2.44 ± 3.9	
IL-6 rs2069837	GG or GA + Val/Met or Met/Met	European	31 (43.1%)	2.65 ± 4.7	***0.006**
	AA + Val/Val		41 (56.9%)	6.60 ± 6.0	

All interactions were performed against BDNF Val66Met. Only subjects with genotypic information for both loci were included in the analysis. Covariates included duration of antipsychotic treatment and baseline weight. Covariate information was missing for 1 person; [a]Values represent the respective interleukin + BDNF genotype; [b]Values represent number (percent) or mean ± standard deviation; ‖Value calculated using Kruskal-Wallis; *Value significant at $p<0.05$ level; IL-1β = interleukin 1 beta, IL-6 = interleukin 6, BDNF = brain-derived neurotrophic factor

FIGURE 4.16: Weight Change (%) Distribution within an Interaction Model for IL-1β rs13032029 and BDNF Val66Met in Europeans on CLZ/OLZ

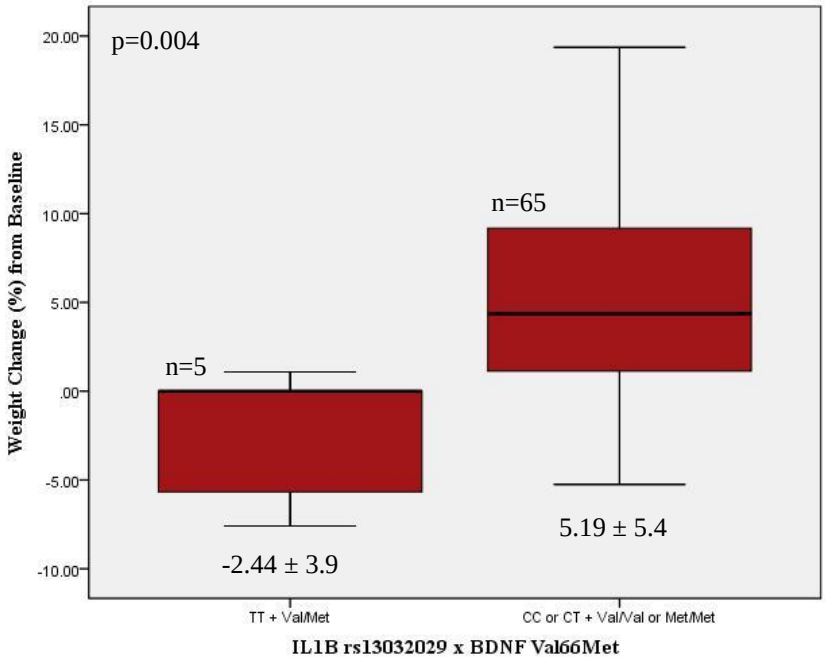


FIGURE 4.17: Weight Change (%) Distribution within an Interaction Model for IL-6 rs2069837 and BDNF Val66Met in Europeans on CLZ/OLZ

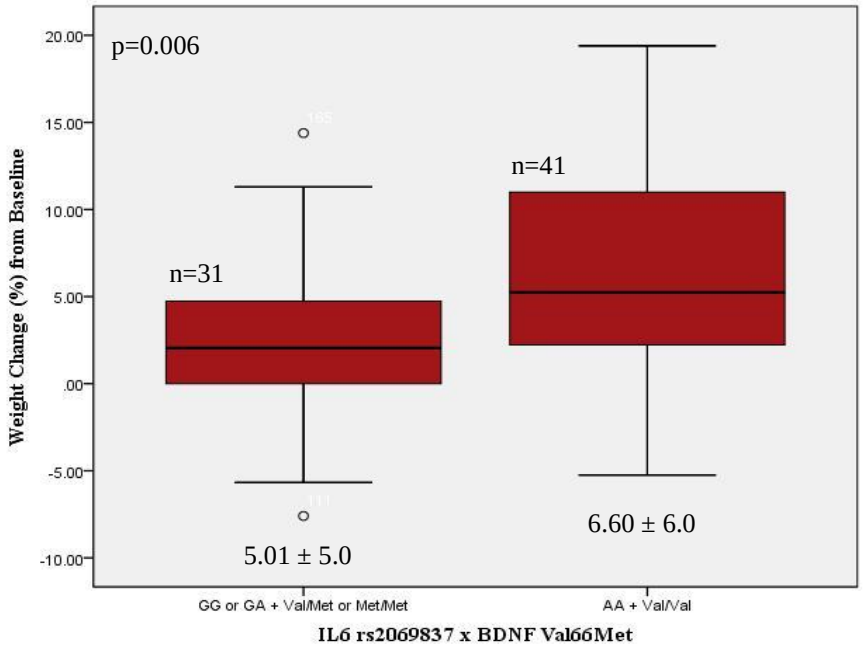
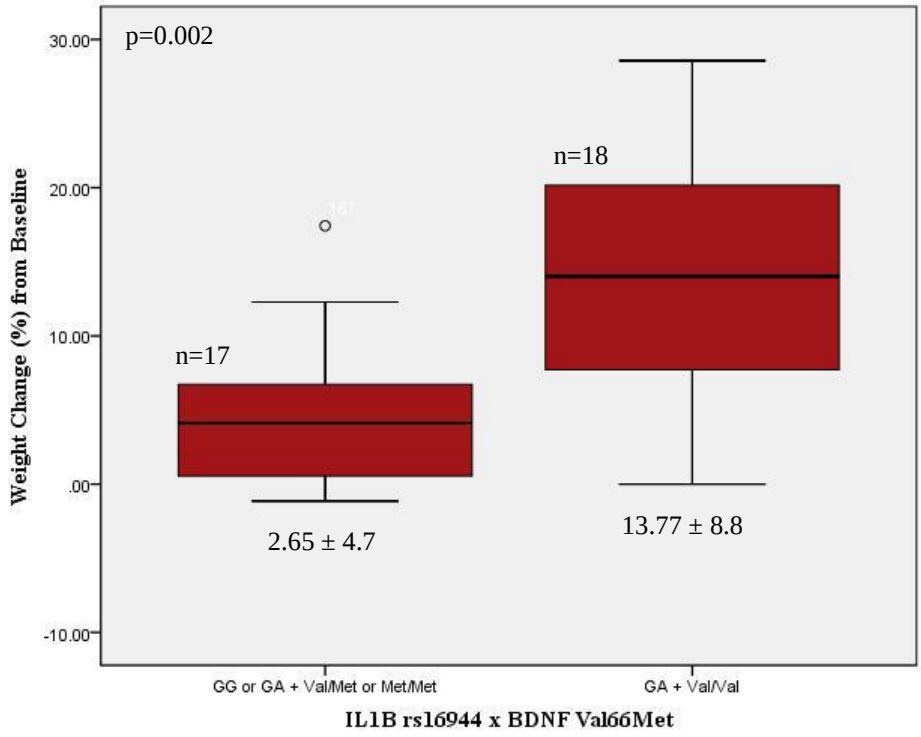


FIGURE 4.18: Weight Change (%) Distribution within an Interaction Model for IL-1β rs16944 and BDNF Val66Met in Africans on CLZ/OLZ



Chapter 5:

CATIE Replication

The purpose of this chapter is to:

- Provide an overview of the CATIE sample
- Describe SNP selection and data extraction
- Describe statistical analyses

5.1. Replication Design

To validate findings from Chapter 4, a replication analysis was performed using genetic information extracted from the Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) sample (described in Section 5.2). AIWG parameters differed from our discovery sample, as information was collected for change in BMI over the course of AP treatment, as a percentage (%) of baseline BMI (herein referred to as *BMI change (%) from baseline*). Despite these methodological differences, the CATIE sample was chosen to evaluate our findings further and because this was the only available replication sample. CATIE participants had the following clinical information collected: age, sex, ethnicity, baseline PANSS, and type of AP medication received including dose and duration of treatment. The effect of genetic variance on AIWG was determined by comparing BMI change (%) from baseline across genotypic groups for each SNP of interest. Study parameters are described in further detail in the below-listed sections.

5.2. CATIE Sample

CATIE was a double-blind clinical trial designed to determine the efficacy of AP treatment. Patients were randomly assigned to one of four SGAs which included olanzapine (7.5

to 30 mg/day), quetiapine (200 to 800 mg/day), ziprasidone (40 to 160 mg/day) or risperidone (1.5 to 6.0 mg/day) or the FGA perphenazine (8 to 32 mg per day), for up to 18 months. Medication dosing was flexible and based on clinical response.

A total of 1,493 patients were recruited between 2001 and 2004 across 57 U.S. sites. Patients were between 18 to 65 years of age with a DSM-IV diagnosis of chronic schizophrenia. Participants were excluded from the study if any of the following criteria were endorsed:

- Diagnosis of schizoaffective disorder
- Mental retardation or other cognitive disorder
- Prior adverse reactions or treatment resistance to the study medications
- Only one past episode of schizophrenic illness

All participants provided written informed consent. The study was approved by all participating institutional ethics review boards. For further details, see Lieberman *et al.* (2005)[468]. For the purpose of this study, analyses were performed on a refined subset of the CATIE sample (n=318). Refinement criteria included the following restrictions:

- No prior exposure to high risk medications for AIWG (e.g. olanzapine or clozapine) lasting longer than 30 days
- No marked obesity (BMI ≥40) at baseline
- No exposure to low risk medications for AIWG (e.g. ziprasidone or perphenazine) during the CATIE trial
- CATIE treatment duration between 14 to 190 days
- No familial relatedness to other CATIE participants
- Only patients of self-reported European or African ancestry[j]
- Heterozygosity of less than four standard deviations from the mean[k]

Demographic and clinical characteristics for this sub-sample are presented in Table 5.1.

[j]Ancestry was further confirmed using genetic markers

[k]Heterozygosity refers to the frequency of heterozygotes for a specified locus. If the level of heterozygosity is higher than would be expected under HWE, this may indicate sample contamination or genotyping error.

5.3. Statistical Covariates of AIWG

Among the total sample, confounding associations were observed between BMI change (%) and baseline BMI (p=0.001) but not participant age (p=0.138), gender (p=0.843), medication dose (p=0.375), duration of treatment (p=0.325) or baseline PANSS score (p=0.147). In the subset of patients of European ancestry treated with olanzapine, no associations were found between any of the aforementioned variables (p>0.05). In the subset of patients of African ancestry treated with olanzapine, an association was observed between BMI change (%) and baseline PANSS score (p=0.047), while all other associations were non-significant (p>0.1). Since baseline BMI and baseline PANSS score influenced the amount of weight gained with AP treatment, they were entered as statistical covariates.

TABLE 5.1: Demographic and Clinical Sample Characteristics

Characteristics	**Total Sample (n=318)**	**Characteristics**	**Total Sample (n=318)**
Sex		Duration (days)	132.84 ± 53.4
Female	76 (23.9%)	*Ethnicity*	
Male	242 (76.1%)	European	207 (65.1%)
Age (years)	41.07 ± 11.1	African	111 (34.9%)
Baseline BMI	28.58 ± 5.4	*Study Medications*	
BMI Change	0.72 ± 2.0	Olanzapine	109 (34.3%); 25.94 ± 49.4 mg/day
BMI Change (%)	2.78 ± 7.5	Quetiapine	108 (34.0%); 557.09 ± 171.5 mg/day
Baseline PANSS	75.29 ± 17.2	Risperidone	101 (31.8%); 3.95 ± 1.2 mg/day

Values represent number (percent) or mean ± standard deviation

5.4. SNP Extraction

Genetic information was extracted from CATIE using Plink[469] version 1.07. Where genetic information was available, original study SNPs were directly investigated using CATIE data. Since genetic data were not available for all original SNPs, high LD proxies were identified 50kbp upstream and 50kbp downstream of each gene region of interest. Variants within the extended gene regions of IL-1β, IL-2 and IL-6 were assessed for pairwise LD with original study SNPs ($r^2 \geq 0.7$) using the SNP Annotation and Proxy Search (SNAP)[470] version 2.2. Final SNPs selected for replication are listed in Table 5.2. The SNP genotyping rate for the replication panel was >96%, and the subject genotyping rate was >83%.

5.5. Hardy-Weinberg Equilibrium and Minor Allele Frequencies

Of the 12 SNPs analyzed in this study, all maintained HWE ($p>0.05$) except IL-6 rs1800795 ($p=0.040$, African sub-sample) which was, therefore, excluded from further analyses. All remaining SNPs had a MAF equal to or greater than 5% in patients of European ancestry. In patients of African ancestry, BDNF Val66Met had a MAF less than 5% but was still analyzed to maintain procedural consistency between ethnic subgroups.

5.6. Statistical Analyses for Replication

BMI change (%) from baseline was compared across genotypic groups according to the testing parameters described in Section 3.5.1. For ANCOVA analyses, baseline BMI and baseline PANSS score were entered as statistical covariates. Gene-gene interaction analyses followed the procedures outlined in Section 3.5.4. Treatment-stratified analyses included only olanzapine cases, as clozapine treatment data were not available from CATIE. Since analyses were performed to replicate findings from Chapter 4, only data pertaining to SNPs (or their high LD proxies) that were significant in our discovery sample (herein referred to as *primary replication SNPs*) are shown. Replication p-values were not corrected for multiple comparisons.

TABLE 5.2: CATIE Replication SNPs

Original SNP	**In CATIE (Y/N)**	**Highest LD Proxy***
IL-1β rs4849127	**N**	**IL-1β rs17042517**
IL-1β rs13032029	N	IL-1β rs4447608
IL-1β rs16944	**N**	**IL-1β rs2723152**
IL-1β rs3136558	N	IL-1β rs11674397
IL-1β rs1143634	**Y**	**IL-1β rs1143634**
IL-1β rs1143643	N	-------
IL-2 rs2069762	N	IL-2 rs11724582
IL-2 rs2069778	N	-------
IL-2 rs2069772	N	IL-2 rs17388568
IL-2 rs2069779	N	-------
IL-2 rs2069776	N	IL-2 rs1479923
IL-6 rs2069827	N	-------
IL-6 rs1800795	Y	IL-6 rs1800795
IL-6 rs2069837	Y	IL-6 rs2069837
IL-6 rs2066992	Y	IL-6 rs2066992
IL-6 rs2069840	N	-------
IL-6 rs2069861	N	-------
IL-6 rs10242595	N	-------
BDNF Val66Met	**Y**	**BDNF Val66Met**

Bold font represents primary replication SNPs. Where an original SNP is analyzed in CATIE, its highest LD proxy is itself; Where a high LD proxy is not listed, r^2<0.6; *Highest LD proxies were included in replication analyses; CATIE = Clinical Antipsychotic Trials of Intervention Effectiveness, SNP = single nucleotide polymorphism, LD = linkage disequilibrium, IL-1β = interleukin 1 beta, IL-2 = interleukin 2; IL-6 = interleukin 6; BDNF = brain-derived neurotrophic factor

5.7. Results from Association Analyses

5.7.1. Total Sample Analyses

In the total sample, none of the primary replication SNPs were associated with AIWG in the CATIE sample (refer to Table 5.3). Additional exploratory analyses across all remaining SNPs identified a significant association involving IL-2 rs11724582 where individuals with the heterozygous GA genotype had the greatest BMI change compared with individuals with either homozygous genotype (GA: 4.13 ± 7.9, AA: 1.90 ± 6.8, GG: 1.96 ± 9.4, p=0.048). Under this model, IL-2 rs11724582 explains around 2.0% of the variance in BMI change (%) from baseline. Post-hoc comparisons revealed that the GA group only statistically differed from the AA (p=0.016) but not the GG (p=0.187) group. A re-analysis of the data was performed with G-allele carriers merged. Results showed that carriers of the G-allele experienced an AP-induced BMI change that was approximately 2% greater than individuals homozygous for the A-allele (GA + GG vs. AA; 3.70 ± 8.2 vs. 1.90 ± 6.8, p=0.037). Under this new model, IL-2 rs11724582 explains around 1.4% of the variance in BMI change (%) from baseline.

5.7.2. Race-Stratified Analyses

In race-stratified sub-samples which separately included patients of European and African ancestry, none of the primary replication SNPs were associated with AIWG in the CATIE sample (refer to Table 5.4 and Table 5.5). Additional exploratory analyses across all remaining SNPs identified a significant association involving IL-2 rs11724582 in patients of African ancestry. Results showed that carriers of the G-allele experienced an AP-induced BMI increase that was approximately 4% greater than individuals homozygous for the A-allele (GA + GG vs. AA; 5.76 ± 8.3 vs. 2.08 ± 7.4, p=0.018). Under this model, IL-2 rs11724582 explains approximately 5.2% of the variance in BMI change (%) from baseline.

5.7.3. Race-and Treatment-Stratified Analyses

In olanzapine-restricted, race-stratified sub-samples which separately included patients of European and African ancestry, none of the primary replication SNPs were associated with AIWG in the CATIE sample (refer to Table 5.4 and Table 5.5). Additional exploratory analyses across all remaining SNPs identified a significant association involving IL-1β rs11674397 in the African sub-sample. Results showed that individuals with the heterozygous CT genotype experienced an AP-induced BMI increase that was approximately 6% greater than individuals homozygous for the C-allele (CT vs. CC; 10.65 ± 8.7 vs. 5.05 ± 8.0, p=0.025). Under this model, IL-1β rs11674397 explains around 12.6% of the variance in BMI change (%) from baseline.

TABLE 5.3: Results from Replication Analysis of SNPs with AIWG in the Total Sample

SNP	Genotype	Frequency[a]	ΔBMI (%)[a]	F Value	P-Value[b]
IL-1β rs17042517	AA	278 (87.7%)	2.87 ± 7.7	0.26	ǂ0.610
	GA	38 (12.0%)	1.95 ± 6.1		
	GG	1 (0.3%)	8.39		
IL-1β rs2723152	CC	172 (54.3%)	3.09 ± 7.7	0.55	0.576
	CT	118 (37.2%)	2.71 ± 7.0		
	TT	27 (8.5%)	1.11 ± 8.3		
IL-1β rs1143634	GG	205 (65.3%)	2.68 ± 7.2	0.20	0.822
	GA	91 (29.0%)	3.13 ± 8.4		
	AA	18 (5.7%)	2.81 ± 6.5		
BDNF Val66Met	Val/Val	243 (76.9%)	2.93 ± 7.5	0.16	0.851
	Val/Met	63 (19.9%)	2.64 ± 7.5		
	Met/Met	10 (3.2%)	0.63 ± 7.9		

Covariate was information missing for one participant; [a]Values represent mean ± standard deviation (number); [b]Uncorrected p-values; ǂP-value calculated under a dominant model of genetic inheritance; SNP = single nucleotide polymorphism, BMI = body-mass index, IL-1β = interleukin 1 beta, BDNF = brain-derived neurotrophic factor

TABLE 5.4: Results from Replication Analysis of SNPs with AIWG in Europeans

SNP	Genotype	ΔBMI (%)[a] All Drugs (n=207)	P-Value[b]	ΔBMI (%)[a] OLZ (n=67)	P-Value[b]	MAF[c]	HWE P-Value[c]
IL-1β rs17042517	AA	2.65 ± 7.5 (181)	0.702	5.49 ± 7.3(56)	0.092	0.06	0.925
	GA	1.83 ± 6.1 (25)		1.22 ± 6.1(10)			
	GG	-----		-----			
IL-1β rs2723152	CC	2.56 ± 7.1 (124)	0.542	4.66 ± 7.4 (42)	ǂ0.611	0.22	0.813
	CT	2.84 ± 7.2 (73)		5.92 ± 6.8 (21)			
	TT	0.11 ± 11.0 (9)		-0.14 ± 8.8 (3)			
IL-1β rs1143634	GG	2.41 ± 6.7 (126)	0.806	4.32 ± 6.9 (38)	0.624	0.23	0.636
	GA	2.68 ± 8.6 (68)		6.06 ± 8.3 (23)			
	AA	3.29 ± 5.6 (12)		3.2 ± 4.5(5)			
BDNF Val66Met	Val/Val	2.74 ± 7.3 (135)	0.908	4.59 ǂ 6.6 (48)	ǂ0.732	0.20	0.466
	Val/Met	2.54 ± 7.4 (60)		6.30 ǂ 8.6 (17)			
	Met/Met	0.63 ± 7.9 (10)		-7.65 (1)			

Covariate information was missing for one participant; [a]Values represent mean ± standard deviation (number); [b]Uncorrected p-values; [c]Values calculated among all patients of European ancestry; ǂP-value calculated under a dominant model of genetic inheritance; SNP = single nucleotide polymorphism, BMI = body-mass index, OLZ = olanzapine, MAF = minor allele frequency, HWE = Hardy-Weinberg equilibrium, IL-1β = interleukin 1 beta, BDNF = brain-derived neurotrophic factor

TABLE 5.5: Results from Replication Analysis of SNPs with AIWG in Africans

SNP	Genotype	ΔBMI (%)[a] All Drugs (n=111)	P Value[b]	ΔBMI (%)[a] OLZ (n=42)	P -Value[b]	MAF[c]	HWE P-Value[c]
IL-1β rs17042517	AA	3.29 ± 8.0 (97)	ǂ0.827	6.42 ± 8.6 (36)	0.428	0.07	0.795
	GA	2.18 ± 6.3 (13)		2.41 ± 4.7 (6)			
	GG	8.39 (1)		-----			
IL-1β rs2723152	CC	4.48 ± 9.0 (48)	0.313	6.52 ± 10.2 (22)	0.926	0.37	0.244
	CT	2.49 ± 6.6 (45)		4.76 ± 6.2 (15)			
	TT	1.61 ± 7.0 (18)		6.20 ± 2.9 (5)			
IL-1β rs1143634	GG	3.11 ± 7.9 (79)	0.661	5.62 ± 8.6 (32)	ǂ0.167	0.16	0.063
	GA	4.46 ± 7.8 (23)		6.88 ±z7.9 (9)			
	AA	1.85 ± 8.6 (6)		3.85 (1)			
BDNF Val66Met	Val/Val	3.17 ± 7.8 (108)	-----	5.63 ± 8.3 (40)	-----	0.014	1.000
	Val/Met	4.69 ± 11.0 (3)		10.24 ± 7.7 (2)			
	Met/Met	-----		-----			

Where p-value not shown, analysis not performed due to <5 subjects in merged comparison group; [a]Values represent mean ± standard deviation (number); [b]Uncorrected p-values; [c]Values calculated among all patients of African ancestry; ǂP-value calculated under a dominant model of genetic inheritance; SNP = single nucleotide polymorphism, BMI = body-mass index, OLZ = olanzapine, MAF = minor allele frequency, HWE = Hardy-Weinberg equilibrium, IL-1β = interleukin 1 beta, BDNF = brain-derived neurotrophic factor

5.8. Results from Gene-Gene Interaction Analyses

Epistatic effects were modelled between variant sites across each of IL-1β, IL-2, and IL-6 and the BDNF Val66Met locus for an association with BMI change (%) from baseline. Analyses were restricted to patients treated with olanzapine, and performed separately for patients of European and African ancestry. In the European sub-sample, none of the primary replication SNPs were found to significantly interact with BDNF Val66Met to predict BMI change (%) from baseline (refer to Table 5.6). No interactions models were found in the African sub-sample. Additional exploratory analyses across all remaining SNPs did not yield any significant findings.

TABLE 5.6: Gene-Gene Interaction against BDNF Val66Met in Europeans on OLZ

SNP	Genotype[a]	Risk Group[b]	Beta	Wald	P-Value	Perm P-Value[c]
IL-1β rs17042517	GA + Met/Met	Low	-12.63	3.10	0.083	0.325
IL-1β rs2723152	CC + Met/Met	Low	-12.63	3.10	0.083	0.391
IL-1β rs1143634	GA + Met/Met	Low	-12.63	3.10	0.083	0.463

All interactions were performed against BDNF Val66Met. [a]Values represent the respective interleukin + BDNF genotype; [b]High risk represents greatest change in BMI (%) from baseline, and low risk represents least change in BMI (%) from baseline; [c]Uncorrected p-values based on 1000 permutations; SNP = single nucleotide polymorphism, OLZ = olanzapine, IL-1β = interleukin 1 beta, BDNF = brain-derived neurotrophic factor

Chapter 6:

Discussion

The purpose of this chapter is to:

- Review main study findings and their relation to study hypotheses
- Discuss how findings integrate into the current literature
- Review study strengths and limitations
- Discuss clinical implications of study findings
- Propose areas for future research

6.1. Main Study Findings

In this sample of chronic schizophrenia patients on AP medication, the authors set out to address four primary study aims pertaining to the pharmacogenetic testing of IL-1β, IL-2, IL-6, and BDNF variants in AIWG. The data revealed the following main findings:

IL-1β variants and BDNF Val66Met are associated with AIWG

In the present study, IL-1β SNPs rs16944 and rs1143634 were significantly involved in mediating the effects of FGA and SGA treatment on weight gain in patients of European ancestry, with the latter SNP remaining significant after correcting for multiple comparisons. Similarly, IL-1β SNPs rs16944 and rs4849127 were associated with AIWG in patients of African ancestry, and remained significant in the African subset treated with olanzapine or clozapine; however, these findings did not remain significant after correction. IL-1β is a critically important inflammatory cytokine that is the proposed 'gatekeeper' of inflammation,

having numerous and diverse roles in regulating injury and infection[471]. Genetic variation at the rs16944 (G/A) locus has been implicated in several diseases that have an inflammatory component including, but not limited to, gastric cancer[472], type II diabetes mellitus[473], osteoarthritis[474], cervical cancer[475], and gastroesphageal reflux disease[476]. Genetic association studies have also provided evidence on the involvement of rs16944 in schizophrenia, with reports that implicate the A-allele in illness endophenotypes like elevated brain activation and brain volume reductions[276,277], and the G-allele in overall disease risk[477,478]. Similarly, the rs16944 variant has been associated with non-remission and impaired emotion processing in MDD, which is another psychiatric condition characterized by aberrant inflammatory signatures[479].

According to functional investigations, rs16944 is a promoter polymorphism involved in the regulation of gene expression, where the A-allele is responsible for high transcriptional activity relative to the G-allele[480]. In this study, genotype effects were observed for rs16944 where GA heterozygotes gained the most weight relative to individuals with either homozygous genotype. The genetic involvement of IL-1β in AIWG is supported by its biological role in regulating food intake[333,374]. Other studies have also identified a relationship between heterozygosity at rs16944 and disease phenotypes. For example, Tanaka *et al.* (2014)[481] found that individuals with the GA genotype had a significantly reduced risk of periodontal disease compared with a GG genotype reference group. Similarly, Zheng *et al.* (2013)[482] found that individuals with the GA genotype had a significantly decreased risk of esophageal squamous cell carcinoma relative to GG homozygous subjects. Based on these findings, we hypothesize that heterozygosity at rs16944 may lead to reduced expression of the IL-1β gene relative to either homozygous genotype. Reduced expression would consequently lower the risk of inflammatory conditions, and increase metabolic disturbances via impaired adiposity signalling.

According to HaploReg[454], rs16944 is in high LD (r^2 =0.98) with a second variant, rs6735739 (T>C), that is approximately 15kbp upstream. The rs6735739 polymorphism serves as a binding site for a hypothalamic transcription factor known as Brain-Specific Homeobox Factor (BSX) which regulates expression of NPY and AgRP[483]. Studies of the adult mouse brain have shown that BSX expression is localized to the hypothalamus, with greatest expression in the ARC and DMH, and some expression in the LHA. Additional evidence indicates that

NPY/AgRP neurons in the ARC express BSX, while POMC neurons do not[483]. BSX mRNA expression is up-regulated in the ARC subsequent to acute ghrelin administration and in response to fasting, while re-feeding decreases expression[484,485]. Overall, various lines of evidence suggest that BSX is involved in hypothalamic control of hyperphagia and locomotor activity[483].

BDNF Val66Met (rs6265) was also significantly involved in mediating AP effects on weight gain in this study, both among patients of European ancestry, and the European subset treated with either clozapine or olanzapine; however, findings did not remain significant after correcting for multiple comparisons. Results showed that carriers of the Met (or T) allele (Met/Met + Met/Val) were protected against excessive AIWG. These findings are consistent with the general obesity literature which has reported on the association between the Met/Met genotype and a lower BMI relative to Val allele carriers[35,434-436]. Investigations of Val66Met and AIWG within the pharmacogenetic literature have yielded mixed findings. In concordance with our results, Lane *et al.* (2006)[74] showed that the Met/Met genotype was associated with lower body weight than the Val allele in acutely exacerbated schizophrenia patients treated with risperidone. However, Zhang *et al.* (2008)[446] showed that the Met/Met genotype was associated with a significantly higher BMI relative to Val allele carriers in schizophrenia patients on long-term AP treatment. Other studies have found no relationship between Val66Met and AIWG in schizophrenia[445,447]. The discrepant susceptibility allele between our study and Zhang *et al.* (2008)[446] may suggest that the risk of AIWG conferred by Val66Met may differ between ethnic groups, as their study sample was exclusively comprised of Chinese patients, while our findings were relevant to patients of European ancestry. Additional support for our findings is derived from reports of the Val/Val genotype being associated with clinical response to APs[447] which, as mentioned in Section 1.3.2, is a risk factor for AIWG. There are also reports of 66Met being associated with eating disorders such as restricting anorexia nervosa[486].

IL-1β and IL-6 variants are associated with CSWG

In our secondary analysis, IL-1β rs4849127 and IL-6 rs1800795 SNPs were associated with CSWG in the total sample. In particular, IL-1β rs4849127*A conferred risk for CSWG, whereas IL-6 rs1800795*G was protective against CSWG. Genotypic analysis of IL-1β rs16944

revealed a significant association between heterozygosity at this locus and risk of CSWG in both the total sample and patients of African ancestry treated with clozapine or olanzapine. These results strengthen the roles of rs4849127 and rs16944 in mediating the genetic risk of AIWG by IL-1β. Significant SNPs from our previous investigations (IL-1β rs1143634 and BDNF Val66Met) may not have been associated with CSWG due to reduced analytic power through dichotomization of the weight change (%) variable.

IL-1β and IL-6 variants interact with BDNF Val66Met among patients treated with clozapine and olanzapine

IL-1β rs13032029 and IL-6 rs2069837 SNPs demonstrated a genetic interaction with BDNF Val66Met in patients of European ancestry. In particular, individuals with the TT genotype at IL-1β rs13032029 and the CT genotype at Val66Met were at low risk for AIWG, whereas carriers of the AA genotype at IL-6 rs2069837 and the CC genotype at Val66Met were at high risk for AIWG. Since these SNPs did not show any significant association with AIWG when analyzed individually, these results suggest that their genetic effects may be mediated through BDNF-dependent pathways. Epistatic effects were also observed among patients of African ancestry between IL-1β rs16944 and Val66Met. Within this interaction, individuals with the GA genotype at IL-1β rs16944 and the Val/Val genotype at Val66Met were identified as high risk for AIWG. The observed genetic interactions between cytokine SNPs and BDNF further support their biological interaction within neuroimmune crosstalk[177-179].

The association of IL-1β variants and BDNF Val66Met did not replicate in the CATIE sample

None of the primary replication SNPs, which included IL-1β rs17042517, rs2723152, and rs1143634 and BDNF Val66Met, had their association with AIWG replicated using the CATIE data across total or subscale analyses. This may have been the result of methodological differences between CATIE and our own study design that may have ultimately led to a loss of power. *AP Treatment.* Our discovery sample included clozapine as a treatment of interest, while

the CATIE study opted for the non-inclusion of clozapine treatment. This may be a major contributor to the observed difference in study results considering that different APs have variable weight-inducing propensities, with clozapine being a high-risk medication[16,17]. Our discovery sample primarily consisted of individuals who had no prior exposure to SGAs. However, SGA-naivety was not an inclusionary criterion for CATIE. At best, the data extracted from CATIE could only be restricted to subjects that had no prior exposure to olanzapine or clozapine that lasted longer than 30 days. In considering that SGAs have considerable weight inducing propensities[16,17], any weight gained outside of the study had the potential to limit within-study weight change, according to the inverse relationship between baseline BMI and AIWG[61,70,71]. *Study subjects.* Our discovery sample allowed for the inclusion of patients with both chronic schizophrenia and schizoaffective disorder whereas CATIE excluded patients with the latter diagnosis. This diagnostic difference could have affected study outcomes as the magnitude of AIWG can differ between schizophrenic subtypes[73-75]. In the same regard, the inclusion of clozapine-treated, and thus, treatment-refractory patients within the discovery sample may have further influenced results. *Weight analysis.* Although the consequences of this methodological difference may be minor, our discovery sample used weight change (%) from baseline as a measure of AIWG, while CATIE used BMI (%) change.

6.2. Strengths and Limitations

To the author's knowledge, this is the first study to demonstrate an association between the IL-1β polymorphisms rs4849127, rs16944, and rs1143634 and increased risk of AIWG. This is also believed to be the first study that has demonstrated epistatic effects between both IL-1β and IL-6 polymorphisms and BDNF Val66Met in mediating AIWG. Investigating interactive effects is highly important in considering that AIWG is a complex, polygenic trait. Our genetic methodology has greater accuracy in assessing cytokine involvement in AIWG since the DNA sequence is not perturbed by the numerous confounds that readily alter cytokine expression in the periphery (refer to Section 1.6.2). Our main AP analyses were also advantageously conducted in a sample of schizophrenic patients that were predominantly SGA, and even clozapine, naive prior to the onset of the study. In addition, pre-selecting SNPs with a known or predicted

immune function increased our statistical power to detect an association compared to employing a hypothesis-free approach. Overall, the goal of identifying peripherally accessible and efficiently analyzed biomarkers of AIWG holds considerable promise for its application in clinical practice (for further details, refer to Section 6.3)

However, our study design is not without limitations. The relatively small sample size and low MAF for some polymorphisms, particularly within race and treatment stratified sub-analyses, may have limited our statistical power to be able to detect small effect sizes. Loss of power would have also resulted from dichotomization of the weight change (%) variable during analysis of CSWG. It is, therefore, possible that we did not have sufficient power to test specific hypotheses within the large number of sub-analyses conducted. This may be a major contributor to the lack of significant relationships between some of our cytokine SNPs and AIWG, and possibly even the over-dominance effect observed for IL-1β rs16944. Despite this consideration, the predominance of sub-analyses that were conducted in this study were secondary investigations that were exploratory in design. In addition, since participants were recruited across three different centers, this may have increased within-sample heterogeneity. In recruiting patients with chronic schizophrenia, duration of illness and possible weight gain from previous treatments may have affected AIWG outcomes. To avoid a population stratification bias, we performed sub-analyses separately restricted to patients of European and African ancestry. However, ancestry was self-reported and not verified genetically. Another limitation is that information on potential risk factors of AIWG (e.g. AP dose, cigarette or cannabis use, dietary status, level of physical activity) was not collected, and therefore, the possible confounding effects of these factors cannot be ruled out. In addition, with inclusion of a placebo-controlled group, we cannot say with absolute certainty that weight gain outcomes are due to AP effects. Finally, a major limitation of our replication analysis was that 74% of all CATIE participants discontinued AP therapy before 18 months. Of these participants, 64% were receiving olanzapine and discontinued treatment predominantly due to AIWG or metabolic outcomes[468].

6.3. Clinical Implications

Although APs offer symptomatic relief for patients diagnosed with schizophrenia or other psychotic disorders, therapeutic benefits may be offset by AIWG. This adverse side effect not only increases the risk of obesity and various metabolic and vascular conditions, but it can also lead to premature mortality and AP non-compliance[18,19]. If patients become non-compliant with treatment or stop treatment prematurely, they put themselves at an elevated risk for symptom recurrence or illness relapse, and both an increased rate and duration of hospital admission[487]. AIWG is quite common, affecting approximately 15% to 72% of schizophrenic patients receiving acute or maintenance treatment[37]. AIWG is also one of the most distressing side effects experienced by patients, particularly females[488]. In recognition of these facts, the American Diabetes Association issued a consensus statement in 2004 which recommended baseline and ongoing metabolic monitoring for all patients receiving high-risk APs[489]. In spite of this recommendation, metabolic screening for AP-treated patients is still alarmingly infrequent, as baseline rates of glucose and lipid testing are only 23% and 8%, respectively[490]. Even metabolic parameters that are relatively simple to assess such as blood pressure and correlates of obesity (e.g. weight, waist circumference) are insufficiently monitored[19]. Although there are numerous factors that can contribute to non-adherence of clinical practice guidelines[491], metabolic monitoring may be improved by minimizing the burden it places on clinicians and patients. This may be partly achieved through the use of more efficient pharmacogenetic paradigms, which would essentially only require a one-time genetic screening for AIWG susceptibility at baseline.

Pharmacogenetic testing can be used to identify inter-individual genetic variation that is associated with, and thus has clinical utility in predicting, patient risk of adverse drug reactions such as AIWG[492]. Isolating relevant genetic variants is critically important when considering that 50% of adverse drug reactions can be attributed to genetic factors, as can 25% to 50% of inappropriate drug responses[493]. Based on the results of this study, variability at IL-1β SNPs rs4849127, rs16944, and rs1143634, and BDNF Val66Met can both individually and, in some cases, synergistically predict AIWG outcomes in AP-treated patients with chronic schizophrenia. These genetic biomarkers offer a potential platform for the design of predictive treatment algorithms that can personalize drug therapy by providing patient-specific information on the

relative safety of a given AP prior to its initial administration[494]. The ultimate goal of pharmacogenetic testing is to therefore optimize treatment outcomes by using an algorithm-based approach to rapidly prescribe APs to patients at a high likelihood of drug tolerability rather than simply using inefficient and costly 'trial and error' methods[495].

The long-term implications of reducing the prevalence of AIWG are numerous. Some of the more clinically relevant aspects pertain to improving patient care and quality of life. For one, this change could lead to significantly decreased rates of AP-induced metabolic and vascular conditions that are typically mediated through increased weight. It could also improve AP adherence, and consequently lower risk of illness relapse and hospitalization. This can have secondary effects on the healthcare system by reducing associated medical expenditures, as hospital costs are more than three times higher in AP non-compliant versus compliant schizophrenia patients[496]. Improved AIWG outcomes can also help minimize obesity stigmatization which has been associated with negative affect and various social disadvantages such as discriminatory hiring practices in employment settings[497]. In addition, clinically validated genetic biomarkers can be used to optimize and expedite drug design and development, respectively, with minimal associated costs. In particular, through a reduction in adverse events and the ability to select more genetically homogenous patient populations, molecular biomarkers can lower the attrition rate of APs during clinical trials, and thus, reduce the overall cost and time of drug development[498,499]. However, before the IL-1β and BDNF polymorphisms identified in this study can be formally incorporated into genetic screening practices, they would need to receive further clinical validation and functional testing.

6.4. Future Directions

The results from the present study contribute to the existing literature by demonstrating the genetic involvement of inflammatory and neurotrophic factors in AIWG. Despite these findings, pharmacogenetic researchers have primarily studied AIWG by investigating the effects of various hypothalamic neurotransmitters and signalling factors. Consequently, there is a paucity of data on the role of inflammatory mediators within this association, with the predominance of available literature only focusing on the effects of TNF-α and leptin[21-23]. To the

author's knowledge, there are no pharmacogenetic studies that have investigated the role of the interleukin family of cytokines in AIWG. Instead, studies of inflammatory involvement are often serologic, and thus, rooted in serum quantification of cytokine protein levels[442-444].

Based on our study findings, it may be promising to investigate a broader collection of immune variants across genes regulating both inflammatory and anti-inflammatory mediators, and their relevant receptors and receptor antagonists. It may also be informative to examine epistatic interactions between immune markers and other neurotrophins beyond BDNF, such as nerve growth factor, based on their involvement in neuroimmune crosstalk. Similarly, with biological interactions occurring between immune markers and the HPA axis[391,392], investigating the interplay between cytokines and both prostaglandins and endocrine hormones like CRH may be useful. Additional relationships of interest include inter-cytokine interactions due to their observed ability to act synergistically[348], in addition to cytokine interactions with hypothalamic signalling factors, neurotransmitters, and neuropeptides[336,365,377,379,380]. In addition to immune factors, the type of weight gain information collected will affect the quality of research. At a minimum, studies investigating AIWG should collect information on weight (in kg), BMI, and waist circumference at baseline, at regular intervals throughout, and at the end of the study. If the study design permits, and if not too burdensome for patients, clinical information on blood pressure and fasting glucose levels should also be obtained. In the end, once significant SNPs have been identified, as in this study, functional investigations of allelic effects are warranted. There would also be significant clinical utility in determining the application of immunogenetic biomarkers within other AP-treated psychiatric disorders reporting AIWG like bipolar disorder[54], treatment-resistant MDD[55], mental retardation and autistic disorder[56], and Tourette's syndrome[57].

The relative success of immunogenetic research in AIWG will be highly dependent on the degree to which various research domains can be comprehensively integrated. Studies of genetic interaction effects are a cornerstone of these initiatives. However, in order to achieve large-scale scientific advances, biomarker data will need to be integrated across various research modalities, such as neuroimaging, proteomics, transcriptomics, clinical assessment, and in the case of our study, molecular genetics. All of these domains have yielded important findings – spanning from studies of brain morphology and activation patterns, PET quantification of TSPO, post-mortem investigations of S100B and astrogliosis, immunogenetics, c-*fos* patterns of

neuronal activation, cytokine knockout models, quantification of cytokine mRNA and protein – that have contributed to characterizing the role of inflammation within psychiatric disease and metabolic disturbance. The inter-disciplinary study of biomarker research may assist in the discovery of consistently validated candidates that can provide promising insights into the biological basis of AIWG. This is similar to the goal behind the ongoing Canadian Biomarker Integration Network in Depression (CAN-BIND) study which aims to use this inter-modality approach to identify predictive candidates of treatment response in MDD[500].

Finally, in considering that APs achieve partial treatment benefits through immuno-suppressive action, it would be logical to examine the effects of cytokine gene variants on therapeutic drug response. In the present study, data on clinical response to APs were collected (but not currently analyzed) for all 188 chronic schizophrenia patients. Clinical response was assessed according to change in BPRS total scores (ΔBPRS) over the course of AP treatment. As a future direction of this project, we intend to examine ΔBPRS across genotypic groups for each SNP from our original panel. Similar to APs, antidepressants also show immunomodulatory properties[195,501,502]. Therefore, examining immunogenetic biomarkers of antidepressant response may also be functionally predictive. Hence, we also intend to examine the relationship between our cytokine SNP panel and response to Duloxetine treatment in an MDD sample. Clinical response has been assessed using the Hamilton Rating Scale for Depression (HAMD) and Montgomery-Asberg Depression Scale (MADRS). It may also be promising to examine the role of cytokine variants in the disease phenotype of schizophrenia, and other psychiatric conditions characterized by up-regulated inflammatory signatures, such as MDD.

6.5. Conclusions

The aim of the present study was to investigate the role of genetic variants across IL-1β, IL-2, IL-6 and BDNF in AIWG. The current literature provides evidence on cytokine and neurotrophin dysregulation within schizophrenia that, through modification by AP medication, can have secondary consequences that lead to hyperphagia and altered energy expenditure. Our data extend these observations by showing that IL-1β SNPs rs4849127, rs16944, and rs1143634, in addition to BDNF Val66Met, were significantly involved in mediating the effects of FGA and

SGA treatment on AIWG in 188 patients with chronic schizophrenia. Additional analyses revealed that IL-1β rs4849127*A, IL-6 rs1800795*G, and heterozygosity at IL-1β rs16944 were associated with CSWG. Epistatic effects between BDNF Val66Met and cytokine SNPs IL-1β rs13032029, IL-1β rs16944, and IL-6 rs2069837 were also observed in patients treated with the high-risk drugs, clozapine and olanzapine. None of the primary replication SNPs, which included IL-1β rs17042517, rs2723152, and rs1143634 and BDNF Val66Met, had their association with AIWG replicated using the CATIE data. Taken together, there is appreciable evidence to support the influence of cytokine genetic variation on AIWG. However, further studies are needed to confirm the predictive utility of these immunogenetic biomarkers and more clearly elucidate the underlying functional mechanisms that support a cytokine model of AIWG.

The scope of future immunogenetic analyses of AIWG should be extended to include additional genes regulating inflammatory and anti-inflammatory factors, and their relevant receptors. Functional gene variants identified from these studies may have promising applications in other AP-treated psychiatric populations, and in elucidating the role of inflammation within treatment response and the pathogenesis of psychiatric phenotypes. Overall, the discovery of clinically validated genetic biomarkers of AIWG may offer a potential platform for the design of predictive treatment algorithms that can personalize drug therapy to reduce the likelihood of adverse drug reactions. Future biomarker initiatives may benefit from the integration of clinically relevant data collected across various research modalities. Nevertheless, immunogenetic research is still in its infancy and requires further investigation.

References

1. Saha S, Chant D, Welham J, McGrath J. A systematic review of the prevalence of schizophrenia. *PLoS medicine.* May 2005;2(5):e141.

2. American Psychiatric Association. *Diagnostic and statistical manual of mental disorders*. 5th edition ed. Arlington, VA: American Psychiatric Publishing; 2013.

3. Andreasen NC, Olsen S. Negative v positive schizophrenia. Definition and validation. *Archives of general psychiatry.* Jul 1982;39(7):789-794.

4. Sharma T, Antonova L. Cognitive function in schizophrenia. Deficits, functional consequences, and future treatment. *The Psychiatric clinics of North America.* Mar 2003;26(1):25-40.

5. Harvey PD. Mood symptoms, cognition, and everyday functioning: in major depression, bipolar disorder, and schizophrenia. *Innovations in clinical neuroscience.* Oct 2011;8(10):14-18.

6. Miyamoto S, Miyake N, Jarskog LF, Fleischhacker WW, Lieberman JA. Pharmacological treatment of schizophrenia: a critical review of the pharmacology and clinical effects of current and future therapeutic agents. *Molecular psychiatry.* Dec 2012;17(12):1206-1227.

7. Arana GW. An overview of side effects caused by typical antipsychotics. *The Journal of clinical psychiatry.* 2000;61 Suppl 8:5-11; discussion 12-13.

8. Kane J, Honigfeld G, Singer J, Meltzer H. Clozapine for the treatment-resistant schizophrenic. A double-blind comparison with chlorpromazine. *Archives of general psychiatry.* Sep 1988;45(9):789-796.

9. Shen WW. A history of antipsychotic drug development. *Comprehensive psychiatry.* Nov-Dec 1999;40(6):407-414.

10. Leucht S, Corves C, Arbter D, Engel RR, Li C, Davis JM. Second-generation versus first-generation antipsychotic drugs for schizophrenia: a meta-analysis. *Lancet.* Jan 3 2009;373(9657):31-41.

11. Petit M, Raniwalla J, Tweed J, Leutenegger E, Dollfus S, Kelly F. A comparison of an atypical and typical antipsychotic, zotepine versus haloperidol in patients with acute

exacerbation of schizophrenia: a parallel-group double-blind trial. *Psychopharmacology bulletin.* 1996;32(1):81-87.

12. Woodward ND, Purdon SE, Meltzer HY, Zald DH. A meta-analysis of neuropsychological change to clozapine, olanzapine, quetiapine, and risperidone in schizophrenia. *Int J Neuropsychopharmacol.* Sep 2005;8(3):457-472.

13. Thornton AE, Van Snellenberg JX, Sepehry AA, Honer W. The impact of atypical antipsychotic medications on long-term memory dysfunction in schizophrenia spectrum disorder: a quantitative review. *J Psychopharmacol.* May 2006;20(3):335-346.

14. Tollefson GD, Sanger TM, Lu Y, Thieme ME. Depressive signs and symptoms in schizophrenia: a prospective blinded trial of olanzapine and haloperidol. *Archives of general psychiatry.* Mar 1998;55(3):250-258.

15. Kapur S, Remington G. Atypical antipsychotics: new directions and new challenges in the treatment of schizophrenia. *Annual review of medicine.* 2001;52:503-517.

16. Lett TA, Wallace TJ, Chowdhury NI, Tiwari AK, Kennedy JL, Muller DJ. Pharmacogenetics of antipsychotic-induced weight gain: review and clinical implications. *Molecular psychiatry.* Mar 2012;17(3):242-266.

17. Allison DB, Mentore JL, Heo M, et al. Antipsychotic-induced weight gain: a comprehensive research synthesis. *The American journal of psychiatry.* Nov 1999;156(11):1686-1696.

18. Bernstein JG. Induction of Obesity by Psychotropic Drugs. *Annals of the New York Academy of Sciences.* 1987;499:203-215.

19. De Hert M, Detraux J, van Winkel R, Yu W, Correll CU. Metabolic and cardiovascular adverse effects associated with antipsychotic drugs. *Nature reviews. Endocrinology.* Feb 2012;8(2):114-126.

20. Muller DJ, Kennedy JL. Genetics of antipsychotic treatment emergent weight gain in schizophrenia. *Pharmacogenomics.* Sep 2006;7(6):863-887.

21. Wang YC, Bai YM, Chen JY, Lin CC, Lai IC, Liou YJ. Genetic association between TNF-alpha -308 G>A polymorphism and longitudinal weight change during clozapine treatment. *Human psychopharmacology.* Jun-Jul 2010;25(4):303-309.

22. Kang SG, Lee HJ, Park YM, et al. Possible association between the -2548A/G polymorphism of the leptin gene and olanzapine-induced weight gain. *Progress in neuro-psychopharmacology & biological psychiatry.* Jan 1 2008;32(1):160-163.

23. Calarge CA, Ellingrod VL, Zimmerman B, Acion L, Sivitz WI, Schlechte JA. Leptin gene -2548G/A variants predict risperidone-associated weight gain in children and adolescents. *Psychiatric genetics.* Dec 2009;19(6):320-327.

24. Pollmacher T, Haack M, Schuld A, Kraus T, Hinze-Selch D. Effects of antipsychotic drugs on cytokine networks. *Journal of psychiatric research.* Nov-Dec 2000;34(6):369-382.

25. Drzyzga L, Obuchowicz E, Marcinowska A, Herman ZS. Cytokines in schizophrenia and the effects of antipsychotic drugs. *Brain, behavior, and immunity.* Nov 2006;20(6):532-545.

26. Chida D, Osaka T, Hashimoto O, Iwakura Y. Combined interleukin-6 and interleukin-1 deficiency causes obesity in young mice. *Diabetes.* Apr 2006;55(4):971-977.

27. Wallenius V, Wallenius K, Ahren B, et al. Interleukin-6-deficient mice develop mature-onset obesity. *Nature medicine.* Jan 2002;8(1):75-79.

28. Finck BN, Johnson RW. Anorexia, weight loss and increased plasma interleukin-6 caused by chronic intracerebroventricular infusion of interleukin-1beta in the rat. *Brain research.* Jul 4 1997;761(2):333-337.

29. Angelucci F, Mathe AA, Aloe L. Brain-derived neurotrophic factor and tyrosine kinase receptor TrkB in rat brain are significantly altered after haloperidol and risperidone administration. *Journal of neuroscience research.* Jun 15 2000;60(6):783-794.

30. Lipska BK, Khaing ZZ, Weickert CS, Weinberger DR. BDNF mRNA expression in rat hippocampus and prefrontal cortex: effects of neonatal ventral hippocampal damage and antipsychotic drugs. *The European journal of neuroscience.* Jul 2001;14(1):135-144.

31. Lebrun B, Bariohay B, Moyse E, Jean A. Brain-derived neurotrophic factor (BDNF) and food intake regulation: a minireview. *Autonomic neuroscience : basic & clinical.* Jun 30 2006;126-127:30-38.

32. Manica-Cattani MF, Bittencourt L, Rocha MI, et al. Association between interleukin-1 beta polymorphism (+3953) and obesity. *Molecular and cellular endocrinology.* Jan 15 2010;314(1):84-89.

33. Berthier MT, Paradis AM, Tchernof A, et al. The interleukin 6-174G/C polymorphism is associated with indices of obesity in men. *Journal of human genetics.* 2003;48(1):14-19.

34. Thorleifsson G, Walters GB, Gudbjartsson DF, et al. Genome-wide association yields new sequence variants at seven loci that associate with measures of obesity. *Nature genetics.* Jan 2009;41(1):18-24.

35. Gunstad J, Schofield P, Paul RH, et al. BDNF Val66Met polymorphism is associated with body mass index in healthy adults. *Neuropsychobiology.* 2006;53(3):153-156.

36. Mitchell AJ, Vancampfort D, De Herdt A, Yu W, De Hert M. Is the prevalence of metabolic syndrome and metabolic abnormalities increased in early schizophrenia? A comparative meta-analysis of first episode, untreated and treated patients. *Schizophrenia bulletin.* Mar 2013;39(2):295-305.

37. De Hert M, Correll CU, Bobes J, et al. Physical illness in patients with severe mental disorders. I. Prevalence, impact of medications and disparities in health care. *World Psychiatry.* Feb 2011;10(1):52-77.

38. Scott D, Happell B. The high prevalence of poor physical health and unhealthy lifestyle behaviours in individuals with severe mental illness. *Issues in mental health nursing.* 2011;32(9):589-597.

39. Alberti KG, Zimmet P, Shaw J. Metabolic syndrome--a new world-wide definition. A Consensus Statement from the International Diabetes Federation. *Diabetic medicine : a journal of the British Diabetic Association.* May 2006;23(5):469-480.

40. Miranda PJ, DeFronzo RA, Califf RM, Guyton JR. Metabolic syndrome: definition, pathophysiology, and mechanisms. *American heart journal.* Jan 2005;149(1):33-45.

41. Kohen D. Diabetes mellitus and schizophrenia: historical perspective. *The British journal of psychiatry. Supplement.* Apr 2004;47:S64-66.

42. Thakore JH, Mann JN, Vlahos I, Martin A, Reznek R. Increased visceral fat distribution in drug-naive and drug-free patients with schizophrenia. *International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity.* Jan 2002;26(1):137-141.

43. Spelman LM, Walsh PI, Sharifi N, Collins P, Thakore JH. Impaired glucose tolerance in first-episode drug-naive patients with schizophrenia. *Diabetic medicine : a journal of the British Diabetic Association.* May 2007;24(5):481-485.

44. Ryan MC, Collins P, Thakore JH. Impaired fasting glucose tolerance in first-episode, drug-naive patients with schizophrenia. *The American journal of psychiatry.* Feb 2003;160(2):284-289.

45. Dasgupta A, Singh OP, Rout JK, Saha T, Mandal S. Insulin resistance and metabolic profile in antipsychotic naive schizophrenia patients. *Progress in neuro-psychopharmacology & biological psychiatry.* Oct 1 2010;34(7):1202-1207.

46. Arranz B, Rosel P, Ramirez N, et al. Insulin resistance and increased leptin concentrations in noncompliant schizophrenia patients but not in antipsychotic-naive first-episode schizophrenia patients. *The Journal of clinical psychiatry.* Oct 2004;65(10):1335-1342.

47. Padmavati R, McCreadie RG, Tirupati S. Low prevalence of obesity and metabolic syndrome in never-treated chronic schizophrenia. *Schizophrenia research.* Aug 2010;121(1-3):199-202.

48. Wright P, Sham PC, Gilvarry CM, et al. Autoimmune diseases in the pedigrees of schizophrenic and control subjects. *Schizophrenia research.* Jul 5 1996;20(3):261-267.

49. Zhang ZJ, Yao ZJ, Liu W, Fang Q, Reynolds GP. Effects of antipsychotics on fat deposition and changes in leptin and insulin levels. Magnetic resonance imaging study of previously untreated people with schizophrenia. *The British journal of psychiatry : the journal of mental science.* Jan 2004;184:58-62.

50. Editorial: Drugs causing weight gain. *British medical journal.* Feb 2 1974;1(5900):168.

51. Planansky K. Changes in weight in patients receiving a tranquilizing drug. *The Psychiatric quarterly.* 1958;32(2):289-303.

52. Haddad P. Weight change with atypical antipsychotics in the treatment of schizophrenia. *J Psychopharmacol.* Nov 2005;19(6 Suppl):16-27.

53. Wetterling T. Bodyweight gain with atypical antipsychotics. A comparative review. *Drug safety : an international journal of medical toxicology and drug experience.* Jan 2001;24(1):59-73.

54. McIntyre RS, Mancini DA, Basile VS, Srinivasan J, Kennedy SH. Antipsychotic-induced weight gain: bipolar disorder and leptin. *Journal of clinical psychopharmacology.* Aug 2003;23(4):323-327.

55. Chen J, Gao K, Kemp DE. Second-generation antipsychotics in major depressive disorder: update and clinical perspective. *Current opinion in psychiatry.* Jan 2011;24(1):10-17.

56. Hellings JA, Zarcone JR, Crandall K, Wallace D, Schroeder SR. Weight gain in a controlled study of risperidone in children, adolescents and adults with mental retardation and autism. *Journal of child and adolescent psychopharmacology.* Fall 2001;11(3):229-238.

57. Ondo WG, Jong D, Davis A. Comparison of weight gain in treatments for Tourette syndrome: tetrabenazine versus neuroleptic drugs. *Journal of child neurology.* Apr 2008;23(4):435-437.

58. Bak M, Fransen A, Janssen J, van Os J, Drukker M. Almost all antipsychotics result in weight gain: a meta-analysis. *PloS one.* 2014;9(4):e94112.

59. Gebhardt S, Haberhausen M, Heinzel-Gutenbrunner M, et al. Antipsychotic-induced body weight gain: predictors and a systematic categorization of the long-term weight course. *Journal of psychiatric research.* Mar 2009;43(6):620-626.

60. Wetterling T, Mussigbrodt HE. Weight gain: side effect of atypical neuroleptics? *Journal of clinical psychopharmacology.* Aug 1999;19(4):316-321.

61. Russell JM, Mackell JA. Bodyweight gain associated with atypical antipsychotics: epidemiology and therapeutic implications. *CNS drugs.* 2001;15(7):537-551.

62. Strassnig M, Miewald J, Keshavan M, Ganguli R. Weight gain in newly diagnosed first-episode psychosis patients and healthy comparisons: one-year analysis. *Schizophrenia research.* Jul 2007;93(1-3):90-98.

63. Safer DJ. A comparison of risperidone-induced weight gain across the age span. *Journal of clinical psychopharmacology.* Aug 2004;24(4):429-436.

64. Briffa D, Meehan T. Weight changes during clozapine treatment. *The Australian and New Zealand journal of psychiatry.* Oct 1998;32(5):718-721.

65. Ratzoni G, Gothelf D, Brand-Gothelf A, et al. Weight gain associated with olanzapine and risperidone in adolescent patients: a comparative prospective study. *Journal of the American Academy of Child and Adolescent Psychiatry.* Mar 2002;41(3):337-343.

66. Basson BR, Kinon BJ, Taylor CC, Szymanski KA, Gilmore JA, Tollefson GD. Factors influencing acute weight change in patients with schizophrenia treated with olanzapine, haloperidol, or risperidone. *The Journal of clinical psychiatry.* Apr 2001;62(4):231-238.

67. Jones B, Basson BR, Walker DJ, Crawford AM, Kinon BJ. Weight change and atypical antipsychotic treatment in patients with schizophrenia. *The Journal of clinical psychiatry.* 2001;62 Suppl 2:41-44.

68. Mushtaq F, Mondelli V, Pariante CM. The metabolic implications of long term cannabis use in patients with psychosis. *Epidemiologia e psichiatria sociale.* Jul-Sep 2008;17(3):221-226.

69. Umbricht DS, Pollack S, Kane JM. Clozapine and weight gain. *The Journal of clinical psychiatry.* Sep 1994;55 Suppl B:157-160.

70. Fleischhaker C, Heiser P, Hennighausen K, et al. Weight gain associated with clozapine, olanzapine and risperidone in children and adolescents. *J Neural Transm.* Feb 2007;114(2):273-280.

71. Cortes B, Becker J, Mories Alvarez MT, Marcos AI, Molina V. Contribution of baseline body mass index and leptin serum level to the prediction of early weight gain with atypical antipsychotics in schizophrenia. *Psychiatry and clinical neurosciences.* Feb 2014;68(2):127-132.

72. Kinon BJ, Kaiser CJ, Ahmed S, Rotelli MD, Kollack-Walker S. Association between early and rapid weight gain and change in weight over one year of olanzapine therapy in patients with schizophrenia and related disorders. *Journal of clinical psychopharmacology.* Jun 2005;25(3):255-258.

73. Saddichha S, Ameen S, Akhtar S. Predictors of antipsychotic-induced weight gain in first-episode psychosis: conclusions from a randomized, double-blind, controlled prospective study of olanzapine, risperidone, and haloperidol. *Journal of clinical psychopharmacology.* Feb 2008;28(1):27-31.

74. Lane HY, Liu YC, Huang CL, et al. Risperidone-related weight gain: genetic and nongenetic predictors. *Journal of clinical psychopharmacology.* Apr 2006;26(2):128-134.

75. Lee SY, Park MH, Patkar AA, Pae CU. A retrospective comparison of BMI changes and the potential risk factors among schizophrenic inpatients treated with aripiprazole,

olanzapine, quetiapine or risperidone. *Progress in neuro-psychopharmacology & biological psychiatry.* Mar 30 2011;35(2):490-496.

76. Simon V, van Winkel R, De Hert M. Are weight gain and metabolic side effects of atypical antipsychotics dose dependent? A literature review. *The Journal of clinical psychiatry.* Jul 2009;70(7):1041-1050.

77. Correll CU, Manu P, Olshanskiy V, Napolitano B, Kane JM, Malhotra AK. Cardiometabolic risk of second-generation antipsychotic medications during first-time use in children and adolescents. *JAMA : the journal of the American Medical Association.* Oct 28 2009;302(16):1765-1773.

78. Meltzer HY, Perry E, Jayathilake K. Clozapine-induced weight gain predicts improvement in psychopathology. *Schizophrenia research.* Jan 1 2003;59(1):19-27.

79. McIntyre RS, Jerrell JM. Metabolic and cardiovascular adverse events associated with antipsychotic treatment in children and adolescents. *Archives of pediatrics & adolescent medicine.* Oct 2008;162(10):929-935.

80. Casey DE, Daniel, D.G., Wassef, A.A., Tracy, K.A., Wozniak, P., Sommerville, K.W. Effect of Divalproex Combined with Olanzapine or Risperidone in Patients with an Acute Exacerbation of Schizophrenia. *Neuropsychopharmacology.* 2003;28:182–192.

81. Stahl SM. *Stahl's Essential Psychopharmacology: Neuroscientific Basis and Practical Applications*. Cambridge, New York: Cambridge University Press; 2008.

82. Stahl SM. Describing an Atypical Antipsychotic: Receptor Binding and Its Role in Pathophysiology. *Primary Care Companion Journal of Clinical Psychiatry.* 2003;5(suppl 3):9–13.

83. Prus A. *An Introduction to Drugs and the Neuroscience of Behavior*. Belmont, CA: Cengage Learning; 2013.

84. Coccurello R, Moles A. Potential mechanisms of atypical antipsychotic-induced metabolic derangement: clues for understanding obesity and novel drug design. *Pharmacology & therapeutics.* Sep 2010;127(3):210-251.

85. Correll CU, Lencz T, Malhotra AK. Antipsychotic drugs and obesity. *Trends in molecular medicine.* Feb 2011;17(2):97-107.

86. Leibowitz SF, Wortley KE. Hypothalamic control of energy balance: different peptides, different functions. *Peptides.* Mar 2004;25(3):473-504.

87. Schwartz MW, Baskin DG, Kaiyala KJ, Woods SC. Model for the regulation of energy balance and adiposity by the central nervous system. *The American journal of clinical nutrition.* Apr 1999;69(4):584-596.

88. Wilding JP. Neuropeptides and appetite control. *Diabetic medicine : a journal of the British Diabetic Association.* Aug 2002;19(8):619-627.

89. Lenard NR, Berthoud HR. Central and peripheral regulation of food intake and physical activity: pathways and genes. *Obesity (Silver Spring).* Dec 2008;16 Suppl 3:S11-22.

90. Woods SC, Seeley RJ, Porte D, Jr., Schwartz MW. Signals that regulate food intake and energy homeostasis. *Science.* May 29 1998;280(5368):1378-1383.

91. Wysokinski A, Kloszewska I. Mechanisms of Increased Appetite and Weight Gain Induced by Psychotropic Medications. *Journal of Advanced Clinical Pharmacology.* 2014;1(1):12-33.

92. Grill HJ. Distributed neural control of energy balance: contributions from hindbrain and hypothalamus. *Obesity (Silver Spring).* Aug 2006;14 Suppl 5:216S-221S.

93. Berthoud HR. Mind versus metabolism in the control of food intake and energy balance. *Physiology & behavior.* Jul 2004;81(5):781-793.

94. Velloso LA, Araujo EP, de Souza CT. Diet-induced inflammation of the hypothalamus in obesity. *Neuroimmunomodulation.* 2008;15(3):189-193.

95. Arora S, Anubhuti. Role of neuropeptides in appetite regulation and obesity--a review. *Neuropeptides.* Dec 2006;40(6):375-401.

96. Wang Q, Liu C, Uchida A, et al. Arcuate AgRP neurons mediate orexigenic and glucoregulatory actions of ghrelin. *Molecular metabolism.* Feb 2014;3(1):64-72.

97. Blundell JE. Is there a role for serotonin (5-hydroxytryptamine) in feeding? *International journal of obesity.* 1977;1(1):15-42.

98. Schwartz MW, Woods SC, Porte D, Jr., Seeley RJ, Baskin DG. Central nervous system control of food intake. *Nature.* Apr 6 2000;404(6778):661-671.

99. Oury F, Karsenty G. Towards a serotonin-dependent leptin roadmap in the brain. *Trends in endocrinology and metabolism: TEM.* Sep 2011;22(9):382-387.

100. Bonhaus DW, Weinhardt KK, Taylor M, et al. RS-102221: a novel high affinity and selective, 5-HT2C receptor antagonist. *Neuropharmacology.* Apr-May 1997;36(4-5):621-629.

101. Currie PJ, Saxena N, Tu AY. 5-HT(2A/2C) receptor antagonists in the paraventricular nucleus attenuate the action of DOI on NPY-stimulated eating. *Neuroreport.* Sep 29 1999;10(14):3033-3036.

102. Wang P, Aulakh CS, Hill JL, Murphy DL. Fawn hooded rats are subsensitive to the food intake suppressant effects of 5-HT agonists. *Psychopharmacology.* 1988;94(4):558-562.

103. Dourish CT, Hutson PH, Curzon G. Low doses of the putative serotonin agonist 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) elicit feeding in the rat. *Psychopharmacology.* 1985;86(1-2):197-204.

104. Baptista T, Parada M, Hernandez L. Long term administration of some antipsychotic drugs increases body weight and feeding in rats. Are D2 dopamine receptors involved? *Pharmacology, biochemistry, and behavior.* Jul 1987;27(3):399-405.

105. Hsiao S, Smith GP. Raclopride reduces sucrose preference in rats. *Pharmacology, biochemistry, and behavior.* Jan 1995;50(1):121-125.

106. Brown RE, Stevens DR, Haas HL. The physiology of brain histamine. *Progress in neurobiology.* Apr 2001;63(6):637-672.

107. Lecklin A, Etu-Seppala P, Stark H, Tuomisto L. Effects of intracerebroventricularly infused histamine and selective H1, H2 and H3 agonists on food and water intake and urine flow in Wistar rats. *Brain research.* May 18 1998;793(1-2):279-288.

108. Masaki T, Chiba S, Yasuda T, et al. Involvement of hypothalamic histamine H1 receptor in the regulation of feeding rhythm and obesity. *Diabetes.* Sep 2004;53(9):2250-2260.

109. Kroeze WK, Hufeisen SJ, Popadak BA, et al. H1-histamine receptor affinity predicts short-term weight gain for typical and atypical antipsychotic drugs. *Neuropsychopharmacology.* Mar 2003;28(3):519-526.

110. Moore RY, Bloom FE. Central catecholamine neuron systems: anatomy and physiology of the norepinephrine and epinephrine systems. *Annual review of neuroscience.* 1979;2:113-168.

111. Leibowitz SF, Roossin P, Rosenn M. Chronic norepinephrine injection into the hypothalamic paraventricular nucleus produces hyperphagia and increased body weight in the rat. *Pharmacology, biochemistry, and behavior.* Nov 1984;21(5):801-808.

112. Wellman PJ, Davies BT. Reversal of cirazoline- and phenylpropanolamine-induced anorexia by the alpha 1-receptor antagonist prazosin. *Pharmacology, biochemistry, and behavior.* May 1992;42(1):97-100.

113. Goldman CK, Marino L, Leibowitz SF. Postsynaptic alpha 2-noradrenergic receptors mediate feeding induced by paraventricular nucleus injection of norepinephrine and clonidine. *European journal of pharmacology.* Sep 10 1985;115(1):11-19.

114. Cohen BM, Lipinski JF. In vivo potencies of antipsychotic drugs in blocking alpha 1 noradrenergic and dopamine D2 receptors: implications for drug mechanisms of action. *Life sciences.* Dec 29 1986;39(26):2571-2580.

115. Theisen FM, Gebhardt S, Haberhausen M, et al. Clozapine-induced weight gain: a study in monozygotic twins and same-sex sib pairs. *Psychiatric genetics.* Dec 2005;15(4):285-289.

116. Wehmeier PM, Gebhardt S, Schmidtke J, Remschmidt H, Hebebrand J, Theisen FM. Clozapine: weight gain in a pair of monozygotic twins concordant for schizophrenia and mild mental retardation. *Psychiatry research.* Feb 28 2005;133(2-3):273-276.

117. Templeman LA, Reynolds GP, Arranz B, San L. Polymorphisms of the 5-HT2C receptor and leptin genes are associated with antipsychotic drug-induced weight gain in Caucasian subjects with a first-episode psychosis. *Pharmacogenetics and genomics.* Apr 2005;15(4):195-200.

118. Reynolds GP, Zhang ZJ, Zhang XB. Association of antipsychotic drug-induced weight gain with a 5-HT2C receptor gene polymorphism. *Lancet.* Jun 15 2002;359(9323):2086-2087.

119. Reynolds GP, Zhang Z, Zhang X. Polymorphism of the promoter region of the serotonin 5-HT(2C) receptor gene and clozapine-induced weight gain. *The American journal of psychiatry.* Apr 2003;160(4):677-679.

120. Miller DD, Ellingrod VL, Holman TL, Buckley PF, Arndt S. Clozapine-induced weight gain associated with the 5HT2C receptor -759C/T polymorphism. *American journal of medical genetics. Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics.* Feb 5 2005;133B(1):97-100.

121. Ellingrod VL, Perry PJ, Ringold JC, et al. Weight gain associated with the -759C/T polymorphism of the 5HT2C receptor and olanzapine. *American journal of medical*

genetics. Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics. Apr 5 2005;134B(1):76-78.

122. Muller DJ, Zai CC, Sicard M, et al. Systematic analysis of dopamine receptor genes (DRD1-DRD5) in antipsychotic-induced weight gain. *The pharmacogenomics journal.* Apr 2012;12(2):156-164.

123. Hong CJ, Liou YJ, Bai YM, Chen TT, Wang YC, Tsai SJ. Dopamine receptor D2 gene is associated with weight gain in schizophrenic patients under long-term atypical antipsychotic treatment. *Pharmacogenetics and genomics.* Jun 2010;20(6):359-366.

124. Brandl EJ, Frydrychowicz C, Tiwari AK, et al. Association study of polymorphisms in leptin and leptin receptor genes with antipsychotic-induced body weight gain. *Progress in neuro-psychopharmacology & biological psychiatry.* Aug 7 2012;38(2):134-141.

125. Tiwari AK, Brandl EJ, Weber C, et al. Association of a functional polymorphism in neuropeptide Y with antipsychotic-induced weight gain in schizophrenia patients. *Journal of clinical psychopharmacology.* Feb 2013;33(1):11-17.

126. Sickert L, Muller DJ, Tiwari AK, et al. Association of the alpha 2A adrenergic receptor -1291C/G polymorphism and antipsychotic-induced weight gain in European-Americans. *Pharmacogenomics.* Jul 2009;10(7):1169-1176.

127. Hong CJ, Lin CH, Yu YW, Chang SC, Wang SY, Tsai SJ. Genetic variant of the histamine-1 receptor (glu349asp) and body weight change during clozapine treatment. *Psychiatric genetics.* Sep 2002;12(3):169-171.

128. Ujike H, Nomura A, Morita Y, et al. Multiple genetic factors in olanzapine-induced weight gain in schizophrenia patients: a cohort study. *The Journal of clinical psychiatry.* Sep 2008;69(9):1416-1422.

129. Muller DJ, Chowdhury NI, Zai CC. The pharmacogenetics of antipsychotic-induced adverse events. *Current opinion in psychiatry.* Mar 2013;26(2):144-150.

130. Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P. *Molecular Biology of the Cell*. 4th Edition ed. New York: Garland Science; 2002.

131. Barton GM. A calculated response: control of inflammation by the innate immune system. *The Journal of clinical investigation.* Feb 2008;118(2):413-420.

132. Ishii KJ, Koyama S, Nakagawa A, Coban C, Akira S. Host innate immune receptors and beyond: making sense of microbial infections. *Cell host & microbe.* Jun 12 2008;3(6):352-363.

133. Iwasaki A, Medzhitov R. Regulation of adaptive immunity by the innate immune system. *Science.* Jan 15 2010;327(5963):291-295.

134. Na KS, Jung HY, Kim YK. The role of pro-inflammatory cytokines in the neuroinflammation and neurogenesis of schizophrenia. *Progress in neuro-psychopharmacology & biological psychiatry.* Jan 3 2014;48:277-286.

135. Meyer U, Schwarz MJ, Muller N. Inflammatory processes in schizophrenia: a promising neuroimmunological target for the treatment of negative/cognitive symptoms and beyond. *Pharmacology & therapeutics.* Oct 2011;132(1):96-110.

136. Palm NW, Medzhitov R. Pattern recognition receptors and control of adaptive immunity. *Immunological reviews.* Jan 2009;227(1):221-233.

137. Ransohoff RM, Brown MA. Innate immunity in the central nervous system. *The Journal of clinical investigation.* Apr 2 2012;122(4):1164-1171.

138. Monji A, Kato T, Kanba S. Cytokines and schizophrenia: Microglia hypothesis of schizophrenia. *Psychiatry and clinical neurosciences.* Jun 2009;63(3):257-265.

139. Kreutzberg GW. Microglia: a sensor for pathological events in the CNS. *Trends in neurosciences.* Aug 1996;19(8):312-318.

140. Banati RB, Gehrmann J, Schubert P, Kreutzberg GW. Cytotoxicity of microglia. *Glia.* Jan 1993;7(1):111-118.

141. Bergink V, Gibney SM, Drexhage HA. Autoimmunity, inflammation, and psychosis: a search for peripheral markers. *Biological psychiatry.* Feb 15 2014;75(4):324-331.

142. Najjar S, Pearlman DM, Alper K, Najjar A, Devinsky O. Neuroinflammation and psychiatric illness. *Journal of neuroinflammation.* 2013;10:43.

143. Block ML, Zecca L, Hong JS. Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. *Nature reviews. Neuroscience.* Jan 2007;8(1):57-69.

144. Beumer W, Gibney SM, Drexhage RC, et al. The immune theory of psychiatric diseases: a key role for activated microglia and circulating monocytes. *Journal of leukocyte biology.* Nov 2012;92(5):959-975.

145. Dong Y, Benveniste EN. Immune function of astrocytes. *Glia.* Nov 2001;36(2):180-190.

146. Kirkpatrick B, Miller BJ. Inflammation and schizophrenia. *Schizophrenia bulletin.* Nov 2013;39(6):1174-1179.

147. Tian L, Ma L, Kaarela T, Li Z. Neuroimmune crosstalk in the central nervous system and its significance for neurological diseases. *Journal of neuroinflammation.* 2012;9:155.

148. Muller N, Schwarz MJ. Neuroimmune-endocrine crosstalk in schizophrenia and mood disorders. *Expert review of neurotherapeutics.* Jul 2006;6(7):1017-1038.

149. Szelenyi J. Cytokines and the central nervous system. *Brain research bulletin.* Mar 1 2001;54(4):329-338.

150. Connor TJ, Song C, Leonard BE, Merali Z, Anisman H. An assessment of the effects of central interleukin-1beta, -2, -6, and tumor necrosis factor-alpha administration on some behavioural, neurochemical, endocrine and immune parameters in the rat. *Neuroscience.* Jun 1998;84(3):923-933.

151. Kerschensteiner M, Meinl E, Hohlfeld R. Neuro-immune crosstalk in CNS diseases. *Results and problems in cell differentiation.* 2010;51:197-216.

152. Banks WA. Blood-brain barrier transport of cytokines: a mechanism for neuropathology. *Current pharmaceutical design.* 2005;11(8):973-984.

153. Quan N, Whiteside M, Herkenham M. Time course and localization patterns of interleukin-1beta messenger RNA expression in brain and pituitary after peripheral administration of lipopolysaccharide. *Neuroscience.* Mar 1998;83(1):281-293.

154. Roth J, Harre EM, Rummel C, Gerstberger R, Hubschle T. Signaling the brain in systemic inflammation: role of sensory circumventricular organs. *Frontiers in bioscience : a journal and virtual library.* Jan 1 2004;9:290-300.

155. Johnson AK, Gross PM. Sensory circumventricular organs and brain homeostatic pathways. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology.* May 1993;7(8):678-686.

156. Plotkin SR, Banks WA, Kastin AJ. Comparison of saturable transport and extracellular pathways in the passage of interleukin-1 alpha across the blood-brain barrier. *Journal of neuroimmunology.* Jun 1996;67(1):41-47.

157. Quan N, Banks WA. Brain-immune communication pathways. *Brain, behavior, and immunity.* Aug 2007;21(6):727-735.

158. Verma S, Nakaoke R, Dohgu S, Banks WA. Release of cytokines by brain endothelial cells: A polarized response to lipopolysaccharide. *Brain, behavior, and immunity.* Sep 2006;20(5):449-455.

159. Fabry Z, Fitzsimmons KM, Herlein JA, Moninger TO, Dobbs MB, Hart MN. Production of the cytokines interleukin 1 and 6 by murine brain microvessel endothelium and smooth muscle pericytes. *Journal of neuroimmunology.* Aug 1993;47(1):23-34.

160. Konsman JP, Parnet P, Dantzer R. Cytokine-induced sickness behaviour: mechanisms and implications. *Trends in neurosciences.* Mar 2002;25(3):154-159.

161. Romeo HE, Tio DL, Rahman SU, Chiappelli F, Taylor AN. The glossopharyngeal nerve as a novel pathway in immune-to-brain communication: relevance to neuroimmune surveillance of the oral cavity. *Journal of neuroimmunology.* Apr 2 2001;115(1-2):91-100.

162. Wang X, Wang BR, Zhang XJ, Xu Z, Ding YQ, Ju G. Evidences for vagus nerve in maintenance of immune balance and transmission of immune information from gut to brain in STM-infected rats. *World journal of gastroenterology : WJG.* Jun 2002;8(3):540-545.

163. Dantzer R, Konsman JP, Bluthe RM, Kelley KW. Neural and humoral pathways of communication from the immune system to the brain: parallel or convergent? *Autonomic neuroscience : basic & clinical.* Dec 20 2000;85(1-3):60-65.

164. Goehler LE, Gaykema RP, Hansen MK, Anderson K, Maier SF, Watkins LR. Vagal immune-to-brain communication: a visceral chemosensory pathway. *Autonomic neuroscience : basic & clinical.* Dec 20 2000;85(1-3):49-59.

165. Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW. From inflammation to sickness and depression: when the immune system subjugates the brain. *Nature reviews. Neuroscience.* Jan 2008;9(1):46-56.

166. Tapia-Arancibia L, Rage F, Givalois L, Arancibia S. Physiology of BDNF: focus on hypothalamic function. *Frontiers in neuroendocrinology.* Jul 2004;25(2):77-107.

167. Hennigan A, O'Callaghan RM, Kelly AM. Neurotrophins and their receptors: roles in plasticity, neurodegeneration and neuroprotection. *Biochemical Society transactions.* Apr 2007;35(Pt 2):424-427.

168. Kerschensteiner M, Gallmeier E, Behrens L, et al. Activated human T cells, B cells, and monocytes produce brain-derived neurotrophic factor in vitro and in inflammatory brain lesions: a neuroprotective role of inflammation? *The Journal of experimental medicine.* Mar 1 1999;189(5):865-870.

169. Kerschensteiner M, Meinl E, Hohlfeld R. Neuro-immune crosstalk in CNS diseases. *Neuroscience.* Feb 6 2009;158(3):1122-1132.

170. Garaci E, Caroleo MC, Aloe L, et al. Nerve growth factor is an autocrine factor essential for the survival of macrophages infected with HIV. *Proceedings of the National Academy of Sciences of the United States of America.* Nov 23 1999;96(24):14013-14018.

171. Vargas-Leal V, Bruno R, Derfuss T, Krumbholz M, Hohlfeld R, Meinl E. Expression and function of glial cell line-derived neurotrophic factor family ligands and their receptors on human immune cells. *J Immunol.* Aug 15 2005;175(4):2301-2308.

172. Kerschensteiner M, Stadelmann C, Dechant G, Wekerle H, Hohlfeld R. Neurotrophic cross-talk between the nervous and immune systems: implications for neurological diseases. *Annals of neurology.* Mar 2003;53(3):292-304.

173. Tabakman R, Lecht S, Sephanova S, Arien-Zakay H, Lazarovici P. Interactions between the cells of the immune and nervous system: neurotrophins as neuroprotection mediators in CNS injury. *Progress in brain research.* 2004;146:387-401.

174. Azoulay D, Urshansky N, Karni A. Low and dysregulated BDNF secretion from immune cells of MS patients is related to reduced neuroprotection. *Journal of neuroimmunology.* Mar 2008;195(1-2):186-193.

175. Hohlfeld R, Kerschensteiner M, Stadelmann C, Lassmann H, Wekerle H. The neuroprotective effect of inflammation: implications for the therapy of multiple sclerosis. *Journal of neuroimmunology.* Jul 24 2000;107(2):161-166.

176. Nurjono M, Lee J, Chong SA. A Review of Brain-derived Neurotrophic Factor as a Candidate Biomarker in Schizophrenia. *Clinical psychopharmacology and neuroscience : the official scientific journal of the Korean College of Neuropsychopharmacology.* Aug 2012;10(2):61-70.

177. Miller AH, Maletic V, Raison CL. Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. *Biological psychiatry.* May 1 2009;65(9):732-741.

178. Wu CW, Chen YC, Yu L, et al. Treadmill exercise counteracts the suppressive effects of peripheral lipopolysaccharide on hippocampal neurogenesis and learning and memory. *Journal of neurochemistry.* Dec 2007;103(6):2471-2481.

179. Mondelli V, Cattaneo A, Belvederi Murri M, et al. Stress and inflammation reduce brain-derived neurotrophic factor expression in first-episode psychosis: a pathway to smaller hippocampal volume. *The Journal of clinical psychiatry.* Dec 2011;72(12):1677-1684.

180. Yang J, Harte-Hargrove LC, Siao CJ, et al. proBDNF negatively regulates neuronal remodeling, synaptic transmission, and synaptic plasticity in hippocampus. *Cell reports.* May 8 2014;7(3):796-806.

181. Dinarello CA. Proinflammatory cytokines. *Chest.* Aug 2000;118(2):503-508.

182. Curfs JH, Meis JF, Hoogkamp-Korstanje JA. A primer on cytokines: sources, receptors, effects, and inducers. *Clinical microbiology reviews.* Oct 1997;10(4):742-780.

183. Foster JR. The functions of cytokines and their uses in toxicology. *International journal of experimental pathology.* Jun 2001;82(3):171-192.

184. Potvin S, Stip E, Sepehry AA, Gendron A, Bah R, Kouassi E. Inflammatory cytokine alterations in schizophrenia: a systematic quantitative review. *Biological psychiatry.* Apr 15 2008;63(8):801-808.

185. Waldmann TA. The biology of interleukin-2 and interleukin-15: implications for cancer therapy and vaccine design. *Nature reviews. Immunology.* Aug 2006;6(8):595-601.

186. Kronfol Z, Remick DG. Cytokines and the brain: implications for clinical psychiatry. *The American journal of psychiatry.* May 2000;157(5):683-694.

187. Keller ET, Wanagat J, Ershler WB. Molecular and cellular biology of interleukin-6 and its receptor. *Frontiers in bioscience : a journal and virtual library.* 1996;1:d340-357.

188. Contassot E, Beer HD, French LE. Interleukin-1, inflammasomes, autoinflammation and the skin. *Swiss medical weekly.* 2012;142:w13590.

189. Weber A, Wasiliew P, Kracht M. Interleukin-1 (IL-1) pathway. *Science signaling.* 2010;3(105):cm1.

190. Janssen DG, Caniato RN, Verster JC, Baune BT. A psychoneuroimmunological review on cytokines involved in antidepressant treatment response. *Human psychopharmacology.* Apr 2010;25(3):201-215.

191. Meyer U. Anti-inflammatory signaling in schizophrenia. *Brain, behavior, and immunity.* Nov 2011;25(8):1507-1518.

192. Ouyang W, Kolls JK, Zheng Y. The biological functions of T helper 17 cell effector cytokines in inflammation. *Immunity.* Apr 2008;28(4):454-467.

193. Zakharyan R, Boyajyan A. Inflammatory cytokine network in schizophrenia. *The world journal of biological psychiatry : the official journal of the World Federation of Societies of Biological Psychiatry.* Apr 2014;15(3):174-187.

194. Goldstein BI, Kemp DE, Soczynska JK, McIntyre RS. Inflammation and the phenomenology, pathophysiology, comorbidity, and treatment of bipolar disorder: a systematic review of the literature. *The Journal of clinical psychiatry.* Aug 2009;70(8):1078-1090.

195. Leonard BE. Impact of inflammation on neurotransmitter changes in major depression: an insight into the action of antidepressants. *Progress in neuro-psychopharmacology & biological psychiatry.* Jan 3 2014;48:261-267.

196. Martin C, Tansey KE, Schalkwyk LC, Powell TR. The inflammatory cytokines: molecular biomarkers for major depressive disorder? *Biomarkers in medicine.* Feb 14 2014.

197. Felger JC, Lotrich FE. Inflammatory cytokines in depression: neurobiological mechanisms and therapeutic implications. *Neuroscience.* Aug 29 2013;246:199-229.

198. Fredrikson S, Link H. *Advances in Multiple Sclerosis: Clincial Research and Therapy*: Martin Dunitz Ltd.; 1999.

199. Meyer U. Prenatal poly(i:C) exposure and other developmental immune activation models in rodent systems. *Biological psychiatry.* Feb 15 2014;75(4):307-315.

200. Nawa H, Takei N. Recent progress in animal modeling of immune inflammatory processes in schizophrenia: implication of specific cytokines. *Neuroscience research.* Sep 2006;56(1):2-13.

201. Patterson PH. Immune involvement in schizophrenia and autism: etiology, pathology and animal models. *Behavioural brain research.* Dec 7 2009;204(2):313-321.

202. Jonakait GM. The effects of maternal inflammation on neuronal development: possible mechanisms. *International journal of developmental neuroscience : the official journal of the International Society for Developmental Neuroscience.* Nov 2007;25(7):415-425.

203. Girgis RR, Kumar SS, Brown AS. The cytokine model of schizophrenia: emerging therapeutic strategies. *Biological psychiatry.* Feb 15 2014;75(4):292-299.

204. Smith SE, Li J, Garbett K, Mirnics K, Patterson PH. Maternal immune activation alters fetal brain development through interleukin-6. *The Journal of neuroscience : the official journal of the Society for Neuroscience.* Oct 3 2007;27(40):10695-10702.

205. Cui K, Ashdown H, Luheshi GN, Boksa P. Effects of prenatal immune activation on hippocampal neurogenesis in the rat. *Schizophrenia research.* Sep 2009;113(2-3):288-297.

206. Jiang P, Zhu T, Zhao W, et al. The persistent effects of maternal infection on the offspring's cognitive performance and rates of hippocampal neurogenesis. *Progress in neuro-psychopharmacology & biological psychiatry.* Jul 1 2013;44:279-289.

207. Gilmore JH, Jarskog LF, Vadlamudi S. Maternal poly I:C exposure during pregnancy regulates TNF alpha, BDNF, and NGF expression in neonatal brain and the maternal-fetal unit of the rat. *Journal of neuroimmunology.* Feb 2005;159(1-2):106-112.

208. Fatemi SH, Emamian ES, Sidwell RW, et al. Human influenza viral infection in utero alters glial fibrillary acidic protein immunoreactivity in the developing brains of neonatal mice. *Molecular psychiatry.* 2002;7(6):633-640.

209. Moreno JL, Kurita M, Holloway T, et al. Maternal influenza viral infection causes schizophrenia-like alterations of 5-HT(2)A and mGlu(2) receptors in the adult offspring. *The Journal of neuroscience : the official journal of the Society for Neuroscience.* Feb 2 2011;31(5):1863-1872.

210. Ozawa K, Hashimoto K, Kishimoto T, Shimizu E, Ishikura H, Iyo M. Immune activation during pregnancy in mice leads to dopaminergic hyperfunction and cognitive impairment in the offspring: a neurodevelopmental animal model of schizophrenia. *Biological psychiatry.* Mar 15 2006;59(6):546-554.

211. Fortier ME, Luheshi GN, Boksa P. Effects of prenatal infection on prepulse inhibition in the rat depend on the nature of the infectious agent and the stage of pregnancy. *Behavioural brain research.* Aug 6 2007;181(2):270-277.

212. Winter C, Djodari-Irani A, Sohr R, et al. Prenatal immune activation leads to multiple changes in basal neurotransmitter levels in the adult brain: implications for brain

disorders of neurodevelopmental origin such as schizophrenia. *Int J Neuropsychopharmacol.* May 2009;12(4):513-524.

213. Meyer U, Engler A, Weber L, Schedlowski M, Feldon J. Preliminary evidence for a modulation of fetal dopaminergic development by maternal immune activation during pregnancy. *Neuroscience.* Jun 23 2008;154(2):701-709.

214. Feigenson KA, Kusnecov AW, Silverstein SM. Inflammation and the two-hit hypothesis of schizophrenia. *Neuroscience and biobehavioral reviews.* Jan 2014;38:72-93.

215. Schwarz MJ, Muller N, Riedel M, Ackenheil M. The Th2-hypothesis of schizophrenia: a strategy to identify a subgroup of schizophrenia caused by immune mechanisms. *Medical hypotheses.* Apr 2001;56(4):483-486.

216. Wilke I, Arolt V, Rothermundt M, Weitzsch C, Hornberg M, Kirchner H. Investigations of cytokine production in whole blood cultures of paranoid and residual schizophrenic patients. *European archives of psychiatry and clinical neuroscience.* 1996;246(5):279-284.

217. Singh B, Bera NK, Nayak CR, Chaudhuri TK. Decreased serum levels of interleukin-2 and interleukin-6 in Indian Bengalee schizophrenic patients. *Cytokine.* Jul 2009;47(1):1-5.

218. Cazzullo CL, Scarone S, Grassi B, Vismara C, Trabattoni D, Clerici M. Cytokines production in chronic schizophrenia patients with or without paranoid behaviour. *Progress in neuro-psychopharmacology & biological psychiatry.* Aug 1998;22(6):947-957.

219. Sirota P, Schild K, Elizur A, Djaldetti M, Fishman P. Increased interleukin-1 and interleukin-3 like activity in schizophrenic patients. *Progress in neuro-psychopharmacology & biological psychiatry.* Jan 1995;19(1):75-83.

220. Ganguli R, Yang Z, Shurin G, et al. Serum interleukin-6 concentration in schizophrenia: elevation associated with duration of illness. *Psychiatry research.* Jan 1994;51(1):1-10.

221. Frommberger UH, Bauer J, Haselbauer P, Fraulin A, Riemann D, Berger M. Interleukin-6-(IL-6) plasma levels in depression and schizophrenia: comparison between the acute state and after remission. *European archives of psychiatry and clinical neuroscience.* 1997;247(4):228-233.

222. Xiu MH, Chen da C, Wang D, et al. Elevated interleukin-18 serum levels in chronic schizophrenia: Association with psychopathology. *Journal of psychiatric research.* Aug 2012;46(8):1093-1098.

223. Zhang XY, Zhou DF, Zhang PY, Wu GY, Cao LY, Shen YC. Elevated interleukin-2, interleukin-6 and interleukin-8 serum levels in neuroleptic-free schizophrenia: association with psychopathology. *Schizophrenia research.* Oct 1 2002;57(2-3):247-258.

224. Na KS, Kim YK. Monocytic, Th1 and th2 cytokine alterations in the pathophysiology of schizophrenia. *Neuropsychobiology.* 2007;56(2-3):55-63.

225. Ebrinc S, Top C, Oncul O, Basoglu C, Cavuslu S, Cetin M. Serum interleukin 1 alpha and interleukin 2 levels in patients with schizophrenia. *The Journal of international medical research.* May-Jun 2002;30(3):314-317.

226. Al-Asmari AK, Khan MW. Inflammation and schizophrenia: alterations in cytokine levels and perturbation in antioxidative defense systems. *Human & experimental toxicology.* Feb 2014;33(2):115-122.

227. Song C, Manku MS, Horrobin DF. Long-chain polyunsaturated fatty acids modulate interleukin-1beta-induced changes in behavior, monoaminergic neurotransmitters, and brain inflammation in rats. *The Journal of nutrition.* May 2008;138(5):954-963.

228. Muller N, Schwarz MJ. COX-2 inhibition in schizophrenia and major depression. *Current pharmaceutical design.* 2008;14(14):1452-1465.

229. Cazzullo CL, Sacchetti E, Galluzzo A, et al. Cytokine profiles in drug-naive schizophrenic patients. *Schizophrenia research.* Mar 1 2001;47(2-3):293-298.

230. Kim YK, Myint AM, Verkerk R, Scharpe S, Steinbusch H, Leonard B. Cytokine changes and tryptophan metabolites in medication-naive and medication-free schizophrenic patients. *Neuropsychobiology.* 2009;59(2):123-129.

231. Kim YK, Kim L, Lee MS. Relationships between interleukins, neurotransmitters and psychopathology in drug-free male schizophrenics. *Schizophrenia research.* Sep 1 2000;44(3):165-175.

232. Martinez-Gras I, Garcia-Sanchez F, Guaza C, et al. Altered immune function in unaffected first-degree biological relatives of schizophrenia patients. *Psychiatry research.* Dec 30 2012;200(2-3):1022-1025.

233. Rizos EN, Rontos I, Laskos E, et al. Investigation of serum BDNF levels in drug-naive patients with schizophrenia. *Progress in neuro-psychopharmacology & biological psychiatry.* Jul 1 2008;32(5):1308-1311.

234. Sotiropoulou M, Mantas C, Bozidis P, et al. BDNF serum concentrations in first psychotic episode drug-naive schizophrenic patients: associations with personality and BDNF Val66Met polymorphism. *Life sciences.* Mar 12 2013;92(4-5):305-310.

235. Haack M, Hinze-Selch D, Fenzel T, et al. Plasma levels of cytokines and soluble cytokine receptors in psychiatric patients upon hospital admission: effects of confounding factors and diagnosis. *Journal of psychiatric research.* Sep-Oct 1999;33(5):407-418.

236. Roubenoff R, Harris TB, Abad LW, Wilson PW, Dallal GE, Dinarello CA. Monocyte cytokine production in an elderly population: effect of age and inflammation. *The journals of gerontology. Series A, Biological sciences and medical sciences.* Jan 1998;53(1):M20-26.

237. Stowe RP, Peek MK, Cutchin MP, Goodwin JS. Plasma cytokine levels in a population-based study: relation to age and ethnicity. *The journals of gerontology. Series A, Biological sciences and medical sciences.* Apr 2010;65(4):429-433.

238. O'Brien SM, Fitzgerald P, Scully P, Landers A, Scott LV, Dinan TG. Impact of gender and menstrual cycle phase on plasma cytokine concentrations. *Neuroimmunomodulation.* 2007;14(2):84-90.

239. Vgontzas AN, Zoumakis E, Bixler EO, et al. Adverse effects of modest sleep restriction on sleepiness, performance, and inflammatory cytokines. *The Journal of clinical endocrinology and metabolism.* May 2004;89(5):2119-2126.

240. Ziccardi P, Nappo F, Giugliano G, et al. Reduction of inflammatory cytokine concentrations and improvement of endothelial functions in obese women after weight loss over one year. *Circulation.* Feb 19 2002;105(7):804-809.

241. Tappia PS, Troughton KL, Langley-Evans SC, Grimble RF. Cigarette smoking influences cytokine production and antioxidant defences. *Clin Sci (Lond).* Apr 1995;88(4):485-489.

242. Crews FT, Bechara R, Brown LA, et al. Cytokines and alcohol. *Alcoholism, clinical and experimental research.* Apr 2006;30(4):720-730.

243. Pickup JC, Chusney GD, Thomas SM, Burt D. Plasma interleukin-6, tumour necrosis factor alpha and blood cytokine production in type 2 diabetes. *Life sciences.* Jun 8 2000;67(3):291-300.

244. Wong CK, Ho CY, Li EK, Lam CW. Elevation of proinflammatory cytokine (IL-18, IL-17, IL-12) and Th2 cytokine (IL-4) concentrations in patients with systemic lupus erythematosus. *Lupus.* 2000;9(8):589-593.

245. Ostrowski K, Rohde T, Asp S, Schjerling P, Pedersen BK. Pro- and anti-inflammatory cytokine balance in strenuous exercise in humans. *The Journal of physiology.* Feb 15 1999;515 (Pt 1):287-291.

246. Orban Z, Remaley AT, Sampson M, Trajanoski Z, Chrousos GP. The differential effect of food intake and beta-adrenergic stimulation on adipose-derived hormones and cytokines in man. *The Journal of clinical endocrinology and metabolism.* Jun 1999;84(6):2126-2133.

247. Maes M, Song C, Lin A, et al. The effects of psychological stress on humans: increased production of pro-inflammatory cytokines and a Th1-like response in stress-induced anxiety. *Cytokine.* Apr 1998;10(4):313-318.

248. Muller N, Schwarz MJ. Immune System and Schizophrenia. *Current immunology reviews.* Aug 2010;6(3):213-220.

249. Miller BJ, Buckley P, Seabolt W, Mellor A, Kirkpatrick B. Meta-analysis of cytokine alterations in schizophrenia: clinical status and antipsychotic effects. *Biological psychiatry.* Oct 1 2011;70(7):663-671.

250. Maes M, Bocchio Chiavetto L, Bignotti S, et al. Increased serum interleukin-8 and interleukin-10 in schizophrenic patients resistant to treatment with neuroleptics and the stimulatory effects of clozapine on serum leukemia inhibitory factor receptor. *Schizophrenia research.* Apr 1 2002;54(3):281-291.

251. Avgustin B, Wraber B, Tavcar R. Increased Th1 and Th2 immune reactivity with relative Th2 dominance in patients with acute exacerbation of schizophrenia. *Croatian medical journal.* Apr 2005;46(2):268-274.

252. Drexhage RC, Hoogenboezem TA, Cohen D, et al. An activated set point of T-cell and monocyte inflammatory networks in recent-onset schizophrenia patients involves both pro- and anti-inflammatory forces. *Int J Neuropsychopharmacol.* Jul 2011;14(6):746-755.

253. Muller N, Dobmeier P, Empl M, Riedel M, Schwarz M, Ackenheil M. Soluble IL-6 receptors in the serum and cerebrospinal fluid of paranoid schizophrenic patients. *European psychiatry : the journal of the Association of European Psychiatrists.* 1997;12(6):294-299.

254. Pillai A, Kale A, Joshi S, et al. Decreased BDNF levels in CSF of drug-naive first-episode psychotic subjects: correlation with plasma BDNF and psychopathology. *Int J Neuropsychopharmacol.* May 2010;13(4):535-539.

255. Licinio J, Seibyl JP, Altemus M, Charney DS, Krystal JH. Elevated CSF levels of interleukin-2 in neuroleptic-free schizophrenic patients. *The American journal of psychiatry.* Sep 1993;150(9):1408-1410.

256. Soderlund J, Schroder J, Nordin C, et al. Activation of brain interleukin-1beta in schizophrenia. *Molecular psychiatry.* Dec 2009;14(12):1069-1071.

257. Barak V, Barak Y, Levine J, Nisman B, Roisman I. Changes in interleukin-1 beta and soluble interleukin-2 receptor levels in CSF and serum of schizophrenic patients. *Journal of basic and clinical physiology and pharmacology.* 1995;6(1):61-69.

258. Nikkila HV, Muller K, Ahokas A, Miettinen K, Rimon R, Andersson LC. Accumulation of macrophages in the CSF of schizophrenic patients during acute psychotic episodes. *The American journal of psychiatry.* Nov 1999;156(11):1725-1729.

259. Garver DL, Tamas RL, Holcomb JA. Elevated interleukin-6 in the cerebrospinal fluid of a previously delineated schizophrenia subtype. *Neuropsychopharmacology.* Aug 2003;28(8):1515-1520.

260. Steiner J, Schmitt A, Schroeter ML, et al. S100B is downregulated in the nuclear proteome of schizophrenia corpus callosum. *European archives of psychiatry and clinical neuroscience.* Feb 7 2014.

261. Rothermundt M, Ohrmann P, Abel S, et al. Glial cell activation in a subgroup of patients with schizophrenia indicated by increased S100B serum concentrations and elevated myo-inositol. *Progress in neuro-psychopharmacology & biological psychiatry.* Mar 30 2007;31(2):361-364.

262. Schroeter ML, Abdul-Khaliq H, Krebs M, Diefenbacher A, Blasig IE. Neuron-specific enolase is unaltered whereas S100B is elevated in serum of patients with schizophrenia--original research and meta-analysis. *Psychiatry research.* May 15 2009;167(1-2):66-72.

263. Rothermundt M, Falkai P, Ponath G, et al. Glial cell dysfunction in schizophrenia indicated by increased S100B in the CSF. *Molecular psychiatry.* Oct 2004;9(10):897-899.

264. Rothermundt M, Missler U, Arolt V, et al. Increased S100B blood levels in unmedicated and treated schizophrenic patients are correlated with negative symptomatology. *Molecular psychiatry.* Jul 2001;6(4):445-449.

265. Falkai P, Honer WG, David S, Bogerts B, Majtenyi C, Bayer TA. No evidence for astrogliosis in brains of schizophrenic patients. A post-mortem study. *Neuropathology and applied neurobiology.* Feb 1999;25(1):48-53.

266. Catts VS, Wong J, Fillman SG, Fung SJ, Weickert CS. Increased expression of astrocyte markers in schizophrenia: Association with neuroinflammation. *The Australian and New Zealand journal of psychiatry.* Apr 17 2014.

267. Radewicz K, Garey LJ, Gentleman SM, Reynolds R. Increase in HLA-DR immunoreactive microglia in frontal and temporal cortex of chronic schizophrenics. *Journal of neuropathology and experimental neurology.* Feb 2000;59(2):137-150.

268. Wierzba-Bobrowicz T, Lewandowska E, Lechowicz W, Stepien T, Pasennik E. Quantitative analysis of activated microglia, ramified and damage of processes in the frontal and temporal lobes of chronic schizophrenics. *Folia neuropathologica / Association of Polish Neuropathologists and Medical Research Centre, Polish Academy of Sciences.* 2005;43(2):81-89.

269. Zhu F, Zhang L, Ding YQ, Zhao J, Zheng Y. Neonatal intrahippocampal injection of lipopolysaccharide induces deficits in social behavior and prepulse inhibition and microglial activation in rats: Implication for a new schizophrenia animal model. *Brain, behavior, and immunity.* May 2014;38:166-174.

270. Doorduin J, de Vries EF, Willemsen AT, de Groot JC, Dierckx RA, Klein HC. Neuroinflammation in schizophrenia-related psychosis: a PET study. *Journal of nuclear medicine : official publication, Society of Nuclear Medicine.* Nov 2009;50(11):1801-1807.

271. Brooks DJ. PET Imaging of Translocator Protein Expression in Neurological Disorders. *PET and SPECT of Neurobiological Systems*: Springer Berlin Heidelberg; 2014.

272. van Berckel BN, Bossong MG, Boellaard R, et al. Microglia activation in recent-onset schizophrenia: a quantitative (R)-[11C]PK11195 positron emission tomography study. *Biological psychiatry.* Nov 1 2008;64(9):820-822.

273. Angelucci F, Brene S, Mathe AA. BDNF in schizophrenia, depression and corresponding animal models. *Molecular psychiatry.* Apr 2005;10(4):345-352.

274. Shi J, Levinson DF, Duan J, et al. Common variants on chromosome 6p22.1 are associated with schizophrenia. *Nature.* Aug 6 2009;460(7256):753-757.

275. Muller N, Myint AM, Schwarz MJ. Inflammation in schizophrenia. *Advances in protein chemistry and structural biology.* 2012;88:49-68.

276. Meisenzahl EM, Rujescu D, Kirner A, et al. Association of an interleukin-1beta genetic polymorphism with altered brain structure in patients with schizophrenia. *The American journal of psychiatry.* Aug 2001;158(8):1316-1319.

277. Fatjo-Vilas M, Pomarol-Clotet E, Salvador R, et al. Effect of the interleukin-1beta gene on dorsolateral prefrontal cortex function in schizophrenia: a genetic neuroimaging study. *Biological psychiatry.* Nov 1 2012;72(9):758-765.

278. Horvath S, Mirnics K. Immune system disturbances in schizophrenia. *Biological psychiatry.* Feb 15 2014;75(4):316-323.

279. Arion D, Unger T, Lewis DA, Levitt P, Mirnics K. Molecular evidence for increased expression of genes related to immune and chaperone function in the prefrontal cortex in schizophrenia. *Biological psychiatry.* Oct 1 2007;62(7):711-721.

280. Di Nicola M, Cattaneo A, Hepgul N, et al. Serum and gene expression profile of cytokines in first-episode psychosis. *Brain, behavior, and immunity.* Jul 2013;31:90-95.

281. Szekeres G, Juhasz A, Rimanoczy A, Keri S, Janka Z. The C270T polymorphism of the brain-derived neurotrophic factor gene is associated with schizophrenia. *Schizophrenia research.* Dec 1 2003;65(1):15-18.

282. Neves-Pereira M, Cheung JK, Pasdar A, et al. BDNF gene is a risk factor for schizophrenia in a Scottish population. *Molecular psychiatry.* Feb 2005;10(2):208-212.

283. Zhang H, Ozbay F, Lappalainen J, et al. Brain derived neurotrophic factor (BDNF) gene variants and Alzheimer's disease, affective disorders, posttraumatic stress disorder, schizophrenia, and substance dependence. *American journal of medical genetics. Part B,*

Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics. Jun 5 2006;141B(4):387-393.

284. Sun RF, Zhu YS, Kuang WJ, Liu Y, Li SB. The G-712A polymorphism of brain-derived neurotrophic factor is associated with major depression but not schizophrenia. *Neuroscience letters.* Feb 1 2011;489(1):34-37.

285. Gratacos M, Gonzalez JR, Mercader JM, de Cid R, Urretavizcaya M, Estivill X. Brain-derived neurotrophic factor Val66Met and psychiatric disorders: meta-analysis of case-control studies confirm association to substance-related disorders, eating disorders, and schizophrenia. *Biological psychiatry.* Apr 1 2007;61(7):911-922.

286. Numata S, Ueno S, Iga J, et al. Brain-derived neurotrophic factor (BDNF) Val66Met polymorphism in schizophrenia is associated with age at onset and symptoms. *Neuroscience letters.* Jun 19 2006;401(1-2):1-5.

287. Lu W, Zhang C, Yi Z, Li Z, Wu Z, Fang Y. Association between BDNF Val66Met polymorphism and cognitive performance in antipsychotic-naive patients with schizophrenia. *Journal of molecular neuroscience : MN.* Jul 2012;47(3):505-510.

288. Ho BC, Milev P, O'Leary DS, Librant A, Andreasen NC, Wassink TH. Cognitive and magnetic resonance imaging brain morphometric correlates of brain-derived neurotrophic factor Val66Met gene polymorphism in patients with schizophrenia and healthy volunteers. *Archives of general psychiatry.* Jul 2006;63(7):731-740.

289. Takahashi T, Suzuki M, Tsunoda M, et al. Association between the brain-derived neurotrophic factor Val66Met polymorphism and brain morphology in a Japanese sample of schizophrenia and healthy comparisons. *Neuroscience letters.* Apr 11 2008;435(1):34-39.

290. Szeszko PR, Lipsky R, Mentschel C, et al. Brain-derived neurotrophic factor val66met polymorphism and volume of the hippocampal formation. *Molecular psychiatry.* Jul 2005;10(7):631-636.

291. Ikegame T, Bundo M, Sunaga F, et al. DNA methylation analysis of BDNF gene promoters in peripheral blood cells of schizophrenia patients. *Neuroscience research.* Dec 2013;77(4):208-214.

292. Kordi-Tamandani DM, Sahranavard R, Torkamanzehi A. DNA methylation and expression profiles of the brain-derived neurotrophic factor (BDNF) and dopamine

transporter (DAT1) genes in patients with schizophrenia. *Molecular biology reports.* Dec 2012;39(12):10889-10893.

293. Laruelle M. Schizophrenia: from dopaminergic to glutamatergic interventions. *Current opinion in pharmacology.* Feb 2014;14:97-102.

294. Poels EM, Kegeles LS, Kantrowitz JT, et al. Imaging glutamate in schizophrenia: review of findings and implications for drug discovery. *Molecular psychiatry.* Jan 2014;19(1):20-29.

295. Campbell BM, Charych E, Lee AW, Moller T. Kynurenines in CNS disease: regulation by inflammatory cytokines. *Frontiers in neuroscience.* 2014;8:12.

296. Myint AM. Kynurenines: from the perspective of major psychiatric disorders. *The FEBS journal.* Apr 2012;279(8):1375-1385.

297. Linderholm KR, Skogh E, Olsson SK, et al. Increased levels of kynurenine and kynurenic acid in the CSF of patients with schizophrenia. *Schizophrenia bulletin.* May 2012;38(3):426-432.

298. Erhardt S, Blennow K, Nordin C, Skogh E, Lindstrom LH, Engberg G. Kynurenic acid levels are elevated in the cerebrospinal fluid of patients with schizophrenia. *Neuroscience letters.* Nov 2 2001;313(1-2):96-98.

299. Nilsson LK, Linderholm KR, Engberg G, et al. Elevated levels of kynurenic acid in the cerebrospinal fluid of male patients with schizophrenia. *Schizophrenia research.* Dec 15 2005;80(2-3):315-322.

300. Schwarcz R, Rassoulpour A, Wu HQ, Medoff D, Tamminga CA, Roberts RC. Increased cortical kynurenate content in schizophrenia. *Biological psychiatry.* Oct 1 2001;50(7):521-530.

301. Beneyto M, Kristiansen LV, Oni-Orisan A, McCullumsmith RE, Meador-Woodruff JH. Abnormal glutamate receptor expression in the medial temporal lobe in schizophrenia and mood disorders. *Neuropsychopharmacology.* Sep 2007;32(9):1888-1902.

302. Olney JW, Farber NB. Glutamate receptor dysfunction and schizophrenia. *Archives of general psychiatry.* Dec 1995;52(12):998-1007.

303. Erhardt S, Engberg G. Increased phasic activity of dopaminergic neurones in the rat ventral tegmental area following pharmacologically elevated levels of endogenous kynurenic acid. *Acta physiologica Scandinavica.* May 2002;175(1):45-53.

304. Bubenikova-Valesova V, Horacek J, Vrajova M, Hoschl C. Models of schizophrenia in humans and animals based on inhibition of NMDA receptors. *Neuroscience and biobehavioral reviews.* Jul 2008;32(5):1014-1023.

305. Felger JC, Miller AH. Cytokine effects on the basal ganglia and dopamine function: the subcortical source of inflammatory malaise. *Frontiers in neuroendocrinology.* Aug 2012;33(3):315-327.

306. Song C, Li X, Kang Z, Kadotomi Y. Omega-3 fatty acid ethyl-eicosapentaenoate attenuates IL-1beta-induced changes in dopamine and metabolites in the shell of the nucleus accumbens: involved with PLA2 activity and corticosterone secretion. *Neuropsychopharmacology.* Mar 2007;32(3):736-744.

307. Hayley S, Wall P, Anisman H. Sensitization to the neuroendocrine, central monoamine and behavioural effects of murine tumor necrosis factor-alpha: peripheral and central mechanisms. *The European journal of neuroscience.* Mar 2002;15(6):1061-1076.

308. Fumagalli F, Molteni R, Roceri M, et al. Effect of antipsychotic drugs on brain-derived neurotrophic factor expression under reduced N-methyl-D-aspartate receptor activity. *Journal of neuroscience research.* Jun 1 2003;72(5):622-628.

309. Finck BN, Kelley KW, Dantzer R, Johnson RW. In vivo and in vitro evidence for the involvement of tumor necrosis factor-alpha in the induction of leptin by lipopolysaccharide. *Endocrinology.* May 1998;139(5):2278-2283.

310. Kuppers E, Beyer C. Dopamine regulates brain-derived neurotrophic factor (BDNF) expression in cultured embryonic mouse striatal cells. *Neuroreport.* May 8 2001;12(6):1175-1179.

311. Ling SH, Tang YL, Jiang F, et al. Plasma S-100B protein in Chinese patients with schizophrenia: comparison with healthy controls and effect of antipsychotics treatment. *Journal of psychiatric research.* Jan-Feb 2007;41(1-2):36-42.

312. Crowle AJ, Douvas GS, May MH. Chlorpromazine: a drug potentially useful for treating mycobacterial infections. *Chemotherapy.* 1992;38(6):410-419.

313. Moots RJ, Al-Saffar Z, Hutchinson D, et al. Old drug, new tricks: haloperidol inhibits secretion of proinflammatory cytokines. *Annals of the rheumatic diseases.* Sep 1999;58(9):585-587.

314. Zhang XY, Zhou DF, Cao LY, Wu GY, Shen YC. Cortisol and cytokines in chronic and treatment-resistant patients with schizophrenia: association with psychopathology and response to antipsychotics. *Neuropsychopharmacology.* Aug 2005;30(8):1532-1538.

315. Mata I, Crespo-Facorro B, Perez-Iglesias R, et al. Association between the interleukin-1 receptor antagonist gene and negative symptom improvement during antipsychotic treatment. *American journal of medical genetics. Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics.* Dec 5 2006;141B(8):939-943.

316. Mundo E, Altamura AC, Vismara S, et al. MCP-1 gene (SCYA2) and schizophrenia: a case-control association study. *American journal of medical genetics. Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics.* Jan 5 2005;132B(1):1-4.

317. Zai G, Muller DJ, Volavka J, et al. Family and case-control association study of the tumor necrosis factor-alpha (TNF-alpha) gene with schizophrenia and response to antipsychotic medication. *Psychopharmacology.* Oct 2006;188(2):171-182.

318. Sommer IE, de Witte L, Begemann M, Kahn RS. Nonsteroidal anti-inflammatory drugs in schizophrenia: ready for practice or a good start? A meta-analysis. *The Journal of clinical psychiatry.* Apr 2012;73(4):414-419.

319. Qurashi I, Collins J, Chaudhry I, Husain N. Promising use of minocycline augmentation with clozapine in treatment-resistant schizophrenia. *J Psychopharmacol.* Mar 19 2014.

320. Bulut M, Savas HA, Altindag A, Virit O, Dalkilic A. Beneficial effects of N-acetylcysteine in treatment resistant schizophrenia. *The world journal of biological psychiatry : the official journal of the World Federation of Societies of Biological Psychiatry.* 2009;10(4 Pt 2):626-628.

321. Liu F, Guo X, Wu R, et al. Minocycline supplementation for treatment of negative symptoms in early-phase schizophrenia: a double blind, randomized, controlled trial. *Schizophrenia research.* Mar 2014;153(1-3):169-176.

322. Muller N, Riedel M, Scheppach C, et al. Beneficial antipsychotic effects of celecoxib add-on therapy compared to risperidone alone in schizophrenia. *The American journal of psychiatry.* Jun 2002;159(6):1029-1034.

323. Laan W, Grobbee DE, Selten JP, Heijnen CJ, Kahn RS, Burger H. Adjuvant aspirin therapy reduces symptoms of schizophrenia spectrum disorders: results from a randomized, double-blind, placebo-controlled trial. *The Journal of clinical psychiatry.* May 2010;71(5):520-527.

324. Sommer IE, van Westrhenen R, Begemann MJ, de Witte LD, Leucht S, Kahn RS. Efficacy of anti-inflammatory agents to improve symptoms in patients with schizophrenia: an update. *Schizophrenia bulletin.* Jan 2014;40(1):181-191.

325. Peet M, Brind J, Ramchand CN, Shah S, Vankar GK. Two double-blind placebo-controlled pilot studies of eicosapentaenoic acid in the treatment of schizophrenia. *Schizophrenia research.* Apr 30 2001;49(3):243-251.

326. Tan YL, Zhou DF, Cao LY, Zou YZ, Zhang XY. Decreased BDNF in serum of patients with chronic schizophrenia on long-term treatment with antipsychotics. *Neuroscience letters.* Jul 1-8 2005;382(1-2):27-32.

327. Xiu MH, Hui L, Dang YF, et al. Decreased serum BDNF levels in chronic institutionalized schizophrenia on long-term treatment with typical and atypical antipsychotics. *Progress in neuro-psychopharmacology & biological psychiatry.* Nov 13 2009;33(8):1508-1512.

328. Grillo RW, Ottoni GL, Leke R, Souza DO, Portela LV, Lara DR. Reduced serum BDNF levels in schizophrenic patients on clozapine or typical antipsychotics. *Journal of psychiatric research.* Jan-Feb 2007;41(1-2):31-35.

329. Pirildar S, Gonul AS, Taneli F, Akdeniz F. Low serum levels of brain-derived neurotrophic factor in patients with schizophrenia do not elevate after antipsychotic treatment. *Progress in neuro-psychopharmacology & biological psychiatry.* Jul 2004;28(4):709-713.

330. Rizos EN, Papadopoulou A, Laskos E, et al. Reduced serum BDNF levels in patients with chronic schizophrenic disorder in relapse, who were treated with typical or atypical antipsychotics. *The world journal of biological psychiatry : the official journal of the World Federation of Societies of Biological Psychiatry.* Mar 2010;11(2 Pt 2):251-255.

331. Pillai A, Terry AV, Jr., Mahadik SP. Differential effects of long-term treatment with typical and atypical antipsychotics on NGF and BDNF levels in rat striatum and hippocampus. *Schizophrenia research.* Feb 15 2006;82(1):95-106.

332. Lee AH, Lange C, Ricken R, Hellweg R, Lang UE. Reduced brain-derived neurotrophic factor serum concentrations in acute schizophrenic patients increase during antipsychotic treatment. *Journal of clinical psychopharmacology.* Jun 2011;31(3):334-336.

333. Guijarro A, Laviano A, Meguid MM. Hypothalamic integration of immune function and metabolism. *Progress in brain research.* 2006;153:367-405.

334. Johnson RW. Immune and endocrine regulation of food intake in sick animals. *Domestic animal endocrinology.* Sep 1998;15(5):309-319.

335. Kushner I. Regulation of the Acute Phase Response by Cytokines. In: Oppenheim JJ, Rossio J, Gearing AJH, eds. *Clinical Applications of Cytokines: Role in Pathogenesis, Diagnosis, and Therapy*. New York, NY: Oxford University Press; 1993.

336. Langhans W. Anorexia of infection: current prospects. *Nutrition.* Oct 2000;16(10):996-1005.

337. Exton MS. Infection-induced anorexia: active host defence strategy. *Appetite.* Dec 1997;29(3):369-383.

338. Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. *The New England journal of medicine.* Feb 11 1999;340(6):448-454.

339. Kanra GY, Ozen H, Kara A. Infection and anorexia. *The Turkish journal of pediatrics.* Oct-Dec 2006;48(4):279-287.

340. Murray MJ, Murray AB. Anorexia of infection as a mechanism of host defense. *The American journal of clinical nutrition.* Mar 1979;32(3):593-596.

341. Wing EJ, Young JB. Acute starvation protects mice against Listeria monocytogenes. *Infection and immunity.* Jun 1980;28(3):771-776.

342. Yang ZJ, Meguid MM. Continuous systemic interleukin-1 alpha infusion suppresses food intake without increasing lateral hypothalamic dopamine activity. *Brain research bulletin.* 1995;36(4):417-420.

343. Plata-Salaman CR, Oomura Y, Kai Y. Tumor necrosis factor and interleukin-1 beta: suppression of food intake by direct action in the central nervous system. *Brain research.* May 10 1988;448(1):106-114.

344. Plata-Salaman CR, Borkoski JP. Interleukin-8 modulates feeding by direct action in the central nervous system. *The American journal of physiology.* Oct 1993;265(4 Pt 2):R877-882.

345. Reichenberg A, Kraus T, Haack M, Schuld A, Pollmacher T, Yirmiya R. Endotoxin-induced changes in food consumption in healthy volunteers are associated with TNF-alpha and IL-6 secretion. *Psychoneuroendocrinology.* Nov 2002;27(8):945-956.

346. Turrin NP, Flynn MC, Plata-Salaman CR. Neuropeptide Y counteracts interferon-alpha-induced anorexia. *Neuroimmunomodulation.* Sep-Oct 1999;6(5):361-366.

347. Sudom K, Turrin NP, Hayley S, Anisman H. Influence of chronic interleukin-2 infusion and stressors on sickness behaviors and neurochemical change in mice. *Neuroimmunomodulation.* 2004;11(5):341-350.

348. Yang ZJ, Koseki M, Meguid MM, Gleason JR, Debonis D. Synergistic effect of rhTNF-alpha and rhIL-1 alpha in inducing anorexia in rats. *The American journal of physiology.* Oct 1994;267(4 Pt 2):R1056-1064.

349. Debonis D, Meguid MM, Laviano A, Yang ZJ, Gleason JR. Temporal changes in meal number and meal size relationship in response to rHu IL-1 alpha. *Neuroreport.* Sep 11 1995;6(13):1752-1756.

350. Bret-Dibat JL, Creminon C, Couraud JY, Kelley KW, Dantzer R, Kent S. Systemic capsaicin pretreatment fails to block the decrease in food-motivated behavior induced by lipopolysaccharide and interleukin-1beta. *Brain research bulletin.* 1997;42(6):443-449.

351. Plata-Salaman CR. Meal patterns in response to the intracerebroventricular administration of interleukin-1 beta in rats. *Physiology & behavior.* Apr 1994;55(4):727-733.

352. Aubert A, Goodall G, Dantzer R. Compared effects of cold ambient temperature and cytokines on macronutrient intake in rats. *Physiology & behavior.* May 1995;57(5):869-873.

353. Tazi A, Dantzer R, Crestani F, Le Moal M. Interleukin-1 induces conditioned taste aversion in rats: a possible explanation for its pituitary-adrenal stimulating activity. *Brain research.* Nov 15 1988;473(2):369-371.

354. Bluthe RM, Michaud B, Poli V, Dantzer R. Role of IL-6 in cytokine-induced sickness behavior: a study with IL-6 deficient mice. *Physiology & behavior.* Aug-Sep 2000;70(3-4):367-373.

355. Arsenijevic D, Garcia I, Vesin C, et al. Differential roles of tumor necrosis factor-alpha and interferon-gamma in mouse hypermetabolic and anorectic responses induced by LPS. *European cytokine network.* Dec 2000;11(4):662-668.

356. Uehara A, Ishikawa Y, Okumura T, et al. Indomethacin blocks the anorexic action of interleukin-1. *European journal of pharmacology.* Nov 7 1989;170(3):257-260.

357. Harden LM, du Plessis I, Poole S, Laburn HP. Interleukin-6 and leptin mediate lipopolysaccharide-induced fever and sickness behavior. *Physiology & behavior.* Sep 30 2006;89(2):146-155.

358. Laye S, Gheusi G, Cremona S, et al. Endogenous brain IL-1 mediates LPS-induced anorexia and hypothalamic cytokine expression. *American journal of physiology. Regulatory, integrative and comparative physiology.* Jul 2000;279(1):R93-98.

359. Porter MH, Arnold M, Langhans W. TNF-alpha tolerance blocks LPS-induced hypophagia but LPS tolerance fails to prevent TNF-alpha-induced hypophagia. *The American journal of physiology.* Mar 1998;274(3 Pt 2):R741-745.

360. Mrosovsky N, Molony LA, Conn CA, Kluger MJ. Anorexic effects of interleukin 1 in the rat. *The American journal of physiology.* Dec 1989;257(6 Pt 2):R1315-1321.

361. Weingarten HP. Cytokines and food intake: the relevance of the immune system to the student of ingestive behavior. *Neuroscience and biobehavioral reviews.* 1996;20(1):163-170.

362. Anforth HR, Bluthe RM, Bristow A, et al. Biological activity and brain actions of recombinant rat interleukin-1alpha and interleukin-1beta. *European cytokine network.* Sep 1998;9(3):279-288.

363. Bret-Dibat JL, Bluthe RM, Kent S, Kelley KW, Dantzer R. Lipopolysaccharide and interleukin-1 depress food-motivated behavior in mice by a vagal-mediated mechanism. *Brain, behavior, and immunity.* Sep 1995;9(3):242-246.

364. Schwartz GJ, Plata-Salaman CR, Langhans W. Subdiaphragmatic vagal deafferentation fails to block feeding-suppressive effects of LPS and IL-1 beta in rats. *The American journal of physiology.* Sep 1997;273(3 Pt 2):R1193-1198.

365. Berkowitz DE, Brown D, Lee KM, et al. Endotoxin-induced alteration in the expression of leptin and beta3-adrenergic receptor in adipose tissue. *The American journal of physiology.* Jun 1998;274(6 Pt 1):E992-997.

366. Kirchgessner TG, Uysal KT, Wiesbrock SM, Marino MW, Hotamisligil GS. Tumor necrosis factor-alpha contributes to obesity-related hyperleptinemia by regulating leptin release from adipocytes. *The Journal of clinical investigation.* Dec 1 1997;100(11):2777-2782.

367. Grunfeld C, Zhao C, Fuller J, et al. Endotoxin and cytokines induce expression of leptin, the ob gene product, in hamsters. *The Journal of clinical investigation.* May 1 1996;97(9):2152-2157.

368. Sarraf P, Frederich RC, Turner EM, et al. Multiple cytokines and acute inflammation raise mouse leptin levels: potential role in inflammatory anorexia. *The Journal of experimental medicine.* Jan 6 1997;185(1):171-175.

369. Plata-Salaman CR. Central nervous system mechanisms contributing to the cachexia-anorexia syndrome. *Nutrition.* Oct 2000;16(10):1009-1012.

370. McCarthy DO. Cytokines and the anorexia of infection: potential mechanisms and treatments. *Biological research for nursing.* Apr 2000;1(4):287-298.

371. Langhans W, Hrupka B. Interleukins and tumor necrosis factor as inhibitors of food intake. *Neuropeptides.* Oct 1999;33(5):415-424.

372. Yabuuchi K, Minami M, Katsumata S, Satoh M. Localization of type I interleukin-1 receptor mRNA in the rat brain. *Brain research. Molecular brain research.* Nov 1994;27(1):27-36.

373. Amaral ME, Barbuio R, Milanski M, et al. Tumor necrosis factor-alpha activates signal transduction in hypothalamus and modulates the expression of pro-inflammatory proteins and orexigenic/anorexigenic neurotransmitters. *Journal of neurochemistry.* Jul 2006;98(1):203-212.

374. Kent S, Rodriguez F, Kelley KW, Dantzer R. Reduction in food and water intake induced by microinjection of interleukin-1 beta in the ventromedial hypothalamus of the rat. *Physiology & behavior.* Nov 1994;56(5):1031-1036.

375. Laviano A, Renvyle T, Meguid MM, Yang ZJ, Cangiano C, Rossi Fanelli F. Relationship between interleukin-1 and cancer anorexia. *Nutrition.* Sep-Oct 1995;11(5 Suppl):680-683.

376. Laviano A, Gleason JR, Meguid MM, Yang ZJ, Cangiano C, Rossi Fanelli F. Effects of intra-VMN mianserin and IL-1ra on meal number in anorectic tumor-bearing rats.

Journal of investigative medicine : the official publication of the American Federation for Clinical Research. Jan 2000;48(1):40-48.

377. Gayle D, Ilyin SE, Plata-Salaman CR. Central nervous system IL-1 beta system and neuropeptide Y mRNAs during IL-1 beta-induced anorexia in rats. *Brain research bulletin.* 1997;44(3):311-317.

378. Sonti G, Ilyin SE, Plata-Salaman CR. Neuropeptide Y blocks and reverses interleukin-1 beta-induced anorexia in rats. *Peptides.* 1996;17(3):517-520.

379. Wang L, Basa NR, Shaikh A, et al. LPS inhibits fasted plasma ghrelin levels in rats: role of IL-1 and PGs and functional implications. *American journal of physiology. Gastrointestinal and liver physiology.* Oct 2006;291(4):G611-620.

380. Meguid MM, Fetissov SO, Varma M, et al. Hypothalamic dopamine and serotonin in the regulation of food intake. *Nutrition.* Oct 2000;16(10):843-857.

381. Doggett NS, Jawaharlal K. Anorectic activity of prostaglandin precursors. *British journal of pharmacology.* Jul 1977;60(3):417-423.

382. Levine AS, Morley JE. The effect of prostaglandins (PGE2 and PGF2 alpha) on food intake in rats. *Pharmacology, biochemistry, and behavior.* Nov 1981;15(5):735-738.

383. Lal J. Possible role of prostaglandins in the regulation of food intake in the newborn rat. *Archives internationales de pharmacodynamie et de therapie.* Nov 1984;272(1):140-149.

384. Fargeas MJ, Fioramonti J, Bueno L. Prostaglandin E2: a neuromodulator in the central control of gastrointestinal motility and feeding behavior by calcitonin. *Science.* Sep 7 1984;225(4666):1050-1052.

385. Stein J, Zeuzem S, Uphoff K, Laube H. Effects of prostaglandins and indomethacin on gastric emptying in the rat. *Prostaglandins.* Jan 1994;47(1):31-40.

386. Ruwart MJ, Rush BD. The effects of PGF2 alpha, PGE2 and 16, 16 dimethyl PGE2 on gastric emptying and small intestinal transit in rat. *Prostaglandins.* Dec 1984;28(6):915-928.

387. Lacroix S, Vallieres L, Rivest S. C-fos mRNA pattern and corticotropin-releasing factor neuronal activity throughout the brain of rats injected centrally with a prostaglandin of E2 type. *Journal of neuroimmunology.* Nov 1996;70(2):163-179.

388. Johnson PM, Vogt SK, Burney MW, Muglia LJ. COX-2 inhibition attenuates anorexia during systemic inflammation without impairing cytokine production. *American journal of physiology. Endocrinology and metabolism.* Mar 2002;282(3):E650-656.

389. Swiergiel AH, Dunn AJ. Cyclooxygenase 1 is not essential for hypophagic responses to interleukin-1 and endotoxin in mice. *Pharmacology, biochemistry, and behavior.* Jul-Aug 2001;69(3-4):659-663.

390. Swiergiel AH, Dunn AJ. Distinct roles for cyclooxygenases 1 and 2 in interleukin-1-induced behavioral changes. *The Journal of pharmacology and experimental therapeutics.* Sep 2002;302(3):1031-1036.

391. Ziemssen T, Kern S. Psychoneuroimmunology--cross-talk between the immune and nervous systems. *Journal of neurology.* May 2007;254 Suppl 2:II8-11.

392. Besedovsky HO, del Rey A, Klusman I, Furukawa H, Monge Arditi G, Kabiersch A. Cytokines as modulators of the hypothalamus-pituitary-adrenal axis. *The Journal of steroid biochemistry and molecular biology.* 1991;40(4-6):613-618.

393. Berkenbosch F, van Oers J, del Rey A, Tilders F, Besedovsky H. Corticotropin-releasing factor-producing neurons in the rat activated by interleukin-1. *Science.* Oct 23 1987;238(4826):524-526.

394. Sapolsky R, Rivier C, Yamamoto G, Plotsky P, Vale W. Interleukin-1 stimulates the secretion of hypothalamic corticotropin-releasing factor. *Science.* Oct 23 1987;238(4826):522-524.

395. Navarra P, Tsagarakis S, Faria MS, Rees LH, Besser GM, Grossman AB. Interleukins-1 and -6 stimulate the release of corticotropin-releasing hormone-41 from rat hypothalamus in vitro via the eicosanoid cyclooxygenase pathway. *Endocrinology.* Jan 1991;128(1):37-44.

396. Bernardini R, Kamilaris TC, Calogero AE, et al. Interactions between tumor necrosis factor-alpha, hypothalamic corticotropin-releasing hormone, and adrenocorticotropin secretion in the rat. *Endocrinology.* Jun 1990;126(6):2876-2881.

397. Shibasaki T, Kim YS, Yamauchi N, et al. Antagonistic effect of somatostatin on corticotropin-releasing factor-induced anorexia in the rat. *Life sciences.* 1988;42(3):329-334.

398. Uehara A, Sekiya C, Takasugi Y, Namiki M, Arimura A. Anorexia induced by interleukin 1: involvement of corticotropin-releasing factor. *The American journal of physiology.* Sep 1989;257(3 Pt 2):R613-617.

399. Wisse BE. The inflammatory syndrome: the role of adipose tissue cytokines in metabolic disorders linked to obesity. *Journal of the American Society of Nephrology : JASN.* Nov 2004;15(11):2792-2800.

400. Fontana L. Neuroendocrine factors in the regulation of inflammation: excessive adiposity and calorie restriction. *Experimental gerontology.* Jan-Feb 2009;44(1-2):41-45.

401. Ye J, Keller JN. Regulation of energy metabolism by inflammation: a feedback response in obesity and calorie restriction. *Aging.* Jun 2010;2(6):361-368.

402. Bastard JP, Jardel C, Bruckert E, et al. Elevated levels of interleukin 6 are reduced in serum and subcutaneous adipose tissue of obese women after weight loss. *The Journal of clinical endocrinology and metabolism.* Sep 2000;85(9):3338-3342.

403. Moschen AR, Molnar C, Geiger S, et al. Anti-inflammatory effects of excessive weight loss: potent suppression of adipose interleukin 6 and tumour necrosis factor alpha expression. *Gut.* Sep 2010;59(9):1259-1264.

404. Monteiro R, Azevedo I. Chronic inflammation in obesity and the metabolic syndrome. *Mediators of inflammation.* 2010;2010.

405. Bastard JP, Maachi M, Lagathu C, et al. Recent advances in the relationship between obesity, inflammation, and insulin resistance. *European cytokine network.* Mar 2006;17(1):4-12.

406. O'Rourke RW, Kay T, Lyle EA, et al. Alterations in peripheral blood lymphocyte cytokine expression in obesity. *Clinical and experimental immunology.* Oct 2006;146(1):39-46.

407. Garcia MC, Wernstedt I, Berndtsson A, et al. Mature-onset obesity in interleukin-1 receptor I knockout mice. *Diabetes.* May 2006;55(5):1205-1213.

408. McGillicuddy FC, Reynolds CM, Finucane O, et al. Long-term exposure to a high-fat diet results in the development of glucose intolerance and insulin resistance in interleukin-1 receptor I-deficient mice. *American journal of physiology. Endocrinology and metabolism.* Oct 1 2013;305(7):E834-844.

409. Netea MG, Joosten LA, Lewis E, et al. Deficiency of interleukin-18 in mice leads to hyperphagia, obesity and insulin resistance. *Nature medicine.* Jun 2006;12(6):650-656.

410. Matsuki T, Horai R, Sudo K, Iwakura Y. IL-1 plays an important role in lipid metabolism by regulating insulin levels under physiological conditions. *The Journal of experimental medicine.* Sep 15 2003;198(6):877-888.

411. Somm E, Henrichot E, Pernin A, et al. Decreased fat mass in interleukin-1 receptor antagonist-deficient mice: impact on adipogenesis, food intake, and energy expenditure. *Diabetes.* Dec 2005;54(12):3503-3509.

412. Horai R, Asano M, Sudo K, et al. Production of mice deficient in genes for interleukin (IL)-1alpha, IL-1beta, IL-1alpha/beta, and IL-1 receptor antagonist shows that IL-1beta is crucial in turpentine-induced fever development and glucocorticoid secretion. *The Journal of experimental medicine.* May 4 1998;187(9):1463-1475.

413. Andersson N, Strandberg L, Nilsson S, et al. Variants of the interleukin-1 receptor antagonist gene are associated with fat mass in men. *Int J Obes (Lond).* May 2009;33(5):525-533.

414. Um JY, Rim HK, Kim SJ, Kim HL, Hong SH. Functional polymorphism of IL-1 alpha and its potential role in obesity in humans and mice. *PloS one.* 2011;6(12):e29524.

415. Lee JH, Kwon YD, Hong SH, Jeong HJ, Kim HM, Um JY. Interleukin-1 beta gene polymorphism and traditional constitution in obese women. *The International journal of neuroscience.* Jun 2008;118(6):793-805.

416. Strandberg L, Lorentzon M, Hellqvist A, et al. Interleukin-1 system gene polymorphisms are associated with fat mass in young men. *The Journal of clinical endocrinology and metabolism.* Jul 2006;91(7):2749-2754.

417. Strandberg L, Mellstrom D, Ljunggren O, et al. IL6 and IL1B polymorphisms are associated with fat mass in older men: the MrOS Study Sweden. *Obesity (Silver Spring).* Mar 2008;16(3):710-713.

418. Stephens JW, Hurel SJ, Cooper JA, Acharya J, Miller GJ, Humphries SE. A common functional variant in the interleukin-6 gene is associated with increased body mass index in subjects with type 2 diabetes mellitus. *Molecular genetics and metabolism.* Jun 2004;82(2):180-186.

419. Goyenechea E, Parra D, Martinez JA. Impact of interleukin 6 -174G>C polymorphism on obesity-related metabolic disorders in people with excess in body weight. *Metabolism: clinical and experimental.* Dec 2007;56(12):1643-1648.

420. Andersson N, Strandberg L, Nilsson S, et al. A variant near the interleukin-6 gene is associated with fat mass in Caucasian men. *Int J Obes (Lond).* Jun 2010;34(6):1011-1019.

421. Qi L, Zhang C, van Dam RM, Hu FB. Interleukin-6 genetic variability and adiposity: associations in two prospective cohorts and systematic review in 26,944 individuals. *The Journal of clinical endocrinology and metabolism.* Sep 2007;92(9):3618-3625.

422. Wolford JK, Colligan PB, Gruber JD, Bogardus C. Variants in the interleukin 6 receptor gene are associated with obesity in Pima Indians. *Molecular genetics and metabolism.* Nov 2003;80(3):338-343.

423. Nawa H, Carnahan J, Gall C. BDNF protein measured by a novel enzyme immunoassay in normal brain and after seizure: partial disagreement with mRNA levels. *The European journal of neuroscience.* Jul 1 1995;7(7):1527-1535.

424. Rios M. BDNF and the central control of feeding: accidental bystander or essential player? *Trends in neurosciences.* Feb 2013;36(2):83-90.

425. Nawa H, Bessho Y, Carnahan J, Nakanishi S, Mizuno K. Regulation of neuropeptide expression in cultured cerebral cortical neurons by brain-derived neurotrophic factor. *Journal of neurochemistry.* Feb 1993;60(2):772-775.

426. Barnea A, Cho G, Lu G, Mathis M. Brain-derived neurotrophic factor induces functional expression and phenotypic differentiation of cultured fetal neuropeptide Y-producing neurons. *Journal of neuroscience research.* Dec 1995;42(5):638-647.

427. Rios M, Fan G, Fekete C, et al. Conditional deletion of brain-derived neurotrophic factor in the postnatal brain leads to obesity and hyperactivity. *Mol Endocrinol.* Oct 2001;15(10):1748-1757.

428. Pelleymounter MA, Cullen MJ, Wellman CL. Characteristics of BDNF-induced weight loss. *Experimental neurology.* Feb 1995;131(2):229-238.

429. Lyons WE, Mamounas LA, Ricaurte GA, et al. Brain-derived neurotrophic factor-deficient mice develop aggressiveness and hyperphagia in conjunction with brain

serotonergic abnormalities. *Proceedings of the National Academy of Sciences of the United States of America.* Dec 21 1999;96(26):15239-15244.

430. Bariohay B, Lebrun B, Moyse E, Jean A. Brain-derived neurotrophic factor plays a role as an anorexigenic factor in the dorsal vagal complex. *Endocrinology.* Dec 2005;146(12):5612-5620.

431. Wang C, Bomberg E, Billington CJ, Levine AS, Kotz CM. Brain-derived neurotrophic factor (BDNF) in the hypothalamic ventromedial nucleus increases energy expenditure. *Brain research.* Jun 8 2010;1336:66-77.

432. Monteleone P, Tortorella A, Martiadis V, Serritella C, Fuschino A, Maj M. Opposite changes in the serum brain-derived neurotrophic factor in anorexia nervosa and obesity. *Psychosomatic medicine.* Sep-Oct 2004;66(5):744-748.

433. Kernie SG, Liebl DJ, Parada LF. BDNF regulates eating behavior and locomotor activity in mice. *The EMBO journal.* Mar 15 2000;19(6):1290-1300.

434. Hong KW, Lim JE, Go MJ, et al. Recapitulation of the association of the Val66Met polymorphism of BDNF gene with BMI in Koreans. *Obesity (Silver Spring).* Sep 2012;20(9):1871-1875.

435. Shugart YY, Chen L, Day IN, et al. Two British women studies replicated the association between the Val66Met polymorphism in the brain-derived neurotrophic factor (BDNF) and BMI. *European journal of human genetics : EJHG.* Aug 2009;17(8):1050-1055.

436. Timpano KR, Schmidt NB, Wheaton MG, Wendland JR, Murphy DL. Consideration of the BDNF gene in relation to two phenotypes: hoarding and obesity. *Journal of abnormal psychology.* Aug 2011;120(3):700-707.

437. Beckers S, Peeters A, Zegers D, Mertens I, Van Gaal L, Van Hul W. Association of the BDNF Val66Met variation with obesity in women. *Molecular genetics and metabolism.* Sep-Oct 2008;95(1-2):110-112.

438. Skledar M, Nikolac M, Dodig-Curkovic K, Curkovic M, Borovecki F, Pivac N. Association between brain-derived neurotrophic factor Val66Met and obesity in children and adolescents. *Progress in neuro-psychopharmacology & biological psychiatry.* Jan 10 2012;36(1):136-140.

439. Nikolac Perkovic M, Mustapic M, Pavlovic M, et al. Lack of association between brain-derived neurotrophic factor Val66Met polymorphism and body mass index change over time in healthy adults. *Neuroscience letters.* Jun 17 2013;545:127-131.

440. Nakazato M, Hashimoto K, Shimizu E, et al. Decreased levels of serum brain-derived neurotrophic factor in female patients with eating disorders. *Biological psychiatry.* Aug 15 2003;54(4):485-490.

441. Saito S, Watanabe K, Hashimoto E, Saito T. Low serum BDNF and food intake regulation: a possible new explanation of the pathophysiology of eating disorders. *Progress in neuro-psychopharmacology & biological psychiatry.* Mar 17 2009;33(2):312-316.

442. Kluge M, Schuld A, Schacht A, et al. Effects of clozapine and olanzapine on cytokine systems are closely linked to weight gain and drug-induced fever. *Psychoneuroendocrinology.* Jan 2009;34(1):118-128.

443. Song X, Fan X, Li X, et al. Changes in pro-inflammatory cytokines and body weight during 6-month risperidone treatment in drug naive, first-episode schizophrenia. *Psychopharmacology.* Jan 2014;231(2):319-325.

444. Klemettila JP, Kampman O, Seppala N, et al. Cytokine and adipokine alterations in patients with schizophrenia treated with clozapine. *Psychiatry research.* May 9 2014.

445. Tsai A, Liou YJ, Hong CJ, Wu CL, Tsai SJ, Bai YM. Association study of brain-derived neurotrophic factor gene polymorphisms and body weight change in schizophrenic patients under long-term atypical antipsychotic treatment. *Neuromolecular medicine.* Dec 2011;13(4):328-333.

446. Zhang XY, Zhou DF, Wu GY, et al. BDNF levels and genotype are associated with antipsychotic-induced weight gain in patients with chronic schizophrenia. *Neuropsychopharmacology.* Aug 2008;33(9):2200-2205.

447. Zai GC, Zai CC, Chowdhury NI, et al. The role of brain-derived neurotrophic factor (BDNF) gene variants in antipsychotic response and antipsychotic-induced weight gain. *Progress in neuro-psychopharmacology & biological psychiatry.* Oct 1 2012;39(1):96-101.

448. Zhang XY, Tan YL, Zhou DF, et al. Serum BDNF levels and weight gain in schizophrenic patients on long-term treatment with antipsychotics. *Journal of psychiatric research.* Dec 2007;41(12):997-1004.

449. Kay SR, Fiszbein A, Opler LA. The positive and negative syndrome scale (PANSS) for schizophrenia. *Schizophrenia bulletin.* 1987;13(2):261-276.

450. Volavka J, Czobor P, Sheitman B, et al. Clozapine, olanzapine, risperidone, and haloperidol in the treatment of patients with chronic schizophrenia and schizoaffective disorder. *The American journal of psychiatry.* Feb 2002;159(2):255-262.

451. Neale BM, Ferreira MA, Medland SE, Posthuma D. *Statistical Genetics: Gene Mapping through Linkage and Association*. New York: NY: Taylor & Francis Group; 2008.

452. Bernstein BE, Birney E, Dunham I, Green ED, Gunter C, Snyder M. An integrated encyclopedia of DNA elements in the human genome. *Nature.* Sep 6 2012;489(7414):57-74.

453. Kent WJ, Sugnet CW, Furey TS, et al. The human genome browser at UCSC. *Genome research.* Jun 2002;12(6):996-1006.

454. Ward LD, Kellis M. HaploReg: a resource for exploring chromatin states, conservation, and regulatory motif alterations within sets of genetically linked variants. *Nucleic acids research.* Jan 2012;40(Database issue):D930-934.

455. Boyle AP, Hong EL, Hariharan M, et al. Annotation of functional variation in personal genomes using RegulomeDB. *Genome research.* Sep 2012;22(9):1790-1797.

456. International HapMap Consortium. A haplotype map of the human genome. *Nature.* Oct 27 2005;437(7063):1299-1320.

457. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics.* Jan 15 2005;21(2):263-265.

458. Hardy GH. Mendelian Proportions in a Mixed Population. *Science.* Jul 10 1908;28(706):49-50.

459. Weinberg W. Über den Nachweis der Vererbung beim Menschen. *Jahresh. Ver. Vaterl. Naturkd. Württemb.* . 1908;64:369–382.

460. Lahiri DK, Nurnberger JI, Jr. A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. *Nucleic acids research.* Oct 11 1991;19(19):5444.

461. Overall JE, Gorham DR. The Brief Psychiatric Rating Scale. *Psychological Reports.* 1962;10:799-812.

462. Sachs GS, Guille C. Weight gain associated with use of psychotropic medications. *The Journal of clinical psychiatry.* 1999;60 Suppl 21:16-19.

463. Dudbridge F. Likelihood-based association analysis for nuclear families and unrelated subjects with missing genotype data. *Human heredity.* 2008;66(2):87-98.

464. Calle ML, Urrea V, Malats N, Van Steen K. mbmdr: an R package for exploring gene-gene interactions associated with binary or quantitative traits. *Bioinformatics.* Sep 1 2010;26(17):2198-2199.

465. Nyholt DR. A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. *American journal of human genetics.* Apr 2004;74(4):765-769.

466. Li J, Ji L. Adjusting multiple testing in multilocus analyses using the eigenvalues of a correlation matrix. *Heredity.* Sep 2005;95(3):221-227.

467. Gauderman WJ, Morrison JM. QUANTO 1.1: A computer program for power and sample size calculations for genetic-epidemiology studies. 2006.

468. Lieberman JA, Stroup TS, McEvoy JP, et al. Effectiveness of antipsychotic drugs in patients with chronic schizophrenia. *The New England journal of medicine.* Sep 22 2005;353(12):1209-1223.

469. Purcell S, Neale B, Todd-Brown K, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *American journal of human genetics.* Sep 2007;81(3):559-575.

470. Johnson AD, Handsaker RE, Pulit SL, Nizzari MM, O'Donnell CJ, de Bakker PI. SNAP: a web-based tool for identification and annotation of proxy SNPs using HapMap. *Bioinformatics.* Dec 15 2008;24(24):2938-2939.

471. Dinarello CA. A clinical perspective of IL-1β as the gatekeeper of inflammation. *European Journal of Immunology.* 2011;41:1203-1217.

472. van der Ploeg AH, Kumpf O, Seelow E, et al. The course of gastric cancer following surgery is associated with genetic variations of the interleukin-1 receptor antagonist and interleukin-1beta. *Gastric cancer : official journal of the International Gastric Cancer Association and the Japanese Gastric Cancer Association.* Feb 21 2014.

473. Achyut BR, Srivastava A, Bhattacharya S, Mittal B. Genetic association of interleukin-1beta (-511C/T) and interleukin-1 receptor antagonist (86 bp repeat) polymorphisms with Type 2 diabetes mellitus in North Indians. *Clinica chimica acta; international journal of clinical chemistry.* Feb 2007;377(1-2):163-169.

474. Meulenbelt I, Seymour AB, Nieuwland M, Huizinga TW, van Duijn CM, Slagboom PE. Association of the interleukin-1 gene cluster with radiographic signs of osteoarthritis of the hip. *Arthritis and rheumatism.* Apr 2004;50(4):1179-1186.

475. Singh H, Sachan R, Goel H, Mittal B. Genetic variants of interleukin-1RN and interleukin-1beta genes and risk of cervical cancer. *BJOG : an international journal of obstetrics and gynaecology.* Apr 2008;115(5):633-638.

476. Chourasia D, Achyut BR, Tripathi S, Mittal B, Mittal RD, Ghoshal UC. Genotypic and functional roles of IL-1B and IL-1RN on the risk of gastroesophageal reflux disease: the presence of IL-1B-511*T/IL-1RN*1 (T1) haplotype may protect against the disease. *The American journal of gastroenterology.* Nov 2009;104(11):2704-2713.

477. Shirts BH, Wood J, Yolken RH, Nimgaonkar VL. Association study of IL10, IL1beta, and IL1RN and schizophrenia using tag SNPs from a comprehensive database: suggestive association with rs16944 at IL1beta. *Schizophrenia research.* Dec 2006;88(1-3):235-244.

478. Zanardini R, Bocchio-Chiavetto L, Scassellati C, et al. Association between IL-1beta -511C/T and IL-1RA (86bp)n repeats polymorphisms and schizophrenia. *Journal of psychiatric research.* Nov-Dec 2003;37(6):457-462.

479. Baune BT, Dannlowski U, Domschke K, et al. The interleukin 1 beta (IL1B) gene is associated with failure to achieve remission and impaired emotion processing in major depression. *Biological psychiatry.* Mar 15 2010;67(6):543-549.

480. Chen H, Wilkins LM, Aziz N, et al. Single nucleotide polymorphisms in the human interleukin-1B gene affect transcription according to haplotype context. *Human molecular genetics.* Feb 15 2006;15(4):519-529.

481. Tanaka K, Miyake Y, Hanioka T, Arakawa M. Relationship between IL1 gene polymorphisms and periodontal disease in Japanese women. *DNA and cell biology.* Apr 2014;33(4):227-233.

482. Zheng L, Yin J, Wang L, et al. Interleukin 1B rs16944 G>A polymorphism was associated with a decreased risk of esophageal cancer in a Chinese population. *Clinical biochemistry.* Oct 2013;46(15):1469-1473.

483. Sakkou M, Wiedmer P, Anlag K, et al. A role for brain-specific homeobox factor Bsx in the control of hyperphagia and locomotory behavior. *Cell metabolism.* Jun 2007;5(6):450-463.

484. Lage R, Vazquez MJ, Varela L, et al. Ghrelin effects on neuropeptides in the rat hypothalamus depend on fatty acid metabolism actions on BSX but not on gender. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology.* Aug 2010;24(8):2670-2679.

485. Nogueiras R, Lopez M, Lage R, et al. Bsx, a novel hypothalamic factor linking feeding with locomotor activity, is regulated by energy availability. *Endocrinology.* Jun 2008;149(6):3009-3015.

486. Ribases M, Gratacos M, Armengol L, et al. Met66 in the brain-derived neurotrophic factor (BDNF) precursor is associated with anorexia nervosa restrictive type. *Molecular psychiatry.* Aug 2003;8(8):745-751.

487. Perkins DO. Predictors of noncompliance in patients with schizophrenia. *The Journal of clinical psychiatry.* Dec 2002;63(12):1121-1128.

488. Fakhoury WK, Wright D, Wallace M. Prevalence and extent of distress of adverse effects of antipsychotics among callers to a United Kingdom National Mental Health Helpline. *International clinical psychopharmacology.* May 2001;16(3):153-162.

489. Consensus development conference on antipsychotic drugs and obesity and diabetes. *Diabetes care.* Feb 2004;27(2):596-601.

490. Morrato EH, Newcomer JW, Kamat S, Baser O, Harnett J, Cuffel B. Metabolic screening after the American Diabetes Association's consensus statement on antipsychotic drugs and diabetes. *Diabetes care.* Jun 2009;32(6):1037-1042.

491. Cabana MD, Rand CS, Powe NR, et al. Why don't physicians follow clinical practice guidelines? A framework for improvement. *JAMA : the journal of the American Medical Association.* Oct 20 1999;282(15):1458-1465.

492. Meyer UA. Pharmacogenetics and adverse drug reactions. *Lancet.* Nov 11 2000;356(9242):1667-1671.

493. Spear BB, Heath-Chiozzi M, Huff J. Clinical application of pharmacogenetics. *Trends in molecular medicine.* May 2001;7(5):201-204.

494. Ensom MH, Chang TK, Patel P. Pharmacogenetics: the therapeutic drug monitoring of the future? *Clinical pharmacokinetics.* 2001;40(11):783-802.

495. Roses AD. Pharmacogenetics and the practice of medicine. *Nature.* Jun 15 2000;405(6788):857-865.

496. Gilmer TP, Dolder CR, Lacro JP, et al. Adherence to treatment with antipsychotic medication and health care costs among Medicaid beneficiaries with schizophrenia. *The American journal of psychiatry.* Apr 2004;161(4):692-699.

497. MacLean L, Edwards N, Garrard M, Sims-Jones N, Clinton K, Ashley L. Obesity, stigma and public health planning. *Health promotion international.* Mar 2009;24(1):88-93.

498. Frank R, Hargreaves R. Clinical biomarkers in drug discovery and development. *Nature reviews. Drug discovery.* Jul 2003;2(7):566-580.

499. Palmer AM. The utility of biomarkers in CNS drug development. *Drug discovery today.* Mar 2014;19(3):201-203.

500. Kennedy SH, Downar J, Evans KR, et al. The Canadian Biomarker Integration Network in Depression (CAN-BIND): advances in response prediction. *Current pharmaceutical design.* 2012;18(36):5976-5989.

501. Alboni S, Benatti C, Montanari C, Tascedda F, Brunello N. Chronic antidepressant treatments resulted in altered expression of genes involved in inflammation in the rat hypothalamus. *European journal of pharmacology.* Dec 5 2013;721(1-3):158-167.

502. Powell TR, Schalkwyk LC, Heffernan AL, et al. Tumor necrosis factor and its targets in the inflammatory cytokine pathway are identified as putative transcriptomic biomarkers for escitalopram response. *European neuropsychopharmacology : the journal of the European College of Neuropsychopharmacology.* Sep 2013;23(9):1105-1114.

Appendix

APPENDIX A: Haplotype Analysis in Patients of European Ancestry on CLZ/OLZ

Test Markers	Overall P-Value[b]	Haplotype	Frequency[a]	χ^2	Haplotype P-Value[b]
IL-1β rs1143643 IL-1β rs1143634 IL-1β rs3136558	0.615	C-A-G	28 (21.2%)	0.05	0.826
		C-G-A	46 (34.9%)	0.95	0.330
		T-G-A	58 (43.9%)	0.57	0.451
IL-1β rs1143634 IL-1β rs3136558 IL-1β rs16944	0.479	A-G-G	28 (20.9%)	0.04	0.835
		G-A-A	37 (27.6%)	1.45	0.228
		G-A-G	69 (51.5%)	0.83	0.363
IL-1β rs3136558 IL-1β rs16944 IL-1β rs13032029	0.640	A-A-C	41 (30.1%)	0.86	0.355
		A-G-T	68 (50.0%)	0.58	0.447
		G-G-C	27 (19.9%)	0.01	0.910
IL-1β rs16944 IL-1β rs13032029 IL-1β rs4849127	0.588	A-C-A	12 (8.3%)	1.92	0.166
		A-C-G	32 (22.2%)	0.01	0.909
		G-C-G	30 (20.8%)	<0.01	0.999
		G-T-G	70 (48.6%)	0.45	0.502
IL-2 rs2069776 IL-2 rs2069772 IL-2 rs2069778	0.794	C-T-A	17 (12.5%)	<0.01	0.952
		C-T-G	15 (11.0%)	0.02	0.881
		T-C-G	44 (32.4%)	0.74	0.389
		T-T-G	60 (44.1%)	0.89	0.345
IL-2 rs2069772 IL-2 rs2069778 IL-2 rs2069762	0.796	C-G-A	43 (31.2%)	0.57	0.451
		T-A-A	18 (13.0%)	0.05	0.827
		T-G-A	33 (23.9%)	<0.01	0.979
		T-G-C	44 (31.9%)	0.87	0.352
IL-6 rs2069827 IL-6 rs1800795 IL-6rs2069837	0.701	G-C-A	68 (47.9%)	0.25	0.618
		G-C-G	11 (7.7%)	1.29	0.255
		G-G-A	50 (35.2%)	0.03	0.874
		T-G-A	13 (9.2%)	0.01	0.943

IL-6 rs1800795 IL-6 rs2069837 IL-6 rs2066992	0.641	C-A-G	56 (40.6%)	0.51	0.476
		C-A-T	9 (6.5%)	<0.01	0.998
		C-G-G	11 (8.0%)	1.41	0.235
		G-A-G	62 (44.9%)	<0.01	0.954
IL-6 rs2069837 IL-6 rs2066992 IL-6 rs2069840	0.691	A-G-C	83 (58.5%)	<0.01	0.968
		A-G-G	39 (27.5%)	0.34	0.562
		A-T-C	9 (6.3%)	0.01	0.941
		G-G-C	11 (7.7%)	1.24	0.266
IL-6 rs2066992 IL-6 rs2069840 IL-6 rs2069861	0.946	G-C-C	75 (52.8%)	0.15	0.702
		G-C-T	19 (13.4%)	0.06	0.800
		G-G-C	39 (27.5%)	0.34	0.562
		T-C-C	9 (6.3%)	0.01	0.941
IL-6 rs2069840 IL-6 rs2069861 IL-6 rs10242595	0.890	C-C-A	36 (24.1%)	0.08	0.777
		C-C-G	50 (34.0%)	0.01	0.913
		C-T-G	19 (12.8%)	0.03	0.869
		G-C-A	9 (6.3%)	0.58	0.447
		G-C-G	34 (22.8%)	0.51	0.475

[a]Values refer to frequency among haplotype options for the specified test markers; [b]Uncorrected p-values; IL-1β = interleukin 1 beta, IL-2 = interleukin 2, IL-6 = interleukin

APPENDIX B: Genetic Predictors of Antipsychotic-Induced CSWG in Europeans

SNP	Genotype	CSWG (n= 35)[a]	Non-CSWG (n= 93)[a]	OR	95% CI	χ^2	P -Value[b]
IL-1β rs4849127	AA/GA	6 (17.1%)	11 (11.8%)	1.54	0.52 – 4.55	-----	ǂ0.559
	GG	29 (82.9%)	82 (88.2%)				
IL-1β rs13032029	TT/CT	26 (78.8%)	69 (74.2%)	1.29	0.50 – 3.36	0.28	0.599
	CC	7 (21.2%)	24 (25.8%)				
IL-1β rs16944	AA/GA	18 (51.4%)	42 (45.7%)	1.26	0.58 – 2.75	0.34	0.560
	GG	17 (48.6%)	50 (54.3%)				
IL-1β rs3136558	GG/GA	16 (45.7%)	36 (38.7%)	1.33	0.61 – 2.92	0.52	0.472
	AA	19 (54.3%)	57 (61.3%)				
IL-1β rs1143634	AA/GA	16 (45.7%)	37 (39.8%)	1.28	0.58 – 2.79	0.37	0.544
	GG	19 (54.3%)	56 (60.2%)				
IL-1β rs1143643	TT/CT	23 (67.6%)	66 (71.7%)	0.82	0.35 – 1.93	0.20	0.654
	CC	11 (32.4%)	26 (28.3%)				
IL-2 rs2069762	CC/CA	19 (54.3%)	55 (59.1%)	0.82	0.38 – 1.80	0.25	0.620
	AA	16 (45.7%)	38 (40.9%)				
IL-2 rs2069778	AA/GA	9 (25.7%)	19 (21.1%)	1.29	0.52 – 3.22	0.31	0.579
	GG	26 (74.3%)	71 (78.9%)				
IL-2 rs2069772	CC/CT	17 (50.0%)	47 (50.5%)	0.98	0.45 – 2.15	<0.01	0.957
	TT	17 (50.0%)	46 (49.5%)				

IL-2 rs2069776	CC/CT	16 (45.7%)	35 (38.5%)	1.35	0.61 – 2.96	0.55	0.458
	TT	19 (54.3%)	56 (61.5%)				
IL-6 rs2069827	TT/GT	7 (20.0%)	18 (19.4%)	1.04	0.39 – 2.76	<0.01	0.935
	GG	28 (80.0%)	75 (80.6%)				
IL-6 rs1800795	GG/CG	21 (60.0%)	64 (70.3%)	0.63	0.28 – 1.43	1.23	0.268
	CC	14 (40.0%)	27 (29.7%)				
IL-6 rs2069837	GG/GA	6 (17.1%)	15 (16.1%)	1.08	0.38 – 3.04	0.02	0.890
	AA	29 (82.9%)	78 (83.9%)				
IL-6 rs2066992	TT/GT	4 (11.4%)	9 (10.1%)	1.15	0.33 – 4.00	-----	ǂ1.000
	GG	31 (88.6%)	80 (89.9%)				
IL-6 rs2069840	GG/CG	17 (48.6%)	47 (50.5%)	0.92	0.43 – 2.01	0.04	0.843
	CC	18 (51.4%)	46 (49.5%)				
IL-6 rs2069861	TT/CT	6 (17.6%)	22 (23.7%)	0.69	0.25 – 1.89	0.52	0.470
	CC	28 (82.4%)	71 (76.3%)				
IL-6 rs10242595	AA/GA	18 (51.4%)	44 (47.8%)	1.16	0.53 – 2.52	0.13	0.717
	GG	17 (48.6%)	48 (52.2%)				
BDNF Val66Met	Met/Met + Val/Met	7 (20.0%)	32 (35.2%)	0.46	0.18 – 1.17	2.72	0.099
	Val/Val	28 (80.0%)	59 (64.8%)				

[a]Values represent number (percent) or mean ± standard deviation; [b]Uncorrected p-values; ǂValue calculated using Fisher Exact test; SNP = single nucleotide polymorphism, CSWG = clinically significant weight gain, ≥7% weight change from baseline, OR = odds ratio, CI = confidence interval, IL-1β = interleukin 1 beta, IL-2 = interleukin 2, IL-6 = interleukin 6, BDNF = brain-derived neurotrophic factor

APPENDIX C: Genetic Predictors of Antipsychotic-Induced CSWG in Africans

SNP	Genotype	CSWG (n= 24)[a]	Non-CSWG (n= 26)[a]	OR	95% CI	χ^2	P -Value[b]
IL-1β rs4849127	AA/GA	13 (54.2%)	12 (46.2%)	1.38	0.45 – 4.20	0.321	0.571
	GG	11 (45.8%)	14 (53.8%)				
IL-1β rs13032029	TT/CT	11 (45.8%)	12 (46.2%)	0.99	0.32 – 3.01	<0.01	0.982
	CC	13 (54.2%)	14 (53.8%)				
IL-1β rs16944	AA/GA	19 (79.2%)	19 (73.1%)	1.40	0.38 – 5.20	0.25	0.614
	GG	5 (20.8%)	7 (26.9%)				
IL-1β rs3136558	GG/GA	5 (20.8%)	8 (30.8%)	0.59	0.16 – 2.15	0.64	0.424
	AA	19 (79.2%)	18 (69.2%)				
IL-1β rs1143634	AA/GA	11 (45.8%)	7 (26.9%)	2.30	0.71 – 7.49	1.94	0.164
	GG	13 (54.2%)	19 (73.1%)				
IL-1β rs1143643	TT/CT	8 (33.3%)	10 (38.5%)	0.80	0.25 – 2.55	0.14	0.706
	CC	16 (66.7%)	16 (61.5%)				
IL-2 rs2069762	CC/CA	4 (17.4%)	4 (15.4%)	1.16	0.25 – 5.27	-----	ǂ1.000
	AA	19 (82.6%)	22 (84.6%)				
IL-2 rs2069778	AA/GA	1 (4.2%)	3 (11.5%)	0.33	0.03 – 3.45	-----	ǂ0.611
	GG	23 (95.8%)	23 (88.5%)				
IL-2 rs2069772	CC/CT	5 (21.7%)	3 (11.5%)	2.13	0.45 – 10.12	-----	ǂ0.448
	TT	18 (78.3%)	23 (88.5%)				

IL-2 rs2069776	CC/CT	7 (29.2%)	6 (24.0%)	1.30	0.37 – 4.65	0.17	0.682
	TT	17 (70.8%)	19 (76.0%)				
IL-6 rs2069827	TT/GT	1 (4.2%)	1 (3.8%)	1.09	0.06 – 18.40	-----	ǂ1.000
	GG	23 (95.8%)	25 (96.2%)				
IL-6 rs1800795	GG/CG	4 (16.7%)	4 (16.0%)	1.05	0.23 – 4.78	-----	ǂ1.000
	CC	20 (83.3%)	21 (84.0%)				
IL-6 rs2069837	GG/GA	7 (30.4%)	5 (19.2%)	1.84	0.49 – 6.87	0.83	0.363
	AA	16 (69.6%)	21 (80.8%)				
IL-6 rs2066992	TT/GT	3 (13.0%)	5 (19.2%)	0.63	0.13 – 2.99	-----	ǂ0.706
	GG	20 (87.0%)	21 (80.8%)				
IL-6 rs2069840	GG/CG	8 (33.3%)	8 (30.8%)	1.13	0.34 – 3.70	0.04	0.846
	CC	16 (66.7%)	18 (69.2%)				
IL-6 rs2069861	TT/CT	2 (8.3%)	1 (3.8%)	2.27	0.19 – 26.81	-----	ǂ0.602
	CC	22 (91.7%)	25 (96.2%)				
IL-6 rs10242595	GG/GA	16 (66.7%)	19 (73.1%)	0.74	0.22 – 2.48	0.24	0.621
	AA	8 (33.3%)	7 (26.9%)				
BDNF Val66Met	Met/Met + Val/Met	3 (13.0%)	2 (7.7%)	1.80	0.27 – 11.86	-----	ǂ0.655
	Val/Val	20 (87.0%)	24 (92.3%)				

[a]Values represent number (percent) or mean ± standard deviation; [b]Uncorrected p-values; ǂValue calculated using Fisher Exact test; SNP = single nucleotide polymorphism, CSWG = clinically significant weight gain, ≥7% weight change from baseline, OR = odds ratio, CI = confidence interval, IL-1β =interleukin 1 beta, IL-2 = interleukin 2, IL-6 = interleukin 6, BDNF = brain-derived neurotrophic factor

APPENDIX D: Genetic Predictors of Antipsychotic-Induced CSWG in Europeans on CLZ/OLZ

SNP	Genotype	CSWG (n= 23)[a]	Non-CSWG (n= 51)[a]	OR	95% CI	χ^2	P -Value[b]
IL-1β rs4849127	AA/GA	5 (21.7%)	7 (13.7%)	1.75	0.49 – 6.23	-----	ǂ0.498
	GG	18 (78.3%)	44 (86.3%)				
IL-1β rs13032029	TT/CT	16 (76.2%)	35 (68.6%)	1.46	0.46 – 4.69	0.41	0.521
	CC	5 (23.8%)	16 (31.4%)				
IL-1β rs16944	AA/GA	14 (60.9%)	25 (49.0%)	1.62	0.59 – 4.40	0.89	0.345
	GG	9 (39.1%)	26 (51.0%)				
IL-1β rs3136558	GG/GA	9 (39.1%)	20 (39.2%)	1.00	0.36 – 2.73	<0.01	0.994
	AA	14 (60.9%)	31 (60.8%)				
IL-1β rs1143634	AA/GA	10 (43.5%)	21 (41.2%)	1.10	0.41 - 2.97	0.04	0.853
	GG	13 (56.5%)	30 (58.8%)				
IL-1β rs1143643	TT/CT	14 (60.9%)	32 (64.0%)	0.88	0.32 – 2.42	0.07	0.797
	CC	9 (39.1%)	18 (36.0%)				
IL-2 rs2069762	CC/CA	15 (65.2%)	25 (49.0%)	1.95	0.70 – 5.40	1.68	0.196
	AA	8 (34.8%)	26 (51.0%)				
IL-2 rs2069778	AA/GA	6 (26.1%)	13 (26.5%)	0.98	0.32 – 3.01	<0.01	0.968
	GG	17 (73.9%)	36 (73.5%)				
IL-2 rs2069772	CC/CT	11 (50.0%)	28 (54.9%)	0.82	0.30 – 2.24	0.15	0.700
	TT	11 (50.0%)	23 (45.1%)				

IL-2 rs2069776	CC/CT	11 (47.8%)	21 (42.9%)	1.22	0.45 – 3.31	0.16	0.692
	TT	12 (52.2%)	28 (57.1%)				
IL-6 rs2069827	TT/GT	5 (21.7%)	8 (15.7%)	1.49	0.43 – 5.19	-----	ǂ0.526
	GG	18 (78.3%)	43 (84.3%)				
IL-6 rs1800795	GG/CG	17 (73.9%)	34 (69.4%)	1.25	0.41 – 3.80	0.16	0.694
	CC	6 (26.1%)	15 (30.6%)				
IL-6 rs2069837	GG/GA	1 (4.3%)	10 (19.6%)	0.19	0.02 – 1.55	-----	ǂ0.156
	AA	22 (95.7%)	41 (80.4%)				
IL-6 rs2066992	TT/GT	3 (13.0%)	6 (12.5%)	1.05	0.24 – 4.63	-----	ǂ1.000
	GG	20 (87.0%)	42 (87.5%)				
IL-6 rs2069840	GG/CG	11 (47.8%)	26 (51.0%)	0.88	0.33 – 2.36	0.06	0.802
	CC	12 (52.2%)	25 (49.0%)				
IL-6 rs2069861	TT/CT	6 (26.1%)	12 (23.5%)	1.15	0.37 – 3.56	0.06	0.812
	CC	17 (73.9%)	39 (76.5%)				
IL-6 rs10242595	AA/GA	10 (43.5%)	27 (52.9%)	0.68	0.25 – 1.84	0.57	0.451
	GG	13 (56.5%)	24 (47.1%)				
BDNF Val66Met	Met/Met + Val/Met	4 (17.4%)	18 (36.7%)	0.36	0.11 – 1.23	2.76	0.097
	Val/Val	19 (82.6%)	31 (63.3%)				

[a]Values represent number (percent) or mean ± standard deviation; [b]Uncorrected p-values; ǂValue calculated using Fisher Exact test; SNP = single nucleotide polymorphism, CSWG = clinically significant weight gain, ≥7% weight change from baseline, OR = odds ratio, CI = confidence interval, IL-1β = interleukin 1 beta, IL-2 = interleukin 2, IL-6 = interleukin 6, BDNF = brain-derived neurotrophic factor

APPENDIX E: Genetic Predictors of Antipsychotic-Induced CSWG in Africans on CLZ/OLZ

SNP	Genotype	CSWG (n= 20)[a]	Non-CSWG (n= 17)[a]	OR	95% CI	χ^2	P -Value[b]
IL-1β rs4849127	AA/GA	12 (60.0%)	8 (47.1%)	1.69	0.46 – 6.23	0.62	0.431
	GG	8 (40.0%)	9 (52.9%)				
IL-1β rs13032029	TT/CT	10 (50.0%)	7 (41.2%)	1.43	0.39 – 5.26	0.29	0.591
	CC	10 (50.0%)	10 (58.8%)				
IL-1β rs16944	AA/GA	17 (85.0%)	13 (76.5%)	1.74	0.33 – 9.19	-----	ǂ0.680
	GG	3 (15.0%)	4 (23.5%)				
IL-1β rs3136558	GG/GA	3 (15.0%)	5 (29.4%)	0.42	0.09 – 2.12	-----	ǂ0.428
	AA	17 (85.0%)	12 (70.6%)				
IL-1β rs1143634	AA/GA	8 (40.0%)	6 (35.3%)	1.22	0.32 – 4.66	0.09	0.769
	GG	12 (60.0%)	11 (64.7%)				
IL-1β rs1143643	TT/CT	7 (35.0%)	6 (35.3%)	0.99	0.26 – 3.82	<0.01	0.985
	CC	13 (65.0%)	11 (64.7%)				
IL-2 rs2069762	CC/CA	3 (15.8%)	2 (11.8%)	1.41	0.21 – 9.62	-----	ǂ1.000
	AA	16 (84.2%)	15 (88.2%)				
IL-2 rs2069778	AA/GA	-----	1 (5.9%)	-----	-----	-----	-----
	GG	20 (100%)	16 (94.1%)				
IL-2 rs2069772	CC/CT	5 (26.3%)	1 (5.9%)	5.71	0.59 – 54.96	-----	ǂ0.182
	TT	14 (73.7%)	16 (94.1%)				

IL-2 rs2069776	CC/CT	6 (30.0%)	3 (18.8%)	1.86	0.38 – 9.00	-----	ǂ0.700
	TT	14 (70.0%)	13 (81.2%)				
IL-6 rs2069827	TT/GT	-----	-----	-----	-----	-----	-----
	GG	20 (100%)	17 (100%)				
IL-6 rs1800795	GG/CG	2 (10.0%)	1 (6.2%)	1.67	0.14 – 20.23	-----	ǂ1.000
	CC	18 (90.0%)	15 (93.8%)				
IL-6 rs2069837	GG/GA	5 (26.3%)	3 (17.6%)	1.67	0.33 – 8.35	-----	ǂ0.695
	AA	14 (73.7%)	14 (82.4%)				
IL-6 rs2066992	TT/GT	2 (10.5%)	3 (17.6%)	0.55	0.08 – 3.76	-----	ǂ0.650
	GG	17 (89.5%)	14 (82.4%)				
IL-6 rs2069840	GG/CG	5 (25.0%)	5 (29.4%)	0.80	0.19 – 3.42	-----	ǂ1.000
	CC	15 (75.0%)	12 (70.6%)				
IL-6 rs2069861	TT/CT	1 (5.0%)	1 (5.9%)	0.84	0.05 – 14.57	-----	ǂ1.000
	CC	19 (95.0%)	16 (94.1%)				
IL-6 rs10242595	GG/GA	12 (60.0%)	13 (76.5%)	0.46	0.11 – 1.94	1.14	0.286
	AA	8 (40.0%)	4 (23.5%)				
BDNF Val66Met	Met/Met + Val/Met	-----	1 (5.9%)	-----	-----	-----	-----
	Val/Val	19 (100%)	16 (94.1%)				

[a]Values represent number (percent) or mean ± standard deviation; [b]Uncorrected p-values; ǂValue calculated using Fisher Exact test; SNP = single nucleotide polymorphism, CSWG = clinically significant weight gain, ≥7% weight change from baseline, OR = odds ratio, CI = confidence interval, IL-1β = interleukin 1 beta, IL-2 = interleukin 2, IL-6 = interleukin 6, BDNF = brain-derived neurotrophic factor