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Antipsychotic Induced Weight Gain and Genetic Variation

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List of Abbreviations

A

ADE *adverse drug event*

AgRP *agouti related peptide*

ALFA *Allele Frequency Aggregator*

ALL *Acute Lymphoblastic Leukemia*

APD *antipsychotic drug*

C

CART *cocaine and amphetamine regulated transcript*

CCK *cholecystokinin*

CGI *Clinical Global Impression*

D

DMSO *dimethyl sulfoxide*

DNA *deoxyribonucleic acid*

DSM *Diagnostic and Statistical Manual of Mental Disorders*

E

EPS *extrapyramidal side effects*

F

FGA *first generation antipsychotic*

FRET *fluorescence resonance energy transfer*

FTO *fat mass and obesity-associated gene*

G

GABA γ-aminobutyric acid

GCP *good clinical practice*

GLP1 *glucagon-like peptide 1*

H

HWE *Hardy-Weinberg equilibrium*

I

ICD *International Statistical Classification of Diseases and Related Health Problems*

N

NDMAR *N-methyl-D-aspartate receptor*

NPY *neuropeptide Y*

NTC *no template control*

O

OXM *oxyntomodulin*

P

PANSS *Positive and Negative Syndrome Scale*

PCR *polymerase chain reaction*

POMC *proopiomelanocortin*

PYY *peptide YY*

S

SERM *selective estrogen receptor modulator*

SGA *second generation antipsychotic*

SNP *single nucleotide polymorphism*

T

TGA *third generation antipsychotic*

TPMT *thiopurine methyltransferase*

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1. Introduction: Pharmacogenetics and Psychiatry

1.1. Developments in Pharmacogenetic Research

Pharmacogenetics is "the study of variability in drug response due to heredity" (Nebert, 1999, p. 248) and therefore describes interindividual differences in effects, side effects, and metabolism of pharmacotherapeutics depending on genetic variation. Although clinical guidelines for choosing patients' most appropriate treatment based on genetic testing might in many settings heavily improve their outcome (Bradley et al., 2018), this practice remains widely unused despite some decades of research (McInnes & Altman, 2020). The potential of a more individual treatment approach with less adverse drug events (ADE) seems to be enormous from an economic point of view as well considering that, according to Stark et al. (2011), in 2007 approximately 2.1 million patients nationwide would have had an ADE during their ambulatory treatment, which is ~4.4% of patients that took medication that year. Moreover, presumably 3.8% would require health services, probably leading to a total cost of about 816 million Euros – an enormous challenge for health care providers (Stark et al., 2011) that has the potentiality to be lowered by pharmacogenetic approaches (Brixner et al., 2016). However, the cost effectiveness (greatly influencing momentum for research) heavily depends on many variables, inter alia the prevalence of the genotype in the population or its effect on outcome parameters, and predictions are harder to make if the clinical data lacks robustness or is not reported appropriately (Plumpton et al., 2016). Poor evidence for cost effectiveness might partly explain why the development of pharmacogenetic applications for example in antidepressant treatment is at a standstill (Fabbri & Serretti, 2020).

Modern pharmacogenetic research began in the last century, supposedly with Snyder's characterization of the 'phenylthiourea nontaster' as an autosomal recessive trait (Snyder, 1932), yet the term was later introduced in 1959 (Vogel). The invention of the polymerase chain reaction (PCR) in the 1980s (Mullis & Faloona, 1987), being the onset of modern molecular biology, vastly augmented and accelerated the generation of knowledge in this field. Today, large biobanks can be a tool to both confirm already established pharmacogenetic interactions as well as detect hitherto unknown associations by linking genetic information to longitudinal clinical data (McInnes & Altman, 2020), though randomized controlled trials remain the gold standard. The basis for pharmacogenetic research is as the term itself suggests DNA (deoxyribonucleic acid), or rather interindividual differences within it which are called mutations. The human genome differs approximately every 500-1000 bases, therefore humans share about 99.9% of common DNA (Roses, 2000). In general, there are three categories of mutations. Genomic mutations are a consequence of an incorrect mitosis or meiosis and

result in an altered number of chromosomes. Chromosome mutations imply a structural change in individual chromosomes and gene and point mutations affect a single gene or nucleotide. (Koch et al., 2014a) A short summary of different types of mutations can be found in Table 1.

Table 1: Overview over changes in DNA (Koch et al., 2014a)

Category of mutation	Subtypes	Explanation
Genomic mutation	Aneuploidy	One chromosome is lost or an additional one is gained, leading to monosomy or trisomy
	Monoploidy	Each chromosome exists only once
	Polyploidy	Each chromosome exists more than twice
Chromosome mutation	Deletion	A fragment of a chromosome - is lost
	Insertion	- inserted at a wrong location
	Inversion	- or in incorrect orientation
	Translocation	An exchange of parts between (non-homologous) chromosomes leads to reciprocal- or Robertsonian translocation
	Duplication	A part of a chromosome is duplicated
Gene and point mutation		Gene mutations apply to a single gene and point mutations to a single nucleotide

A point mutation that occurs in at least 1% of the population is termed 'single nucleotide polymorphism' (SNP). Those SNPs represent by far the most interindividual genetic differences (~90%). Today, there are millions of SNPs known and of these some 10,000 are located within the coding region, possibly leading to a different amino acid sequence or even a premature stop codon. Others can be found in regulatory regions that may or may not result in altered gene transcription respectively protein expression. (Koch et al., 2014b)

While a large portion of mutations, especially at nucleotide level, can occur unnoticed, they may as well cause diseases. Those that are the result of the mutation of a single gene are referred to as monogenic and can be divided into three main categories – dominant, recessive, and X-linked. Polygenic diseases arise from the cumulative contribution of many polymorphic genes often having only modest individual influence (Lvovs et al., 2012). The latter are very common! For example, the worldwide prevalence of diabetes in adults was roughly 8.8% in 2015 and will rise up to 10.4% until 2040, according to estimates (Ogurtsova et al., 2017). Schizophrenia, another disease expected to have a polygenic contribution to its etiology and of importance in this dissertation, affects about one percent of people (Freedman, 2003).

Mutations are not only expected to cause or contribute to illnesses, but also have an impact on their therapy. In general, the interaction between organism and drug can be divided into two groups: pharmacokinetics and pharmacodynamics. Pharmacokinetics (in simple words) describes the effects that the organism has on an administered drug: How it is absorbed, distributed within various compartments, its biotransformation and excretion. It enables us to understand the concentration of substances in different tissues as a function of time. Pharmacodynamics on the other hand deals with the physiological and biochemical effects that an administered drug has on the body. Genetic mutations that have an influence on pharmacokinetics often affect enzymes of metabolic clearance, and those regarding pharmacodynamics usually affect receptors, ion channels, and transporter proteins. (Steimer & Potter, 2002) For some examples of mutations influencing pharmacokinetics and -dynamics see Table 2.

Table 2: Mutations with an influence on pharmacokinetics and pharmacodynamics

Mutations affecting pharmacokinetics		
Gene	Variant	Possible consequence
CYP1A2	1D, 1F (polymorphisms)	Altered olanzapine serum concentrations (Czerwensky et al., 2015)
CYP2D6	gene duplication	Higher levels of morphine in ultra-rapid metabolizers treated with codeine (Kirchheiner et al., 2007) Lower fluoxetine / norfluoxetine ratio in patients with higher number of CYP2D6 active genes (Llerena et al., 2004)
CYP2D6	*10 (polymorphism)	Lower levels of the activated form of tamoxifen(Lim et al., 2007)
CYP2C9	CYP2C9*2, CYP2C9*3 (polymorphisms)	Association with increased risk of overanticoagulation in treatment with warfarin(Higashi et al., 2002)
CYP2C19	CYP2C19*1*1 (polymorphism)	Lower plasma concentrations of the active metabolite of clopidogrel in poor metabolizers (Umemura et al., 2008)
Mutations affecting pharmacodynamics		
RYR1	R614C, G2434R, G341R etc.	Mutations in the ryanodine receptor can lead to malignant hyperthermia when treated with certain anesthetics (Jurkat-Rott et al., 2000)

In some fields of modern medicine, pharmacogenetic approaches have already led to great improvements for individual patients. Oncology is one of those areas benefitting from the developments in genetics, oncogenomics, and pharmacology, leading to genetic testing prior to treatment (Miteva-Marcheva et al., 2020). Acute lymphoblastic leukemia (ALL) is most often treated with a regiment that contains thiopurines as mercaptopurine or thioguanine acting as antimetabolites to purines (Franca et al., 2019). These thiopurines including mercaptopurine's prodrug azathioprine are substrates of the thiopurine methyltransferase (TPMT). TPMT deficiency can lead to the accumulation of these drugs resulting in severe, even lethal myelotoxicity. (Franca et al., 2019; Lennard, 2014) Testing for TPMT deficiency is common practice and cost-effective (Lennard, 2014). Another example of the implementation of pharmacogenetics into treatment strategies is breast cancer. Cancer cells that have estrogen receptors can be treated with selective estrogen receptor modulator (SERM) such as tamoxifen that result in a reduced function of the SERM-ER complex and therefore reduced growth stimulation of cells carrying the receptor. Besides, tamoxifen is a prodrug activated by CYP2D6. Less functioning variants of this enzyme might explain why some patients do not profit from a treatment with tamoxifen although they have an ER+ tumor. (Brandt & Petrides, 2014; Lim et al., 2007) Moreover, treatment of 'rarer diseases' has in some cases made significant progress within the last few years. Duchenne muscular dystrophy is a severe muscle disorder caused by different mutations of the dystrophin gene. Approximately 13% of cases are a result of a nonsense mutation. Ataluren, a drug developed to enable ribosomal readthrough of premature stop codons, may slow the rate of decline in walking ability. (Bushby et al., 2014; Campbell et al., 2020)

Pharmacogenetic research in psychiatry dates back to at least the 1990s. Validating therapeutic targets, identifying factors for response, and determining the influence of genetics on side effects became quickly accepted as relevant objectives (Staddon et al., 2002), though individually tailored treatment regiments are not common (A. K. Malhotra et al., 2012) and even in recent years, recommendations for genetic testing prior-to medication remain scarce (Müller & Rizhanovsky, 2020). Experts however are calling for the implementation into clinical practice of those tools available today (Lunenburg & Gasse, 2020) as there has been made good progress especially in regards to pharmacokinetics and genetic interactions. Müller et al. (2018) give a general overview of pharmacogenetics of antidepressants, antipsychotics, and lithium. In case of schizophrenia, pharmacogenetic developments could help tackle the enormous economic and humanistic burden that partly result from ineffective treatment or treatment rich in side effects (Chong et al., 2016; Millier et al., 2014).

1.2. Schizophrenia

1.2.1. Definition, Symptoms, and Classification

"Schizophrenia is a chronic, debilitating psychotic mental disorder" (Freedman, 2003, p. 1738) that shows characteristic alterations in thoughts, perception, affect, and volition. It impairs social interaction and affects both women and men evenly with a lifetime prevalence of 1%. The onset frequently lays between the age of 15 and 35, though 3-4% fall ill when they are younger than 15. Socioeconomic status and education of those affected are usually below average. Patients often show associated somatic and psychiatric comorbidity, have a higher rate of suicide, and their life expectancy is reduced by approximately 15 years. (Rentrop & Müller, 2013)

In general, the symptoms occurring can be classified as psychotic (or 'positive'), deficits (or 'negative'), and cognitive dysfunction. Positive symptoms may include auditory hallucinations (voices that interact with or talk about the patient) and delusions. Negative symptoms manifest in a reduced capability to pay attention, an avolition or diminished drive, flattened affect, and social withdrawal. Cognitive dysfunction in the form of a deficient short term memory and reduced capability to pay attention is also characteristic for schizophrenia (Freedman, 2003). However, the latter is not obligatory and regularly develops only during the course of the illness. (Rentrop & Müller, 2013)

A concept often used in the past century to diagnose schizophrenia and still regularly taught at universities (having a non-theoretical pragmatic character) are Scheider's first (and second) rank symptoms. These clinical findings such as delusional perceptions ('*Wahnwahrnehmung*'), auditory hallucinations in the form of voices commenting on or talking with the patient or the patient's own thoughts ('*Gedankenlautwerden*'), and the feeling of being influenced by the outside ('*Ich-Störung mit Fremdbeeinflussungserleben*') can be found in a substantial percentage of patients. (Marneros, 1984)

Diagnostic criteria for Schizophrenia are specified in the DSM (Diagnostic and Statistical Manual of Mental Disorders) - which is the main authority for psychiatric diagnoses in the United States - and in the International Statistical Classification of Diseases and Related Health Problems (ICD) to which inter alia German psychiatrists mainly refer. The world health organization adopted its latest version -11 in 2019 which became valid on 1st January 2022 (World Health Organisation, 2022). Heres et al. (2022) classified the participants of the SWITCH-Study (see chapter 2.1) in accordance with the DSM-IV, whereby in 2013, the American Psychiatric Association released a version -5 (American Psychiatric Association, 2013). There are six diagnostic criteria in both the DSM-IV and -5. The following table summarizes them and highlights the changes in the DSM-5.

Table 3: Diagnostic Criteria for Schizophrenia in DSM-IV and -5, Modified from Tandon et al. (2013, p. 3)

DSM-IV criteria for schizophrenia	Changes in DSM-5
Criterion A: Characteristic symptoms At least two of the following, each to be present for a significant portion of a one-month interval (or less if treated successfully) (1) Delusions (2) Hallucinations (3) Disorganized speech (4) Grossly disorganized or catatonic behavior (5) Negative symptoms (affective flattening, alogia, avolition) Note: Under certain circumstances only one criterion A is required	Criterion A: Characteristic symptoms At least two of the following, each to be present for a significant portion of a one-month interval (or less if treated successfully). At least one of these should include 1-3 1. Delusions 2. Hallucinations 3. Disorganized speech 4. Grossly disorganized or catatonic behavior 5. Negative symptoms (diminished emotional expression or avolition) [no note]
Criterion B: Social/occupational dysfunction At least one area of functioning (work, interpersonal relations, self-care) is noticeably below the level prior to the onset	Criterion B: Social/occupational dysfunction [no change]
Criterion C: Duration Continuous signs of the disturbance occur for a period of at least 6 months (±prodromal/residual periods), which include at least 1 month of symptoms meeting Criterion A	Criterion C: Duration [no change]
Criterion D: Schizoaffective and major mood disorder exclusion	Criterion D [no change]
Criterion E: Substance/general mood condition exclusion The disturbance is not a direct consequence of a substance (drug, medication) or another medical condition	Criterion E [no change]
Criterion F: Relationship to Global Developmental Delay or Autism Spectrum Disorder An additional diagnosis of schizophrenia in patients with a history of autism spectrum disorder is made only if prominent delusions or hallucinations are present for at least 1 month	Criterion F: Relationship to Global Developmental Delay or Autism Spectrum Disorder An additional diagnosis of schizophrenia in patients with a history of autism spectrum disorder or other communication disorder of childhood onset is made only if prominent delusions or hallucinations are present for at least 1 month

Although the differences between the ICD and DSM classifications of schizophrenia supposedly faded more and more, some noticeable distinctions can still be made and remain with the introduction of the ICD-11 (Schultze-Lutter et al., 2021; Tandon et al., 2013). While the DSM-IV and -5 require a minimum duration of six months, the ICD-10 and -11 demand only one month (Schultze-Lutter et al.,

2021). The criterion of social/occupational dysfunction is completely missing from the ICD-10 (Tandon et al., 2013).

Schizophrenia is a very heterogenic mental disorder that had been classified into various subtypes. Up until the DSM-IV and the ICD-10, these subcategorizations were retained as they were traditionally used in the clinical setting. Since they have a low diagnostic stability, do not show predictive value for response to treatment or course of disease, and are not heritable, they were excluded from the DSM-5 and ICD-11. (Schultze-Lutter et al., 2021; Tandon et al., 2013) In the interest of completeness and since patients in the SWITCH-Study were diagnosed according to the DSM-IV, the subtypes of schizophrenia are explained below (Falkai et al., 2017; Rentrop & Müller, 2013):

- Paranoid Schizophrenia (ICD-10 F20.0)

  Most common subtype, delusions and acoustic hallucinations are predominant; rather good prognosis
- Hebephrenic Schizophrenia (ICD-10 F20.1)

  Onset usually between the age of 15-25, predominantly inappropriate or flattened affect, formal thought disorder, and negative symptoms, less hallucinations and delusions; rather bad prognosis
- Catatonic Schizophrenia (ICD-10 F20.2)

  Defective psychomotor, e.g. mutism and stupor, agitation, rigidity, catalepsy, waxy flexibility; rather good prognosis
- Undifferentiated Schizophrenia (ICD-10 F20.3)

  Disease pattern fits schizophrenia, but not the subtypes F20.1,-.2,-.4,-.5 or covers criteria of more than one
- Post-schizophrenic Depression (ICD-10 F20.4)

  Severe symptoms of depression and schizophrenic residual symptoms after a diagnosed schizophrenia within the last 12 months
- Schizophrenic Residuum (ICD-10 F20.5)

  Predominantly negative symptoms such as psychomotor retardation, flattened affect, loss of motivation, social withdrawal
- Schizophrenia Simplex (ICD-10 F20.6)

  Slow development of characteristic negative symptoms while hallucinations or delusions are not present; diagnosis of this subtype is not recommended

The course of disease is variable. About one third of patients has few or sometimes no recurrences and no residual symptoms. Another third has a relapsing pattern where symptoms recur when antipsychotic medication is discontinued. A further one-third has an unfavorable course with deficient remission and residual symptomatology. (Rentrop & Müller, 2013)

Predictors of the severity of an individual case may help to plan therapeutic and/or prophylactic strategies. While single parameters for themselves have not proven to be of great predictive value, a combination of several may indeed be helpful and reasonable. Indicative for a rather good prognosis are for example the female sex, higher age, no concomitant substance abuse, initially no negative symptoms, no acoustic hallucinations, and a good response to antipsychotics within the first 14 days of treatment. (Falkai et al., 2017)

1.2.2. Etiology and Pathophysiology

The etiology of schizophrenia is expected to be multifactorial and different contributors may have a different impact in each individual patient. Genetic variation and environmental factors during pregnancy lead to structural and functional changes in a person's brain, cause physiological and biochemical features that increase the vulnerability to manifest the disorder. (Falkai et al., 2017) Certain stressors eventually result in the onset of schizophrenia (*vulnerability-stress-model* (Rentrop & Müller, 2013), in the English literature more frequently *diathesis-stress-model* (Fowles, 1992)).

Genetic Factors

Various studies have shown that there is an association between the degree of relatedness to a schizophrenic person and the probability to develop the disease. While dizygotic twins have a concordance rate of ~10%, monozygotic twins are at a much higher risk as the concordance rate is expected to be around 50% (Falkai et al., 2017). Adoption studies revealed that children of an affected parent adopted at an early age by a family with no schizophrenic parents are more likely to develop schizophrenia than vice versa (Tienari, 1990). In recent years, large scale GWAS identified more and more genetic variants that increase the risk to develop schizophrenia. One from 2014 found more than 100 loci of which the majority includes protein-coding genes that are often associated with the treatment of schizophrenia (dopamine receptor D_2-gene, DRD2), or with glutamatergic neurotransmission, synaptic plasticity, and subunits of voltage-gated calcium channels (Ripke et al., 2014). It has been estimated that about 8300 mostly common SNPs contribute to the etiology,

accounting for at least 32% of the variance of liability to schizophrenia (Ripke et al., 2013). While these SNPs for themselves have a modest impact, there are some few mutations that have shown to be strong risk factors. The strongest single known risk factor of these is a deletion at 22q11 (22q11.2 deletion syndrome, DiGeorge syndrome) as presumably 25% of patients with this syndrome are diagnosed with schizophrenia and 1 in 100-200 individuals with schizophrenia is carrying the 22q11.2 deletion (McDonald-McGinn et al., 2015).

Some studies indicated that there are epigenetic factors in schizophrenia: mRNA translation and protein expression are not exclusively determined by the features of the primary DNA. Several forms of epigenetic regulation can dynamically change the structure of the surrounding chromatin, resulting in a regulation of genes. Mechanisms are 1) direct methylation of DNA by DNA methyltransferases; 2) chemical modification of the associated histones; 3) interchangeable isoforms of histone molecules; 4) nucleosome remodelers regulating access to DNA. (Föcking et al., 2019) For instance, one analysis that investigated DNA methylation quantitative trait loci concludes that a major proportion of genetic variants increasing schizophrenia risk is associated with DNA methylation (Hannon et al., 2016).

Morphologic, Structural, and Functional Features

Analyses of postmortem brains of patients suffering from schizophrenia have revealed structural changes and/or abnormalities compared to controls (Falkai et al., 2017). Saia-Cereda et al. (2015) identified alterations in protein expression in the corpus callosum, the largest accumulation of white matter in the human brain that connects the two hemispheres. Recent investigations did not find differences in mean numbers of neurons and neuron density in parts of the hippocampus, though decreases in the number of oligodendrocytes might lead to a worsened synaptic connectivity (Schmitt et al., 2009). In contrast to diseases of the brain than involve a loss of neurons, schizophrenia is therefore not considered a neurodegenerative disease (Falkai et al., 2017; Schmitt et al., 2009).

Brain imaging has been a tool for clarifying structural differences in schizophrenic brains for more than 40 year (Falkai et al., 2017). For example, van Erp et al. (2016) discovered brain volume abnormalities using MRI scans of the brain. They found smaller hippocampi, amygdalae, thalami, and brain volumes compared to the control group.

Biochemical Hypotheses

A central hypothesis of the pathophysiology of schizophrenia had been the dopamine theory that postulated excessive dopaminergic transmission (Falkai et al., 2017). This was because drugs with antipsychotic properties were dopamine receptor antagonists and drugs that can cause schizophrenia-like symptoms increased dopaminergic transmission (Falkai et al., 2017; Freedman, 2003). Davis et al. (1991) suggested in a modified hypothesis that a frontal hypodopaminergia causes negative symptoms while a striatal hyperdopaminergia leads to positive symptoms (Howes & Kapur, 2009). Howes and Kapur (2009) concluded in their 'Version III' of the dopamine theory that multiple hits contribute to a dopamine dysregulation at the presynaptic control level. These theories, however, are limited by some observations and have led to a search for other neurotransmitters involved in the pathophysiology of schizophrenia: Levels of dopamine metabolites and receptors are generally within normal values when measured in patients pre- and post-treatment (Freedman, 2003).

Another theory focuses on glutamatergic synaptic transmission. Certain substances such as ketamine (a narcotic) and phencyclidine (a recreational drug also known as 'angel dust') blocking the N-methyl-D-aspartate receptor (NDMAR) are known to induce schizophrenia-like psychosis (Javitt et al., 2012). Although the NDMAR should be a rather simple target for pharmacological treatment and several treatment studies have been performed, these findings are yet to be used in clinical practice (Falkai et al., 2017; Javitt et al., 2012).

An integrative approach sees the disruption in dopaminergic and glutamatergic synaptic transmission because of malfunctioning GABAergic (γ-aminobutyric acid) systems (hypothesis of disinhibition). A defective regulation during brain development in adolescence causes an increase in excitative and reduction in inhibitive functions that ultimately create an excitation-inhibition-disequilibrium. (Falkai et al., 2017)

Environmental and Psychosocial Stressors

The debate about the influence of environmental factors in the etiology of schizophrenia arose as epigenetic mechanism became better understood (Falkai et al., 2017). A meta-analysis from Cannon et al. (2002) identified obstetric complications that were associated with an increased risk for schizophrenia. They summarized them into three groups that were 1) complications of pregnancy (for example diabetes, preeclampsia) 2) abnormal fetal development (such as low birth weight) and 3) complication of delivery (for instance asphyxia). Another meta-analysis found an association of prenatal maternal infection with schizophrenia and brain abnormalities relevant to schizophrenia (Khandaker et al., 2013).

Psychosocial stressors that have been documented to be important to the manifestation of the disease can be subsumed into critical life events, traumata, and everyday stressors, though these can be found and their influence can be seen in various others mental diseases (Falkai et al., 2017).

1.2.3. Treatment

The modern pharmacological treatment of schizophrenia started some 70 years ago when chlorpromazine was first introduced into clinical practice in France in 1952 and was perceived as a 'miracle drug' due to its higher effectiveness compared to previous therapies (Ban, 2007). The many antipsychotic drugs (APD)developed since then have been historically categorized as first-generation (or typical) and second-generation (or atypical) (Falkai et al., 2017). First-generation APDs (FGA) are characterized by their high affinity to D_2-receptors where they act as antagonists. They are effective in reducing positive symptoms. However, a substantial percentage of patients has either only a small or even no response at all. Furthermore, typical side effects that result from the blockade of dopamine receptors are extrapyramidal (EPS or hyperprolactinemia. Second generation antipsychotics (SGA) on the other hand are characterized by their lower affinity for D_2-receptors and greater affinities for different other neuroreceptors. The prototype of SGAs, clozapine, did not cause EPS and proved more effective in treatment-resistant schizophrenia. Newer SGAs such as olanzapine provided nearly similar benefits compared to FGAs with an important improvement: They did not share the risk of agranulocytosis that is associated with clozapine. (Miyamoto et al., 2005) Besides, Leucht, Corves, et al. (2009) found in a meta-analysis that four SGAs (amisulpride, clozapine, olanzapine, and risperidone) were more efficacious in treating negative symptoms than FGAs while five were not.

Table 4: Antipsychotic Substances and Their Receptor Affinities, Modified from Müller and Benkert (2021, pp. 259-260)

		Receptor-Affinity						
Antipsychotic	Category	D_1	D_2	D_3	5-HT_2	M_1	α_1	H_1
Amisulpride	**SGA**	**0**	**+++**	**+++**	**0**	**0**	**0**	**0**
Aripiprazole	SGA/TGA[1]	0	+++	+++	++	0	+	+
Clozapine	SGA	++	+	++	+++	+++	+	+++
Haloperidol	FGA	++	+++	++	+	0	++	0
Olanzapine	**SGA**	**++**	**+++**	**++**	**+++**	**++**	**++**	**+++**
Quetiapine	SGA	+	+	+	+	0	+	++
Risperidone	SGA	++	+++	++	+++	0	++	+
Ziprasidone	SGA	+	++	++	+++	0	+	++

Weight gain associated with antipsychotics in **bold letters** was analyzed in this dissertation; [1]TGA: Aripiprazole has been named third generation antipsychotic (Keltner & Johnson, 2002) as it is a D_2 partial agonist; 0, +, ++, +++: receptor affinity;

Multimodal Treatment Approach

The treatment of patients with antipsychotic diseases should be comprehensive, multiprofessional, multidimensional, and consist of different components (Deutsche Gesellschaft für Psychiatrie und Psychotherapie, 2019; Falkai et al., 2017). Antipsychotic drugs are useful in all phases of the disease and are generally recommended by German and international guidelines (Deutsche Gesellschaft für Psychiatrie und Psychotherapie, 2019). They should be given as soon as the diagnosis has been made. Moreover, patients benefit from psychosocial interventions (first and foremost cognitive behavioral therapy) and social support. Choosing the right antipsychotic drug is a very individual decision and depends on symptoms, risk for side effects, interactions with other medication, patients' medical history, and preference. (Falkai et al., 2017)

Weight Gain as a Common Side Effect of SGAs

Although SGAs are in general less likely to cause EPS than FGAs, this does not mean that they have less adverse effects. Pharmacotherapy with second-generation APDs is often associated with a notable risk for metabolic complications. Studies have indicated that up to 40% of patients suffer from significant weight gain or even develop metabolic syndrome. These pose great risk for cardiovascular diseases, reduce quality of life and adherence, and presumably increase the probability to develop diabetes and certain carcinomas. (Müller & Benkert, 2021) A meta-analysis from Leucht et al. (2013) compared some of the most often used antipsychotics in regards to their weight gain inducing capabilities and showed

that olanzapine performed significantly worse than most other substances; others with relatively high increase in body weight were for example clozapine, zotepine, chlorpromazine, and quetiapine.

These metabolic side effects show why parameters such as body weight, blood glucose, blood pressure, and waist circumference need to be monitored carefully when treating patients with SGAs, so that appropriate measures can be quickly taken to address them (Falkai et al., 2017). A weight gain of more than 7% and a BMI higher than 25 kg/m² respectively 30 kg/m² (then classifying as obesity) is often considered to be significant and relevant (Deutsche Gesellschaft für Psychiatrie und Psychotherapie, 2019; Falkai et al., 2017; Müller & Benkert, 2021). Dietary measures, nutrition counseling, and physical exercise in combination with a suitable antipsychotic substance may prevent increase in body weight. In some cases, a reduction in dosage can also be reasonable and helpful. In other cases, physicians must switch the antipsychotic medication, though then there might occur other side effects as EPS or the psychiatric condition might worsen. (Falkai et al., 2017; Müller et al., 2018) There are few recommendations for a pharmacological weight reduction (Müller & Benkert, 2021). A meta-analysis concluded that the antidiabetic drug metformin supports a BMI reduction and lowers the insulin resistance index (de Silva et al., 2016). Another one investigated the effect of antagonist of the histamine H2 receptor ranitidine (Gu et al., 2018), some studies focused on the antidepressant reboxetine (Poyurovsky et al., 2007), and some on the steroidal antiprogesterone mifepristone (Gross et al., 2009).

The German S3 guideline on schizophrenia recommends psychotherapeutic and psychosocial interventions to prevent weight gain prior to a pharmacological treatment or at the latest if a relative weight increase of >7% has occurred. In case of strong weight gain while treatment with antipsychotics remains necessary, metformin or topiramate should be offered (“recommendation level A”), though their use would be off-label. (Deutsche Gesellschaft für Psychiatrie und Psychotherapie, 2019)

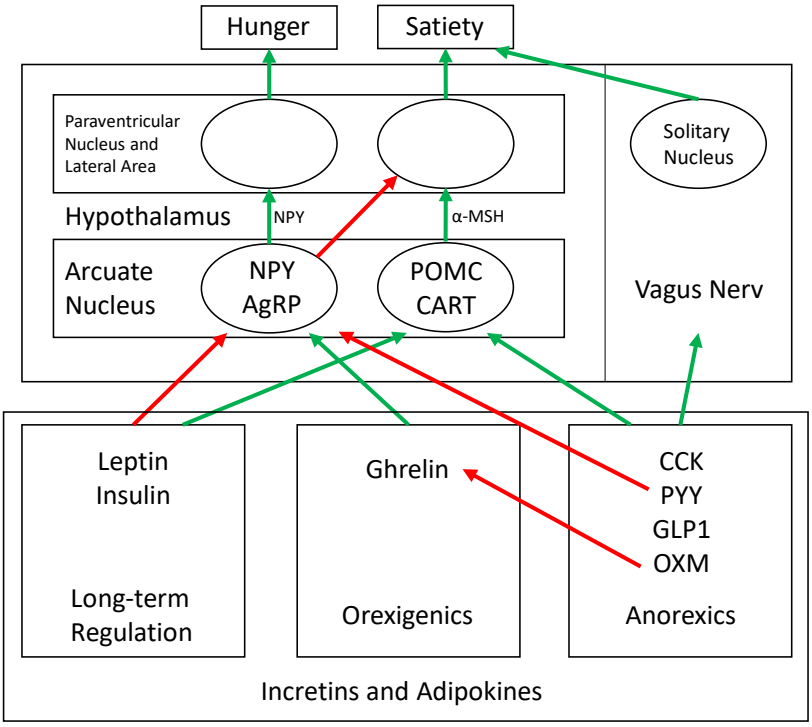
1.3. Candidate Genes

1.3.1. Physiological Weight Regulation and Antipsychotic Drugs

Physiological weight regulation is the complex interaction of factors that increase hunger and therefore food intake or increase satiety and consequently reduce food intake to match the energy consumption caused by normal physiological processes and exercise. The brain acts as the central processing unit of afferent signals which can be neural or humoral, while the efferent signal is (simplified) the motoric process of eating. It is an astonishing fact that, although the energy needs of an individual can vary enormously and food intake can be very infrequent, many tend to have the same

body weight over the course their life. (Löffler, 2014) In contrast to that, antipsychotic drugs can induce significant weight gain within a short period of time (see Chapter 1.2.3), which suggests that they disrupt this physiological equilibrium. To explore possible genetic causes or differences linked to high AIWG, the mechanisms of weight regulation are illustrated in the following.

Figure 1: Physiological Weight Regulation, Adapted from Löffler (2014, p. 480)



Abbreviations: NPY neuropeptide Y; AgRP agouti related peptide; POMC proopiomelanocortin; CART cocaine and amphetamine regulated transcript; CCK cholecystokinin; PYY peptide YY; GLP1 glucagon-like peptide 1; OXM oxyntomodulin; green arrows represent a stimulating, red arrows a suppressing effect

The arcuate nucleus as part of the hypothalamus is believed to be the integrating unit that receives information from energy depots and the gastrointestinal tract. Appetite stimulating neurons produce NPY or AgRP as their neuropeptide, appetite suppressant neurons POMC or CART. Superior nuclei in the hypothalamus such as the paraventricular nucleus or the lateral area receive their afferent information from the arcuate nucleus, integrate these with further signals, and eventually cause the sensations that are commonly known as hunger or satiety. (Löffler, 2014)

The gastrointestinal tract reacts to certain nutritional components by releasing peptide hormones which are called incretins. The only appetite stimulating incretin is ghrelin. Synthesized by enteroendocrine cells of the stomach, it stimulates NPY and AgRP producing neurons in the hypothalamus. CCK is a peptide hormone produced by cells of the ileum, responding to low or non-existent levels of fatty acids, amino acids, and peptides. By stimulating the vagus nerv it acts as an appetite suppressant; its influence on the arcuate nucleus is not clear. PYY supposedly suppresses

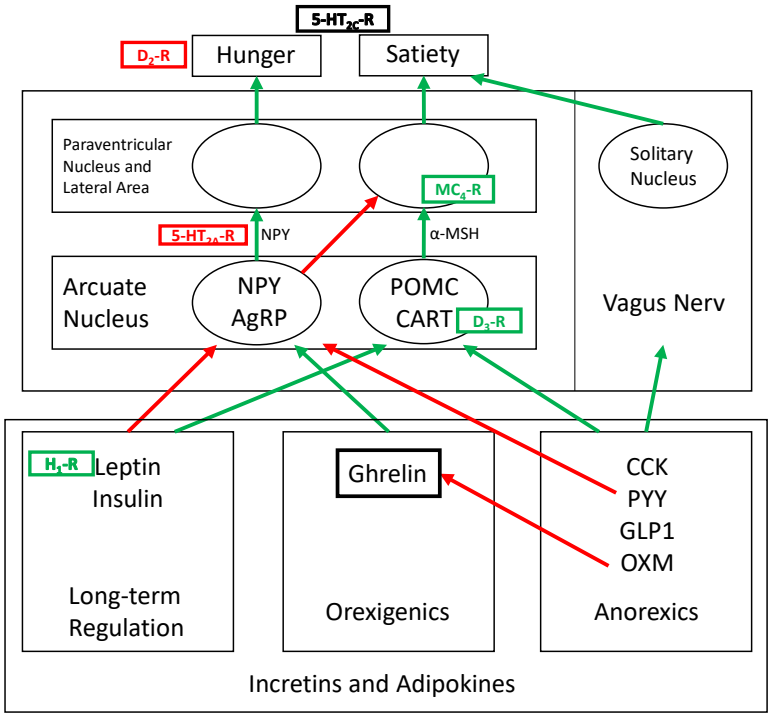
appetite stimulating neurons in the arcuate nucleus and thus lowers appetite. The exact molecular mechanism of GLP1 and OXM is not fully understood, though OXM seems to inhibit cells that produce ghrelin; furthermore, receptors in the arcuate nucleus indicate that they have a direct influence on the hypothalamus. (Löffler, 2014)

The long-term regulation of the energy homoeostasis involves signaling peptides that are produces by adipose tissue. One important of these adipokines is leptin, whose blood levels correlate with body fat mass. Leptin inhibits appetite stimulating and activates appetite suppressing neurons in the arcuate nucleus. Additionally, it can induce energy consumption in some peripheral tissues. Insulin, which is not produces by fat tissue but is an indicator of the metabolic status, has some similar effects in the hypothalamus. (Löffler, 2014)

Mechanisms of Antipsychotic Induced Weight Gain

As illustrated above, a disruption of the physiological weight regulation may be a reason why antipsychotic substances cause significant weight increase.

Figure 2: Potential Mechanisms for (Atypical) Antipsychotic Induced Weight Gain (Roerig et al., 2011), Figure adapted from Löffler (2014, p. 480)



Abbreviations: D_2-R: Dopamine Receptor D_2; D_3-R: Dopamine Receptor D_3; H_1-R: Histamine Receptor H_1; 5-HT_{2A}-R: Serotonin Receptor 5-HT_{2A}; 5-HT_{2C}-R: Serotonin Receptor 5-HT_{2C}; MC_4-R: Melanocortin 4 receptor; Also see Figure 1 for the remaining abbreviations; α-MSH is a stimulating ligand of the MC_4-R (Mutch & Clément, 2006); green arrows represent a stimulating, red arrows a suppressing effect

Serotonin receptors are known to influence weight regulation. The 5-HT_{2A} receptor might decrease orexigenic signals of NPY, which is why an antagonism to it could potentially contribute to AIWG. The exact mechanism of the 5-HT_{2C} receptor is not entirely clear, though antagonism to it is believed to increase food intake. (Roerig et al., 2011) A polymorphism within the promoter region of the 5-HT_{2C} gene has been strongly associated with increased weight gain under antipsychotic treatment, undermining the relevance of the 5-HT_{2C} receptor (Reynolds et al., 2006). Another receptor that is very likely to play a significant role in antipsychotic induced weight gain is the histamine receptor H1. A blockade might increase hypothalamic levels of the monophosphate-activated protein kinase and functioning H1-receptors are required for the mediating anorexic effects of leptin. (Roerig et al., 2011) Epigenetic-induced alterations on genes coding for leptin or leptin receptors caused by SGAs might also contribute to AIWG (Endomba et al., 2020). Kroeze et al. (2003) reported a statistically significant correlation between weight gain and the affinities for the H1, α1A, 5-HT2C, and 5-HT6 receptors, though affinity to the H1-receptor had the strongest correlation (Spearman $\rho=-0.72$; $p<0.01$), and they even suggested that new atypical antipsychotics should be screened for their H1-affinity. Various dopamine receptors might also play a role in AIWG. Activation of the D2-receptor would normally

lower food intake, thus atypical antipsychotic would cause the opposite, and D3-receptors play a role in expression of CART (Roerig et al., 2011). Levels of incretins such as ghrelin and their downstream signaling might directly be influenced by antipsychotics, though exact mechanisms and substance-dependent differences are not entirely understood, and it is not fully clear whether elevated ghrelin levels are cause or consequence of AIWG (Roerig et al., 2011; Zhang et al., 2013).

1.3.2. rs9939609 (FTO)

The fat mass and obesity-associated gene (FTO) has been strongly associated with obesity in general and obesity-related traits (da Silva et al., 2018; Dina et al., 2007; Frayling et al., 2007; Scuteri et al., 2007). It encodes for a demethylase that might modify rRNA and function as a transcription factor (Scuteri et al., 2007; Zhao et al., 2014), and is highly expressed in areas of the brain that play a crucial role in energy homeostasis like the hypothalamus (Gerken et al., 2007). Furthermore, it is potentially involved in leptin-signaling (Wang et al., 2011), though the exact mechanisms for its effect on weight remain unclear.

One of the SNPs that have been reported to increase risk for obesity is the rs9939609 (Tanofsky-Kraff et al., 2009). The exchange of a thymine with an adenine (National Center for Biotechnology Information, 2021a) has been reported to influence antipsychotic induced weight gain in some studies (Schröder et al., 2019; Song et al., 2014), yet others did not show a significant influence on AIWG or only in patients receiving chronic treatment (Reynolds et al., 2013; Shing et al., 2014). A meta-analysis from 2016 did not find a significant impact of the FTO variant in both AA vs. T and TT vs. A (Zhang et al., 2016). More data could set a base for future meta-analyses and therefore help determining, whether and how strong an influence of the rs9939609 on weight gain under antipsychotic treatment is.

1.3.3. rs17782313 (MC4R)

The rs17782313 single nucleotide polymorphism is a substitution of thymine with cytosine located 188 kb downstream of the melanocortin-4 receptor gene (*MC4R*) (Loos et al., 2008) with a minor allele frequency of approximately 23.6% in the European population (National Center for Biotechnology Information, 2021b). It has been described that the SNP leads to a loss of function of the receptor which usually decreases food intake when stimulated (Balt et al., 2011; Fan & Tao, 2009)(Figure 2). In

a large genome wide association study (GWAS), the rs17782313 SNP showed the strongest association with BMI. Data from a total of 77,228 individuals lead to the assumption that each copy of the C-allele was equivalent to an additional 0.22 kg/m² in BMI (difference in BMI of 0.049 Z-score units, $p=2.8x10^{-15}$). (Loos et al., 2008) A later GWAS focused on weight gain in pediatric patients associated with 12 weeks of first exposure to antipsychotic drugs and found 20 SNPs at a single locus near the *MC4R* gene and confirmed those findings in three replication cohorts (Anil K. Malhotra et al., 2012), underlining the importance of mutations in or near the *MC4R* gene.

While many studies have analyzed the influence of the rs17782313 on body weight and obesity, there are only few that investigated its connection with antipsychotic induced weight gain. Chowdhury et al. (2013) found a non-significant trend for the C-allele and higher AIWG in European-ancestry patients. Czerwensky et al. (2013) showed a significant association of the rs17782313 with higher weight gain under antipsychotic treatment. A recent study with a high number of participants (n=1991) could not replicate Czerwensky's findings, though its participants were Han-Chinese and not Caucasian. The meta-analysis mentioned above included Czerwensky's and Chowdhury's publications and did not report an overall significant effect (Zhang et al., 2016). These not completely consistent results show that there is need for further research.

1.4. Motivation and Goals

Weight gain that is primarily caused by SGAs can be quite significant (Leucht et al., 2013) and moreover directly affects treatment adherence and quality of life. Weiden et al. (2004) reported that obese patients had a more than twice as high likelihood of missing their medication compared to patients with a normal BMI when asked for their compliance and identified subjective distress from weight gain and BMI status as negative predictors. Low adherence rates are also associated with enormous healthcare costs and utilization (Ascher-Svanum et al., 2010; Gilmer et al., 2004; Offord et al., 2013; Weiden & Olfson, 1995). Furthermore, a large portion of schizophrenia patients does suffer from obesity and has reportedly lower weight related and general health related quality of life than the non-obese comparison group, according to Kolotkin et al. (2008).

Guidelines for genetic testing prior to antipsychotic treatment do apparently not play an important role in national treatment guideline so far, exemplarily in the German S3 guidelines on schizophrenia (Deutsche Gesellschaft für Psychiatrie und Psychotherapie, 2019). As this dissertation aims to produce valid data and support the development of such, the objectives were the following

- to show that weight gain was a notable side effect of antipsychotics in the analyzed cohort in the first place and scrutinize respectively confirm possible predictors and variates besides genetic features contributing to or confounding later statistical models
- to investigate how antipsychotic induced weight gain depended on the substance administered to the patients as described by meta-analyses as the design of the trial (comparing treatment strategies in a multi-center double-blind controlled setting, for more details see chapter 2.1) was particularly suitable for a direct comparison
- to explore a possible correlation between certain mutations and antipsychotic induced weight gain that has been described in earlier publications, as authors have highlighted the need for more data on pharmacogenetic interactions that would provide the basis for the implementation of future applications (Müller et al., 2018), the main objective
- to verify known covariables and calculate a statistical model to assess the magnitude of the influence that single mutations have on antipsychotic induced weight gain
- to examine whether the antipsychotic agent itself affects the correlation of weight gain and genetic mutations, an aspect that up until now has been neglected far too often. Again, the study design with only two different treatment groups to which patients were randomized provided a good setting

The findings gained from the statistical analysis are intended to contribute to a personalized, pharmacogenetic approach to antipsychotic treatment in the future and help reduce side effects, namely weight gain, under which many patients suffer. By that, there is a chance to improve their quality of life and adherence to treatment, which might go along with lower health care costs society has to bear.

2. Material and Methods

2.1. The SWITCH-Study

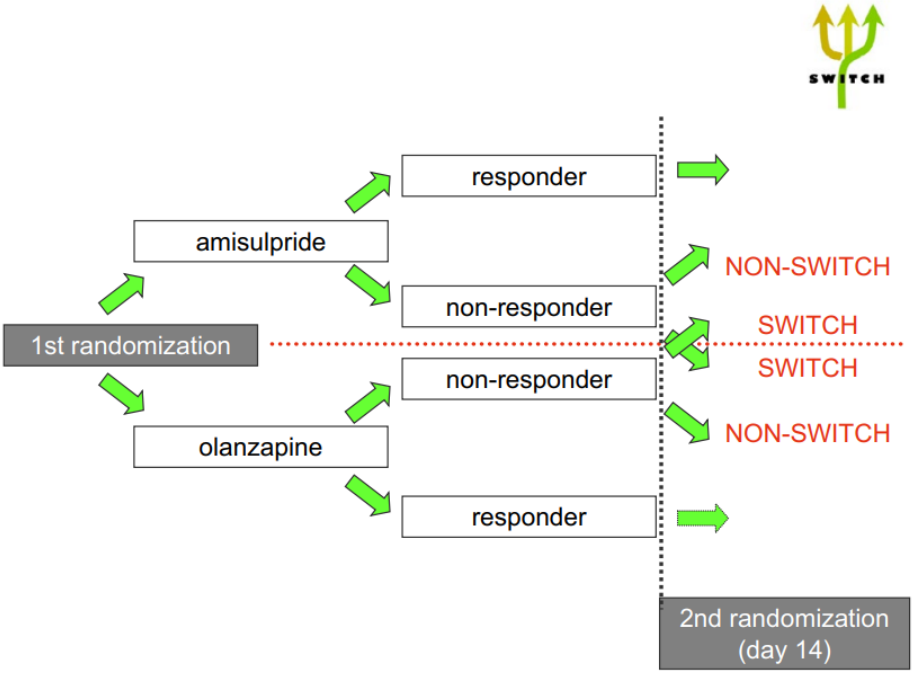
Study Design

The basis for this investigation into antipsychotic associated weight gain was the multicentric, controlled, double-blinded SWITCH study (Heres et al., 2016; Heres et al., 2022) which was conducted at 17 sites in Germany and 15 sites in Romania. Its primary endpoint was the number of patients responding poorly to antipsychotic treatment after a duration of two weeks and the number of patients that reached symptomatic remission after the completion of the trial which lasted eight weeks in total. The study hypothesis was that patients with a poor response after two weeks and a switch in their medication had a higher rate of symptomatic remission than those with a poor response and no switch.

At the beginning, patients were randomly assigned to one of the two medication groups which received either olanzapine or amisulpride for two weeks (phase I). At the end of phase I, their response was measured and, if not sufficient, they were randomized again, either into a non-switch group (continuing their medication) or into a switch group (changing the study drug). Neither the patients nor the treating physicians knew, which medication was assigned to whom. This was also the case for the second randomization as patients and physicians did not know if or to which group patients were switched in case of non-response.

Subjects that experienced an adequate response to their phase I medication continued their treatment until the end of the trial as they were not relevant for the primary endpoint of the study. Their increase of body weight was especially relevant for this analysis though as it allowed an analysis of AIWG for each drug separately.

Figure 3: Design of the SWITCH Study, from Heres et al. (2016, p. 515)



The design of the SWITCH Study: After two weeks of treatment with the originally assigned drug, the patients were evaluated regarding their response to their treatment. 'Responders' continued their medication, 'non-responders' were randomized into a non-switch and a switch group

Inclusion and Exclusion Criteria

Patients that met the inclusion criteria and were treated at one of the 32 sites could be enrolled in this trial. They had to give their written consent in accordance with the Declaration of Helsinki and the International Committee on the Harmonization of Good Clinical Practice (ICH-GCP). As the SWITCH Study was multicentric and took place in different countries, local ethic committees or review boards in conformity with domestic legislation approved the protocol Table 5 shows inclusion and exclusion criteria.

The pharmacogenetic testing was not a mandatory part of the SWITCH trial, thus patients had to give their written consent separately. If they rejected, they did not suffer any disadvantage and were not excluded from the primary study. In addition, they could withdraw their consent at any time, which would lead to a disposal of their genetic samples.

Table 5: Inclusion and Exclusion-Criteria of the SWITCH Study (Heres et al., 2016; Heres et al., 2022)

Inclusion criteria	Exclusion criteria
Age 18-65	Contraindications to one of the study drugs or known intolerance
Diagnosis of schizophrenia, schizoaffective disorder, or schizophreniform disorder (see chapter 1.2.1)	No clinical change in the current episode within the last 4 weeks despite adequate treatment
PANSS total score ≥75 at screening and baseline	Non-response following a 6-8-week treatment attempt
CGI-I rating ≥4 at inclusion	Treatment with one of the study medications in the 2 weeks prior to study entry
Increase in the level of care within 5 working days prior to enrollment	≥25% PANSS total score reduction from screening to baseline
	Impending risk of suicide or endangerment of others
	Depot antipsychotic medication within one injection cycle
	Pregnancy or lactation period, or the intent to conceive within the next 3 months
	Diagnosed substance dependency according to DSM-IV in the 3 months previous to the trial
	Other relevant medical findings and previous enrollment in the trial

Abbreviations: PANSS*: Positive and Negative Syndrome Scale*; CGI-I: *Clinical Global Impression-Improvement*; DSM-IV: *Diagnostic and Statistical Manual of Mental Disorders* (see chapter 1.2.1)

Medication and Concomitant Drug Therapy

Patients took either amisulpride or olanzapine or both consecutively as their study medication (compare Figure 3). These antipsychotic substances are traditionally viewed as 'second generation' (SGA) but differ significantly in their receptor binding profiles as amisulpride is a selective dopamine antagonist and olanzapine blocks central serotonin receptors more than dopamine receptors (see chapter 1.2.3) (Heres et al., 2016). Previous meta-analyses showed that both these substances have similar antipsychotic efficacies and comparable side effects (Davis et al., 2003; Leucht, Komossa, et al., 2009; Leucht et al., 2002) which was especially important as FGAs might have led to an unblinding through their EPS. In addition, both can cause significant weight gain which was the central aspect for this analysis.

The initial dosing in phase I was 600-800 mg/d of amisulpride or 15-20 mg/d of olanzapine targeted on day 3; afterwards doses of 200-800 mg/d of amisulpride or 5-20 mg/d of olanzapine were allowed yet decreases should be considered only if bad tolerability occurred. The 'SWITCH-groups' were treated accordingly in phase II. Concomitant medication was strictly regulated to avoid biased results and drug interactions. Other antipsychotic agents, newly begun antidepressant medication, or mood stabilizers

were not allowed. Rescue medication according to protocol were lorazepam, diazepam, zolpidem, lormetazepam, zopiclone, temazepam, and biperiden, which were permitted for the symptomatic treatment of side effects, agitation, and sleep disturbances. (Heres et al., 2016)

Data Collection

During the course of the study, there were 7 visits in total (screening on day -3 to1, baseline on day 1, end of phase I on day 14, fourth visit on day 21, fifth visit on day 28, sixth visit on day 42, end of phase II on day 56) and 4 contacts as a follow up (on day 56-86). At screening, participants underwent routine clinical testing, females were tested for pregnancy, researchers conducted a diagnostic M.I.N.I. interview, and interviewed patients using the PANSS and the CGI. Later, there were blood samples drawn for analysis of drug serum levels (on days 14, 28, 56), genetic testing (usually day 14), their body weight and height was monitored, side effects and quality of life were assessed, and the PANSS and CGI were carried out at any of the 7 visits. (Heres et al., 2016)

The Positive and Negative Syndrome Scale (PANSS) is an instrument that rates positive symptoms and negative symptoms of schizophrenia, and general psychopathology (Leucht et al., 2005). The Clinical Global Impression (CGI) is a scale is intended to assess the effectiveness of a particular treatment by evaluating severity of illness, global improvement, and efficacy index (Haro et al., 2003). In the primary analysis, remission was the primary outcome.

2.2. Statistical Analysis

The statistical analysis was performed using IBM SPSS Statistics for Windows, version 21.0 (IBM Corp., Armonk, N.Y., USA). Two-tailed P=0.05 were viewed as statistically significant, though a correction for multiple testing was not included as this analysis has an explanatory character. Normal distribution of variables was determined by graphic analysis and by running the Kolmogorov-Smirnov and Shapiro-Wilk test. When confronted with diverging results, the Shapiro-Wilk test was usually given preference as it presumably has the highest power for all types of distribution and sample sizes (Razali & Wah, 2011). In normally distributed variables, homogeneity of variance was asserted using Levene's test. If equal variances could be assumed, variables were compared by T-Test, otherwise by Welch-Test. For variables with more than two groups, we performed an analysis for variance (ANOVA). Non-normally distributed variables were analyzed with Mann-Whitney-U-Test respectively Kruskal-Wallis-Test.

Qualitative variables were analyzed with the Chi²-test if the requirements were fulfilled. Those were (StatistikGuru, 2015a):

- Nominally scaled variables
- Independent measurements
- No expected cell frequencies below 5

We determined the combined effects of possible covariates and the known confounders sex, age, baseline body weight, and smoking status(Gebhardt et al., 2009) using a stepwise multiple linear regression analysis. We included these parameters when the 95% confidence interval of their standard error did not include zero and the objective function value fell by more than 3.84, which implicates a significance (probability) of the F value of 0.05, as similarly done in for instance Czerwensky et al. (2013); Laika et al. (2010). Some results included in this dissertation were published in Schreyer et al. (2023) using this statistical model. Linear correlation between two variables was determined by Pearson correlation coefficient. When calculating a Pearson correlation coefficient, the following requirements were applied (StatistikGuru, 2015b):

- Metrically scaled variables
- No (extreme) outliers, verified by box plots
- Linear correlation, verified by scatter diagram
- Bivariate normal distribution: According to the central limit theorem, this is the case for samples n≥30 (Döring & Bortz, 2016)

Hardy-Weinberg equilibrium (HWE) was determined by comparing the results from the Allele Frequency Aggregator (ALFA) project (National Center for Biotechnology Information, 2020; Phan et al., 2020) with the distribution in the study population using the chi-squared test. A $p > 0.05$ showed that Hardy-Weinberg equilibrium was not violated.

2.3. Genotyping

Both SNPs that were of interest in this dissertation had been genotyped before in a cost and time efficient way using the Roche LightCycler 2.0. Therefore, after extracting the DNA the PCR protocols were performed in accordance. This chapter explains those steps.

DNA Extraction

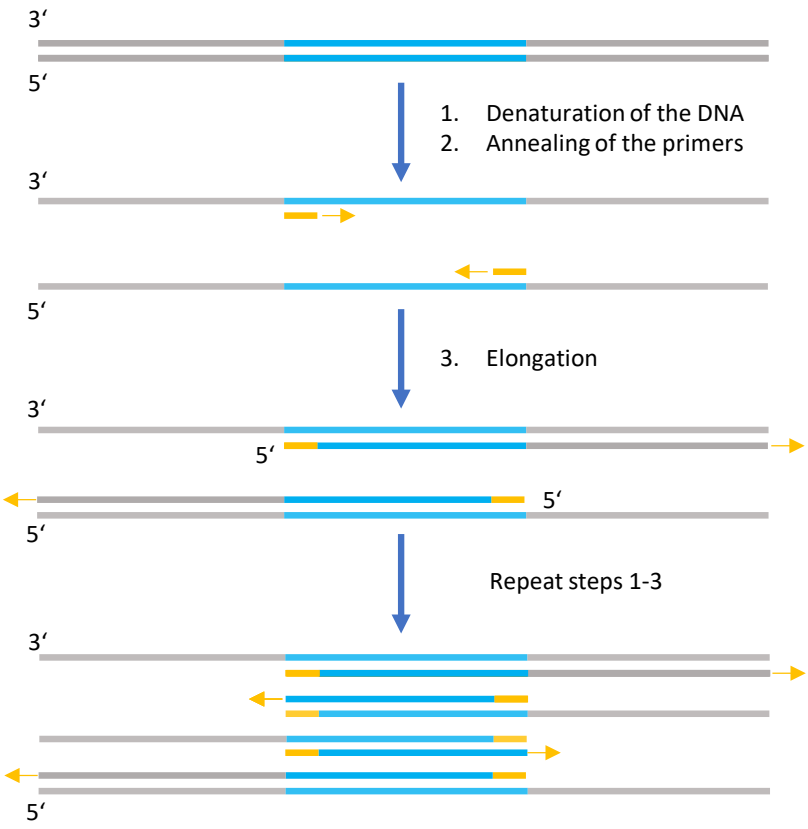
At the end of phase I (day 14), blood samples were drawn from patients that gave their written consent to pharmacogenetic testing with a 9 ml EDTA tube and stored at -70°C. After thawing, we extracted DNA using the QIAmp® DNA Blood Mini Kit according to the manufacturer's instructions. Remaining material was refrozen immediately. Each sample was extracted twice, one aliquot was stored at -70°C for later usage while one was stored at 3-8°C for immediate analysis.

Probe Based Polymerase Chain Reaction on the LightCycler® 2.0

The PCR has been a standard tool for molecular biology research and biosciences for the last thirtysomething years. Mullis and Faloona (1987) first described it when they presented a method to amplify or alter specific DNA sequences. The PCR basically consists of the following steps, which can be repeated as often as desired depending, inter alia, on the amount of DNA in the sample and the concentration of the product needed (Brix et al., 2014):

- **Denaturation**
  An increase in temperature to approximately 90°C separates the DNA double strands into single strands by breaking hydrogen bonds
- **Annealing**
  A lowering of the temperature to about 50-60°C leads to an annealing of the primers to each single strand of the DNA. Primers are short DNA fragments that are complementary to the 3' ends of the DNA sequence that is to be amplified
- **Elongation**
  Adding a thermostable DNA polymerase and desocyribonucleoside triphosphates while increasing the temperature to the polymerase's optimum results in the synthesis of new DNA that is complementary to the template

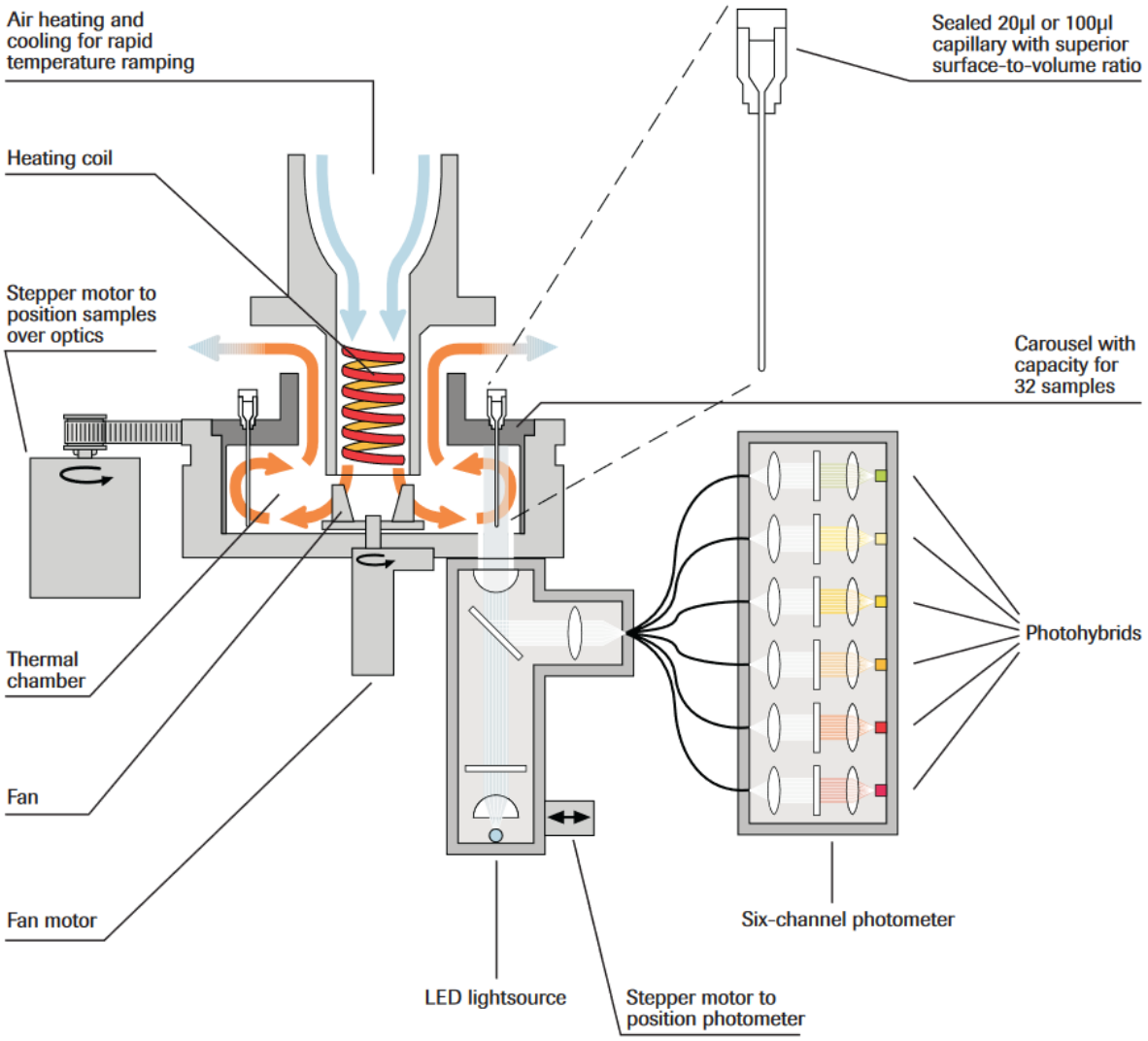
Figure 4: Schematic Illustration of the Amplification of DNA by PCR; Modified from Brix et al. (2014, p. 666)



The sequence of interest is in blue, the primers are in orange. After denaturation and annealing (steps 1 and 2), a thermostable DNA polymerase produces a new strand that is complementary to the template (step 3).

By repeating these steps 20 times, a 10^6-fold amplification may be easily achieved, taking around 1.5-2 hours in time (Brix et al., 2014). Known SNPs can now be detected using specific restriction endonucleases: These bacterial enzymes find certain DNA sequences and, if chosen so that the SNP lies within that region, produce DNA fragments of different sizes (depending on whether new restriction endonuclease recognition sites are created or destroyed). They can then be identified by gel electrophoresis. (Newton et al., 1989; Ota et al., 2007) Whenever there are no known restriction endonucleases for a region with a given point mutation, the allele-specific PCR enables the distinction of carriers of these in a rapid and reliable fashion. This method requires a pair of two separate reactions in which DNA is amplified with a common primer and with a normal or a mutant primer. The mutant primer binds to the region in which the SNP is located while the normal primer anneals to the wildtype sequence. A gel electrophoresis of the reaction products then shows whether the sample contains the mutant variate or the wildtype variate or both. (Newton et al., 1989)

Figure 5: The LightCycler® 2.0 Instrument; from Roche Applied Science (2005, p. 36)

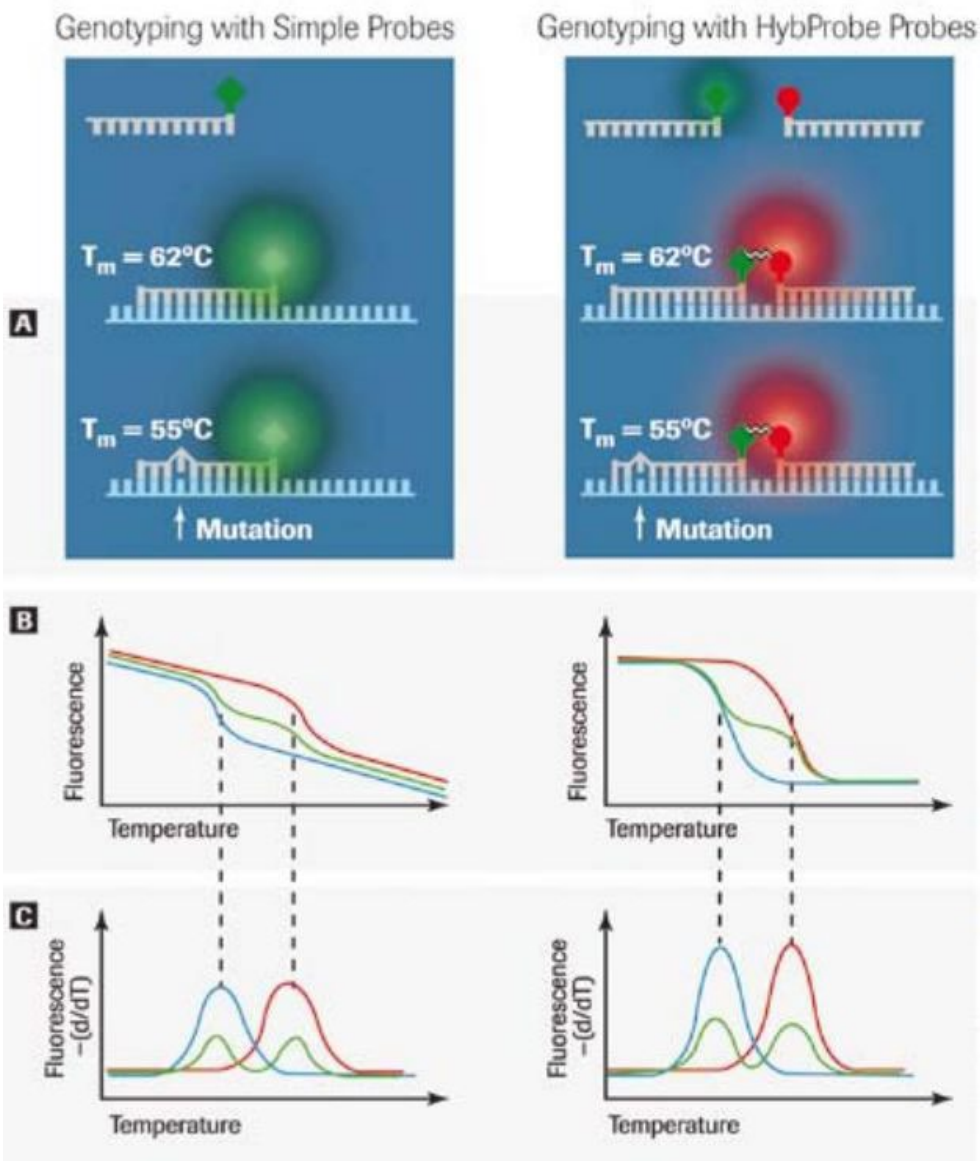


The probe-based polymerase chain reaction on the LightCycler® 2.0 is an improved PCR method that provides a simple and fast single step technique for genotyping genetic polymorphisms. It uses high ramp rates for the heating and cooling process (up to 20°C/s) and thereby results in shorter thermal cycles (20-60s) and overall reaction time. By integrating fluorescent probe melting curve analysis at the end of the PCR, additional steps such as restriction fragment length polymorphism or gel electrophoresis become redundant. (Czerwensky, 2014; Müller et al., 2003; Popp et al., 2003) Compared to conventional PCR devices, the LightCycler® 2.0 (Figure 5) abandons thermal blocks but uses air for heating and cooling, thus enabling rapid cycling. During photometric measurement, a stepper rotor ensures the correct positioning of the glass capillaries into the focus of the photometer optics (see Roche Applied Roche Applied Science (2005) for a comprehensive description).

In this dissertation, we used either HybProbe probes or SimpleProbe probes. HybProbe probes are sequence specific oligonucleotides that use a detection principle called FRET (fluorescence resonance energy transfer). FRET is a phenomenon that occurs between two dye molecules. When excited by

light, a donor molecule transfers energy to an acceptor molecule by dipole-dipole interaction. The acceptor then emits light of a different wave length which can be measured. (Didenko, 2001) HybProbe probes are designed pairwise, one labeled with the acceptor dye (fluorescein), one labeled with the donor dye (LightCycler Red 610 or LightCycler Red 670 or LightCycler Red 705). These probes must bind to the DNA in close proximity as the FRET phenomenon decreases with the sixth power of distance and normally requires a distance of no more than 1-5 base pairs. (Roche Applied Science, 2004)

Figure 6: Mutation Detection using SimpleProbe Probes or HybProbe Probes and FRET, (Roche Applied Science, 2008, p. 233)



A: Destabilizing mismatches cause a reduction of melting temperature; **B**: Melting Curve; **C**: Derivative melting peaks The melting temperature of each samples reveals whether it is classified wild type, heterozygote, or mutant. Red curve: homozygous wild type; green curve: heterozygous sample; blue curve: homozygous mutant. See next paragraph.

After amplifying the DNA template, the sample is cooled to lower temperature and then slowly re-heated (at ramp rates of 0.1°C/s). As the hydrogen bonds between the probes and the DNA dissolve,

they separate from their target region, increasing the distance between acceptor and donor dyes and therefore lowering the measured fluorescence. If there is a mismatch under the sensor probe (hence a point mutation), the hydrogen bonds are weaker and the probe dissolves at lower temperatures (Figure 6 **A**) (Roche Applied Roche Applied Science, 2004). When converting these fluorescence signals (Figure 6 **B)** to melting peaks (providing a suitable graph for visual analysis, Figure 6 **C**), the negative derivative of the fluorescence with respect to temperature is plotted against temperature (-dF/dT versus dT) (Popp et al., 2003).

A SimpleProbe probe is a specific oligonucleotide that causes a fluorescence signal when binding to the DNA. (Roche Applied Roche Applied Science, 2004). If not annealed to DNA, a quencher that is connected to the fluorescent dye prevents the emission of light (Roche Applied Science, 2008). The detection process is similar to the above, though not based on FRET as there is only one fluorescent.

Procedure

For each PCR reaction, the reagents (DNAse-free water, $MgCL_2$, primers, probes, DMSO(dimethyl sulfoxide), and DNA polymerase (LightCycler DNA Master Mix)) were vortexed and centrifuged. The total volume of the stock was calculated as the number of samples n+4, as each run included a wildtype, a mutant, and a no template control (NTC). Every LightCycler glass capillary was filled with 19 µl of stock and each one except NTC with 1 µl of DNA. The capillaries were then sealed with the designated cups using the transfer pin. Subsequently, the carousel was put into the LightCycler centrifuge adaptor and briefly centrifuged, pushing the liquid from the upper conical part to the bottom (see Figure 5 for the capillary). (Czerwensky, 2014)

Primers, Probes, and PCR Protocol

The genotyping of the rs17782313 near the MC4R gene and of the rs9939609 within the FTO gene was performed as developed by and described in Czerwensky (2014). The PCR protocols, reagents, primers, and probes are listed in the following tables.

Table 6: Method Description rs17782313, from Czerwensky (2014, p. 73)

PCR Protocol rs17782313				
95°C 90s; 35x [95°C 10s – 58°C 20s – 72°C 30s]; 52°C -67°C with a ramp rate of 0.1°C/s				
Reagents		Volume in µl	Concentration	Concentration converted into 20 µl stock solution
Sterile water		12.6		
$MgCl_2$		1.4	25 mM	2,75 mM
Primer	Forward MC4R	0.5	25 µM	625 nM
	Reverse MC4R	0.5	25 µM	625 nM
HybProbe Probes	Sen MC4R	0.5	3 µM	75 nM
	Anc MC4R	0.5	3 µM	75 nM
DMSO		1		
LightCycler DNA Master Mix		2	10x	0.5x

Table 7: Method Description rs9939609, from Czerwensky (2014, p. 79)

PCR Protocol rs9939609				
95°C 90s; 35x [95°C 10s – 59°C 20s – 72°C 30s]; 52°C -67°C with a ramp rate of 0.1°C/s				
Reagents		Volume in µl	Concentration	Concentration converted into 20 µl stock solution
Sterile water		13.5		
$MgCl_2$		1.4	25 mM	2,75 mM
Primer	Forward FTO	0.5	25 µM	625 nM
	Reverse FTO	0.3	25 µM	375 nM
SimpleProbe Probe	simple FTO	0.3	3 µM	45 nM
DMSO		1		
LightCycler DNA Master Mix		2	10x	0.5x

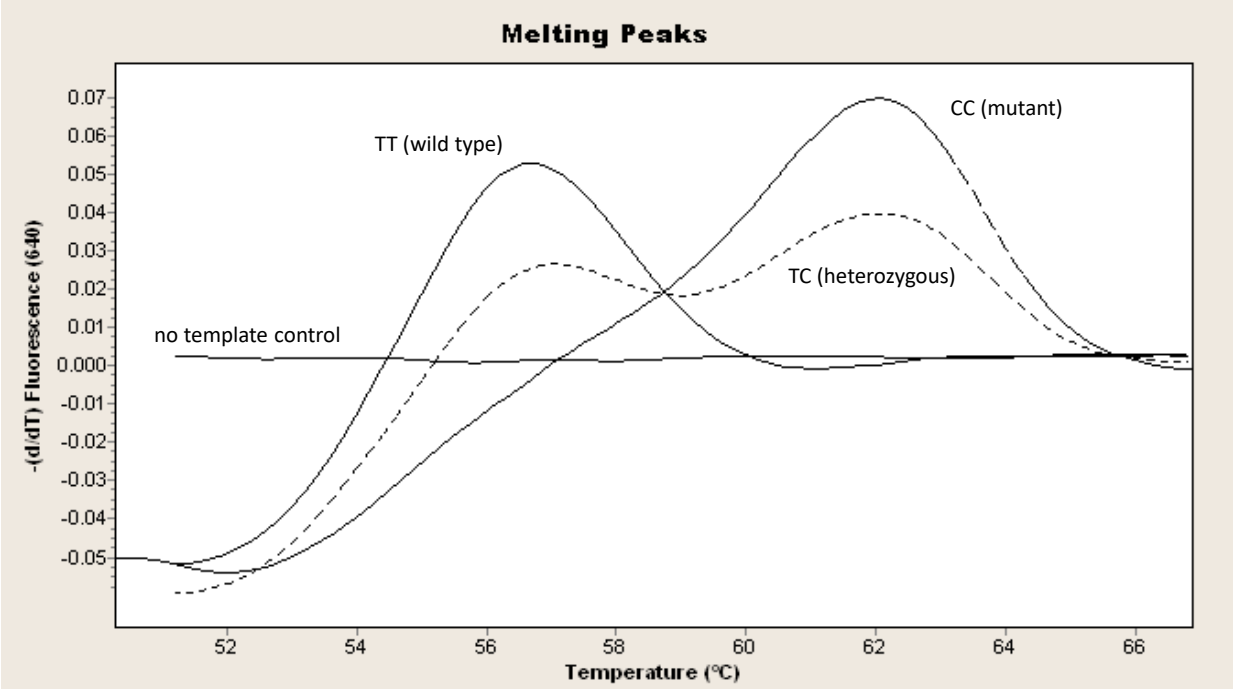
Table 8: Primers and Probes, from Czerwensky (2014, pp. 238-239)

Name	Sequence	Number of bp	Amplicon length in bp	Melting Temperature Tm in °C
Primers and Probes rs17782313				
Forward MC4R	TTGTGTGCCAGAGGAAACAG	20	320	59.9
Reverse MC4R	ACCTCAATCCCAGATGCTAAA	21		59.2
Sen MC4R	GAGATTGTATCC**C**GATGGAAATGACAAGAA-Fluorescein	30		63.6
Anc MC4R	LCRed640-GCTTCAGGGGG AAGGTGACATTTAAGTTGG–P	30		69.7
Primers and Probe rs9939609				
Forward FTO	GGTGGTACGCTGCTATGGTT	20	353	60.0
Reverse FTO	TGCTCTCCCACTCCATTTCT	20		59.8
Simple FTO	CTTGCGACTGCTGTGAAT**X***TT**A**GTGATGC-Phosphate	28		65.1

The base in bold and underlined marks the location of the point mutation; marked with * and in bold plus underlined is the fluorescence marked thymine base

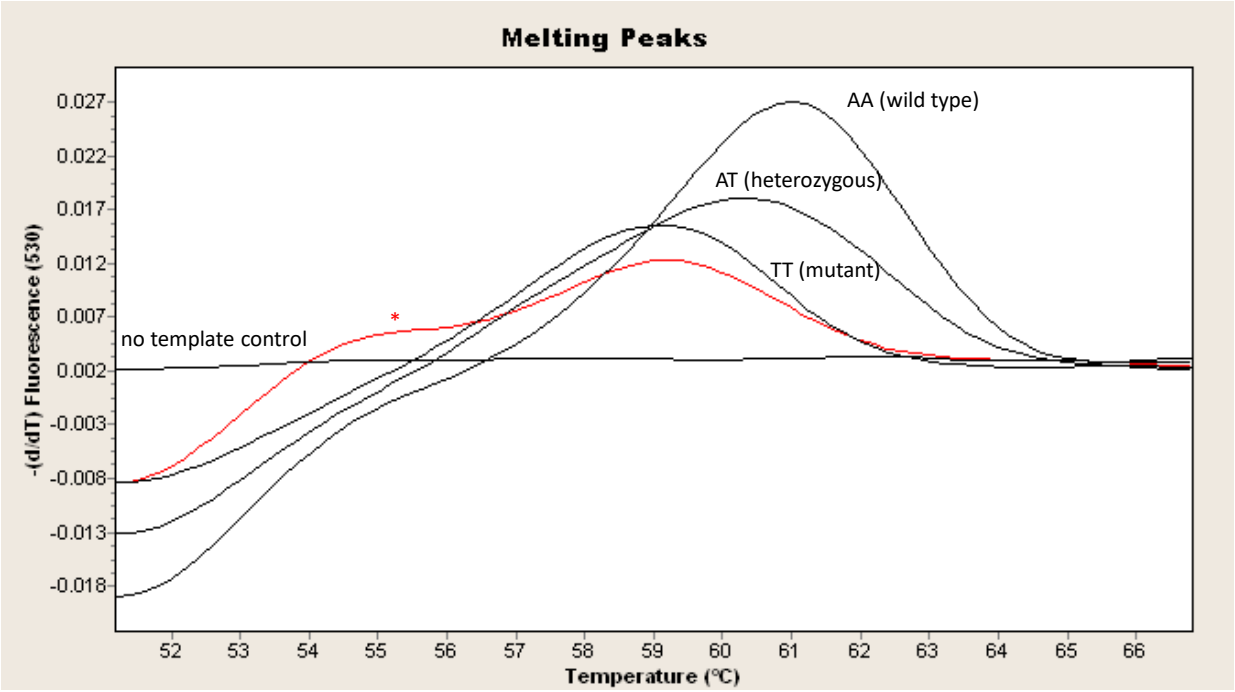
The analysis of the fluorescence signal gave the following melting peaks as a result, which are in line with Czerwensky's (2014) findings:

Figure 7: Melting Curve rs17782313



Some of the samples had an additional peak or slightly different peaks in their melting curve. Czerwensky (2014) identified these as an interaction of the rs9939609 mutation with the rs76804286 SNP. Depicted in Figure 8 is the combination of a homozygous mutant of the rs9939609 polymorphism with a heterozygous mutant of the rs76804286 polymorphism.

Figure 8: Melting Curve rs9939609



The peak marked with * is a combination of the rs9939609 homozygous mutation and the rs76804286 heterozygous mutation as described in (Czerwensky, 2014).

Reagents and Equipment

Table 9 gives an overview over the devices, reagents, and software used.

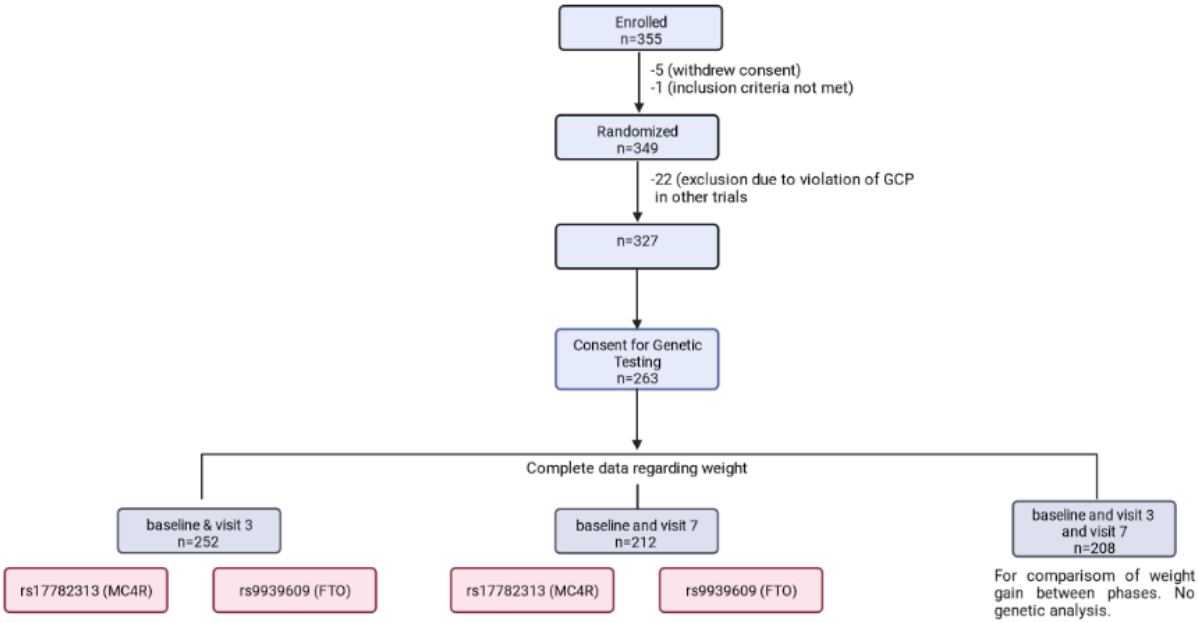
Table 9: Reagents and Equipment

Reagent	**Manufacturer**
Dimethyl Sulfoxide (DMSO)	PeqLab, VWR International, Radnor, PA, USA
Hybrid Probes and Simple Probe	TIB MOLBIOL, Berlin, Germany
Ethanol	Merck KGaA, Darmstadt, Germany
LightCycler-DNA Master HybProbe	Roche Diagnostics, Mannheim, Germany
LightCycler® FastStart DNA Master HybProbe	Roche Diagnostics, Mannheim, Germany
MgCl2 (25mM)	Roche Diagnostics, Mannheim, Germany
Nuclease-free Water	Qiagen, Venlo, Netherlands
Primer	Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA
QIAmp® DNA Blood Mini Kit	Qiagen, Venlo, Netherlands
Device	**Manufacturer**
Bio Vortex V1	Biosan, Riga, Latvia
Centrifuge 5415 C	Eppendorf SE, Hamburg, Germany
LightCycler 2.0	Roche Diagnostics, Mannheim, Germany
Thermoblock UNITEK® HBS-130	PeqLab, VWR International, Radnor, PA, USA
Software	**Manufacturer**
BioRender	BioRender, Toronto, Ontario, Canada
LightCycler Software 4.05	Roche Diagnostics, Mannheim, Germany
GraphPad Prism	GraphPad Software, San Diego, CA, USA
SPSS Statistics for Windows Version 21.0	IBM Corp., Armonk, N.Y., USA

3. Results

3.1. Study Population

Figure 9: The Study Population; created in BioRender.com



The study population.

Of the total 355 individuals enrolled, five reconsidered their consent and one failed to meet the inclusion criteria. Of the remaining 349 patients that were randomized, 22 were treated at a facility which was involved in an investigation into misconduct in obtaining patients' consent in a different, later conducted study. In accordance with Heres et al. (2022), we excluded these from analysis. Of the remaining 327 individuals, 263 gave their consent to genetic testing and an EDTA sample was collected. Data regarding body weight was available for 252 individuals for baseline and visit 3, for 212 subjects for baseline and for visit 7, and for 208 patients for baseline, visit 3, and visit 7. One person's body height was missing.

Weight gain of a first-episode subgroup was not analyzed since of the 252 patients with clinical data only 37 would have qualified. Consequently, the statistical analysis of antipsychotic induced weight gain and the SNPs rs17782313 and rs9939609 involved two subpopulations (Figure 9). In these two subgroups, patients treated with either only amisulpride or only olanzapine were analyzed separately as well.

3.2. Weight Gain depending on Study Medication

Weight Gain in Phase I

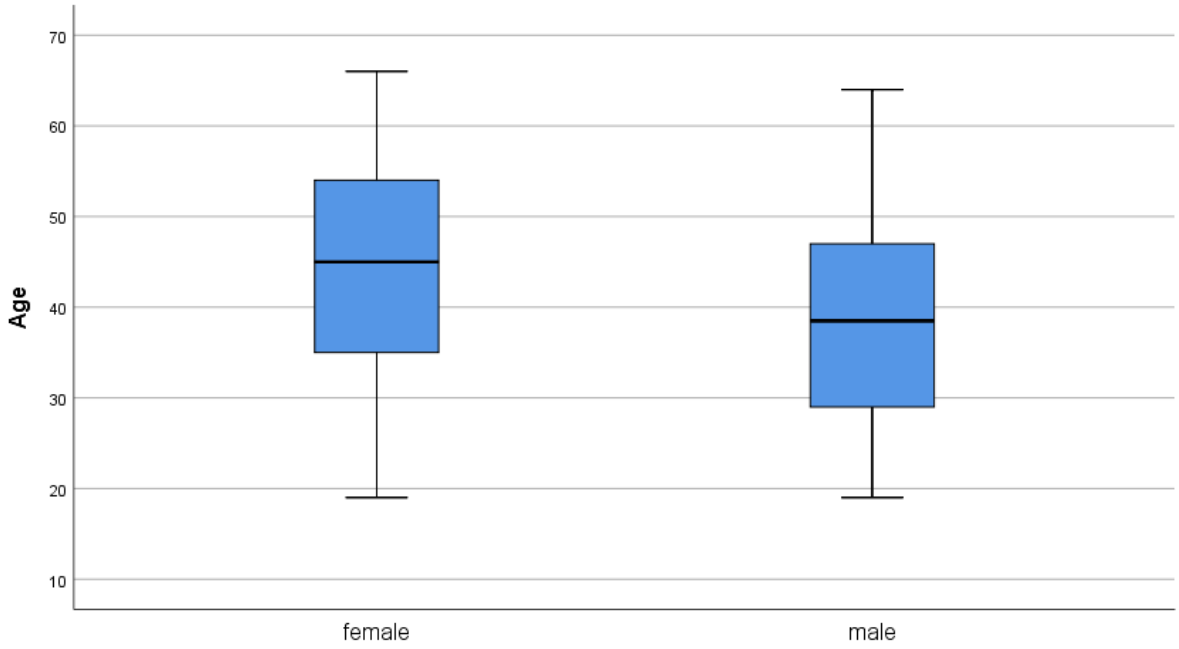
Table 10: Demographic and Clinical Data, Phase I, Data Published in Schreyer et al. (2023)

	Whole Study Population	Amisulpride in Phase I	Olanzapine in Phase I
Participants	n=252*	n=129	n=123*
Female/Male, %	48/52	47/53	49/51
Mean Age, years	42	42	41
f/m	44/39; p<0.01	46/39	43/39
Caucasian Decent, %	97.6	96.9	98.4
Smoker, %	54.6	55.8	53.3 (51.1)
First Episode	n=37	n=15	n=22
Baseline weight, kg	75.19±16.42	75.27±16.78	75.10±16.11
Weight after 2 weeks, kg	76.00±16.17 (75.87±16.29)	75.88±16.34 (75.29±16.29)	76.13±16.03
Weight gain, kg	0.81±2.29 (0.77±2.21)	0.61±2.07 (0.58±2.00)	1.03±2.48
Mean body height, cm	171±10	170±10	171±11
Females, cm	163±6	163±6	163±7
Males, cm	178±7	177±7	179±7
*Baseline BMI, kg/m²	25.78±5.40	25.98±5.36	25.59±5.45
BMI after 2 weeks	26.06±5.27	26.18±5.18	25.92±5.39
BMI gain, kg/m²	0.27±0.76	0.21±0.69	0.35±0.82

Results plus standard deviation; *one patient's height was not available; all calculations concerning body height and BMI therefore include one individual less

There was clinical data available for phase I for 252 patients that gave their consent to genetic testing (for one of these individuals body height was missing). Approximately 48% were female and the mean age was 42 years. Female participants were on average 44 years old while their male counterparts were 39 years old (Figure 10). That difference was of statistical significance ($p<0.01$). 97.6% of all patients had Caucasian ancestry. The percentage of people who smoke was comparable in both groups, yet slightly higher in patients treated with amisulpride – 55.8% vs 53.8%.

Figure 10: Age of the Study Participants



Overall, the baseline BMI was 25.78±5.40 kg/m² and baseline weight was 75.19±16.42 kg. The amisulpride group and the olanzapine group did not differ significantly in baseline BMI (p=0.39) or baseline body weight (p=0.83).

The total weight gain in the first two weeks was 0.81±2.29 kg and the BMI increased by 0.27± 0.76 kg/m². Members of the olanzapine group had a 1.68-fold higher increase in BMI and a 1.68-fold higher increase in body weight, though these were not statistically significant (p=0.11 for both). Males had a slightly higher gain in body weight while the BMI increase was higher in females. Both these observations lacked significance. More than half of the participants of the SWITCH Study were smokers (54.6%). Those who did not smoke suffered from 1.58-fold higher BMI gain and 1.58-fold higher increase in body weight. Although the statistical tests did not show a statistically significant relationship between smoking status and change in physique, the relatively low p-values might indicate a possible association (see Table 11).

Table 11: Comparison of Weight Gain between the Study Medications, the Sexes, and the Smoking Statuses

Medication			Ratio* [Olanzapine/Amisulpride]	p-Value
ΔBMI, kg/m²	Amisulpride	0.21±0.69	1.68	0.11
	Olanzapine	0.35±0.82		
ΔWeight, kg	Amisulpride	0.61±2.07	1.68	0.11
	Olanzapine	1.03±2.48		
Sexes			Ratio* [Female/Male]	p-Value
ΔBMI, kg/m²	Female	0.30±0.73	1.16	0.70
	Male	0.25±0.80		
ΔWeight, kg	Female	0.79±1.96	0.95	0.98
	Male	0.83±2.56		
Smoking Status			Ratio* [Non- Smoker/Smoker]	p-Value
ΔBMI, kg/m²	Non-Smoker	0.34±0.80	1.58	0.08
	Smoker	0.22±0.73		
ΔWeight, kg	Non-Smoker	1.01±2.40	1.58	0.09
	Smoker	0.64±2.19		

*The ratios of each two groups were calculated with more accurate results from SPSS, which explains why they do not perfectly match the rounded values from the left; results ± standard deviation

A possible correlation between weight gain and baseline body weight, baseline BMI, or age was determined by Pearson correlation coefficient.

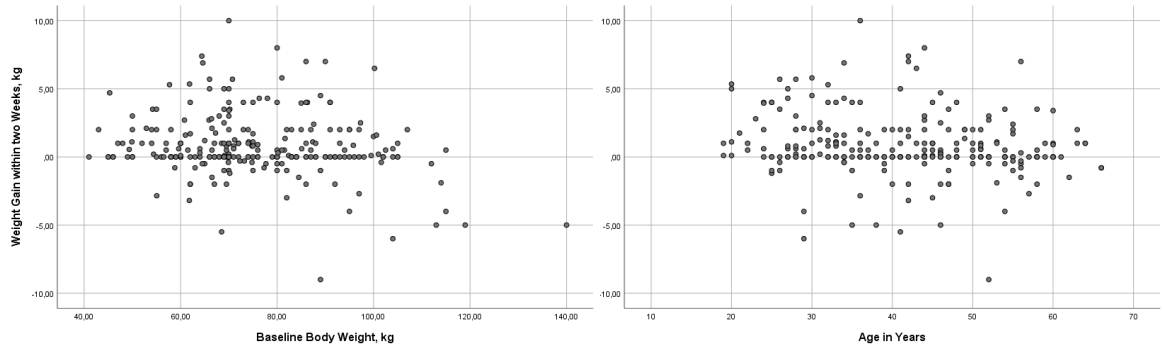
Table 12: Pearson Correlation Coefficient

	Weight Gain	
	r	p
Baseline Weight	-0.180	<0.01
Baseline BMI	-0.231	<0.01
Age	-0.180	<0.01

Abbreviation **r**: Pearson correlation coefficient

Baseline weight, BMI, and age did significantly correlate with the weight gain occurring within the first two weeks of treatment as all p-values were <0.01.

Figure 11: Scatter Plot visualizing Pearson Correlation



However, as Figure 11 indicates, the correlation between weight gain and baseline weight, baseline BMI, and age was low. A higher increase in body weight was associated with lower weight and BMI at inclusion and lower age (negative correlation).

Weight Gain throughout the Entire Study (Phases I and II)

For the entire course of the study, including visit 1 (baseline), visit 3 (end of phase I), and visit 7 (end of phase II), complete data from 208 patients was available. Of these, 84 were treated exclusively with amisulpride, 75 received only olanzapine, 23 took amisulpride in phase I and olanzapine in phase II, and 26 were treated vice versa. The clinical data is summarized in Table 13.

Table 13: Complete Demographic and Clinical Data, Entire Study, Data in Parts Published in Schreyer et al. (2023)

	Whole Study Population	Amisulpride non-switch	Olanzapine non-switch	Amisulpride-Olanzapine switch	Olanzapine-Amisulpride switch
Participants	n=208	n=84	n=75	n=23	n=26
Female/Male, %	50/50	43/57	56/44	52/48	58/42
Mean Age, years;	42	42	41	44	41
Caucasian Decent, %	97.1	96.4	98.7	95.7	96.2
Smoker, %	53.4	56.0	50.7	56.5	50.0
First Episode	n=29	n=12	n=13	n=0	n=4
Baseline Weight, kg	75.36±16.66	76.66±17.54	76.37±17.24	71.73±12.09	71.45±15.20
Weight Gain Phase I, kg	0.80±2.29	0.78±1.87	0.98±2.63	-0.03±2.67	1.12±2.02
Weight Gain Phase II	0.90±2.90	0.59±2.51	1.43±3.60	0.43±2.45	0.76±1.63
Total Weight Gain	1.70±3.67	1.37±3.48	2.41±4.23	0.40±3.01	1.87±2.66
Baseline BMI, kg/m²	25.99±5.48	26.21±5.65	26.30±5.76	25.39±4.38	24.96±5.14
BMI Gain Phase I, kg/m²	0.27±0.76	0.26±0.62	0.33±0.87	-0.02±0.90	0.40±0.72
BMI Gain Phase II, kg/m²	0.29±0.95	0.18±0.84	0.47±1.17	0.12±0.79	0.26±0.64
Total BMI Gain, kg/m²	0.56±1.21	0.44±1.16	0.79±1.46	0.10±1.06	0.66±0.95

Results ± standard deviation

Compared to the study population with complete data for phase I (Table 10), the baseline weight and weight gain within the first two weeks (and many other variables) of participants who completed the whole study were quite consistent - 75.36±16.66 vs 75.19±16.42 respectively 0.80±2.29 vs 0.81±2.29 (Table 10 and Table 12). This indicates that these two groups are comparable.

During phase I, patients gained an average of 0.80±2.29 kg and during phase II 0.90±2.90 kg. Although these increases in weight are not that different, it should be noted that phase I lasted 14 days and phase II 42. Therefore, a "per day" gain would be 2.7-fold (2.9-fold) in the first phase compared to phase II. Members of the amisulpride non-switch group gained an average 1.37±3.48 kg compared to 2.41±4.23 kg in the olanzapine-only arm. Nevertheless, this difference was not statistically significant (p=0.14).

In phase II, those treated with olanzapine only (non-switch) had a 3.29-fold higher weight gain than those who had switched from amisulpride to olanzapine. The difference, however, did not reach statistical significance – p=0.22. Vice versa, patients that had switched from olanzapine to amisulpride had a 1.3-fold increase in body weight compared to those receiving only amisulpride. This observation lacked significance as well (p=0.82). Even though not proven statistically, it is noticeable, that for each medication in phase II, participants gained more weight if they had been treated with olanzapine in phase I.

While in phase I there were hints of an association between the smoking statuses and change in body weight, this could not be replicated for the entire course of the study – for weight gain p was 0.80 and for BMI increase p was 0.74 (see Table 14).

In contrast to phase I (see above), males had a 1.95-fold higher weight gain than females within the entire eight weeks of the trial. This observation just missed significance (p=0.07).

Table 14: Comparison of Weight Gain between Smoking Statuses, entire Course of Study

Smoking Status			Ratio* [Non- Smoker/Smoker]	p-Value
ΔBMI, kg/m²	Non-Smoker	0.59±1.41	1.10	0.74
	Smoker	0.53±1.02		
ΔWeight, kg	Non-Smoker	1.81±4.17	1.13	0.80
	Smoker	1.61±3.19		
Sexes			Ratio* [male/female]	p-Value
ΔBMI, kg/m²	female	0.42±1.15	1.64	0.26
	male	0.69±1.27		
ΔWeight, kg	female	1.16±3.09	1.95	0.07
	male	2.26±4.13		

*The ratios of each two groups were calculated with more accurate results from SPSS, which explains why they do not perfectly match the rounded values from the left; results ± standard deviation

3.3. The Influence of MC4R

Data displayed in this chapter has been in parts published in Schreyer et al. (2023).

The rs17782313 genotypes were determined successfully in all cases. The clinical data for patients that participated only in phase I and the data for those taking part in the entire study can be found in 3.2. Here it should be mentioned that for the entire course of the study and the rs17782313 genotype the number of participants was slightly higher than in 3.2. In the previous chapter, patients were included, when their body weight was measured at baseline, visit 3, and visit 7. For this chapter, only baseline and visit 3 **or** visit 7 was of interest (see also 3.1). In a first step, variables for weight gain and BMI increase were analyzed depending on the rs17782313 genotype. In a second step, the non-switch/'one antipsychotic only' subpopulations were scrutinized. As the C-allele has been associated with increased risk for AIWG (see chapter 1.3.3), a statistical comparison of C-allele carriers with T-homozygotes was added in the following tables (p*).

Hardy-Weinberg Equilibrium

As the HAPMAP site was taken down in 2016 due to security flaws (National Center for Biotechnology Information, 2016), we obtained reference data from ALFA (Phan et al., 2020). The current European reference population for the rs17782313 consists of approximately 232.000 individuals with a minor allele frequency of C=0.231114, release version 20201027095038 (National Center for Biotechnology Information, 2021b).

Table 15: HWE for the rs17782313; Reference Distribution from National Center for Biotechnology Information (2021b), Data Published in Schreyer et al. (2023)

	TT	TC	CC	Chi-square Test	
n=252	155	84	13	p=0.739	χ=0.604
[Phase I]	61.5%	33.3%	5.2%		
n=212	130	70	12	p=0.743	χ=0.593
[Phase I + II]	61.3%	33.0%	5.7%		
ALFA (European)	59.1%	35.5%	5.3%		

The chi-squared test (Table 15) showed that there was no statistically significant difference between the study populations and the reference data from ALFA as all p were >0.05. Thus, the distribution of the observed genotypes was in Hardy-Weinberg equilibrium.

Weight Gain depending on rs17782313 in Phase I

Table 16: Analysis of Variance: rs17782313 and Phase I, Data Published in Schreyer et al. (2023)

Whole Study Population	TT	TC	CC	p	p*
Phase I, n=252	n=155[#]	n=84	n=13		
Baseline Weight, kg	73.53	79.08	68.76	**0.021**	0.077
Weight after 2 Weeks, kg	74.32	79.85	70.14	**0.016**	**0.043**
Absolute Weight Gain, kg	0.79	0.77	1.38	0.670	0.823
Relative Weight Gain, %	1.23	1.10	1.92	0.637	0.971
Baseline BMI, kg/m²	25.47	26.47	25.09	0.360	0.192
BMI after 2 Weeks, kg/m²	25.74	26.71	25.56	0.330	0.167
Absolute BMI Gain, kg/m²	0.27	0.25	0.47	0.575	0.839
Amisulpride Subpopulation	TT	TC	CC	p	p*
Phase I, n=129	n=83	n=41	n=5		
Baseline Weight, kg	72.68	81.56	66.66	**0.008**	**0.011**
Weight after 2 Weeks, kg	73.27	82.09	68.24	**0.005**	**0.007**
Absolute Weight Gain, kg	0.59	0.53	1.58	0.407	0.738
Relative Weight Gain, %	0.95	0.85	2.58	0.440	0.883
Baseline BMI, kg/m²	25.39	27.28	25.06	0.121	0.060
BMI after 2 Weeks, kg/m²	25.59	27.46	25.63	0.086	**0.036**
Absolute BMI Gain, kg/m²	0.20	0.17	0.57	0.390	0.824
Olanzapine Subpopulation	TT	TC	CC	p	p*
Phase I, n=123	n=72[#]	n=43	n=8		
Baseline Weight, kg	74.51	76.71	70.08	0.543	0.967
Weight after 2 Weeks, kg	75.53	77.70	71.33	0.545	0.692
Absolute Weight Gain, kg	1.03	0.99	1.25	0.997	0.936
Relative Weight Gain, %	1.57	1.33	1.50	0.964	0.798
Baseline BMI, kg/m²	25.57	25.68	25.11	0.985	0.921
BMI after 2 Weeks, kg/m²	25.92	26.00	25.51	0.967	0.801
Absolute BMI Gain, kg/m²	0.36	0.32	0.41	0.989	0.945

*Comparison of C-allele carriers with the TT-genotype; [#]for one patient there was no data for height

In the whole study population that took part in phase I, there was a statistically significant difference in baseline weight and weight after two weeks as TC-carriers had the highest numbers and CC-carriers the lowest. This phenomenon could not be observed when comparing carriers of the C-allele with the TT-genotype. Patients homozygous for the C-allele gained a total of 1.38 kg or 1.92%, TC-carriers gained 0.77 kg or 1.10%, and participants homozygous for the wildtype T-allele had an increase in body

weight of 0.70 kg or 1.23%. Neither when comparing the three genotypes nor the C-allele carriers with homozygous T-carriers, a significant difference was observed.

In the amisulpride subpopulation, baseline weight and weight at the end of phase I differed significantly – p=0.008 respectively p=0.005. The comparison of TT-carriers with C-allele carriers also showed a significant difference in BMI after 14 days. CC-Carriers gained an average 1.58 kg or 2.58% compared to a weight gain of 0.53 kg or 0.85% respectively 0.59 kg or 0.95% in TC and TT-carriers – 1.79-fold and 1.74-fold. This observation lacked statistical significance as p was 0.407.

In the olanzapine subpopulation, no differences in baseline variables or increase in weight or BMI could be shown.

Multiple linear regression analyses with relative weight gain as dependent variable and the known confounders age, sex, baseline body weight, and smoking status were conducted to determine the influence of the MC4R genotype. In all three medication groups, no significant association could be shown ($0.343<P<0.844$).

Weight Gain depending on rs17782313 in the entire Course of the Study

In a next step, the weight gain depending on the rs17782313 genotype of all participants finishing phase II was analyzed. The subgroups in this case only included patients that belonged to the non-switch arms and received only either olanzapine or amisulpride during the whole trial.

Table 17: Analysis of Variance: rs17782313 and Entire Course of Study, Data Published in Schreyer et al. (2023)

Whole Study Population	TT[#]	TC	CC	p	p*
Entire Study, n=212	n=130	n=70	n=12		
Baseline Weight, kg	77.26	78.75	68.32	0.059	0.275
Weight after 8 Weeks, kg	75.49	81.09	72.23	**0.039**	0.117
Absolute Weight Gain, kg	1.23	2.34	3.91	0.174	0.063
Relative Weight Gain, %	1.89	3.23	5.37	0.256	0.112
Baseline BMI, kg/m²	25.90	26.36	24.64	0.643	0.682
BMI after 8 Weeks, kg/m²	26.30	27.12	25.93	0.438	0.308
Absolute BMI Gain, kg/m²	0.40	0.75	1.29	0.179	0.072
Amisulpride Subpopulation	TT	TC	CC	p	p*
Entire Study, n=85	n=55	n=26	n=4		
Baseline Weight, kg	75.88	80.29	64.80	0.216	0.557
Weight after 8 Weeks, kg	76.57	82.57	68.40	0.156	0.278
Absolute Weight Gain, kg	0.69	2.28	3.60	0.063	**0.043**
Relative Weight Gain, %	1.33	3.11	5.97	0.177	0.103
Baseline BMI, kg/m²	26.30	26.52	23.71	0.650	0.974
BMI after 8 Weeks, kg/m²	26.52	27.25	24.98	0.681	0.696
Absolute BMI Gain, kg/m²	0.22	0.72	1.27	0.060	**0.028**
Olanzapine Subpopulation	TT[#]	TC	CC	p	p*
Entire Study, n=75	n=43	n=24	n=8		
Baseline Weight, kg	75.76	79.57	70.08	0.383	0.910
Weight after 8 Weeks, kg	77.55	82.56	74.14	0.486	0.949
Absolute Weight Gain, kg	1.79	2.99	4.06	0.676	0.495
Relative Weight Gain, %	2.60	3.92	5.08	0.791	0.566
Baseline BMI, kg/m²	26.61	26.16	25.11	0.716	0.419
BMI after 8 Weeks, kg/m²	27.21	27.13	26.41	0.811	0.567
Absolute BMI Gain, kg/m²	0.60	0.97	1.30	0.754	0.544

Comparison of C-allele carriers with the TT-genotype; [#]for one patient there was no data for height

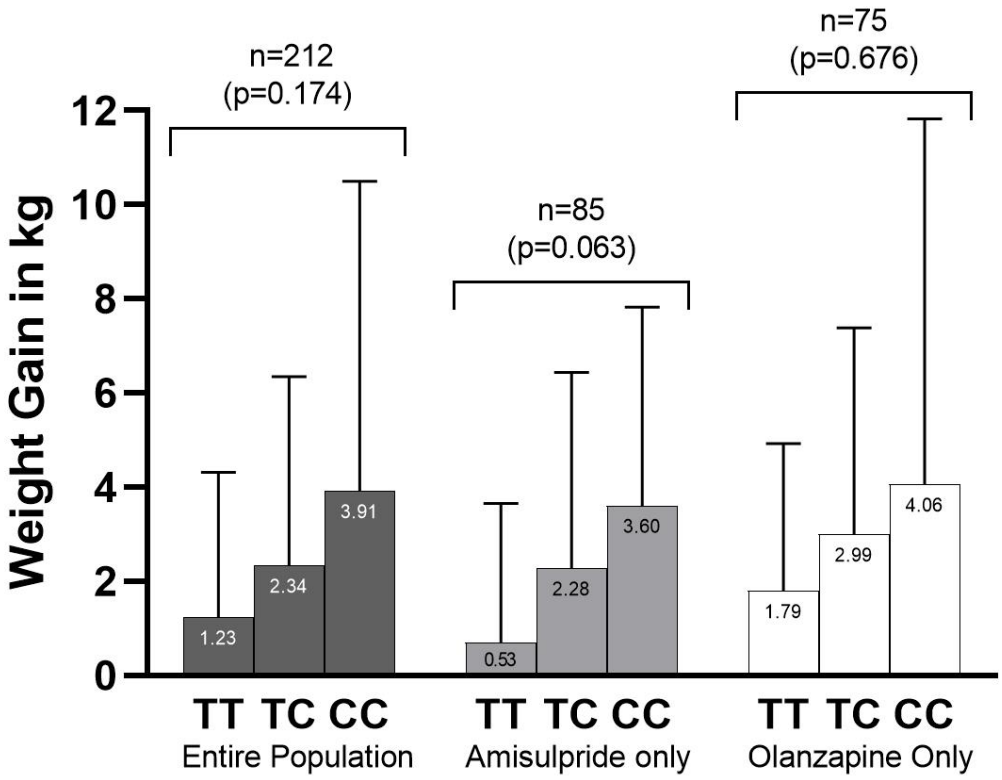
In the whole study population that finished the trial, there was a statistically significant difference in body weight after 8 weeks – p=0.039. The baseline characteristics of the subpopulations did not differ.

Within the entire 8 weeks of the trial, CC-carriers had a total weight gain of 5.37 kg compared to 3.23 kg (TC-carriers) and 1.23 kg (TT-carriers). Patients carrying the C-allele had a combined increase in body weight of 2.57 kg. Although 2.1-fold higher than TT-carriers, the statistical test missed significance – p=0.063 (see Figure 12 and Figure 13).

In the amisulpride subpopulation, C-carriers gained an average 2.46 kg while TT-carriers only experienced an increase in body weight of 0.69 kg. This observation was significant – p=0.043 (see Figure 13). The increase in BMI showed a significant difference as well – 1.27 kg/m² vs 0.72 kg/m² vs 0.22 kg/m² and p=0.028.

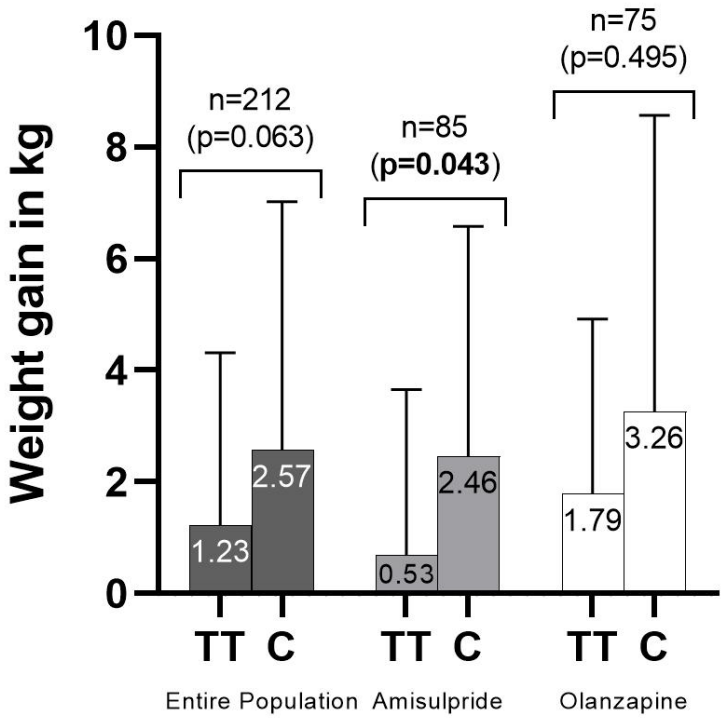
In the olanzapine subpopulation, participants carrying the C-allele had a mean increase in body weight of 3.26 kg (2.35 kg) vs 1.79 kg (1.75 kg) of the TT-genotype. However, neither weight gain nor BMI increase was statistically significant in patients treated with olanzapine only (see Figure 12 and Figure 13 for weight gain).

Figure 12: Absolute Weight Gain After 8 Weeks depending on rs17782313 Genotype, Entire Population and Subpopulations, Data published in Schreyer et al. (2023)



Bar graph comparing the absolute weight gain after 8 weeks of treatment. Each (sub)group with rs11782313 genotypes. Weight gain + SD, statistical tests compare all three genotypes.

Figure 13: Absolute Weight Gain After 8 Weeks depending on rs17782313 Genotype Comparing TT with C-Carriers, Entire Population and Subpopulations, adapted from Schreyer et al. (2023)



Bar graph comparing the absolute weight gain after 8 weeks of treatment. In all medication groups TT-genotype is compared with C-carriers (TC+CC). Weight gain + SD, statistical tests compare TT with C (TC+CC).

To adjust for known confounders, multiple linear regressions with relative weight gain as dependent variable and the MC4R genotype, sex, baseline body weight, age, and smoking status as cofactors were calculated. In all patients that took part in phase I and II regardless of medication lower age (β=-0.191; p=0.004), male sex (β=0.193; p=0.004), lower baseline body weight (β=-0.233; p<0.001), non-smoking status (β=0.107; p=0.110), and the C-*MC4R*-allele (β=0.201; p=0.002) were shown to be relevant factors.[1] The overall model reached levels of conventional statistical significance, F(5, 206)=8.244, p<0.001. Its R^2 was 0.167 (adjusted R^2=0.147). In patients that were treated with amisulpride only during the entire eight weeks lower age (β=-0.198; P=0.049), male sex (β=0.250; P=0.015), lower baseline body weight (β=-0.408; P<0.001), non-smoking status (β=0.059; P=0.529), and the C-*MC4R*-allele (β=0.244; P=0.012) could again be included being relevant factors. The overall model reached once more statistical significance, F(5, 79)=7.372, P<0.001. Its R^2 was higher, 0.318 (adjusted R^2=0.275). In patients that were treated with olanzapine only during the entire trial, we identified no relevant

[1] Relevant factor does not equal statistical significance, as criteria for inclusion into the model are defined differently, see 2.2 Statistical Analysis

factor, and none was significant (age, baseline body weight, smoking status, sex: p>0.129, *MC4R*: β=0.169; p=0.144), the overall model was insignificant (p=0.199). Its R^2 would be 0.098, its adjusted R^2 0.033. (Schreyer et al., 2023)

For a good comparison with Czerwensky et al. (2013), who did not include smoking habits, a stepwise multiple linear regression without the smoking status was calculated for all patients regardless of medication as well. The overall model was statistically significant – F(4, 207), p<0.001. Its R^2 was 0.156 and its adjusted R^2 was 0.140. All variables were included and significant (*MC4R*: β=0.200; p=0.002; age: β=-0.202, p=0.002; sex: β=0.162, p=0.016; baseline body weight: β=-0.227, p=0.001).

3.4. The Role of FTO

All samples were genotyped successfully. As in the previous chapter, two study populations were analyzed. First, all patients for whom there was complete data regarding body weight at baseline and end of phase I (visit 3), then all participants with complete data for baseline and end of the trial (visit 7). As the A-allele has been associated with higher AIWG (see chapter 1.3.2), a statistical comparison of A-allele carriers with T-homozygotes was included in the following tables.

Hardy-Weinberg Equilibrium

For the rs9939609, the European reference group from the ALFA project (National Center for Biotechnology Information, 2021a; Phan et al., 2020) was smaller than for the rs17782313 (n=73.860), the minor allele frequency is A=0.41025 – European reference group, release version 20201027095038. Still, for all subgroups the distribution of the rs9939609 mutation was in Hardy-Weinberg equilibrium as all p were >0.05 (Table 18).

Table 18: HWE for the rs9939609, Reference Distribution from National Center for Biotechnology Information (2021a)

	AA	AT	TT	Chi-Square Test	
n= 252	48	108	96	p=0.210	χ=3.126
[Phase I]	19.0%	42.9%	38.1%		
n= 212	38	92	82	p=0.338	χ=2.169
[Phase I + II]	17.9%	43.4%	38.7%		
ALFA (European)	16.8%	48.4%	34.8%		

Weight Gain depending on rs9939609 in Phase I

Table 19: Analysis of Variance: rs9939609 and Phase I

Whole Study Population#	AA	AT	TT	p	p*
Phase I, n=252	n=48	n=108	n=96		
Baseline Weight, kg	79.96	74.83	73.06	0.167	0.172
Weight after 2 Weeks, kg	80.79	75.63	73.89	0.127	0.129
Absolute Weight Gain, kg	0.83	0.80	0.83	0.962	0.809
Relative Weight Gain, %	1.26	1.25	1.18	0.966	0.797
Baseline BMI, kg/m²	27.28	26.03	24.75	0.101	0.052
BMI after 2 Weeks, kg/m²	27.54	26.31	25.02	0.065	**0.034**
Absolute BMI Gain, kg/m²	0.27	0.28	0.27	0.941	0.749
Amisulpride Subpopulation	AA	AT	TT	p	p*
Phase I, n=129	n=27	n=54	n=48		
Baseline Weight, kg	82.24	76.11	70.40	**0.011**	**0.006**
Weight after 2 Weeks, kg	82.54	76.63	71.28	**0.014**	**0.010**
Absolute Weight Gain, kg	0.31	0.52	0.88	0.766	0.515
Relative Weight Gain, %	0.58	0.87	1.33	0.720	0.496
Baseline BMI, kg/m²	28.12	26.70	23.96	**0.002**	**<0.001**
BMI after 2 Weeks, kg/m²	28.23	26.88	24.26	**0.002**	**<0.001**
Absolute BMI Gain, kg/m²	0.10	0.17	0.30	0.758	0.510
Olanzapine Subpopulation	AA#	AT	TT	p	p*
Phase I, n=123	n=21	n=54	n=48		
Baseline Weight, kg	77.04	73.54	75.72	0.803	0.508
Weight after 2 Weeks, kg	78.53	74.62	76.49	0.619	0.794
Absolute Weight Gain, kg	1.50	1.08	0.77	0.520	0.346
Relative Weight Gain, %	2.13	1.62	1.03	0.489	0.315
Baseline BMI, kg/m²	26.19	25.35	25.56	0.978	0.835
BMI after 2 Weeks, kg/m²	26.66	25.74	25.81	0.996	0.935
Absolute BMI Gain, kg/m²	0.47	0.39	0.24	0.458	0.282

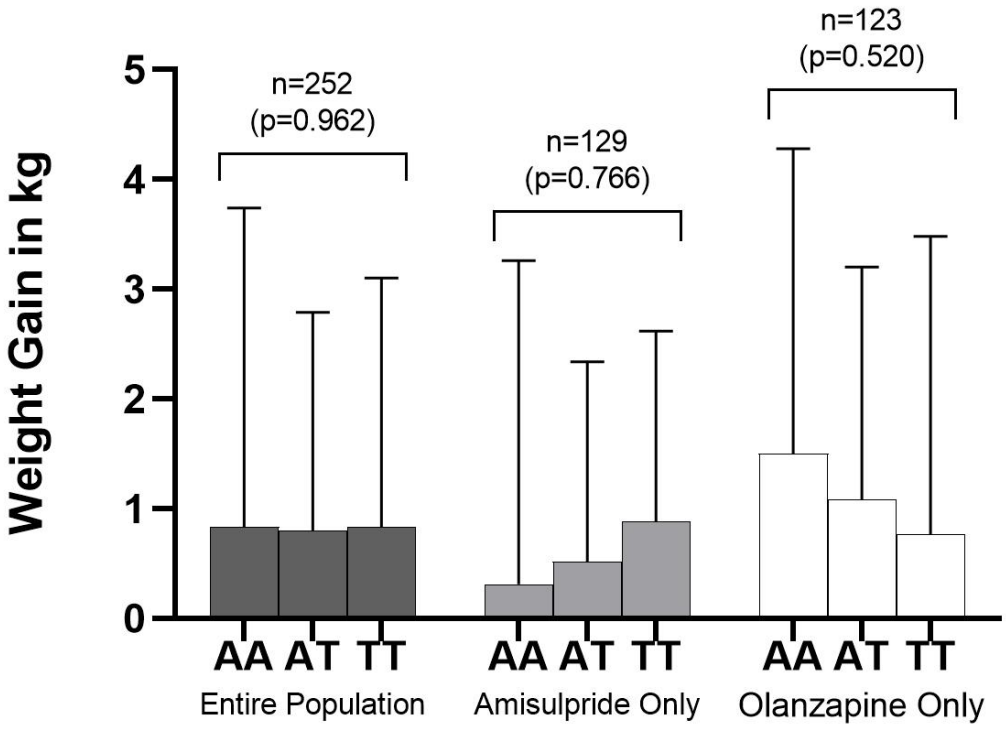
*Comparison of A-allele carriers with the TT-genotype; #for one patient there was no data for height

In the entire study population that participated in phase I, baseline weight and weight after 2 weeks did not differ statistically between the three genotypes, although in raw figures AA-carriers had the highest and TT-carriers the lowest: 79.96 kg vs. 74.83 kg vs. 73.89kg for baseline and 80.79kg vs. 75.63 kg vs. 73.89 kg for visit 3. BMI at the end of phase I did show a significant difference among the genotypes when comparing A-allele carriers with TT homozygous participants – p=0.034. The mean absolute weight gain was nearly identical in the three groups – 0.83 kg vs 0.80 kg vs. 0.83 kg – reflected in a high p-value >0.9.

In the olanzapine subpopulation, neither weight nor BMI were statistically different at baseline and end of phase I. AA-carriers gained an average 1.50 kg compared to a weight increase of 1.08 kg in the AT group and 0.77 kg in TT-carriers. No statistically relevant difference in absolute and relative weight gain, and in absolute BMI increase could be seen.

Between the three rs9939609 genotypes that received amisulpride only in phase I, baseline and visit 3 body weight and BMI differed significantly – all $p < 0.02$. Patients homozygous for the T-allele had a mean increase in body weight of 0.88 kg compared to 0.52 kg (AT) and 0.31 kg (AA). As in patients treated with olanzapine, this observation lacked significance, though it should be mentioned, that in the olanzapine arm AA-carriers had the highest weight and BMI gain in contrast to TT-carriers in the amisulpride group (Figure 14).

Figure 14: Weight Gain in Phase I, Depending on rs9939609 Genotype and Medication



Bar graph comparing the absolute weight gain after 2 weeks of treatment (phase I). Each (sub)group with rs9939609 genotypes. Weight gain + SD, statistical tests compare all three genotypes.

Stepwise multiple linear regressions did not report the rs9939609 as a significant factor for relative weight gain in phase I in neither all patients nor those treated with only amisulpride nor those receiving olanzapine ($p>0.211$).

Weight Gain depending on rs9939609 in the entire Course of the Study

Table 20: Analysis of Variance: rs9939609 and Entire Course of Study

Whole Study Population#	AA	AT	TT	p	p*
Entire Study, n=212	n=38	n=92	n=82		
Baseline Weight, kg	80.01	75.37	73.32	0.119	0.199
Weight after 8 Weeks, kg	81.73	76.99	75.21	0.130	**0.049**
Absolute Weight Gain, kg	1.72	1.63	1.89	0.906	0.741
Relative Weight Gain, %	2.45	2.50	2.59	0.930	0.759
Baseline BMI, kg/m²	27.40	26.23	25.03	0.199	0.093
BMI after 8 Weeks, kg/m²	27.92	26.78	25.64	0.197	0.093
Absolute BMI Gain, kg/m²	0.53	0.55	0.61	0.939	0.823
Amisulpride Subpopulation	AA	AT	TT	p	p*
Entire Study, n=85	n=14	n=37	n=34		
Baseline Weight, kg	83.79	79.47	70.79	**0.026**	**0.006**
Weight after 8 Weeks, kg	84.36	80.71	72.49	**0.032**	**0.007**
Absolute Weight Gain, kg	0.56	1.24	1.70	0.590	0.406
Relative Weight Gain, %	1.13	2.06	2.54	0.567	0.328
Baseline BMI, kg/m²	28.17	27.54	24.05	**0.011**	**0.001**
BMI after 8 Weeks, kg/m²	28.27	27.95	24.62	**0.010**	**0.001**
Absolute BMI Gain, kg/m²	0.10	0.41	0.57	0.442	0.340
Olanzapine Subpopulation	AA	AT	TT	p	p*
Entire Study, n=75	n=13	n=36	n=26		
Baseline Weight, kg	82.32	72.99	78.09	0.204	0.361
Weight after 8 Weeks, kg	84.97	74.96	81.00	0.161	0.240
Absolute Weight Gain, kg	2.65	1.97	2.91	0.560	0.806
Relative Weight Gain, %	3.51	2.91	3.69	0.694	0.802
Baseline BMI, kg/m²	27.53	25.62	26.64	0.560	0.603
BMI after 8 Weeks, kg/m²	28.41	26.31	27.55	0.483	0.541
Absolute BMI Gain, kg/m²	0.88	0.68	0.91	0.799	0.945

*Comparison of A-allele carriers with the TT-genotype; #for one patient there was no data for height

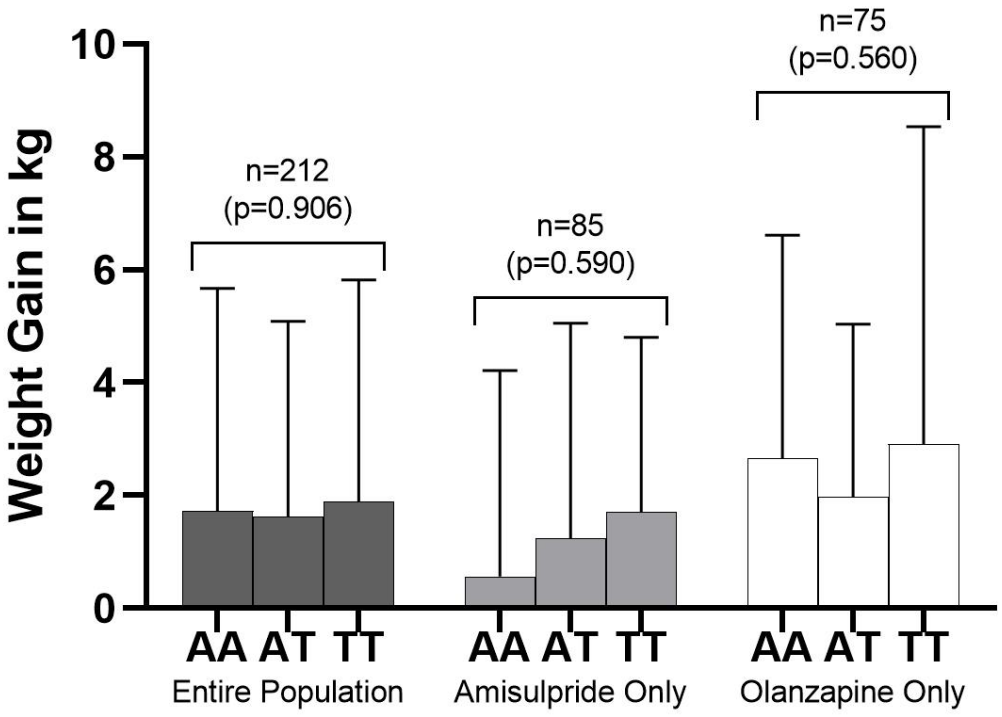
Like the participants that finished phase I, baseline weight and BMI and body weight and BMI in patients that underwent 8 weeks of treatment did not differ significantly, although again AA-carriers had the highest and TT-carriers the lowest. All three rs9939609 genotypes had a comparable relative weight gain between 2.45% and 2.59%, p=0.567.

In the olanzapine non-switch arm, neither mean baseline and final weight and BMI, nor average increase in body weight and BMI were significantly different comparing the FTO variants. After 8 weeks

as opposed to 2 weeks though, TT-carriers gained most weight, second highest increase in weight occurred in AA-carriers and AT-carriers gained the least – 2.91 kg vs. 2.65 kg vs. 1.97 kg; p=0.560.

Patients that were exclusively treated with amisulpride significantly differed in baseline weight and BMI, and in BMI and body weight after 8 weeks depending on their rs9939609 genotype. As in the higher number of patients that finished phase I, AA-carriers weighed the most and those homozygous for the T-allele the least – 83.79 kg vs. 79.47 kg vs 70.79 kg; p=0.026. Analogous to those participating in phase I, TT-carriers gained the most weight, second were heterozygous individuals and AA-carriers had the smallest weight gain, though this observation was again not significant– 1.70 kg vs. 1.24 kg vs. 0.56 kg; p=0.590.

Figure 15: Weight Gain in the Entire Course of the Study, Depending on Medication and rs9939609 Genotype



Bar graph comparing the absolute weight gain after 8 weeks of treatment (entire trial). Each (sub)group with rs9939609 genotypes. Weight gain + SD, statistical tests compare all three genotypes.

The inverse interaction between administered drug and amount of weight gain, that was notable in phase I, could not be seen over the entire course of the trial (Figure 15).

Multiple linear regressions again could not identify the rs9939609 genotype as significant factors for relative weight gain in all three medication groups (p>0.401).

4. Discussion

4.1. Weight Gain and Influencing Factors

Participants of the trial were on average 42 years old, though females were significantly older than males – 44 years compared to 39 years, $p<0.01$. As AIWG decreases with advancing age (Lee et al., 2011; Safer, 2004), there might be some restrictions for a direct comparison of male and female patients in this sample. Furthermore, the number of first episode patients was small – n=37 – this could mean that females had been experiencing antipsychotic treatment for a longer part of their life than their male counterparts. In phase I, females had a 1.16-fold increase in BMI while their absolute weight gain was 0.95-fold, both $p>0.05$. This diverging observation is simply explained by the lower mean body height of females. After the full eight weeks of treatment, males gained an average 2.26 kg while females experienced a mean weight gain of 1.16 kg. The higher weight gain observed in males (1.95-fold) nearly was statistically significant as $p=0.07$. However, the role of male or female sex remains unclear as there are studies that predicted females to experience higher BMI increase (Gebhardt et al., 2009; Lau et al., 2016; Lee et al., 2011), some reported that men gained more weight (Czerwensky, 2014), and others did not find a correlation between sex and AIWG (Ascher-Svanum et al., 2005). In the SWITCH Study, a tendency for higher weight gain in males could be seen after 8 weeks, but not after 2 weeks of treatment with second generation antipsychotics, which might lead to the assumption that the patients' sex affects very early AIWG less than that during the later course of treatment, yet no study could be found supporting this claim and there remains a need for further data.

In phase I, 54.6% of the patients did smoke, 55.8% in the amisulpride arm and 53.3% in the olanzapine arm. In the non-smoker group, an average increase of body weight of 1.01 kg and of BMI of 0.34 kg/m² occurred. This was 1.58-fold higher than in those who consumed tobacco. Although barely not statistically significant – for BMI increase p was 0.08 and for weight gain p was 0.09 – there seemed to be a trend for higher weight and BMI gain in non-smokers. Previous studies however are quite inconsistent in this matter. Lasser et al. (2004) reported that non-smokers have a higher weight gain when treated with risperidone, but not with olanzapine. Lau et al. (2016) discovered an opposite effect of smoking habits in their study population. The analysis of the population finishing the entire 8 weeks of the trial though did not show the same association that was seen during phase I as those who did not smoke had only a 1.13-fold increase in body weight compared to smokers.

The average weight gain in phase I was 0.81 kg for the 252 individuals (0.80 kg for the 208 completers). In the second phase, the 208 completers gained 0.90 kg. As phase I only lasted 14 days as opposed to the 42 days of phase II, a mean "per day weight gain" would be 2.7-fold higher in phase I. A meta-

analysis from 2010 concluded that SGA-induced weight gain is the highest within the first few weeks of treatment, gradually decreases, and then reaches a plateau after some time, in the case of olanzapine after 4-9 months (Rummel-Kluge et al., 2010). Although the rate of pretreated patients was rather high, there was a decrease of average daily weight gain observable in this sample over the course of the trial.

To identify risk factors for increased weight gain under SGA treatment, a Pearson correlation was conducted. As described in 2.2, there are several requirements to be fulfilled when using Pearson correlation, for instance no extreme outliers. While at first glance there are some individuals that experienced rather high increases in their body weight, we did not consider them to be (too) extreme and thus did not exclude them from further analysis. Several studies have shown that a significant part of patients suffer from 'rapid weight gain'. Ascher-Svanum et al. (2005) estimated that 15% of those receiving olanzapine would gain 12.1 kg after six weeks – on average. As indicated above, even within the first some weeks there was a trend for decreasing per-day rates of weight gain, these cases of very high early weight increase do therefore not seem unrealistic, which justifies their inclusion into analysis as 'true outliers' (StatistikGuru, 2015c). Within the first two weeks of antipsychotic treatment, low baseline weight – $r=-0.180$, $p<0.01$ – low baseline BMI – $r=-0.231$, $p<0.01$– and younger age – $r=-0.180$, $p<0.01$ – did significantly correlate with higher absolute increase in body weight. According to Cohen (1988), these product moments represent a small effect size respectively correlation. Those findings are in line with many previous reports (Ascher-Svanum et al., 2005; Kinon et al., 2001; Lee et al., 2011; Safer, 2004; Vandenberghe et al., 2015). Czerwensky (2014) reported similar correlation coefficients of $r=-0.298$, $p>0.001$ for baseline BMI and $r=-0.122$, $p>0.022$ for younger age with relative weight gain over a period of four weeks.

In phase I, the 129 patients that had been receiving amisulpride as antipsychotic treatment gained an average of 0.61 kg respectively experienced an increase in BMI of 0.21 kg/m². Compared to that, the 123 individuals in the olanzapine arm showed an average weight gain of 1.03 kg respectively increase in BMI of 0.35 kg/m². There was a trend for higher change in body weight and BMI in the olanzapine group as it was 1.68-fold compared to the amisulpride arm. This trend missed significance in the statistical analysis for BMI increase and weight gain, p was 0.11 for both. During the entire course of the study, the statistical difference between the 84 patients treated with amisulpride only and the 75 members of the olanzapine non-switch group was slightly smaller. The former experienced an increase in body weight of 1.37 kg while the latter suffered from a mean weight gain of 2.41 kg ($p=0.14$). In accordance with that, Rummel-Kluge et al. (2010) and Leucht et al. (2013) concluded in meta-analyses that olanzapine caused significantly higher weight gain than amisulpride. A meta-analysis from Allison et al. (1999) using a fixed effects model estimated that ten weeks of treatment with olanzapine would

cause an average weight increase of 3.51 kg, though there was no information available regarding the share of pretreated individuals, age distribution, and sex. Younger, first-episode patients experience higher AIWG (Alvarez-Jimenez et al., 2008) and the number of first-episode participants was quite low - app. 14.3% - which is why a direct comparison with other populations or meta-analyses might have limited explanatory power. Still, the number reported by Allison is comparable to the 2.41 kg found in the SWITCH population. Leucht et al. (2004) used a regression model and estimated an increase in body weight of 0.80 kg after ten weeks of treatment with amisulpride, which is also 'in the same ballpark'.

4.2. rs17782313

Data displayed in this chapter has been in parts published in Schreyer et al. (2023).

The distribution of the rs17782313 polymorphism in both groups of participants, either finishing the entire trial or with data for phase I only, was in Hardy-Weinberg equilibrium. Previous studies including a large GWAS have proposed that the C-allele correlates with higher baseline body weight (Beckers et al., 2011; Loos et al., 2008; Xi et al., 2012; Yu et al., 2020). In this patient sample, TC-carriers had a higher baseline body weight than TT-carriers, though patients homozygous for the C-allele had the lowest baseline body weight – 73.53 kg vs. 79.08 kg vs. 68.76 kg, p=0.021 for all in phase I; 77.26 kg vs. 78.75 kg vs. 68.32 kg, p=0.059 for those taking part in phase I and phase II. The same observation could be made in the amisulpride and olanzapine subgroups. While the comparison of TT-carriers with TC-carriers is in accordance with earlier publications as mentioned above, the diverging findings regarding CC-carriers might be partly due to the small number of individuals with this genotype – in total 13 – following the relatively low minor allele frequency of app. 23.1% in the European population (National Center for Biotechnology Information, 2021b). In addition, there might be more factors that have an influence on baseline body weight in this study population, limiting direct comparisons with healthy population-based studies: First and foremost, the high percentage of patients pretreated with antipsychotic substances. Furthermore, it should be mentioned that the differences between the genotypes were not statistically significant in all other subgroups except the entire population and the amisulpride arm in phase I.

In phase I, CC-carriers experienced higher increase in both absolute weight and BMI than T-carriers - 1.38 kg vs. 0.77 kg vs. 0.79 kg, p=0.670 respectively 0.47 kg/m² vs. 0.25 kg/m² vs. 0.27 kg/m², p=0.575. Similar to that, in the amisulpride group as well as the olanzapine arm CC-carriers gained more weight than T-carriers, yet participants heterozygous for the C-allele gained less than TT-carriers. None of

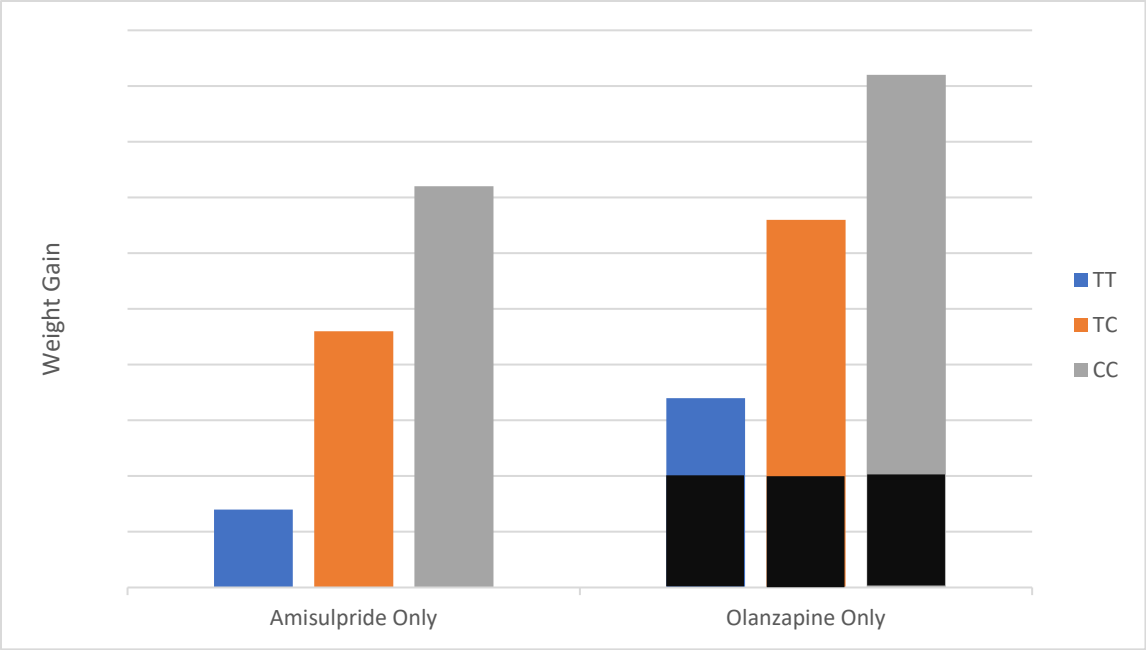
these comparisons were statistically significant. A correction for the known confounders age, baseline weight, smoking habits, and sex did not alter this observation regarding relative or absolute weight gain. Of those patients participating in the entire trial, CC-carriers gained 3.91 kg or 5.37%, TC-carriers had an increase of 2.34 kg or 3.23%, and TT-carriers experienced an increase in body weight of 1.23kg or 1.89%. This trend barely reached statistical significance when comparing C-carriers with TT-homozygous patients as p=0.063. In the amisulpride subpopulation, both weight gain as well as BMI increase were statistically different between CC-carriers and T-carriers – p=0.043 for weight gain and p= 0.028 for BMI increase. No significant influence of the rs17782313 could be seen in the olanzapine arm, yet there was a trend visible. An ANCOVA with the same confounders as above showed significant results for the entire study population and the amisulpride group. To date, there are few studies that have investigated the impact of the rs17782313 on AIWG. Czerwensky et al. (2013) reported that the mutation near the MC4R gene significantly increased weight gain and BMI increase in their entire population as well as in an adjusted subpopulation without additional weight gain-inducing comedication after four weeks of treatment. Chowdhury et al. (2013) at least observed a non-significant trend (p=0.09) in clozapine- and olanzapine-treated patients after up to 14 weeks. Zhang et al. (2019) though did not find an association of this SNP with increased BMI gains neither in their entire population nor in any subgroups after six weeks of antipsychotic therapy, though the comparison with this study population might have limited power as theirs consisted mainly of Han Chinese. Antipsychotic-induced weight gain is a common side effect of SGA that is known to occur early (Ascher-Svanum et al., 2005), and it is also a generally accepted fact that genetic variation plays a role in it (Lett et al., 2012; Roerig et al., 2011). Interestingly, in this population the rs17782313 mutation had a nearly significant influence on AIWG after eight weeks of treatment with either amisulpride or olanzapine or both consecutively (meaning a switch after two weeks) and a significant influence in the amisulpride non-switch subpopulation. Yet the same cohort did not show comparable differences between the genotypes after only two weeks of receiving either one of the two drugs. Very short observation intervals might be too short to see influence of genetic variation. Moreover, a 'ceiling effect' could have played a role in patients treated with olanzapine for a course of eight weeks (Kinon et al., 2005). However, this finding must be interpreted carefully as not all the patients that participated in phase I finished the entire trial and the total number of CC-homozygous individuals was low (13).(Schreyer et al., 2023)

Age, baseline body weight, smoking habits, and sex have been described as modifying parameters of AIWG (see 4.1). An overall model including the rs17782313 genotype was calculated using a multiple linear regression. The overall model was significant ($p<0.001$) and all factors were included (rs17782313: $\beta=0.201$; $p=0.002$). The R^2 of the overall model was 0.167, which means that 16.7% of the relative weight gain variation might be explained by this model. Czerwensky et al. (2013) used

similar statistical tools but did not include smoking habits and found an R^2 of 0.197 for the remaining factors, though determined in an adjusted subpopulation. When not including smoking habits in the same calculation in the SWITCH population, the R^2 would be 0.156, which is a bit lower but still fairly similar to Czerwensky's findings. It is quite noticeable that a single SNP (in combination with some easily determinable baseline characteristics) might already explain a significant amount of weight gain variation. (Schreyer et al., 2023)

In the amisulpride non-switch arm, CC-carriers experienced a 1.58-times higher increase in body weight than TC-carriers and a 5.25-times higher weight gain than TT-carriers. Compared to that, those relative differences were smaller in the olanzapine non-switch arm as CC-carriers gained only 1.36-times more weight than TC-carriers and 2.27-times more weight than TT-carriers. Czerwensky (2014) already reported a trend for the rs17782313 polymorphism and weight gain in a subsample treated with "low-risk" SGAs (risperidone, quetiapine, amisulpride, or paliperidone) but a lower correlation in patients treated with "high-risk" SGAs (clozapine or olanzapine). He noted that the missed significance in Chowdhury et al. (2013) could potentially be explained by a high share of patients receiving clozapine or olanzapine. Unknown mechanisms may exist enabling the rs17782313 SNP to enhance weight gain under SGA treatment differently depending on the medication. The C-allele of the rs17782313 polymorphism might increase body weight as it probably leads to a loss of function of the MC4R which normally lowers food intake when being stimulated (Balt et al., 2011; Fan & Tao, 2009). Olanzapine acts as an antagonist to the 5-HT_{2C} receptor (Müller & Benkert, 2021), resulting in reduced levels of α-MSH by blocking POMC neurons (Balt et al., 2011; Xu et al., 2008), and α-MSH acts as the natural, endogenous stimulating ligand of the MC4R. When being treated with olanzapine, AIWG might occur in all three rs17782313 genotypes through the α-MSH-modulated pathway and C-allele carriers could accumulate weight gain through an additive effect (since the receptor works less effective and levels of α-MSH are reduced). Contrary to that, amisulpride (being an antagonist to dopamine receptors) supposedly does not reduce levels of α-MSH and therefore fails to produce additional AIWG in all three genotypes. The hypothetical model that olanzapine causes comparable extra weight-increase in all allele-carriers could explain lower inter-allelic differences that occurred in the olanzapine arm as they would be 'flattened' and so to speak 'concealed'. Still, one should also consider a 'ceiling effect' in weight gain under olanzapine that might be involved (Kinon et al., 2005). (Schreyer et al., 2023)

Figure 16: Flattened Inter-Allelic Differences in Weight Gain in Olanzapine-Treated Patients



Weight gain induced via reduced levels of α-MSH through antagonism to the 5-HT_{2C} receptor (depicted in black) might flatten inter-allelic differences in olanzapine-but not amisulpride-treated patients. **Schematic depiction** of a possible cause of higher relative inter-allelic differences in weight gain.

Of course, this simplified explanation has some flaws to it. Firstly, the low number of CC-homozygous patients, especially in the medication subgroups that finished the trial – 4 in the amisulpride arm and 8 in the olanzapine arm – may have led to results that simply arose from statistical chance. Secondly, the influence and mechanism of the rs17782313 polymorphism on AIWG are, to date, not entirely understood as recent publications have shown additional pathways to the above explaining variance in body weight that might also play a role in antipsychotic induced weight gain. (Schreyer et al., 2023) Magno et al. (2020) found elevated plasma levels of ghrelin in the postprandial period in obese women carrying the risk-allele. As individual SGAs might have varying influence on circulating ghrelin levels (Zhang et al., 2013), AIWG depending on the rs17782313 SNP might also be mediated via an additive ghrelin-mediated mechanism, though evidence for substance-dependent ghrelin levels is scarce.

4.3. rs9939609

For the rs9939609, the distribution of genotypes in both all the patients from phase I as well as in those finishing the entire trial did not deviate from Hardy-Weinberg equilibrium as well. The A-allele has been associated with higher BMI and fat mass multiple times in the past (González-Sánchez et al., 2009;

Tanofsky-Kraff et al., 2009). Accordingly in the entire study population that took part in phase I and in participants that participated in the entire experiment, patients homozygous for the A-allele had the highest baseline body weight and BMI, second were AT-carriers, and TT-carriers had the lowest respectively. While this observation was only a trend and not statistically significant, it should again be mentioned that a high number of pretreated participants could perhaps have had distorted baseline characteristics compared to first episode patients. Interestingly though, TT-carriers in the olanzapine subpopulations showed higher baseline weight and BMI compared to AT-carriers. As patients were randomly assigned to one of the two medication groups before treatment initiation and genotypes in both medication arms combined showed baseline characteristics as described in literature, it can be assumed that the odd observation within the olanzapine arm occurred by chance. Some even say that testing for differences in baseline characteristics in randomized subgroups (not in the entire population though) "serves no purpose and can be misleading" (de Boer et al., 2015, p. 7), and therefore even if there were significant differences within the olanzapine-arm in baseline body weight it should not have consequences on the sub-analysis. Nevertheless, it is important to see if statistical differences in the non-switch arms at the end of the trial had already existed in the beginning.

In phase I as well as over the entire course of the study, absolute weight gain, relative weight gain, and absolute BMI increase did not differ significantly in the three genotypes and no clear trend was visible. An unexpected observation was made within the medication subgroups in phase I though: while in the olanzapine subpopulation AA-carriers gained the most weight, second were AT-carriers, and patients homozygous for the T-allele gained the least, weight increase in the amisulpride subpopulation behaved contrariwise. Both these findings lacked statistical significance. Previous studies have produced inconsistent data regarding weight gain under antipsychotic medication and the rs9939609 polymorphism. Jassim et al. (2011) did not find a statistically significant association in 160 patients of German origin with schizophrenia, Perez-Iglesias et al. (2010) analyzed weight gain in 239 first-episode patients for up to a year and did not reveal significant differences between the FTO genotypes. Moreover, an association study with 218 patients suffering from chronic schizophrenia or schizoaffective disorder receiving mainly clozapine or olanzapine for up to 14 weeks showed only numerically higher weight increase in AA-carriers, but lacked overall significance (Shing et al., 2014). On the other hand, Song et al. (2014) reported a significant association of the SNP with weight gain in 237 Chinese Han patients treated with risperidone for six months after controlling for some confounders, Roffeei et al. (2014) reported an association of the FTO polymorphism with metabolic syndrome in chronic schizophrenia patients receiving antipsychotic treatment, and Schröder et al. (2019) found a significant influence of the risk allele on AIWG in patients treated with olanzapine or clozapine, and in an adjusted subgroup excluding confounding co-medication. One systematic review and meta-analysis from Zhang et al. (2016) found that both the comparison of A-carriers vs. TT-carriers

and AA-carriers vs. T-carriers did not show significant differences in weight gain, though naturally did not include the findings of Schröder et al.

The analysis of this cohort treated with either amisulpride or olanzapine only, or both consecutively did not yield a significant association between the FTO rs9939609 SNP and weight gain that some of the previous studies have reported. The polymorphism within the first intron of the FTO gene remains a promising candidate for future investigations though as its effect on obesity is well documented and recent studies brought more light into its underlying mechanisms on weight regulation. Zhou et al. (2017) suggested that FTO genotype alone may not be sufficient to predict obesity but the combination with its methylation status increases predictive capacity as shown in their Australian population. The FTO variant also has the potential to influence the expression of downstream genes. Almén et al. (2012) described multiple sites where the methylation level depended on the rs9939609. Once these molecular mechanisms are better understood, future investigations will have additional tools for analyzing antipsychotic induced weight gain.

5. Conclusion

In participants of a multicenter randomized, controlled, double-blind study that compared two treatment regimes in patients with schizophrenia or schizoaffective disorder, we could show that carriers of the C-allele of the rs17782313 polymorphism experienced higher weight gain compared to TT-carriers after eight weeks of treatment ($p=0.063$). A stepwise multiple linear regression model identified the SNP as a significant factor and estimated an R^2 of 0.167 which means that the overall model explained 16.7% of relative weight gain variation ($p<0.001$). An analysis of the amisulpride arm showed significantly higher BMI and weight increase after eight weeks ($p=0.028$ respectively $p=0.043$) but not two weeks ($p=0.824$ respectively $p=0.738$) when comparing C with TT. (Schreyer et al., 2023)

Pharmacogenetics can be an instrument for personalized medicine by identifying and analyzing interindividual genetic differences and linking them to altered pharmacokinetics and dynamics of certain drugs. Although it promises to improve inter-alia response and overall outcome of pharmacological treatment, routine genetic testing remains to be implemented into many fields of modern medicine.

Schizophrenia is a common psychiatric disease affecting up to 1% of people and often becoming chronic and relapsing. While second-generation antipsychotics are effective in reducing both positive and negative symptoms and go along with less motoric side effects than their first-generation counterparts, significant weight gain is a common and serious adverse effect, often causing discontinuation of treatment. A more individual therapy strategy based on genetic (pre)testing could increase compliance rates by predicting weight gain and by choosing the antipsychotic agent accordingly.

So far, some genetic markers that might be predictors of increased weight gain under treatment with antipsychotics have been identified. The rs17782313 SNP near the MC4R gene and the rs9939609 SNP within the first intron of the FTO gene are known to influence baseline body weight and BMI. Regarding antipsychotic-induced weight gain, previous studies including a meta-analysis came to inconclusive or ambiguous results, revealing the need for further data. Moreover, direct comparisons of specific drugs and their potential interaction with gene variants remain scarce.

In this dissertation 252 participants of the multi-center, controlled double-blinded SWITCH Study were analyzed. They were being treated for schizophrenia or schizoaffective disorder for a duration of up to eight weeks and received either olanzapine or amisulpride, or both consecutively. We genotyped patients for these two SNPs using a probe-based rapid cycle polymerase chain reaction, a simple and

fast single-step technique for determining single nucleotide polymorphisms. The statistical analysis was completed with IBM SPSS Statistics for Windows, version 21.0 (IBM Corp., Armonk, N.Y., USA).

For the rs17782313 near the MC4R gene, we observed a non-significant trend for higher weight gain linked to the C-allele after eight weeks of treatment (p=0.063), yet not to the same extend after the first two weeks of the trial. A stepwise multiple linear regression model identified the SNP as a significant factor and estimated an R^2 of 0.167 which means that the overall model explained 16.7% of relative weight gain variation (p<0.001). An analysis of the amisulpride arm showed significantly higher BMI and weight increase after eight weeks but not two weeks when comparing C with TT. Although the trend withing the olanzapine arm seemed to be similar, the level of significance was not. These findings conform earlier studies, yet there remains a need for more detailed data. To date, we don't fully understand substance dependent influence of SNPs on AIWG. In the future, a deeper knowledge could help design predictive tools that might not only foresee weight gain but also help choosing the most suitable antipsychotic regarding metabolic side effects. (Schreyer et al., 2023)

The rs9939609 in the FTO gene was not significantly linked to increased weight gain within the SWITCH-Study's patients. Only in phase I, the olanzapine arm showed a non-significant trend for higher AIWG and the A-allele, though strangely the observation within the amisulpride arm was vice-versa. As previous studies are not conclusive, more data could set a basis for future meta-analyses.

There are some limitations to these findings. Firstly, a relatively high number of subjects was pretreated as less than 15% had their first episode. It is a known fact that individuals who had received antipsychotics before gain less additional weight than the unexposed. Nevertheless, in clinical settings a vast majority of patients suffers from chronic schizophrenia and results might therefore still be of high relevance for clinical practice. Secondly, not all participants who completed phase I finished the entire trial. A direct comparison of weight gain within the first two weeks with that after eight weeks might not be completely valid. However, only few studies investigated the course of AIWG with regard to related polymorphisms, and this dissertation saw a significant association of AIWG with an SNP after eight weeks, but not after two weeks. Thirdly, the minor allele frequency especially of the rs17782313 was very low. In the eight-weeks-amisulpride-subpopulation, only five CC-carriers remained. As significant results for CC-carriers might have arisen from very few outliers, the combined risk allele-carriers (C-carriers) were compared with TT-homozygous individuals as well, which led to even lower p-values.

Future investigations into weight gain associated with antipsychotic pharmacotherapy will most likely include SNPs that have not been in the focus of pharmacogenetic research, and progress in molecular biology, laboratory methods, and bioinformatics will enable studies of larger scale that include multiple

polymorphisms and analyze both independent and combined effects of many risk-alleles. Moreover, deeper knowledge of the exact molecular mechanisms that are behind certain mutations can be additional instruments in determining the effect of known SNPs. The statistical model that was designed to predict the effect of the rs17782313 in combination with some baseline characteristics – a stepwise multiple linear regression - already explained 16.7% of weight gain variation. This gives rise to the hope that later models with more mutations will be able to predict variation in AIWG significantly better and will be useful in choosing antipsychotic agents in clinical practice.

Publication

Schreyer, K. F., Leucht, S., Heres, S., & Steimer, W. (2023). Genetic association of the rs17782313 polymorphism with antipsychotic-induced weight gain. Psychopharmacology. https://doi.org/10.1007/s00213-023-06331-9

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