Personalized pharmacotherapy of psychosis

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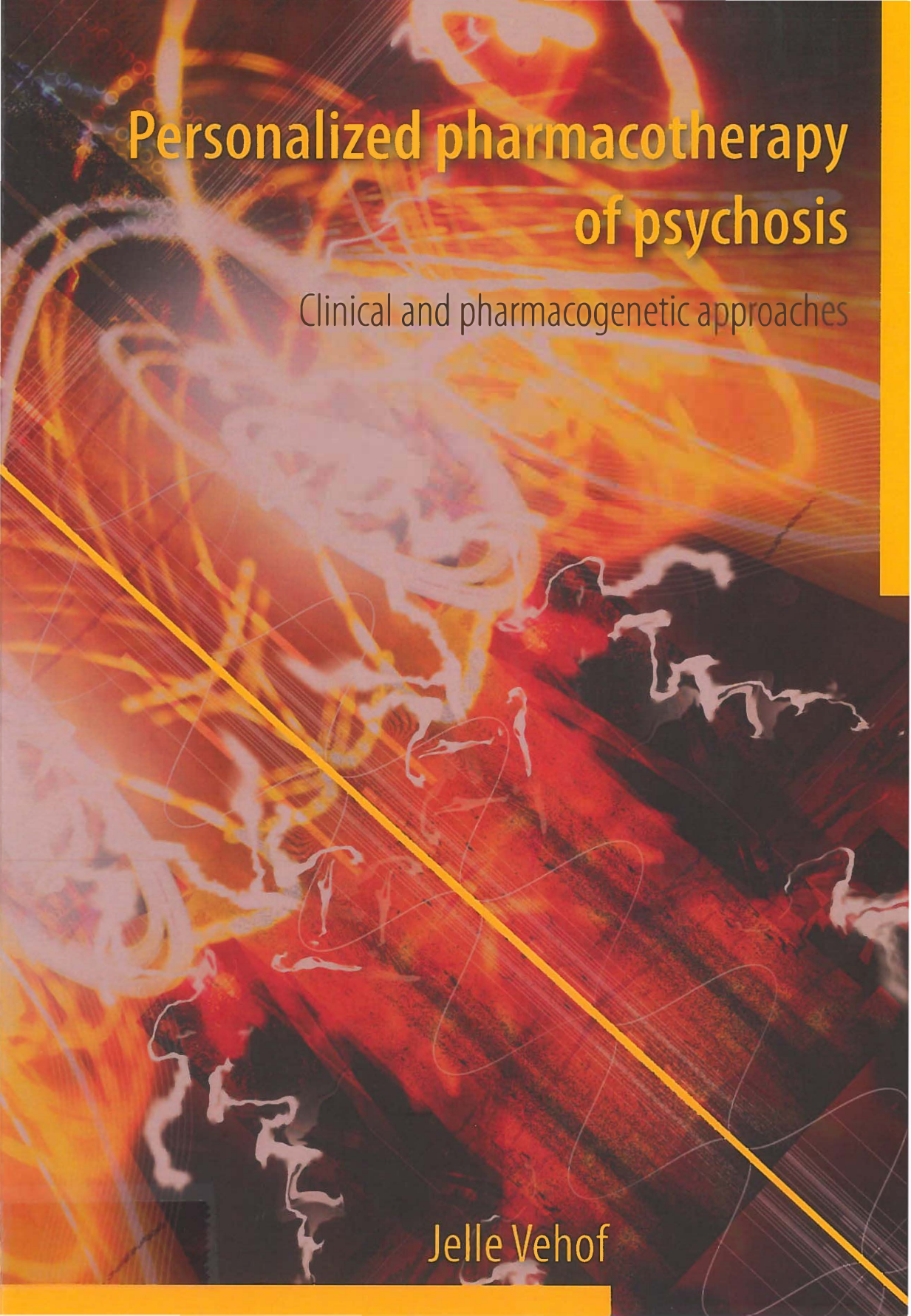
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Clinical and pharmacogenetic approaches
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Stellingen behorende bij het proefschrift *Propositions belonging to the thesis*

Personalized pharmacotherapy of psychosis. Clinical and pharmacogenetic approaches.

1. Depot antipsychotica worden te weinig voorgeschreven.

2. De farmacogenetica van antipsychotica lijkt vooralsnog meer een uitdaging voor onderzoekers dan een belofte voor patiënten.

3. Het *a posteriori* bepalen van in- en exclusiecriteria, statistische tests en covariabelen waarvoor gecorrigeerd moet worden doet de farmacogenetica geen goed.

4. Genetische studies waarin slechts een of twee polymorfismen onderzocht worden en geen associatie gevonden wordt zijn weinigzeggend.

5. De rol van histamine en muscarine receptoren bij gewichtstoename door antipsychotica wordt te weinig onderzocht.

6. Een uiteindelijk model dat respons en bijwerkingen op antipsychotica kan voorspellen moet per etniciteit afzonderlijk ontworpen worden.

7. Promoveren is voor negentig procent monnikenwerk, al doet het feit dat zo weinig monniken gepromoveerd zijn anders vermoeden.

8. The eye sees only what the mind is prepared to comprehend. (Henri Bergson)

9. Few of us have lost our minds, but most of us have long ago lost our bodies. (Ken Wilber)

10. Fulltime werken loont veel te weinig in vergelijking met parttime 1 dag vrij: een 'werk/vrije tijd ratio' van 2,5 versus 1,33 bij een nettoloon van 2,5 versus 2,1.

RIJKSUNIVERSITEIT GRONINGEN

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Clinical and Pharmacogenetic Approaches

Proefschrift

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Contents

Chapter 1

General Introduction and Aims of the Thesis

Jelle Vehof

In 1952 the first antipsychotic, chlorpromazine, was introduced. It heralded a revolution in psychiatry (1). Since then pharmacotherapy is the key in the treatment of psychoses. Now, almost 60 years later, numerous other antipsychotic agents have been introduced. A traditional classification into two groups has been made. The first generation (classical or typical) antipsychotics (FGA) have strong affinities for dopamine receptors. The newer (atypical) second generation antipsychotics (SGA) have a multitarget profile with affinity for dopamine, serotonin, histamine, muscarine, adrenergic and other receptors (2). However, the mechanisms of action of antipsychotic drugs are not fully understood (2).

Antipsychotics have proven to be particularly effective in treating the positive symptoms of a psychosis, leading to reduced hallucinations and delusions, and diminished thought disorder and disorganized behaviour. Although cutting down the positive symptoms is a big step in the treatment of psychoses, the pharmacotherapy of psychosis is still far from optimal. First, not all patients taking antipsychotic medication respond well, a reasonable degree of clinical improvement after current antipsychotic therapy is reached in only some 50% of patients (2, 3) and the two-year relapse risk on antipsychotics is still around 30% (4). Second, negative symptoms like poverty of speech and thought, anhedonia and social withdrawal, are still difficult to treat, despite the claim that the SGA tend to reduce these symptoms as well (5). Third, the side-effects of antipsychotics are devastating and may lead to physical disabilities, morbidity and even mortality (6, 7). An important class of side-effects, especially in the FGA, is movement disorders. Antipsychotic-induced movement disorders can occur acutely, starting in the first days after initiation (or increase in dose) of the antipsychotic. The most prevalent acute movement disorders are parkinsonism, akathisia and dystonia. Late-onset movement disorders, like tardive dyskinesia, can appear after months or years of antipsychotic treatment. A second class of important side-effects, especially in the SGA, is metabolic disturbances.

Antipsychotic treatment is known to cause weight gain and obesity, and it increases the risk for dyslipidemia, diabetes, accelerated cardiovascular disease and premature death (3, 8-15). Fourth, antipsychotic-induced side-effects, the lack of response and behaviour as a consequence of the psychotic disorder itself commonly lead to lack of compliance (16, 17). Low compliance is a major problem in many psychiatric drugs (18, 19) but especially antipsychotic therapy, with relapse of psychosis as a consequence (3, 20, 21). For that reason, depot (or long-acting) antipsychotics have been developed, which are injected every one to four weeks depending on the

formulation (22). Their aim is to improve compliance and to reach more stable plasma levels of the antipsychotic, with reduced relapse rates and side-effects as a consequence (22-26). Clinical studies have shown the advantages of a depot (27), but the use of depot medication is still limited (28, 29). Some clinicians associate depots with increased risk of certain side-effects and some are influenced by the idea that depots are not acceptable for a patient (30, 31), while there is in general no evidence for these ideas (32-34).

In clinical practice, it is very hard to predict who will respond to antipsychotic therapy or who is at risk for a certain side-effect. There is a large inter-individual variability in response to drug treatment (35). Therefore, better personalization of pharmacotherapy from the start could be of great value in antipsychotic treatment. This means prescribing the most optimal drug with regard to effectiveness side-effect ratio, in the most optimal dose, based on the individual characteristics of a patient. Clinical, demographic and environmental factors can help predict response (36-39). For example, male gender and early age of onset have been correlated with poor response with clozapine, whereas paranoid symptoms and presence of movement disorders with previous antipsychotics have been correlated with good response (3). However, nongenetic factors have not shown to be of much value in predicting clinical response in practice, influencing only a small part of the variability (40). Based on this and the large inter-individual and inter-racial differences in response, genetic factors have been suggested to play a role. A few case-reports, and some small twin studies and same-sex sibling concordance studies have been performed which added evidence that genetic factors are involved in antipsychotic efficacy (41, 42), antipsychotic-induced weight gain (43, 44), and tardive dyskinesia (45, 46). However, no large twin studies have been performed, which makes quantification of the genetic and environmental influences difficult if not impossible. Nevertheless, pharmacogenetics, the study of drug response as related to variation in DNA sequence, might be an important addition to optimize pharmacotherapy. Moreover, it has the potential to detect the molecular substrates of antipsychotic drugs, which, as said before, have no clear mechanism of action.

The concept of pharmacogenetics started in the 1950s, with discoveries that certain drug responses (primaquine, isoniazid, and suxamethonium chloride) were influenced by genetic factors (47-49). Late 1980s, with the start of the Human Genome Project (50) and the identification of a common genetic defect that influences debrisoquine metabolism in humans (51), the interest in this area accelerated. By now, pharmacogenetic research has expanded to most fields in medicine, particularly

in complex diseases, like schizophrenia, where pharmacotherapy is expensive or inadequate. The great expectations in the beginning of the pharmacogenetics of antipsychotics, including the concept of individually tailored treatment, have not yet been fulfilled, but modest successes have been made. The biggest success of the pharmacogenetics of antipsychotics is in the field of the pharmacokinetics, which is the process by which the antipsychotic is absorbed, distributed, metabolized and eliminated from the body. The cytochrome P450 CYP2D6 is an important enzymatic pathway for haloperidol and risperidone (52). Almost a hundred mutations in the gene coding for this enzyme have been described, with four of them being responsible for most inactive alleles in Caucasians (53). Patients with a genetic profile leading to a poor metabolizer status for the CYP2D6 require a lower antipsychotic dose than patients who have an extensive or ultrarapid metabolizer status (54, 55). Similar results, although less clear, have been found for CYP1A2 (56-61), the main metabolic pathway of clozapine and olanzapine (62, 63), and for some other enzymes that are important for antipsychotic breakdown (for a review, see Fleeman *et al.* and Arranz and De Leon (35, 40)). These studies have shown that genetically determined metabolic alterations may affect plasma levels of antipsychotics and, as a result, may induce side-effects (64-66). However, the relationship with efficacy is less clear (67, 68). Thus, pre-treatment determination of the metabolizer status of a patient may improve antipsychotic treatment. It has been estimated that this could lead to reduced side-effects (10-20%) and improved efficacy (10-15%), mostly as a result of increased compliance (69). However, clinical studies that compared outcomes in patients with and without pre-treatment genetic determination have not yet been performed (35).

Neurotransmitter receptors involved in the pharmacodynamics of antipsychotics have been studied as well. Most research has been performed on variants in dopamine and serotonin receptor genes. Pharmacogenetic studies helped confirm the hypothesis that antipsychotics are partly mediated by dopamine blockade. Polymorphisms in dopamine D2 (Taq1A and -141 C Ins/Del) and D3 (Ser9Gly) receptor genes have been repeatedly associated with treatment response (70, 71) and antipsychotic induced movement disorders (72, 73). Similarly, polymorphisms in serotonin receptors have been associated with treatment response, movement disorders and metabolic parameters (40). For example, the -759-T/C polymorphism of the serotonin 2C receptor gene influences antipsychotic induced weight gain (74-78). However, several attempts to replicate above findings in different populations have failed, making generalization and application to general practice difficult (79-82). Genes coding for other neurotransmitter systems, like the adrenergic, glutamate and

histamine systems, and more recently genes coding for proteins important in the regulation of neural metabolism, development and functionality have been investigated but associations with response are limited and often not well replicated (40, 83).

An attempt to combine individually associated genetic variants (in serotonin 2A and 2C, histamine H2, serotonin transporter genes) to predict clozapine response in refractory patients has shown promising results (84), but, again, replication in a population with different clinical characteristics failed (85). A test on adverse drug reactions (PGxPredict:CLOZAPINE) was taken off the market within one year because of insufficient sensitivity and specificity (86). A few pharmacogenetic tests are available at the moment, of which the test for determination of the CYP2D6 status of a patient is clinically the most important. Its use is still limited in the Netherlands, with only few psychiatrists requesting the test, mostly after initiation of pharmacotherapy which was not effective or troubled by side-effects. Pretreatment genetic determination is practically not used in the Netherlands.

Further research is needed to improve the concept of personalized medicine based on a patient's genetic profile. The validation of promising variants in different clinical settings and populations is required. Also, attempts to detect associations with new genetic variants in known and unknown pathways of antipsychotics could help to unravel the exact mechanism of antipsychotics and make the treatment of psychoses less problematic.

The present thesis aims to contribute to personalized pharmacotherapy of psychosis. It starts with two studies, both of which aim to give more insight in characteristics that predict which antipsychotics are prescribed in clinical practice. These studies have a focus on risperidone long-acting injectable (RLAI), the first SGA depot. The greater part of this thesis, however, is formed by several pharmacogenetic studies appearing in subsequent chapters. These have the purpose to improve and extend pharmacogenetic knowledge in antipsychotic therapy, concerning response as well as side-effects.

In **chapter 2.1**, we aimed to determine predictors for the prescription of 1) depot versus oral antipsychotics, and 2) RLAI versus FGA depot in a sample of chronic users of antipsychotics. In **chapter 2.2**, we analyzed the adoption and persistence of RLAI therapy after its introduction in the Netherlands in 2003 compared with the adoption and persistence of existing FGA depot drugs, as an example of the diffusion of a new drug in the Netherlands. Both studies made use of the InterAction DataBase

(www.iadb.nl). The InterAction DataBase is a joint effort of the Department of PharmacoEpidemiology and PharmacoEconomics of the University of Groningen together with pharmacists from community pharmacies. It contains prescription data with information on users and prescribers, which is stored anonymously. The data are collected from more than 50 community pharmacies from the north of the Netherlands. The catchment population of these pharmacies is approximately 500,000 people.

In **chapter 3.1**, we studied polymorphisms of the histamine H1 and muscarine acetylcholine M3 receptor for an association with metabolic side-effects of antipsychotics. In **chapter 3.2**, we investigated the association between a polymorphism in the adrenergic α-2a receptor and the metabolic syndrome. In **chapter 3.3**, we performed a replication study on the association between serotonin 2C receptor (HTRC2) polymorphisms and the metabolic syndrome. In **chapter 3.4**, we examined a polymorphism in the *ROBO1* gene for an association with BMI as proxy for antipsychotic-induced weight gain. All four studies were performed with patients included from the ongoing Pharmacotherapy Monitoring and Outcome Survey (PHAMOUS) in the Netherlands (www.phamous.eu). PHAMOUS is a large longitudinal cohort study setup by the Rob Giel Onderzoekcentre, GGz Fryslan, Lentis, GGz Drenthe and the University Centre Psychiatry/UMCG. Patients with a psychotic disorder using antipsychotics undergo a yearly somatic screening, combined with Routine Outcome Assessments including such instruments as the HONoS, PANSS and MANSA. Antipsychotic medication, side-effects and psychosocial functioning are evaluated and the goal is to optimize treatment and care. The PHAMOUS population, accounting for approximately half of the patients, was pooled with two other populations of patients on antipsychotic medication in the Netherlands.

In **chapter 4**, we endeavoured to replicate previously findings from literature that found an association between polymorphisms in dopamine system related genes and antipsychotic-induced movement disorders. In **chapter 5**, we performed a similar study as in chapter 8, but now on antipsychotic response. These two studies were performed as part of the Genetic Risk and Outcome of Psychosis (GROUP) study (www.group-project.nl). This study is a longitudinal, observational cohort study from a consortium of four academic psychiatric centers in the Netherlands (AMC, UM, UMCG and UMCU) with their affiliated mental health care institutions, covering a catchment area of more than 10 million inhabitants. A population-based cohort of approximately 1000 patients with a recent developed non-affective

psychotic disorder was created. In addition, a cohort of subjects at risk (brothers/sisters (n=1000)), parents (n=900) and controls (n=600) was formed. The assessments will take place with a 3 year and 6 year follow-up. The goal of GROUP is to gain insight in (the interaction between) vulnerability and protective factors in the development and variation in the course of a psychotic disorder.

In **chapter 6**, the general discussion, the studies in this thesis are put into a broader perspective. In this chapter we focused on difficulties and pitfalls in performing and interpreting pharmacogenetic studies and we give recommendations for future research. Finally, the thesis is concluded with a summary and some final remarks.

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Chapter 2.1

Predictors for Starting Depot Administration of Risperidone in Chronic Users of Antipsychotics

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ABSTRACT

Introduction: Risperidone long-acting injectable (RLAI), the first second-generation depot antipsychotic, has extensively been studied before introduction. Thereafter, questions about the type of patients actually treated with RLAI in daily practice remain to be answered for making valid antipsychotic treatment comparisons involving RLAI in observational studies. We aimed to determine in chronic antipsychotic users who switched treatment, predictors for the prescription of (1) depot versus oral antipsychotics and (2) RLAI versus first-generation antipsychotics (FGAs) depot.
Methods: We used pharmacy dispensing data from 53 community pharmacies in the northeast of the Netherlands containing approximately 500,000 persons. Chronic antipsychotic users were defined and followed up for a switch in antipsychotic treatment within the first period that RLAI was on the market. Multivariable analysis was performed to relate patient, prescriber, and medication characteristics to prescription of a new antipsychotic drug.
Results: Predictors for switching to depot versus oral antipsychotics were male sex, previous use of depot antipsychotics, recent anticholinergic drug use, and a gap in antipsychotic dispensation history. Predictors for switching to RLAI versus FGA depot were previous use of depot and consulting a specialist.
Conclusion: The results suggest that, compared with oral antipsychotics, patients receiving a depot are less compliant users, with more extrapyramidal side effects. Compared with FGA depot, patients receiving RLAI tend to be more severely ill patients. We conclude that RLAI may be partly channelled to patients as a last resort, which may have important consequences for the interpretation of observational effectiveness comparisons between RLAI and other antipsychotics in daily practice.

INTRODUCTION

Schizophrenia, with a lifetime prevalence of approximately 1.0%, (1) is one of the most devastating mental illnesses with severe physical, social, and economic consequences (2). Primary cost drivers in schizophrenia are relapse and rehospitalisation and are closely related to low compliance with therapy (3–6).

Since the introduction in the 1950s of the now called first-generation antipsychotics (FGAs), medication is the cornerstone in the treatment of schizophrenia. Second-generation antipsychotics (SGAs), which were developed in the 1990s, were initially believed to be superior in medication adherence because of their lower rate of neurological side effects (7). Meta-analyses have shown that, with SGAs, dropout rates are not lower than with first-generation ones (8). The supposed superiority may partly be based on registration studies that compare SGA with higher than nowadays recommended doses of FGAs (8, 9). The rates of relapse are modestly but significantly lower with the newer second-generation drugs (10).

A depot antipsychotic aims at promoting compliance in people with particularly severe mental illnesses, thereby enhancing relapse prevention (11–16). Several studies showed advantages of a depot regarding rates and durations of rehospitalisation compared with oral antipsychotics (11). Guidelines recommend considering depot antipsychotics in patients with repeated nonadherence (17). Until the 2000s, only FGAs, such as haloperidol and zuclopenthixol, were available as long-acting depots. Risperidone long-acting injectable (RLAI) is the first and, at the time of our study, only SGA in depot formulation and is available in the Netherlands since May 2003.

In the efficacy studies on RLAI, the type of patients may have represented a selection of the population that will eventually be treated in routine clinical practice. Therefore, it is largely unknown what the real-life benefits and risks are compared with other antipsychotics with similar indications. Such postmarketing comparisons between medications are almost always made using observational study designs. Consequently, adjustment for the type of patients who receive the medications under study is essential for reasons of validity. In addition, models used for pharmacoeconomic evaluation were often based on assumptions rather than actual data about drug prescription in daily practice (18, 19). Thus, questions about the type of patients who are actually treated with RLAI need to be answered.

The aim of the present study was 2-fold. First, we aimed to determine predictors for the prescription of depot versus oral antipsychotics in patients who had a medication switch during long-term antipsychotic treatment. Second, within the patients receiving a depot antipsychotic, we aimed to determine predictors for the

prescription of RLAI versus FGA depot. For the analyses of these predictors, data on pharmacy-based prescription drug histories from the target population were used.

MATERIALS AND METHODS

The present study was performed using data from the InterAction DataBase (www.iadb.nl). This database provides anonymous data on drug prescription from 53 pharmacists in a dynamic population of approximately 500,000 residents of the northern and eastern provinces of the Netherlands from 1994 onward. Besides demographical data, such as date of birth and sex, several aspects of pharmacotherapy can be derived from the prescription records. Diagnoses are not included in this database.

Chronic users of antipsychotics were included in the study population. In InterAction DataBase, men and women younger than 65 years on May 1, 2001, were defined as chronic users if they received at least 1 prescription for an antipsychotic drug in each year of the 2-year period from May 1, 2001, to April 30, 2003. By doing so, we aimed to include representatives of our target population, that is, chronic schizophrenic patients. Because the actual diagnoses were unknown, we tried to achieve this by making restrictions as to age and comedication. A maximum age was set to exclude elderly getting antipsychotic drugs for indications other than schizophrenia, for example, delirium. A lower age limit was set at 12 years to prevent inclusion of children treated with antipsychotics for, among others, attentiondeficit/hyperactivity disorder. Lithium users were excluded to exclude patients with bipolar disorder.

We followed up chronic antipsychotic users over time from May 1, 2003, until December 31, 2005, for a switch to a not previously used oral antipsychotic or depot antipsychotic, the latter being FGA depot or RLAI. A switch to a not previously used specific antipsychotic was defined if the first prescription of that antipsychotic occurred from May 1, 2003, onward, and was not prescribed in the period May 1, 2001, to April 30, 2003. The oral and depot preparations of a specific antipsychotic drug were analyzed as different antipsychotics. Thus, a patient who always used oral haloperidol and switched to haloperidol depot after May 1, 2003, was considered as switching to a not previously used antipsychotic, that is, FGA depot. The first prescription date of the new treatment was defined as the index date. In case of more than 1 switch to a new antipsychotic drug per patient in the follow-up period, one of these treatments was randomly selected for the analysis. One-time use of the corresponding oral formulation just before a depot antipsychotic was started, which is common when starting a depot antipsychotic, was not counted as a switch to a new oral antipsychotic.

For each new user, we assessed several potential predictors of use. First, we assessed sex, age at index date, and the prescriber who initiated the new treatment (general practitioner [GP] or specialist). Second, as a marker for recent disease severity, use of psychotropic comedication (anxiolytics [ATC N05B], hypnotics/sedatives [ATC N05C], and antidepressants [ATC N06A]) and, as a marker for extrapyramidal side effects, use of anticholinergic drugs (ATCN04A) were determined in the 3 months preceding the index date. Third, in the 2 years preceding the index date, the number of different antipsychotic drugs used, use of depot antipsychotics, and the presence of a gap of 3 months or more in antipsychotic dispensing history were determined. A gap of 3 months or more in dispensing occurrences of antipsychotic drugs was used as a measure for reduced compliance. The size of the gap was based on the fact that antipsychotics are prescribed for, at most, 3 months in the Netherlands.

We first studied potential predictors of a switch to an oral antipsychotic as compared with a switch to a depot antipsychotic. Next, within the group of depot users, we studied predictors of a switch to RLAI as compared with a switch to FGA depot. Odds ratios (ORs) with 95% confidence intervals (95% CIs) were calculated as measures of relative risk. Multivariable logistic regression analysis was used to adjust for age and sex. Of note is that, in this study, predictors of switching to a new therapy itself are ruled out because the results are conditional on switching.

RESULTS

We identified a total of 2491 eligible subjects as chronic antipsychotic users, and they formed our study cohort. During observation period, a total of 652 users (26.2%) switched to a not previously used antipsychotic drug. Forty patients (6.1%) of these 652 switched to an oral as well as a depot antipsychotic. After random assignment of the patients who switched more than once, 110 patients were classified as switching to a depot antipsychotic, and 542 patients were classified as switching to an oral antipsychotic. Figure 1 shows a flowchart describing the study population.

Risperidone long-acting injectable accounted for approximately half (n = 56) of all new depot users. From the new users of FGA depot (n = 54), zuclopenthixol (n = 20) was the most frequently dispended depot antipsychotic, followed by haloperidol (n = 12), flupenthixol (n = 10), fluphenazine (n = 6), perphenazine (n = 3), bromperidol (n = 2), and fluspirilene (n = 1). Table 1 summarizes the characteristics of the new users of the different groups of antipsychotics.

Figure 1. Flowchart of the study population, including the 2 comparisons of the study: depot versus oral antipsychotics, and RLAI versus first-generation depot antipsychotics (FGA depot).

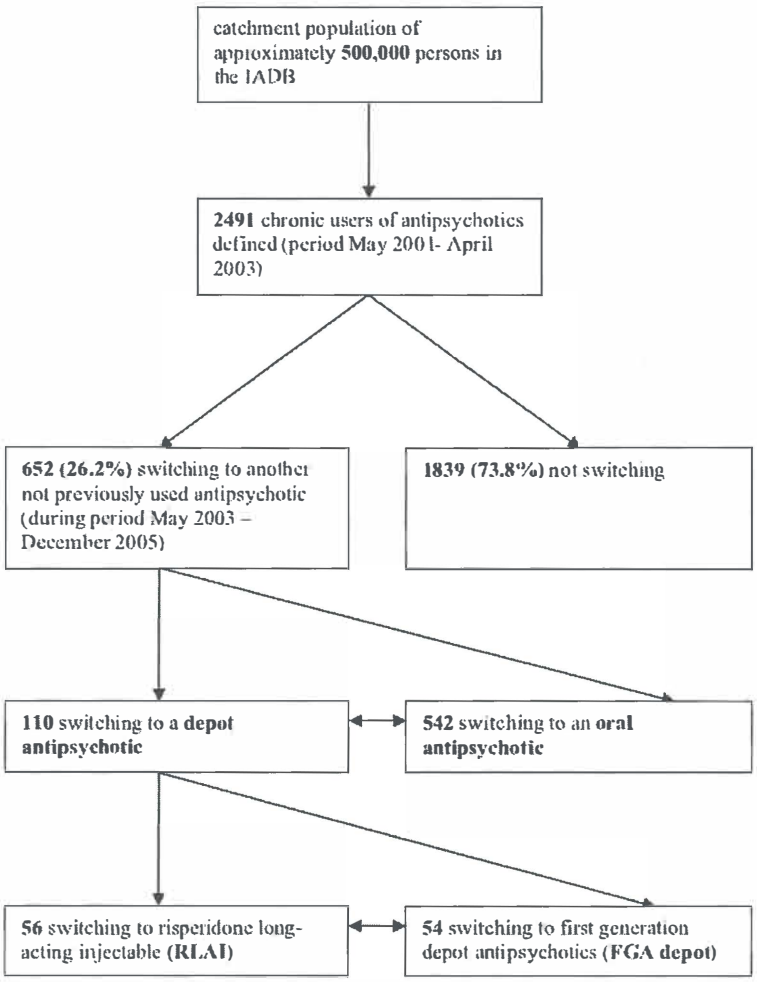

Table 1. Frequency of the characteristics of switchers to oral and to depot antipsychotics.

	Oral Antipsychotic (n = 542)		Depot Antipsychotic (n = 110)		RLAI (n = 56)		FGA Depot (n = 54)	
	n	%	n	%	n	%	n	%
Sex								
Female	247	45.6	43	39.1	22	39.3	21	38.9
Age category, yrs								
<30	103	19.0	26	23.6	15	26.8	11	20.4
30–50	313	57.7	49	44.5	20	35.7	29	53.7
51+	126	23.2	35	31.8	21	37.5	14	25.9
Prescriber								
GP	153	28.2	25	22.7	6	10.7	19	35.2
Specialist	389	71.8	85	77.3	50	89.3	35	64.8
3 mo preceding new antipsychotic								
Comedication								
Use of sedatives/hypnotics	135	24.9	17	15.5	10	17.9	7	13.0
Use of anxiolytics	222	41.0	37	33.6	18	32.1	19	35.2
Use of antidepressants	216	39.9	22	20.0	12	21.4	10	18.5
Any of psychotropic medication above	348	64.2	49	44.5	25	44.6	24	44.4
Use of anticholinergics	59	10.9	32	29.1	19	33.9	13	24.1
2 yrs preceding new antipsychotic								
Prior use of depot	41	7.6	37	33.6	25	44.6	12	22.2
No. different antipsychotics used								
1	361	66.6	61	55.4	31	55.3	30	55.6
2 or more	181	33.4	49	44.6	25	44.7	24	44.4
Gap of ≥3 mo	216	39.9	69	62.7	32	57.1	37	68.5

Depot users are divided in RLAI and first-generation depot antipsychotics (FGA depot).

Table 2. Predictors for switching to 1) a new treatment with a depot versus an oral antipsychotic, and 2) a new treatment with RLAI versus FGA depot in chronic antipsychotic users.

	Depot (n = 110) vs Oral Antipsychotics (n = 542)				RLAI (n = 56) vs FGA Depot (n = 54)			
	Univariable		Multivariable		Univariable		Multivariable	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Sex								
Female	**0.54**	0.35–0.82			1.02	0.47–2.19		
Age category, yrs								
<30	1.0				1.0			
30–50	0.62	0.37–1.05			0.51	0.19–1.33		
51+	1.10	0.62–1.95			1.10	0.39–3.08		
Prescriber								
GP	1.0		1.0		1.0		1.0	
Specialist	1.34	0.82–2.17	1.44	0.88–2.36	**4.52**	1.64–12.50	**4.88**	1.72–13.70
3 mo preceding new antipsychotic								
Comedication								
Use of sedatives/hypnotics	**0.55**	0.32–0.96	0.62	0.35–1.10	1.46	0.51–4.16	1.32	0.42–4.11
Use of anxiolytics	0.73	0.48–1.12	0.78	0.51–1.21	0.87	0.40–1.93	0.87	0.39–1.96
Use of antidepressants	**0.38**	0.23–0.62	**0.41**	0.25–0.68	1.20	0.47–3.06	1.11	0.43–2.93
Any of psychotropic medication above	**0.45**	0.30–0.68	**0.49**	0.32–0.75	1.00	0.48–2.14	1.00	0.48–2.14
Use of anticholinergics	**3.36**	2.05–5.50	**3.52**	2.12–5.84	1.62	0.70–3.73	1.78	0.75–4.23
2 yrs preceding new antipsychotic								
Prior use of depot	**6.19**	3.73–10.29	**5.78**	3.44–9.71	**2.82**	1.23–6.47	**2.76**	1.17–6.49
No. different used antipsychotics								
1	1.0		1.0		1.0		1.0	
2 or more	**1.60**	1.06–2.43	**1.63**	1.07–2.48	1.00	0.48–2.14	0.99	0.46–2.13
Gap of ≥3 mo	**2.54**	1.66–3.88	**2.49**	1.62–3.85	0.75	0.24–2.33	0.63	0.28–1.45

Multivariable analysis was corrected for age and sex. Statistically significant predictors are shown in bold font.

In Table 2, ORs for the predictors of new users of depot antipsychotics as compared with new users of oral antipsychotics are displayed. The age and prescriber distribution did not differ significantly between these users. Compared with new users of oral antipsychotics, users of depot antipsychotics less often were female (OR, 0.54; 95% CI, 0.35–0.82) and more often received an anticholinergic drug before the index date (OR, 3.52; 95% CI, 2.12–5.84). Depot antipsychotics were approximately 2 times less frequently prescribed to patients who recently used psychotropic comedication (OR, 0.49; 95% CI, 0.32–0.75), especially antidepressants (OR, 0.41; 95% CI, 0.25–0.68). Depot antipsychotics were more often prescribed to patients who had a gap of 3 or more months in their prescription data (OR, 2.49; 95% CI, 1.62–3.85). Finally, depot antipsychotics were around 6 times more frequently prescribed to patients who had been prescribed a depot antipsychotic before (OR, 5.78; 95% CI, 3.44–9.71) and to patients in which the number of different used oral antipsychotics before was higher (OR, 1.63; 95% CI, 1.07–2.48).

Also in Table 2, ORs from the predictors of new users of RLAI as compared with new users of FGA depot are shown. Compared with FGA depot, RLAI was more often prescribed by specialists (OR, 4.88; 95% CI, 1.72–13.70) and to patients who had been prescribed a depot before (OR, 2.76; 95% CI, 1.17–6.49). There was no significant difference in the other characteristics.

DISCUSSION

In our study, chronic antipsychotic drug users who switched to a depot formulation, more often were male, had more frequently used anticholinergic drugs before, had less often used psychotropic comedication before, and had more gaps in their antipsychotic prescription history compared with those who switched to an oral antipsychotic. Furthermore, depot antipsychotics were predominantly prescribed to patients who had used depot antipsychotics before. Users of RLAI had similar profiles as FGA depot users, except for 2 characteristics. Patients who used a depot antipsychotic before, and who consulted a psychiatrist rather than a GP, were more likely to be prescribed RLAI than FGA depot.

Some potential limitations of our study should be mentioned. We used gaps of 3 months or more in antipsychotic drug history as a proxy for medication compliance. This is, however, a somewhat dual parameter, because it can point to noncompliance or it can point to (re)hospitalization. Data of hospital prescriptions, however, were not available. Nevertheless, both causes of a gap indicate deterioration of the patient. Another reason for a gap in prescription could be the result of "targeted treatment" in which a patient stops his medication after a certain psychosis-free period and starts again when he or she has a new psychosis. A gap from this cause may be indicative of a temporary improvement of the patient. However, we consider a gap of 3 months or more on average being a measure of noncompliance. Unfortunately,

unambiguous information on compliance to therapy and underlying disease cannot be derived from pharmacy prescription data. Furthermore, we were unable to assess other relevant prognostic clinical characteristics such as the number of prior psychotic episodes, direct clinical measures such as the Clinical Global Impression scale or Positive and Negative Syndrome Scale, or the reason for switching. Although we had no data on the diagnoses of the patients in our study, the far majority must have been experiencing schizophrenia (20). Finally, we limited the study population to those subjects receiving antipsychotic medication during a 2-year period to select chronic users. Because first-episode patients who have been clinically stable for 1 year may have undergone a trial of discontinuation of antipsychotics, which is put forward as an option in Dutch guidelines, these patients may be underrepresented in our study.

Our results indicate that depot antipsychotics are prescribed to patients who had adherence problems toward their oral medication and more frequent or more severe extrapyramidal side effects. These findings are in line with our a priori expectations. First, following the guidelines (17), extrapyramidal side effects are one of the main reasons to change antipsychotic treatment. One of the possible benefits of a depot antipsychotic is that a stable, low dose can be sustained, with less side effects as a result (16, 21). Indeed, the higher prescription rate of anticholinergics in the 3 months before the switch in users of depot is in line with this notion. Second, a gap in prescription history may be a sign of noncompliance, which is the main reason to switch to a depot antipsychotic. With a depot antipsychotic, compliance to therapy can be improved (11–15). Thus, a higher number of prescription gaps is what we expected in the group of new users of depot antipsychotics compared with oral antipsychotics. Interestingly, male patients are also more likely to be prescribed depot antipsychotics than female patients. An explanation could be that men with schizophrenia have a poorer prognosis and outcome than women (1), needing more different medication strategies. It can be hypothesized that men are thought to have a lower compliance than women, although this cannot be confirmed by empirical evidence (22). One could expect that new users of depot antipsychotics are not only less compliant patients but also more severely ill patients than new users of oral antipsychotics. However, this expectation was not supported by our data because the use of psychotropic comedication was not associated with switching to depot antipsychotics.

The difference in prior use of depot between RLAI and FGA depot users suggests that RLAI is especially prescribed to patients not responding satisfactorily to FGA depot, the latter being suggestive of more severe illness. Also, patients with schizophrenia treated with first generation depot antipsychotics have been shown to use more alcohol and illicit substances and to show higher levels of psychopathology (23). Our finding that switching to RLAI is more likely than to FGA depot when a specialist is the prescriber is also in line with channeling of RLAI to the more severely

ill patients. An alternative explanation for the difference in prescriber between FGA depot and RLAI that cannot be excluded is that specialists were more familiar with the existence of RLAI after its introduction than were GPs. Also corroborating our findings is the study of Niaz and Haddad (24), where patients prescribed RLAI had significantly higher baseline rates of drug misuse, unemployment, and forensic markers than control patients prescribed oral antipsychotics. The increasing evidence suggesting that RLAI is channeled to the more severely ill patient may have important consequences for the validity of comparisons between RLAI and other groups of antipsychotics in observational studies (25).

In our study, a relatively small number of chronic antipsychotic users switched to a depot antipsychotic. This concurs with observations by others (21, 26) that depot antipsychotics, despite their potential advantages, are still not much prescribed in the treatment of schizophrenia. This limited use of depot antipsychotic medication may be due to the introduction of the oral SGAs in the 1990s (21), leading to a less awareness of its diminished relapse rates, its reduced durations of hospitalizations (11, 16), and its well acceptance by experienced patients (27). Heres et al (26) showed in their study on attitudes of psychiatrists toward antipsychotic depot medication that the main reason not to choose a FGA depot was the fear of extrapyramidal side effects. The main reason for not prescribing RLAI was the assumed sufficient compliance with an oral SGA.

In conclusion, depot antipsychotics are preferentially prescribed to patients with adherence problems and more extrapyramidal side effects, as compared with oral antipsychotics. This is in accordance with therapeutic guidelines (17). Our data further indicate that, within depot users, RLAI is largely channeled to the more severely ill patients who tried a depot before, that is, RLAI is used as a last resort for many users. These observations could have important consequences for interpreting observational comparisons between groups of antipsychotics.

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Chapter 2.2

Diffusion of a New Drug: a Comparative Analysis of Adoption, Treatment Complexity, and Persistence of Risperidone Long-acting Injectable Therapy in the Netherlands

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ABSTRACT

Introduction: The study's objective was to analyze the adoption and persistence of risperidone long-acting injectable (RLAI) therapy after its introduction in the Netherlands in 2003 compared with the adoption and persistence of existing first-generation antipsychotic (FGA) depot drugs as an example of the diffusion of a new drug in the Netherlands.
Methods: Data on antipsychotic use were obtained from the InterAction DataBase (IADB.nl), a database containing pharmacy dispensing records of patients in the northern Netherlands, from May 20, 2003, to December 31, 2006. Treatment complexity for patients prescribed RLAI was analyzed on the basis of psychotropic comedication at baseline and during treatment, as well as on the number of previous antipsychotic therapies. Differences in treatment complexity between patients using RLAI and those using FGA depot drugs were estimated using parametric regressions. To evaluate persistence, survival analysis techniques were applied to estimate the probability of patients continuing the use of RLAI or FGA depot drugs over time.
Results: Data on 435 patients who were treated with depot antipsychotics were extracted from the IADB.nl. Patients had a mean (SD) age of 40.7 (13.8) years, and 65% of them were male. The results of this analysis indicated that persistence for patients prescribed RLAI was significantly lower compared with other depot antipsychotics (RLAI vs zuclopenthixol, $P = 0.002$; RLAI vs all other depot antipsychotics, $P = 0.009$). At the initiation of treatment, patients prescribed RLAI had more previous psychotropic comedication and had, on average, ~5 and ~1.5 times more prior depot drug therapies compared with zuclopenthixol and any other FGA depot drug, respectively.
Conclusion: The findings of this study suggest that RLAI has been prescribed more often for difficult-to-treat patients than have other available depot antipsychotics. This may explain the low adoption and poor persistence observed in the first few years after the introduction of RLAI. Further research with more extensive data should be pursued to obtain better understanding of the current diffusion of RLAI in daily clinical practice.

INTRODUCTION

Over the past several decades, increasing research attention has been focused on the diffusion process of new drugs in the pharmaceutical market. Some studies centered on analyzing the effects of product, marketing, and pricing characteristics on the diffusion process (1, 2), while others have focused on the effects of clinical implications of daily drug use. Comedication, adverse drug reactions, the *channeling effect* (the selective prescribing of new drugs to the most severe patients in whom treatment with older drugs has failed), established treatment guidelines, and negative scientific evidence may affect the diffusion process significantly (3) and could lead to drug withdrawal from the market (4). In this respect, postmarketing analysis is of critical importance.

In the antipsychotic market, Hamann et al (5) investigated the recent entrance of aripiprazole to the market and its early adoption by German psychiatrists. In this study, semistructured interviews of 50 German hospital psychiatrists were performed 3 months before and 3 months after the launch of aripiprazole in the German market, to examine the psychiatrists' awareness, perceptions, and prescribing patterns of the drug. Adoption was found to be nearly instantaneous, attributed mainly to heavy marketing campaigns and increased public demand. The study serves as an illustration of the rapid implementation of an innovative drug. Valenstein et al (6) evaluated, through generalized estimating equations on detailed registry data from the Veteran Affairs (VA) National Psychosis Registry, the influence of patient demographic (eg, age, sex, race) and clinical characteristics (eg, diabetes, hospitalization) on the diffusion of ziprasidone in the VA population after its introduction in 2002. This study reported that physicians were possibly more eager to prescribe ziprasidone to patients with more severe psychiatric disorders, indicating that the new treatment may have been reserved for patients in whom other treatments had already failed. Finally, research by Domino et al (7) presented a more theoretic approach to the factors affecting antipsychotic drug diffusion. Through an extended literature review of studies related to the parameters influencing the diffusion of new antipsychotic medications, the authors concluded that patient, insurance, and market characteristics seem to be the main factors that affect diffusion, but they do so in an unknown proportion of patients and in an unpredictable pattern.

Schizophrenia, antipsychotics, and risperidone long-acting injectable therapy

Schizophrenia is a devastating disease that affects ~0.5% of the population worldwide (8). It affects patients' ability to function in daily life and therefore presents a major challenge for public health, with physical, social, and economic consequences. Schizophrenia ranks among the 10 major causes of disability in

developed countries (9). Mortality is also relevant for the disease, as the lifetime suicide risk in patients with schizophrenia is ~10% (10).

The most effective treatment for schizophrenia is antipsychotic drug therapy (11, 12). Antipsychotic drugs are classified into 2 groups: typical or first-generation antipsychotics (FGAs), and atypical or second-generation antipsychotics (SGAs). The 2 groups differ in several ways, particularly in their profiles of adverse drug reactions. The most prevalent adverse drug reaction reported in FGA therapy is extrapyramidal adverse events; SGAs are more often associated with metabolic adverse events (13, 14).

Long-acting depot antipsychotics are characterized by consistent drug delivery and related reduced risk of overdose. They also enhance adherence, preventing the occurrence of nonadherence-related relapses (15). While several FGAs are available in a depot formulation (eg, haloperidol, zuclopenthixol), risperidone long-acting injectable (RLAI) is the first SGA depot drug to be approved in the Netherlands and subsequently approved by the Dutch Ministry of Health, Welfare and Sport to be introduced into the Dutch drug reimbursement system.

Owing to the full reimbursement of RLAI, it is expected that patients will be indifferent regarding the financial cost of their treatments. Physicians are also expected to not be influenced by price differences, because there is no incentive for them to prescribe less expensive drugs unless they are personally costconscious. Therefore, the prices of the various antipsychotic formulations were not expected to have an effect on the utilization patterns analyzed in the present study.

PATIENTS AND METHODS

Research questions and general approach

Because RLAI is the first SGA depot drug available in the Netherlands, it is a potentially valuable addition to the treatment of schizophrenia (16). However, little is known about the adoption of this drug in daily clinical practice. The present comparative analysis, conducted nearly 5 years after the introduction of RLAI to the Dutch market in May 2003, evaluated the adoption of this drug into daily clinical practice in the northern Netherlands. The research question was: Did adherence of patients treated with RLAI differ from that of patients treated with FGA depot drugs? To answer this question, we evaluated the response of patients and prescribers to the introduction of RLAI by examining treatment persistence and complexity in patients prescribed RLAI compared with those using FGA depot drugs. The following factors were analyzed: the number of previous therapy switches (to provide an indication of treatment complexity); psychotropic comedication of patients prescribed depot at treatment initiation as well as during

treatment (to further investigate treatment complexity); and the persistence of patients prescribed RLAI compared with those using other depot antipsychotics.

Outpatient pharmacy data were retrieved from the InterAction DataBase (www.iadb.nl) (17) which contains pharmacy prescriptions for the entire population of the major cities and some regional centers in the northern Netherlands. The population covered by the IADB.nl is ~500,000 persons (of ~16,500,000 total Dutch inhabitants). IADB.nl has been used in previous research on antipsychotic drug use (18). The database allows analyses at both the prescription and the patient levels.

Patients receiving antipsychotic depot treatment for schizophrenia were selected by searching the database for prescriptions for depot antipsychotics. Patients with concomitant lithium use were excluded, as they were assumed to have bipolar disorder rather than schizophrenia (19). Further distinction between users of antipsychotic medication was not possible because the IADB.nl lacks information on diagnostic indications. Patients who filled only 1 RLAI prescription were excluded from the analysis, as RLAI reaches its therapeutic level only after at least prescriptions (21 days). Patients who were prescribed FGA depot drugs only 1 time were also excluded from the analysis to achieve comparable data.

The time interval of the analysis spans from the date of the first RLAI prescription in the IADB.nl (May 20, 2003) until the last possible follow-up date (December 31, 2006). Each drug group includes only those patients who started use of the respective antipsychotic depot drug after the date RLAI became available.

Categorization

Prescriptions for antipsychotic depot formulations were categorized into 3 groups. Patients prescribed RLAI formed 1 group, and patients prescribed FGA depot drugs were divided into 2 groups: those taking zuclopenthixol depot prescriptions and those taking other FGA depot prescriptions (bromperidol, flufenazine, flupentixol, fluspirileen, haloperidol, and perphenazine).

Treatment complexity, adoption, and channeling

To investigate whether there was a channeling of RLAI to more difficult-to-treat patients, the differences in treatment complexity among patients prescribed depot antipsychotics were analyzed. The mean number of antipsychotic medications taken between the date of first identified antipsychotic prescription in the database and the date of initiation of depot treatment was identified, as was the amount of psychotropic comedication prescribed during treatment with a depot formulation. Comparisons were made within the RLAI group (early vs later users) as well as between the RLAI group and the 2 groups of patients using FGA depot drugs. Our

inference of treatment complexity was based on prescription information, which included identifying patients who were difficult to treat and who experienced adverse events while taking FGA depot drugs, as well as those with actual comorbidities (eg, depression).

First, an inception cohort was constructed for every drug group considered, including those patients whose depot antipsychotic treatment was initiated during the study period. To avoid biased estimates, patients who had no history of prescribed medication of any type before their first antipsychotic prescription were omitted. A *switch* was defined as any change of treatment between antipsychotic drug groups, but not between formulations of the same drug (eg, a change from risperidone oral to risperidone depot would not be considered a switch). The number of therapy switches before the first depot antipsychotic prescription for each patient was measured. The significance of the difference in the number of therapy switches between drug groups was statistically tested using a Poisson regression.

To test the hypothesis that the first patients prescribed RLAI were the more difficult-to-treat ones, 2 RLAI user cohorts were created. The first cohort consisted of all patients with an RLAI prescription within the first 6 months after the introduction of the drug. The second cohort included RLAI users with a first prescription ≥6 months after the introduction of RLAI. Poisson regression was applied to estimate the difference between the mean number of previous therapy switches for the 2 cohorts.

Additionally, concomitant psychotropic drug use (eg, anxiolytics, sedatives/hypnotics, antidepressants) was analyzed for the time period just before and during treatment with depot antipsychotics as an indicator of treatment complexity. The number of defined daily doses (DDDs) was counted for coprescribed psychotropic drugs in the year before the initiation of depot antipsychotic use. The DDDs per year of coprescribed psychotropic drugs after the patient began depot drug therapy was also measured. If a depot antipsychotic was used for >1 year, the mean number of DDDs per year was used for analysis. For patients who were followed for <1 year after starting depot antipsychotic use, concomitant psychotropic drug use for the entire year was extrapolated to achieve comparable data. All patients initiating depot antipsychotic use in the last 3 months of the study period, or who had a duration of treatment <3 months, were excluded from the comedication analysis to avoid nonvalid extrapolations.

Because of the high proportion of patients with no comedication in some of the psychotropic drug groups, the analysis was divided. One analysis was conducted of the proportion of patients who received comedication, and one was conducted of the number of comedication DDDs. First, the percentage of patients who received psychotropic comedication in each drug group was calculated. Then, the differences between these percentages among drug groups were analyzed using logistic

regression modeling, correcting for patient demographic characteristics and previous SGA use.

As the numbers of DDDs of psychotropic comedications within the drug groups were not always normally distributed (often skewed to the right), they were first transformed according to the appropriate distribution and then the expected value of the respective distribution was calculated as an estimate of the mean (20). Parametric regression models were used to test the significance of the difference in concomitant psychotropic drug use for the different depot medications (21, 22). The generalized *F* distribution was assumed for the error term of the models. The flexibility of this distribution enabled reduction in the dependence of the model on the specific assumption for the error distribution (23). Although the tests of significance were performed on the transformed data, the estimates of the mean are presented in the original scale (21).

Based on the concomitant drug use of participants in the Clinical Antipsychotic Trials of Intervention Effectiveness study (24), it was suggested that gender characteristics and previous SGA use may be predictors of concomitant psychotropic drug use. Therefore, the number of SGA DDDs for every depot antipsychotic user for the year before initiation of depot treatment was calculated. This information was included together with demographic information for every patient as control variables in the regression models.

Persistence

Persistence was defined as days of medication use, calculated as the period between the first and the last prescription plus the duration of the last prescription. All prescriptions were assumed to appropriately follow the 2008 Dutch pharmacoeconomic guidelines from the Health Insurance Executive Board (25). The persistence of patients receiving RLAI was compared with that of patients receiving FGA depot drugs, by applying survival analysis techniques. Kaplan Meier curves were used to visualize potential differences in the probability of a patient remaining on a specific drug over time (26). Comparisons between persistence for different groups were made using accelerated failure time (AFT) models (27). The AFT models included an indicator variable, distinguishing between the different drug groups, along with covariates controlling for demographic characteristics of the patients. Propensity scores were added in the AFT models to correct for the nonrandom, uncontrolled assignment of patients to different depot antipsychotics (28). The propensity scores were calculated using a logistic regression that included, as covariates, user demographic information (age and sex), type of prescribing physician at the initiation of treatment (general practitioner or specialist), and amount of concomitant psychotropic drug use (anxiolytics, antidepressants, and/or sedatives) before first depot antipsychotic use. Patients who were still active users at

the end of the study period and patients who were lost in follow-up for other reasons (eg, died, moved) were considered as censored. The software R, version 2.10.0 (R Development Core Team, Vienna, Austria), was used for the statistical analysis.

RESULTS

In this study period, the IADB.nl included 313,191 prescriptions for antipsychotic drugs for 17,746 patients. Of these prescriptions, 2816 were for depot formulations. These prescriptions were addressed to 435 patients and were prescribed by 135 different physicians.

Adoption

The study sample included 192 patients using RLAI, of which 10 patients had concomitant prescriptions for lithium (Table 1). The number of concomitant lithium users was approximately the same across all depot drug users, ranging from 3% to 10%. Patients were started on treatment with RLAI, or were switched to it, by 46 different physicians. A similar number of physicians (42 and 48) were responsible for treatment initiation with zuclopenthixol depot or with any other FGA depot drug, respectively. Forty-three of 192 patients received an RLAI prescription only once. None of the patients prescribed RLAI had concomitant prescriptions for another depot formulation.

Treatment complexity

The mean number of previous antipsychotic therapy switches for the 2 groups of RLAI patients was analyzed. Patients in the first group started RLAI therapy within the first 6 months after market introduction of RLAI; patients in the second group started ≥6 months after the introduction of RLAI. Poisson regression analysis yielded a significant difference between the 2 groups. Patients prescribed RLAI within the first 6 months had a mean of 2.11 previous different types of antipsychotic medication, whereas the second group had 1.44 ($P = 0.008$). This may indicate that, over time, the prescription pattern for RLAI changed from predominantly difficult to-treat patients to less difficult-to-treat patients.

The number of previous therapy switches for patients prescribed RLAI was compared with that of patients prescribed other depot formulations. Poisson regression found that there was not enough evidence to reject the hypothesis that patients receiving RLAI had the same number of previous antipsychotic therapy switches (1.64 switches per patient) compared with patients receiving zuclopenthixol (1.56 switches per patient; P = NS). However, a significant difference was found for the comparison against the patients taking any other depot antipsychotic (2.06 switches per patient; $P = 0.03$).

Table 1. Demographic characteristics of patients per depot antipsychotic drug in the study analysis period from May 20, 2003 through December 31, 2006.

Depot Drug	New Depot Patient Records	Excluded Lithium Patient Records	Excluded One-Time Users	Analyzed Records Sample	Male, %	Age, Mean (SD), y
RLAI	192	10	43	139	69	39.5 (12.9)
Zuclopenthixol	139	4	43	92	65	40.4 (14.0)
Haloperidol	63	2	29	32	68	45.3 (16.5)
Other depot drugs	87	5	21	61	51	41.7 (12.9)
All FGA depot drugs	289	11	93	185	61	41.9 (14.3)

RLAI = risperidone long-acting injectable; FGA = first-generation antipsychotic.

Table 2. Comedication before and after switching to risperidone long-acting injectable (RLAI) or first generation antipsychotic (FGA) depot drug.

	Anxiolytics		Sedatives		Antidepressants	
	DDD*	%†	DDD*	%†	DDD*	%†
Before RLAI	282	41	223	28	396	24
After RLAI	289	50	268	30	386	24
Before FGA	158	34	82	17	192	18
After FGA	265	49	109	26	314	25
Statistical comparisons of differences, *P*‡						
Before vs After RLAI	0.582	0.160§	0.604§	0.756§	0.502§	0.998§
Before vs After FGA	0.018§	0.011§	<0.001§	0.050§	0.011§	0.165
Before RLAI vs Before FGA	0.242§	0.525§	0.036§	0.045§	0.177	0.574§
After RLAI vs After SGA	0.835§	0.810§	0.029§	0.492§	0.497	0.658§

DDD = defined daily dose; SGA = second-generation antipsychotic.

*The mean of the number of DDDs per year per user.

†The percentage of users prescribed the comedication.

‡The *P* values correspond to the coefficients from the parametric and logistic regressions applied in the concomitant drug use comparisons and the percentages of use comparisons, respectively. All regressions were corrected for age, sex, and prior SGA use.

§Comparisons where the coefficient of prior SGA use was positive and statistically significant at the 5% level.

To further investigate the possibility that RLAI users might be more difficult to treat, these patients' previous experiences with depot medications were evaluated. Patients treated with RLAI had used significantly more depot formulations (0.48 switch per patient) compared with patients treated with zuclopenthixol (0.10 switch per patient; $P < 0.01$) or other depot antipsychotics (0.29 switch per patient; $P = 0.04$).

Comedication

Table 2 presents the differences in psychotropic comedication before and after the start of depot antipsychotic use. Among patients using RLAI, no statistically significant increase in psychotropic comedication was observed before versus after the initiation of treatment. In contrast, patients using FGA depot drugs received a significant increase in the prescription of anxiolytics ($P = 0.011$) and sedatives ($P = 0.050$) after initiation of the depot drug, as was expected due to the adverse-event profile of this class. There was also a significant increase in the number of DDDs after initiation of FGA depot drug use for all psychotropics studied (anxiolytics, $P = 0.018$; sedatives, $P < 0.001$; antidepressants, $P = 0.011$). Of the RLAI and FGA patients, 37% and 29%, respectively, had a follow-up of <1 year and required extrapolation of psychotropic comedication to the entire year.

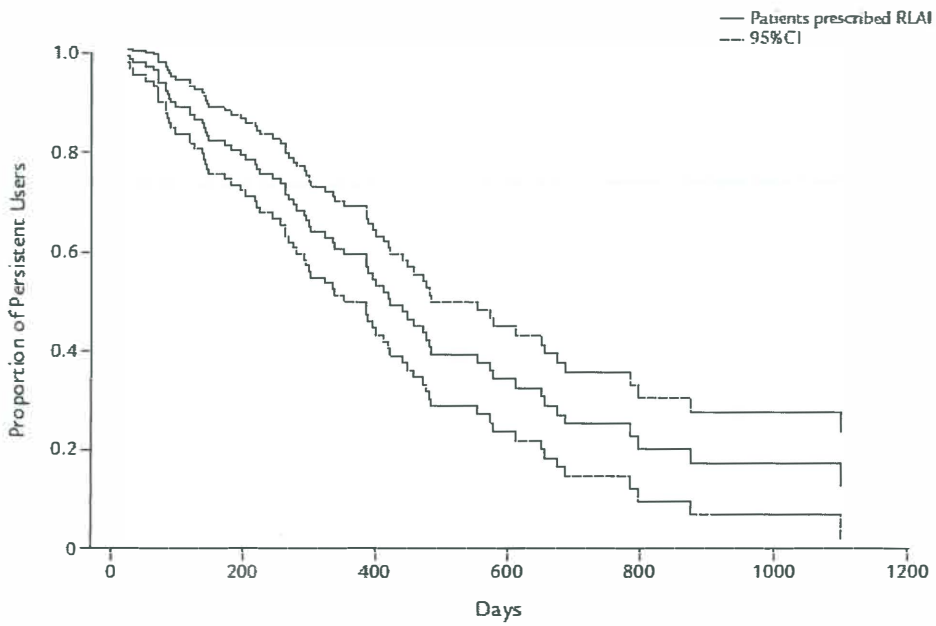
Table 2 also presents the differences in psychotropic comedication patterns between patients using either RLAI or FGA depot drugs. The data revealed that patients using RLAI received more psychotropic comedication than did patients using FGA depot drugs, before the initiation of RLAI or FGA depot treatment. However, this difference in comedication was only statistically significant for the proportion of patients prescribed sedatives and the corresponding number of DDDs ($P = 0.036$ and $P = 0.045$, respectively) and appeared to decrease after the initiation of depot antipsychotic treatment in the 2 groups.

The differences in the amounts of psychotropic comedication seemed to be largely influenced by previous SGA use. The covariate added in the regressions to correct for prior exposure to SGA medication was almost always positive and statistically significant, indicating a positive relationship between SGA use and the amount of psychotropic comedication (Table 2).

Persistence

Figure 1 presents the persistence over time of patients prescribed RLAI. Eighty percent of patients using RLAI were persistent for at least 180 days, with a median duration of treatment of 420 days. Of all patients in the analysis, 51% were censored.

Figure 1. Persistence of patients prescribed risperidone long-acting (RLAI) therapy

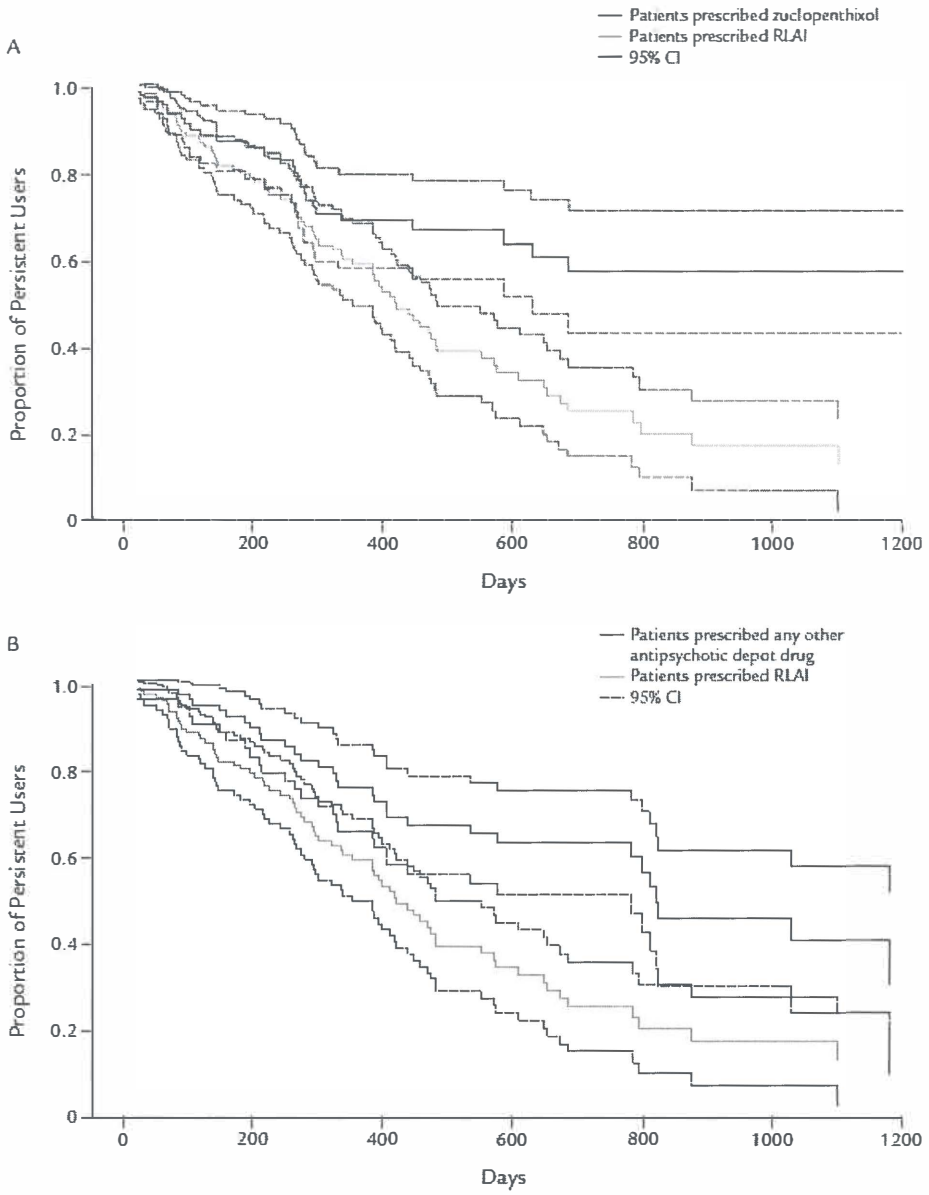


The Kaplan-Meier curves in Figure 2 present the persistence of patients prescribed RLAI versus the persistence of patients prescribed zuclopenthixol depot therapy (92 patients, 29% censored; median estimated treatment duration, 1066 days) and the other forms of depot antipsychotic drugs (93 patients, 33% censored; median estimated treatment duration, 883 days). These data, together with the significant drug group coefficients of the AFT comparison models (RLAI vs zuclopenthixol, P = 0.002; RLAI vs all other depot antipsychotics, P = 0.009), provide evidence to indicate more persistence for all FGA depot drugs compared with RLAI.

DISCUSSION

In the present study, some aspects of RLAI adoption for treating schizophrenia in daily clinical practice in the Netherlands were analyzed. Comparison of previous therapy switches for patients prescribed RLAI versus those prescribed FGA depot drugs found a statistically significant difference in possible indicators of treatment complexity against those patients prescribed non-zuclopenthixol FGA depot drugs. However, care should be taken in the interpretation of this difference, because it may be of minor clinical importance due to its small magnitude. There was also a noteworthy positive relationship between RLAI use and overall experience of depot

Figure 2. Persistence of patients prescribed risperidone long-acting injectable (RLAI) versus patients prescribed (A) zuclopenthixol and (B) any of the other antipsychotic depot drugs.



antipsychotic use. It may be more likely for a patient currently using a depot antipsychotic to be switched to RLAI as an alternative depot drug than for a first-time depot antipsychotic patient to be initiated on RLAI. Patients who were prescribed RLAI immediately on its introduction to the market had a greater number of previous therapy switches, possibly indicating greater treatment complexity in these patients. Therefore, our findings support the hypothesis that patients initiated on depot-drug therapy with RLAI may be generally more difficult to treat or may have faced more intolerable adverse events than have patients starting treatment with FGA depot drugs.

Analysis of psychotropic comedication revealed that patients starting treatment with RLAI received more previous psychotropic comedication than did patients starting treatment with FGA depot drugs. This difference was also influenced by prior exposure to SGA medication. However, after the initiation of treatment, this difference disappeared because of increased comedication use in patients prescribed FGA depot drugs. Psychotropic comedication use is related to the adverse events associated with antipsychotic drugs. Both the FGAs and risperidone have been associated in the literature with anxiety, depression, weight gain, and movement disorders (29).

It is necessary to note some limitations of this analysis of psychotropic comedication use. Along with the number of DDDs, the percentage of patients who used psychotropic comedications was analyzed before and after initiation of a depot antipsychotic. This percentage is likely to be underestimated for the first year after the start of antipsychotic depot therapy, because patients who first used a depot drug in 2006 (the final year of the study) had a lower probability of receiving psychotropic comedication than did others who had at least 1 full year of use during the study time frame. There was also an overestimation of this same percentage: patients who used a depot antipsychotic for >1 year had a higher probability of receiving comedication. Thus, the percentage of patients receiving psychotropic comedication is an imperfect estimate. However, it can still be perceived as an indication of the increase or decrease in the percentage of patients prescribed certain concomitantly used drugs.

In this analysis, patients who received RLAI seemed to be less persistent than were patients prescribed FGA depot drugs. The most plausible reason for this finding is that RLAI may be used as a last resort, which unfavorably affects persistence a priori. Another explanation could be that patients treated with RLAI may have faced intolerable adverse events and therefore discontinued treatment. A final reason for this finding could be the different market characteristics of the various drugs within the period captured by this analysis. At the time point when the estimation of persistence in this study starts, RLAI had just been introduced and the FGA depot drugs had been on the market for a much longer period. Presumably, a more

appropriate means of comparing persistence with RLAI would be against persistence with the other depot antipsychotic drugs at the time of their introduction. Unfortunately, this was not possible because the introduction of these drugs occurred before the establishment of the IADB.nl.

A further limitation of the study is that the IADB.nl does not include prescriptions for medications dispensed in hospitals and psychiatric clinics, particularly not those prescribed by outpatient depot clinics. Therefore, no historical information exists as to whether patients were initiated on treatment in a clinic before their appearance in the IADB.nl. The fact that the IADB.nl does not capture medication that is administered when patients are hospitalized might also affect current persistence estimates. It is possible that patients receiving their medication at an outpatient depot clinic may be in an earlier stage of their disease. Given that RLAI has been used thus far mainly for the most difficult-to-treat patients, this might have particularly affected the results relating to RLAI because patients may be hospitalized more often and for longer periods as the duration of their disease increases. Finally, another limitation of the IADB.nl is the lack of information on personal and demographic characteristics of the prescribing physicians that may possibly influence the selection and use of specific medications.

The conclusions on treatment complexity of patients drawn from our analysis are in accordance with prior studies conducted on treatment complexity in patients prescribed RLAI. In particular, Niaz and Haddad (30), in a mirror-image analysis of 74 RLAI and 46 control patients, found that patients prescribed RLAI had higher drug and alcohol misuse rates (44.6% vs 19.6%, $P < 0.05$; and 45.9% vs 23.9%, $P < 0.05$, respectively) at the time of therapy initiation. They reported that the most common reason for initiation of therapy with RLAI was failure of compliance with previous oral medications (seen in 56.7% of patients taking RLAI), another indicator of treatment complexity. Additionally, Paton and Okocha (31), in a small-sample descriptive study of 50 patients treated with RLAI, reported that failure to comply with other treatments and avoidance of the extrapyramidal adverse effects of FGAs were the main reasons for initiation of therapy with RLAI for 84% of the patients.

This study presents an analysis of the diffusion of a new drug in daily clinical practice. Further research is needed to detect common patterns in such diffusion, from both the methodologic and pharmacologic perspectives. One of the methodologic issues to be tackled is the possible existence of heteroscedasticity among drug groups, and how to adequately correct for it in regression analysis of concomitant drug use. Additionally, investigating other past pharmacotherapeutic introductions in the Netherlands could identify common patterns that may enable better understanding of the use and effects of new drugs, and provide possible predictions for new drug introductions.

CONCLUSIONS

To summarize the main findings of this analysis, patients prescribed RLAI seemed to be less persistent but also more difficult to treat compared with patients prescribed FGA depot drugs. In particular, patients starting RLAI treatment had previously received more psychotropic comedications as well as more depot antipsychotic therapies than did patients starting FGA depot treatment. This supports the hypothesis that a channeling effect has occurred, with RLAI being reserved for more difficult-to-treat patients.

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Chapter 3.1

Association of Genetic Variants of the Histamine H1 and Muscarinic M3 Receptors with BMI and HbA1c Values in Patients on Antipsychotic Medication

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ABSTRACT

Introduction: Antipsychotic affinity for the histamine H1 receptor and the muscarinic M3 receptor have been associated with the side effects weight gain, and development of diabetes, respectively. We investigated polymorphisms of the histamine H1 *(HRH1)* and muscarinic acetylcholine receptor M3 *(CHRM3)* receptor genes for an association with body mass index (BMI) and glycated hemoglobin (HbA1c).
Methods: We included 430 Caucasian patients with a nonaffective psychotic disorder using antipsychotics for at least 3 months. Primary endpoints of the study were crosssectionally measured BMI and HbA1c; secondary endpoints were obesity and hyperglycaemia. Two singlenucleotide polymorphisms (SNPs) in the *HRH1* gene, rs346074 and rs346070, and one SNP in the *CHRM3* gene, rs3738435, were genotyped. Our primary hypothesis in this study was an interaction between genotype on BMI and antipsychotic affinity for the H1 and M3 receptor.
Results: A significant association of interaction between haplotype rs346074–rs346070 and BMI (P = 0.025) and obesity (P = 0.005) in patients using high-H1 affinity antipsychotics versus patients using low-H1 affinity antipsychotics was found. There was no association of *CHRM3* gene variant rs3738435 with BMI, and we observed no association with HbA1c or hyperglycaemia in any of the variants.
Conclusion: This study, for the first time, demonstrates a significant association between *HRH1* variants and BMI in patients with a psychotic disorder using antipsychotics. In future, genotyping of *HRH1* variants may help predicting weight gain in patients using antipsychotics.

INTRODUCTION

The majority of patients with schizophrenia or other psychotic disorder use antipsychotic medication. Antipsychotic treatment, especially the use of clozapine and olanzapine, increases the risk of developing obesity (1-3) and type 2 diabetes mellitus (T2DM) (2, 4-9). The underlying mechanisms of antipsychotic-induced weight gain and diabetes mellitus are unknown, and may involve different pathways. As in the general population, obesity may have an unfavorable impact on glucose homeostasis in patients using antipsychotics. However, several studies have shown elevated serum insulin levels following atypical antipsychotic medication independent of body mass index (BMI) (10-12). This finding suggests that antipsychotics may directly affect glucose homeostasis by mechanisms other than by weight gain alone. There is also a considerable variability among users of the same antipsychotic in weight gain and T2DM (e.g., not all patients on clozapine ultimately develop T2DM). It is plausible that this variability in patient propensity to these side effects is determined by a combination of genetic and environmental factors.

Atypical antipsychotics may differ highly in their affinities for the dopaminergic, serotonergic, histaminergic, adrenergic, and muscarinic acetylcholine receptors (13). Combining receptor affinities and clinical data, several authors have concluded that histamine H1 antagonism showed the best correlation with drug-induced weight gain and diabetes mellitus (14-16). Likewise, antagonism of the muscarine acetylcholine receptor was suggested to play an important role, especially in the development of diabetes mellitus (14, 17). Interactions with serotonergic (5-HT2C and 5-HT6) and adrenergic (alpha1A) receptors were also significantly correlated with metabolic parameters (14, 15). To date, pharmacogenetic studies have shown the most consistent evidence for polymorphisms in the 5-HT2C receptor and leptin genes to be associated with antipsychotic-induced weight gain (18-24) and the metabolic syndrome (25-28). So far, only two studies (29, 30) have reported on histamine H1 polymorphisms and antipsychotic-induced weight gain, both finding no association. Thus, the contribution of genetic variations of the histamine and muscarine acetylcholine receptors on the emergence of weight gain and diabetes in antipsychotic-treated patients remains to be elucidated.

The ventromedial hypothalamus and the paraventricular nucleus of the brain, where H1 receptors are localized in high density (31), play a central role in the development of obesity by regulating energy expenditure and food intake (32). Clozapine, olanzapine, and quetiapine exhibit the highest affinities for the H1 receptor, whereas risperidone and aripiprazole exhibit lower, and ziprasidone and haloperidol exhibit hardly any affinity towards the H1 receptor (13, 33). Clozapine and olanzapine are also known to induce most weight gain, followed by quetiapine and risperidone. Aripiprazole, ziprasidone, and haloperidol are known to cause little or no weight gain at all (16, 33). Tricyclic antidepressants with a high antihistaminergic effect (e.g.

amitriptyline) are found to induce weight gain as well (34). The histamine H1 receptor may therefore play a role in the etiology of medication-induced weight gain.

The M3 receptor is expressed on pancreatic β cells. These receptors seem to play a critical role in regulating insulin release and glucose homeostasis (35). Impaired glucose tolerance and reduced levels of insulin were found in mice with targeted deletions in the *CHRM3* gene (35). This might indicate that antagonism of the β-cell M3 receptor leads to a higher risk of hyperglycemia and developing diabetes in humans. Olanzapine and clozapine, which have the highest binding affinities with the M3 receptor, have been associated with highest risk of developing T2DM (6, 8, 9, 36) and higher levels of glycated hemoglobin (HBA1c) and blood glucose (2, 7, 33). Risperidone, quetiapine, ziprasidone, haloperidol, and aripiprazole have weak to absent M3 receptor antagonistic activity (13, 33) and are associated with lower levels of HbA1c and blood glucose in patients (2, 33).

Out of the known H1 receptor gene *(HRH1)* splice variants, we studied two polymorphisms in the B/K variant, which is by far the most prevalent (95%) in the brain (37). Rs346070 is a single-nucleotide polymorphism (SNP) and may be functional as it is located in the exonic splicing enhancer region. SNP rs346074 is located in the transcription factor binding sites of the *HRH1* gene and may thus affect transcription rates. The muscarinic acetylcholine receptor M3 *(CHRM3)* variant rs3738435 is located in the 5′ untranslated region of the first exon. Its C allele was found to be associated with increased risk of early onset type 2 diabetes and a reduced acute insulin response in a family-based sample of Pima Indians (38).

This is, as far as we know, the first study to examine the pharmacogenetics of genetic variations in genes encoding for the histamine H1 (rs346074 and rs346070) and muscarine M3 receptors (rs3738435) in relation to BMI and HbA1c in Caucasian psychosis patients using antipsychotics. Our primary hypothesis in this cross-sectional study is an interaction between the mentioned variations on BMI and antipsychotic affinity for the H1 and M3 receptor.

MATERIALS AND METHODS

Setting

For this study, three similar psychiatric patient populations from the Netherlands were pooled. The majority of patients were from the ongoing 'Pharmacotherapy Monitoring and Outcome Survey' (PHAMOUS). PHAMOUS is an initiative from the Rob Giel Research centre, including three Mental Health Care Institutions and the University Centre of Psychiatry of Groningen. It combines a yearly somatic screening with routine outcome assessment in patients using antipsychotics included. Subjects included in this study originated from the northern part of the Netherlands. The two

other study populations have been described in detail elsewhere (25, 26, 39). In brief, these populations consisted of patients from a Department of Psychiatric Disorders of a general hospital in the North of the Netherlands (25, 26), and patients from a Mental Health Care Organisation in the West of the Netherlands (39).

Design and patients

A cross-sectional design was used to assess the association between the variants with BMI and HbA1c. Caucasian patients (northern European ancestry) were eligible for inclusion in this study when they met Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria for a non-affective psychotic disorder (schizophrenia, schizophreniform disorder, schizoaffective disorder, delusional disorder, psychotic disorder not otherwise specified (NOS)), were 18 years or older, and used one or more antipsychotics for at least 3 months.

Outcome measures

The primary endpoints of the study were BMI, calculated as body weight (kilogram) divided by height squared (square meter), and the proportion glycated hemoglobin HbA1c (percent). BMI was measured in all patients; HbA1c values were available only in the PHAMOUS population.

Determinants

Primary determinants were the genotypes of the two SNPs in the *HRH1* gene, rs346074 (G/A) and rs346070 (C/T), and one SNP in the *CHRM3* gene, rs3738435 (C/T). Other clinical and demographic (co)variables that were measured in the study were gender, age, patient population, DSM-IV-diagnosis, and antipsychotic medication used at the day of assessment.

Genotyping

The study protocol was approved by the local university hospital medical ethics committee and all participants gave their written informed consent. Genomic DNA was extracted from EDTA whole blood according to standard protocols. Genotyping of rs3738435, rs346070, and rs346074 was conducted blind to the clinical status of the patients. Fluorogenic 5′ exonuclease TaqMan® assays were applied for the genotyping (Made-To-Order assays obtained from Applied Biosystems; C2747428510, C60474110, and C2685588510, respectively). Genotyping success rates were 99% for rs346074 and 100% for rs346070 and rs3738435.

Statistical analysis

To compare BMI and HbA1c values among various users of antipsychotics (i.e., BMI in users of clozapine versus olanzapine versus risperidon versus aripiprazole versus quetiapine versus users of more than one antipsychotic) and between patients using typical versus atypical antipsychotics we applied analysis of variance (ANOVA) and Student's *t* test, respectively. We used linear regression to explore the relationship of BMI and HbA1c with the independent variables age, gender, and patient population.

Departure from Hardy–Weinberg Equilibrium was calculated by a $\chi 2$ test with 1df. We initially considered an additive model for rs346074 *(HRH1)*, and, due to the low numbers of the recessive genotype, a dominant model for rs3738435 *(CHRM3)* and rs346070 *(HRH1)*.

We first compared demographic characteristics between the genotypes of the three variants. To test our primary hypothesis, we applied linear regression to test whether genotype in users of high-affinity antipsychotics has a significantly different outcome on BMI and HbA1c than in users of low-affinity antipsychotics. We used the interaction term affinity x genotype in our model to test this association, where affinity was coded as 1 or 0 when the patient used a high- or a low-affinity antipsychotic, respectively. A pKi > 7 defined a high affinity antipsychotic for a certain receptor (33), the other antipsychotics were considered having a low affinity. We adjusted for age, gender, and patient population in our analyses. Similarly, logistic regression was used to analyze the associations with obesity (BMI > 30 kg/m2) and hyperglycemia (HbA1c ≥ 6.1% or the use of antidiabetics). Additionally, for the two *HRH1* variants haplotype analysis using the haplotype trend regression approach (40) was performed, with haplotypes inferred by the software package PHASE (41, 42). Pairwise linkage disequilibirum (LD) was tested by calculating D′, as well as r^2. All of the analyses were performed using standard software (SPSS 16.0 for Windows). The level of significance was set at 0.05, two-sided.

RESULTS

Subjects

A total of 430 subjects met the inclusion criteria. Table 1 presents their demographic, genetic, and clinical characteristics. Approximately 95% of the patients had a diagnosis within the schizophrenia spectrum, the other patients had a psychotic disorder not otherwise specified (NOS).

Table 1. Demographic, genetic, and unadjusted clinical variables of the total study sample.

Characteristic	Total study sample (n = 430)
Age, mean (range)	38.4 (18–69)
Gender	
•Male	290 (67%)
•Female	140 (33%)
DSMIV-Diagnosis	
•Schizophrenia	333 (77%)
•Schizoaffective disorder	77 (18%)
•Psychotic disorder NOS	20 (5%)
Antipsychotic medication	
•Typical	68 (16%)
•Atypical	362 (84%)
BMI (kg/m^2), mean (SD)	28.0 (5.2)
Weight category	
•Non-obese (BMI < 25)	135 (31%)
•Overweight (BMI 25-30)	157 (37%)
•Obesity (BMI > 30)	138 (32%)
HbA1c (%) (n=221)	
•Mean (SD)	5.78 (1.25)
•Hyperglycaemia (HbA1c ≥ 6.1% or antidiabetic medication)	30 (14%)
Genotype rates	
•*HRH1* rs346074 (GG/GA/AA)	182/189/55
•*HRH1* rs346070 (CC/CT/TT)	286/128/15
•*CHRM3* rs3738435 (TT/TC/CC)	276/137/17

Medication

Patients used monotherapy clozapine (21.9%), olanzapine (22.6%) or risperidon (22.1%), aripiprazole (2.3%), quetiapine (4.2%), typical antipsychotics (14.4%), or had a combination of more than one antipsychotic (12.6%). No substantial differences in BMI (range 27.4–29.3 kg/m2) were found between users of the various antipsychotics (P ANOVA = 0.58) or between different diagnoses. HbA1c values (range 5.5–6.8%) were significantly different between the various antipsychotics (P ANOVA = 0.033). Between users of typical and atypical antipsychotics, no differences in BMI and HbA1c were found (P Student's *t* test = 0.93 and 0.82, respectively). Of all antipsychotics used in our population, clozapine, olanzapine, and quetiapine were defined as high H1 receptor affinity antipsychotics, and clozapine and olanzapine as high M3 receptor affinity antipsychotics.

Association analyses

Genotype distributions were consistent with the Hardy–Weinberg equilibrium (p values 0.59, 0.88, and 1.00 for rs346074, rs346070, and rs3738435, respectively). Age (increase of 0.055 kg/m2 per year, P = 0.021) and gender (increase of 2.97 kg/m2 if female, P < 0.001) were significantly associated with BMI. Patient population was not associated with BMI. HbA1c was not associated with patient population, age, or gender. Demographic characteristics, DSM-IV-diagnosis, and antipsychotic distributions did not differ between genotype groups in all three variants.

In Table 2 the genetic associations with BMI and obesity are depicted. In users with antipsychotics with high H1 affinity, there was a non-significant increase in BMI per A allele of rs346074 and per T allele of rs346070. An opposite trend can be seen in users with a low H1 affinity antipsychotic (see Figure 1). The increased trend in BMI with minor alleles of rs346074 and rs346070 in high H1 affinity antipsychotic users was significantly different from the decreased trend in BMI with minor alleles in low H1 affinity antipsychotic users. The interaction term genotype x affinity tested significant when using an additive or recessive model for the A allele of rs346074 (P = 0.046 and 0.033, respectively), and when using a dominant model for the T allele of rs346070 (P = 0.044).

Logistic regression showed similar results regarding genotype and obesity, but even stronger and more significant. The interaction terms genotype x affinity for rs346074 (OR 2.80, 95% CI 1.23 6.37, P = 0.015) and rs346070 (OR 2.51, 95% CI 1.33–4.74, P = 0.005) were both significant. Thus, for a patient, there is a more than twoand-a-half times higher risk of obesity per minor allele of rs346074 when having a high H1 affinity antipsychotic as compared to when having a low H1 affinity antipsychotic.

Table 2. Mean BMI values and obesity proportions of genotype groups for SNPs rs346074, rs346070, and rs3738435 among 430 antipsychotic users.

Variables	No. of patients	Mean (s.d.)/ proportion			p-value β genotype	p-value β interaction genotype x affinity
HRH1 rs346074	GG/GA/AA	GG	GA	AA		
BMI	182/189/55	28.0 (5.2)	27.8 (5.3)	28.5 (5.0)	0.93	**0.046**
High aff.	83/97/28	27.5 (4.2)	27.7 (5.3)	30.1 (5.3)	0.27	
Low aff.	99/92/27	28.4 (5.9)	27.9 (5.2)	26.8 (4.0)	0.10	
Obesity	182/189/55	34%	30%	31%	0.58	**0.005**
High aff.	83/97/28	25%	30%	46%	0.14	
Low aff.	99/92/27	40%	30%	15%	**0.015**	
HRH1 rs346070	CC/CT/TT	CC	CT	TT		
BMI	286/128/15	28.0 (5.1)	28.2 (5.6)	27.4 (4.8)	0.74	**0.044**
High aff.	139/58/12	27.6 (4,7)	29.0 (5.9)	28.5 (4.2)	0.10	
Low aff.	147/70/3	28.4 (5.5)	27.5 (5.3)	22.9 (4.9)	0.22	
Obesity	286/128/15	34%	29%	20%	0.22	**0.009**
High aff.	139/58/12	28%	38%	25%	0.36	
Low aff.	147/70/3	39%	21%	0%	**0.006**	
CHRM3 rs3738435	TT/TC/CC	TT	TC	CC		
BMI	276/137/17	28.0 (5.2)	27.6 (5.2)	30.4 (5.5)	0.60	0.88
High aff.	127/57/7	27.8 (4.9)	27.8 (4.9)	30.7 (6.1)	0.33	
Low aff.	149/80/10	28.3 (5.5)	27.5 (5.4)	30.2 (5.3)	0.90	
Obesity	276/137/17	31%	32%	53%	0.15	0.56
High aff.	127/57/7	28%	32%	57%	0.16	
Low aff.	149/80/10	34%	33%	50%	0.56	

-BMI (kg/m2, mean and standard deviation) and obesity (%) are given per genotype group, separated in users of antipsychotics with low and high affinity for the histamine H1 receptor (in rs346074 and rs346070 high affinity: clozapine, olanzapine, and quetiapine) and the muscarine M3 receptor (in rs3738435 high affinity: clozapine and olanzapine).

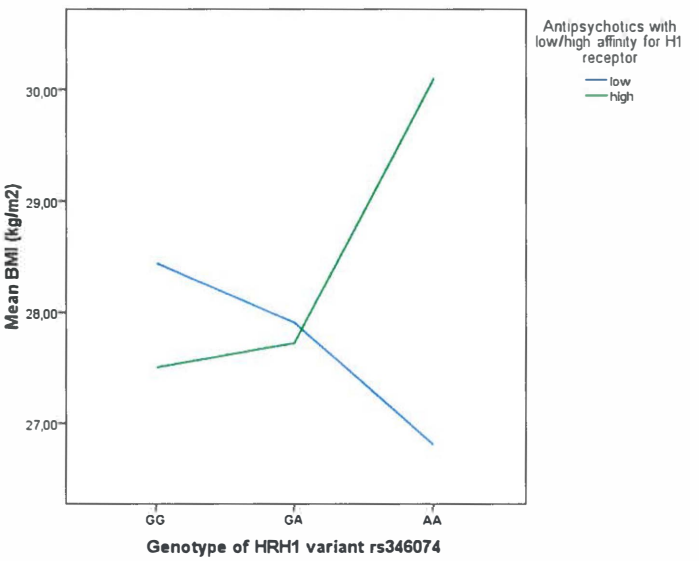
-P-values are given for 1) the β of the variable genotype in linear and logistic regression, and 2) the β of the interaction term genotype x affinity in linear and logistic regression.

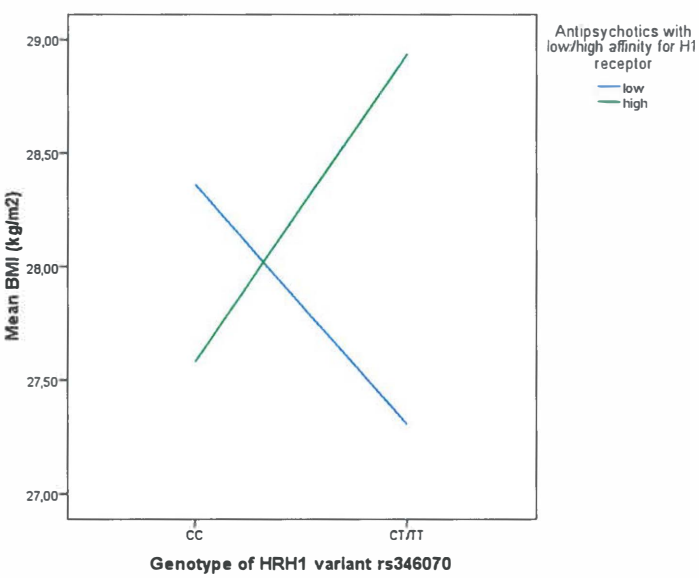
-All results are adjusted for age, gender, and population group.

-Genotype was tested additive in rs346074, and dominant for the minor allele in rs346070 and rs3738435.

-Significant P-values are shown in bold.

Figure 1. *HRH1* variants rs346074 and rs346070 and mean BMI values in users of antipsychotics with and without affinity for the H1 receptor: a significant opposite effect can be seen between genotype and BMI in users of antipsychotics with high versus low affinity for the H1 receptor.





The two *HRH1* SNPs were found to be in substantial LD (D' = 1.00, r2 = 0.42). Haplotype analyses of the two polymorphisms showed similar opposite effects of haplotype on BMI and obesity in low and high H1 affinity antipsychotic users (see Table 3). For each AT-haplotype, having a high H1 affinity antipsychotic means a more than three times higher risk of obesity (P = 0.005) compared to the reference haplotype G-C, than when having a low H1 affinity antipsychotic.

In the total sample of antipsychotic users, *CHRM3* rs3738435 had no effect on BMI. There were no differences in genotype effect on BMI between users of antipsychotics with high and low affinity for the M3 receptor. None of the three SNPs showed any association with HbA1c or hyperglycaemia (see supplemental Table 1).

Table 3. Haplotype analysis on BMI and obesity for rs346074 and rs346070 of the *HRH1* gene.

BMI	**Haplotype (rs346074-rs346070)**	**β in high H1 affinity AP users**	**P-value**	**β in low H1 affinity AP users**	**P-value**	**β of interaction term haplotype * affinity**	**P-value**
	G-C	-		-		-	
	A-C	+0.569	0.378	-0.129	0.85	0.795	0.39
	A-T	+0.941	0.104	-1.093	0.13	2.043	**0.025**
Obesity	**Haplotype (rs346074-rs346070)**	**e^{β} in high H1 affinity AP users**	**P-value**	**e^{β} in low H1 affinity AP users**	**P-value**	**e^{β} of interaction term haplotype * affinity**	**P-value**
	G-C	-		-		-	
	A-C	1.672	0.099	0.795	0.43	2.110	0.07
	A-T	1.256	0.415	0.375	**0.004**	3.331	**0.005**

-The unstandardized coefficients (β) of haplotype in linear regression with BMI and the odds ratios (e^{β}) of haplotype in logistic regression with obesity are given, in high and low H1 affinity antipsychotic (AP) users, respectively.
-Haplotypes A-C and A-T are compared with the most frequent haplotype G-C as a reference. Haplotype G-T was not prevalent.
-All results are adjusted for age, gender, and population group.
-Significant P-values are shown in bold.

DISCUSSION

To the best of our knowledge, this is the first study to examine the pharmacogenetics of histamine H1 (rs346074 and rs346070) and muscarine M3 (rs3738435) receptor variants in relationship to weight gain and hyperglycaemia as proxied by BMI and HbA1c in Caucasian psychosis patients on antipsychotics. We demonstrated significant associations between the *HRH1* gene variants rs346070 and rs346074 and BMI in Caucasian patients with a psychotic disorder when comparing users of high H1 affinity antipsychotics with low H1 affinity antipsychotics. We found no association between the *CHRM3* gene variant rs3738435 and BMI. We observed no association with HbA1c in any of the variants.

Although it has been proposed that histamine H1 receptor antagonism causes weight gain (14, 15), earlier studies on other histamine H1 receptor variants showed no relationship with clozapine induced weight gain (29, 30). Of note, post-hoc analysis in our study showed similar direction and effect size of the risk alleles on BMI in all three high H1 affinity antipsychotics studied (clozapine, olanzapine, and quetiapine), emphasizing the role of the histamine receptor.

Regarding the metabolic consequences of antipsychotic treatment, several receptors other than the H1 receptor are of importance (43), especially the 5-HT2C receptor. Previously, we have shown a significant association between 5-HT2C polymorphism rs1414334 and obesity (44) and the metabolic syndrome (25-27). The association with obesity of this polymorphism also tested significant in the present population (data not shown). We additionally included this polymorphism as a covariate in our regression analysis on obesity. This did not alter the results of the H1 polymorphisms on obesity, implying a 5-HT2C rs1414334 independent, additive effect of our H1 polymorphisms.

Within the hypothalamus, histamine and the H1 receptor are part of the leptin-signaling pathway (45, 46). Leptin is an adipocyte-specific hormone that regulates the mass of adipose tissue through hypothalamic effects on satiety and energy expenditure (47). Polymorphisms in the leptin and leptin receptor gene have been associated with antipsychotic-induced weight gain (19, 22-24). Templeman et al. (22) demonstrated that a genetic variation in the 5-HT2C receptor resulted in different pre-treatment leptin levels. Of note, an interaction between two polymorphisms in the 5-HT2C receptor and leptin gene was showed to influence the risk of metabolic disturbances during antipsychotic treatment (28). Future studies investigating gene–gene interactions between histamine H1, 5-HT2C and leptin genes may help unravel the exact role of the histamine system in antipsychotic-induced weight gain.

Since the biological function of the studied polymorphisms is unknown, one can only speculate about the observed opposite genotype effects on BMI in low and high H1

affinity antipsychotic users. One possible explanation might lay in the LD status of our polymorphisms with one or more other functional polymorphisms. It might be that one of the polymorphisms in LD with our polymorphisms has a large, H1 affinity antipsychotic induced effect, while another polymorphism in LD has a moderate opposite antipsychoticindependent effect. If our results are true-positive associations, then high H1 affinity antipsychotics should be avoided when possible in patients with risk alleles. It would be interesting for future studies to test whether these variants could predict food intake or energy expenditure as well. This might help to understand the pathways of histaminergic mechanisms for atypical antipsychotic-induced weight gain.

Next to antipsychotics, several other risk factors for hyperglycaemia are overrepresented in psychotic patients, such as a positive family history, high BMI, and reduced physical activity. It has been hypothesized that patients with schizophrenia may already have β-cell defects prior to antipsychotic treatment (48). Since several factors, involving multiple metabolic pathways, may contribute to hyperglycaemia in psychosis patients, examining genetic associations with antipsychotic-induced alterations in glucose homeostasis may be difficult to perform.

The present study has some limitations. First, we did not have complete quantitative information on the cumulative exposure to currently and previously used antipsychotics. Therefore, the relationship between BMI and users of antipsychotics with H1 affinity may be partly biased by earlier use of a previous other antipsychotic. However, since all patients used the antipsychotic for at least 3 months, we do not expect this limitation to be a serious deficit. Second, since this study is cross-sectional, we did not have information on BMI or HbA1c before antipsychotic treatment was started, suggesting that results might reflect non antipsychotic-mediated pathways. However, this is very unlikely, since we decided to test the interaction between genotype and antipsychotic affinity for the certain receptor. We found significantly different genotype effects on BMI values between users of antipsychotics with high and low affinity for the H1 receptor. Since one would expect genotype effect on baseline BMI values to be similar between future users of low and high H1 affinity antipsychotics, non-antipsychotic-mediated effects of genotype would not lead to differences in genotype effect on BMI between users with high and low H1 affinity antipsychotics. Also, genotype distributions did not differ between users of low and high H1 affinity antipsychotics, ruling out the possibility of confounding by indication because of genotype. Despite its limitations this study has also several merits. First, compared to previous studies, we have a big sample size (more than 400 patients). Second, we have a very homogeneous group of Caucasian patients of Northern European ancestry, all diagnosed with a nonaffective psychosis.

In conclusion, the *HRH1* gene haplotype consisting of rs346074 and rs346070 might be associated with BMI and obesity in patients using antipsychotics with high

affinity for the histamine H1 receptor. These findings need to be replicated in independent samples. In none of the variants an association with HbA1c or hyperglycaemia was found. Genotyping for *HRH1* variants may help predicting weight gain in patients using atypical antipsychotics. Further longitudinal studies are warranted to investigate the potential role on BMI of the *HRH1* gene.

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Supplemental table 1. Mean HbA1c values and hyperglycaemia proportions of genotype groups for SNPs rs346074, rs346070, and rs3738435 among 221 antipsychotic users.

Variables	No. of patients	Mean (s.d.)/ proportion			p-value β genotype	p-value β interaction genotype x affinity
HRH1 rs346074	GG/GA/AA	GG	GA	AA		
HbA1c	90/99/30	5.7 (1.2)	5.9 (1.4)	5.6 (0.7)	0.96	0.08
High aff.	46/56/19	5.6 (0.9)	6.3 (1.8)	5.6 (0.7)	0.36	
Low aff.	44/43/11	5.9 (1.4)	5.4 (0.5)	5.4 (0.5)	0.08	
Hyperglycaemia	90/99/30	16%	16%	7%	0.42	0.40
High aff.	46/56/19	13%	23%	5%	0.82	
Low aff.	44/43/11	18%	7%	9%	0.25	
HRH1 rs346070	CC/CT/TT	CC	CT	TT		
HbA1c	156/57/7	5.8 (1.4)	5.7 (1.0)	5.4 (0.3)	0.45	0.60
High aff.	84/31/6	5.9 (1.5)	6.0 (1.2)	5.4 (0.4)	0.83	
Low aff.	72/26/1	5.7 (1.2)	5.4 (0.5)	5.2 (-)	0.27	
Hyperglycaemia	156/57/7	17%	10%	0%	0.19	0.83
High aff.	84/31/6	19%	13%	0%	0.47	
Low aff.	72/26/1	14%	8%	0%	0.50	
CHRM3 rs3738435	TT/TC/CC	TT	TC	CC		
HbA1c	146/65/10	5.8 (1.2)	5.7 (1.2)	6.0 (1.2)	0.70	0.37
High aff.	73/32/5	5.9 (1.2)	5.6 (1.3)	5.8 (0.9)	0.29	
Low aff.	73/33/5	5.7 (1.3)	5.7 (1.0)	6.3 (1.6)	0.73	
Hyperglycaemia	146/65/10	15%	12%	20%	0.98	0.39
High aff.	73/32/5	15%	9%	20%	0.53	
Low aff.	73/33/5	15%	15%	20%	0.52	

-HbA1c values (%, mean and standard deviation), and hyperglycaemia (%, proportion) are given per genotype group, separated in users of antipsychotics with low and high affinity for the histamine H1 receptor (in rs346074 and rs346070 high affinity: clozapine, olanzapine, and quetiapine) and the muscarine M3 receptor (in rs3738435 high affinity: clozapine and olanzapine).
-P-values are given for 1) the β of the variable genotype in linear and logistic regression, and 2) the β of the interaction term genotype x affinity in linear and logistic regression.
-All results are adjusted for age and gender.
-Genotype was tested additive in rs346074, and dominant for the minor allele in rs346070 and rs3738435.

Chapter 3.2

Association between the 1291-C/G Polymorphism in the Adrenergic α-2a Receptor and the Metabolic Syndrome

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ABSTRACT

The prevalence of the metabolic syndrome is increased in patients with schizophrenia compared with the general population. The strong interindividual differences in susceptibility to developing the metabolic syndrome suggest that the genetic makeup is a modulating factor. Part of the genetic puzzle can possibly be explained by variations in the gene coding for the adrenergic α-2a receptor *(ADRA2A)* because this receptor plays an important role in lipolysis.

Three studies have found an association between the α-2a 1291-C/G polymorphism and antipsychotic induced weight gain, with conflicting results between whites and Asians. No studies have been published investigating the association between the 1291-C/G polymorphism and the metabolic syndrome.

The primary objective of this cross-sectional study was to investigate the association between the *ADRA2A* 1291-C/G polymorphism and the metabolic syndrome in 470 patients using antipsychotic drugs.

There was no significant association between carriership of the variant 1291-G allele and prevalence of the metabolic syndrome (odds ratio, 0.73; 95% confidence interval, 0.49-1.15). Exploratory analysis showed an association between carriership of the variant 1291-G allele and a reduced prevalence of the metabolic syndrome in patients not currently using antipsychotics (odds ratio, 0.05; 95% confidence interval, 0.003-0.97; P = 0.048).

In conclusion, this study shows that the *ADRA2A* 1291-C/G polymorphism does not seem to be a strong predictor for long-term occurrence of the metabolic syndrome in antipsychotic using patients. Studies investigating this association using a prospective, or retrospective, design, as well as studies investigating this association in a nonpsychiatric population, are warranted.

INTRODUCTION

It has been shown that the prevalence of the metabolic syndrome is increased in patients with schizophrenia compared with the general population (1). Although controversy exists about the causal mechanisms, it is most likely that metabolic adverse effects of antipsychotic drugs including lipid abnormalities, disturbed glucose metabolism, and weight gain are important determinants for this increased prevalence. These metabolic disturbances, like insulin resistance, can still be present for more than a year after treatment with the antipsychotic drugs has ended (2).

The mechanism behind antipsychotic-induced metabolic abnormalities is not entirely clear. The high interindividual differences suggest that genetic makeup is a modulating factor.

One of the potential genetic determinants is genetic variation in the gene coding for the adrenergic α-2 receptor, because of its effects on the breakdown of fat (lipolysis) and the fact that antipsychotics such as clozapine, which are associated with metabolic abnormalities, have a high affinity for the α-2 receptors.

Studies have shown that stimulation of the G protein-coupled α-2 adrenergic receptor leads to an inhibition of lipolysis (3). Weight loss during hypocaloric diets was associated with decreased α-2 adrenoceptor sensitivity (4).

Three different subtypes of the α-2 adrenoceptor have been discovered: α-2a, α-2b, and α-2c (5). Data from the HERITAGE Family study showed an association between the 1291-C/G polymorphism (rs1800544) in the gene coding for the α-2a receptor *(ADRA2A)* and accumulation of (predominantly abdominal) body fat (6). Black male patients carrying the variant 1291-G allele had a higher trunk-to-extremity skin fold ratio than black male patients without the variant allele. No association was found in white subjects. Recently, overexpression of the α-2a receptor and the rs553668 polymorphism in the *ADRA2A* gene have also been associated with type 2 diabetes (7).

To our knowledge, 3 studies have been published investigating the potential role of the *ADRA2A* 1291-C/G polymorphism in explaining interindividual differences in antipsychotic induced weight gain.

The study by Wang et al (8), in 93 Asian patients with a follow-up of 14 (SD, 6) months, showed that patients carrying the 1291-G/G genotype experienced more weight gain during treatment with clozapine than did carriers of the 1291-C/C genotype (8.5 [SD, 7.2] kg vs 2.8 [SD, 6.1] kg, respectively; P = 0.023). The 1291-GG genotype or carriership of the variant G allele was also associated with a more than 7% increase in body weight during treatment with clozapine (odds ratio [OR], 4.21;

95% confidence interval [CI], 1.58-11.19; and OR, 3.45; 95% CI, 1.87-6.35, respectively). The study by Park et al (9), in 62 Asian patients with a minimum follow-up of 3 months, showed that patients carrying the G allele more often experienced a more than 10% increase in body weight during treatment with olanzapine (OR, 2.58; 95% CI, 1.21-5.51). A recent study by Sickert et al (10), in 129 patients with a follow up of 6 to 14 weeks, showed that European Americans carrying the 1291-C allele gained more weight compared with subjects homozygous for the G allele (3.7 [SD, 4.1] kg vs 0.2 [SD, 2.9] kg, respectively; P = 0.01). These results suggest that ethnicity may play a role in the effect of the *ADRA2A* 1291-C/G polymorphism on antipsychotic-induced weight gain, with the 1291-G allele being protective for weight gain in whites and the 1291-C allele being protective for weight gain in Asians. A basis for this discrepancy between ethnicities may lie in a differential gene expression caused by genetic and/or environmental factors.

To our knowledge, no studies investigating the possible association between the *ADRA2A* 1291-C/G polymorphism and prevalence of the metabolic syndrome have been published.

The primary objective of this study was to investigate the association between the *ADRA2A* 1291-C/G polymorphism and prevalence of the metabolic syndrome in patients using antipsychotics. Secondary objectives were associations between the *ADRA2A* 1291-C/G polymorphism and individual parameters contributing to the metabolic syndrome as well as effects of individual antipsychotics.

MATERIALS AND METHODS

Setting

This study included patients from 3 pooled comparable patient populations. Two of these populations (n = 114 and n = 170) were used before in previous studies investigating the association between HTR2c polymorphisms and antipsychoticinduced metabolic syndrome. The study designs of these studies have been described in detail elsewhere (11-13). The third sample (n = 186) came from an ongoing Pharmacotherapy Monitoring and Outcome Survey (PHAMOUS). PHAMOUS is an initiative from the Rob Giel Research Centre, a number of mental health care institutions, and the Department of Pharmacotherapy and Pharmaceutical Care from the University of Groningen. PHAMOUS combines a yearly somatic screening with routine outcome assessment in patients using antipsychotics. Risk factors for cardiovascular and metabolic complications are monitored, and effectiveness of antipsychotic treatment is evaluated in this survey, with the goal of improving health care for people with psychosis. Patients included in this study originated from the northern part of The Netherlands. A detailed description of the study design can be found on www.phamous.eu (Dutch).

Design and patients

A cross-sectional design was used to assess the association between *ADRA2A* 1291-C/G genotype and the metabolic syndrome. Patients were eligible for inclusion in this study if they were 18 years or older and with a diagnosis of schizophrenia, schizoaffective or schizophreniform disorder, or psychotic disorder. After complete description of the study to the patients, written informed consent was obtained, and blood was drawn.

Outcome measures

Primary end point of the study was the presence of the metabolic syndrome. The metabolic syndrome was defined according to the new definition by the National Cholesterol Education Program's Adult Treatment Panel IIIa (ATPIIIa) (14). The metabolic syndrome was diagnosed in all patients when 3 or more of the following 5 metabolic criteria were met: waist circumference 102 cm or greater (male) or 88 cm or greater (female); triglycerides 1.7 mmol/L or greater or use of a fibrate; high-density lipoprotein (HDL) cholesterol less than 1.0 mmol/L (male) or less than 1.3 mmol/L (female) or use of a statin; blood pressure 130/85 mm Hg or greater or use of an antihypertensive drug; and finally fasting glucose 5.6 mmol/L or greater or hemoglobin A1c (HbA1c) greater than 6.1% or use of an antidiabetic. Hemoglobin A1c was used when fasting glucose level datawere not available. The cutoff value used for HbA1c is based on a review by Bennett et al (15). With respect to triglyceridelowering therapy or HDL-increasing therapy, a choice was made to allocate fibrates specifically to the triglyceride category and statins to the HDL category. Allocating both fibrates and statins to both triglyceride and HDL categories would have led to an overestimation of the metabolic syndrome, because treatment with a statin or a fibrate would have led to a diagnosis of the metabolic syndrome almost immediately. Secondary end points were the separate metabolic parameters as mentioned above.

Determinants

Primary determinant was the genotype of the 1291-C/G (rs1800544) polymorphism located in the *ADRA2A* gene. It should be noted with regard to *ADRA2A* polymorphism nomenclature that, for reasons of clarity, we use the nomenclature and nucleotide numbering at the genomic level according to the guidelines of the Human Genome Variation Society (www.hgvs.org) as well as the "traditional" nomenclature and numbering used in previous publications. We regarded the 1291-G allele as the dominant allele, based on the studies by Wang et al (8), Park et al (9), and Sickert et al (10).

DNA isolation and genotyping

Genomic DNA of patients was isolated from EDTA blood using the X-tractor Gene (Corbett Robotics; Corbett Life Science, Westburg, Leusden, The Netherlands) with X-tractor Gene Liquid Sample Reagent Pack (XTR1, Sigma-Aldrich, Westburg, Leusden, The Netherlands).

Rs1800544 was determined with allelic discrimination using a predeveloped assay (C_7611979_10; Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands) according to a standard protocol provided by Applied Biosystems. The reaction was carried out in TaqMan universal polymerase chain reaction master mix (Applied Biosystems) in a TaqMan 7500 apparatus. The genotyping of this assay was conducted blind to the clinical status of the patients.

Data analyses and statistics

The association between the metabolic syndrome or the individual metabolic parameters and the *ADRA2A* genotype, or presence or absence of the *ADRA2A* 1291-G allele, was investigated with logistic regression. Data were investigated for potential confounding effects of age, HTR2c rs1414334 and rs3813929 genotypes, ethnicity, Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) diagnosis, sex, and antipsychotic drugs prescribed. We included these variables in the multivariate model if they were univariately associated with the primary end point metabolic syndrome at a significance level of $P < 0.20$ (16). Unless otherwise stated, results are expressed as adjusted ORs. More exploratory analyses included a stratified analysis for individual antipsychotic drugs used at the moment of evaluation as well as an investigation of the association between the 1291-C/G polymorphism and the metabolic syndrome in a small group of schizophrenic patients not currently using antipsychotic drugs. $P \leq 0.05$ or less was regarded as significant. Data were analyzed using SPSS 17.0 (SPSS Inc, Chicago, Ill).

RESULTS

In total, 497 patients were recruited for this study. Twentyseven patients did not take any antipsychotic drug at the moment of evaluation, and data from these patients were used only in the exploratory analysis. Therefore, the data from 470 patients were used for primary data analysis. Most patients were male (68%), with a diagnosis of schizophrenia (78%) or schizoaffective disorder (17%), with a mean age of 38 (SD, 10) years. Prevalence of the metabolic syndrome was 39%.

Of these patients, 442 were of white origin, 14 were of Asian origin, 9 were of African origin, and the remainder was of unknown origin. Olanzapine (n = 106 [23%]), risperidone (n = 103 [22%]), and clozapine (n = 102 [22%]) were the most frequently

prescribed atypical antipsychotic drugs. The remaining 23% of the patients used aripiprazole (n = 21), quetiapine (n = 12), or typical antipsychotics (n = 69) or used a combination of antipsychotics (n = 57).

The *ADRA2A* 1291-CC genotype was most prevalent (51%), followed by the 1291-CG (41%) and 1291-GG genotypes (8%). Genotype frequencies of the 1291-C/G polymorphism did not deviate from those expected under Hardy-Weinberg equilibrium (P = 0.59). Ethnicity, DSM-IV diagnosis, carriership of the variant HTR2C rs1414334 C allele, age, sex, and prescribed antipsychotic drug were associated with the metabolic syndrome at a P < 0.20 significance level and were therefore included as covariates in the multivariate analysis. We did not find any confounding effects of statin and/or fibrate use. Multivariate analysis using only the data from patients of white origin did not influence the results either.

Table 1 shows that the *ADRA2A* 1291-C/G polymorphism was not significantly associated with an increased risk for the metabolic syndrome in patients using antipsychotics. Analysis based on genotypes as well as carriership of the variant G allele did not show a significant association with the metabolic syndrome, although the point estimate decreases by the number of variant 1291-G alleles.

Table 2 shows that an analysis of the association between the *ADRA2A* polymorphism and the 5 components of the metabolic syndrome showed a trend for an association with lower triglyceride levels. Carriership of the variant G allele was protective for reaching the triglyceride cutoff point of 1.7 mmol/L (OR, 0.67; 95% CI, 0.44-1.00; P = 0.05).

Table 1. *ADRA2A* 1291C/G genotype and metabolic syndrome.

Genotype	Patients (n=408)	Metabolic Syndrome	Crude OR [a] (95% CI, P)	Adjusted OR [a,b] (95% CI, P)
Patients with antipsychotics				
1291-CC	215	43%	1	1
1291-GC	165	36%	0.74 (0.49-1.13; 0.17)	0.77 (0.48-1.23; 0.27)
1291-GG	28	29%	0.54 (0.23-1.27; 0.16)	0.49 (0.18-1.33; 0.16)
1291-GG+GC [c]	193	35%	0.71 (0.48-1.06; 0.095)	0.73 (0.49-1.15; 0.18)
Patients without antipsychotics (n=25) [d]				
1291-GG+GC	9	11% [e]	0.097 (0.01-0.97; 0.047)	0.05 (0.003-0.97; 0.048)

[a] Data were analysed with the common genotype (1291-CC) as reference.

[b] Data were adjusted for age, gender, carriership of variant HTR2c rs1414334 C-allele, ethnicity, DSM-IV diagnosis and prescribed antipsychotic drug.

[c] Analysis for carriership if the variant allele.

[d] Data could only be investigated for an association between carriership of the variant allele and the metabolic syndrome because of sample size.

[e] Compared to 56% in group with 1291-CC genotype.

A stratified analysis for the individual antipsychotic drugs showed no association or trend between carriership of the variant 1291-G alleles and prevalence of the metabolic syndrome in any of the antipsychotics (Table 3).

The exploratory analysis in the group of schizophrenic patients not currently using any antipsychotic drugs (n = 27, with 25 patients evaluable) showed that patients carrying the variant 1291-G allele had a lower chance of having the metabolic syndrome than patients not carrying the variant 1291-G allele (nonadjusted OR, 0.10; 95% CI, 0.01-0.97; P = 0.047). This lowered risk was still significant after correction for the 2 significant (P G 0.2) covariables, age and sex (OR, 0.05; 95% CI, 0.003-0.97; P = 0.048; Table 1).

Table 2. Association between carriership of the variant 1291 G-allele and individual ATPIIIa parameters contributing to the metabolic syndrome.

Determinant[a]	Patients[b]	Crude OR (95% CI, P)	Adjusted OR (95% CI, P) [c]
HDL	440	1.00 (0.69-1.46; 0.99)	1.07 (0.71-1.62; 0.75)
TG	442	0.60 (0.41-0.88; 0.008)	0.67 (0.44-1.00; 0.05)
Waist	446	1.18 (0.82-1.71; 0.38)	1.43 (0.92-2.21; 0.11)
Hypertension	357	0.93 (0.61-1.41; 0.73)	0.95 (0.60-1.52; 0.84)
Glucose	408	0.97 (0.59-1.61; 0.91)	0.87 (0.49-1.55; 0.64)

[a] HDL = HDL-cholesterol < 1.0 mmol/l (male) or < 1.3 mmol/l (female) or use of a statin. TG = triglycerides ≥1.7 mmol/l or use of a fibrate. Waist = waist circumference ≥ 102 cm (male) or ≥ 88 cm (female). Hypertension = blood pressure ≥ 130/85 mmHg or use of an antihypertensive drug. Glucose = fasting glucose ≥ 5.6 mmol/l, or HbA1c > 6.1% or use of an antidiabetic.

[b] Patient number varies because of missing values.

[c] Data were adjusted for age, gender, carriership of variant HTR2c rs1414334 C-allele, ethnicity, DSM-IV diagnosis and prescribed antipsychotic drug.

Table 3. Association between carriership of the variant 1291 G-allele and the metabolic syndrome for individual antipsychotics.

Antipsychotic	N	M.S.	Crude OR (95% CI)	Adjusted OR [a] (95% CI)
Clozapine	91	44%	0.89 (0.47-1.71)	0.99 (0.47-2.07)
Olanzapine	99	33%	0.50 (0.23-1.08)	0.49 (0.18-1.39)
Risperidone	87	31%	1.03 (0.48-2.19)	0.44 (0.15-1.32)
Quetiapine	16	38%	1.30 (0.25-6.74)	n.a.
Aripiprazole	10	30%	0.21 (0.01-3.13)	n.a.
Typical a.p.	56	45%	1.03 (0.46-2.30)	1.37 (0.49-3.89)
Multiple a.p.	49	33%	0.39 (0.13-1.16)	0.32 (0.09-1.22)

[a] Data were adjusted for age, gender, carriership of variant HTR2c rs1414334 C-allele, ethnicity, and *DSM-IV* diagnosis.

MS indicates metabolic syndrome; NA, not applicable.

DISCUSSION

In this study, we did not find a significant association between the 1291-C/G polymorphism in the *ADRA2A* gene and prevalence of the metabolic syndrome in psychiatric patients using antipsychotics. However, we found that the point estimate for an association between the 1291-C/G genotype and the metabolic syndrome decreased as the number of variant 1291-G alleles in the genotype increased (Table 1). This inverse relation is suggestive for a gene-dose effect, although this was not significant possibly because of the limited power of this study. A trend was found for an association between the *ADRA2A* 1291-C/G polymorphism and triglyceride levels, in which the variant 1291-G allele was protective for reaching the triglyceride cutoff point of 1.7 mmol/L (OR, 0.67; 95% CI, 0.44-1.00; P = 0.05). An exploratory analysis in a group of patients not currently using antipsychotics showed a protective effect of carriership of the variant 1291-G allele on prevalence of the metabolic syndrome (OR, 0.05; 95% CI, 0.003-0.97; P = 0.048).

There are some limitations to these results. First, although our sample is relatively large with 470 patients, still only 37 patients carrying the 1291-G/G genotype were included. Because most effect of the 1291-C/G polymorphism is expected in this group (Sickert et al (10), Wang et al (8)), it is possible that the sample size was too small to find significant results.

Second, we recognize that a cross-sectional design has its limitations because data on metabolic parameters of the patients at the initiation of antipsychotic drug treatment were not available to us. Therefore, we were unable to analyze data for changes in these parameters over time related to the use of antipsychotic drugs.
This limitation makes it difficult to compare our results to prospective follow-up studies investigating the association between the *ADRA2A* 1291-C/G polymorphism and metabolic disturbances (weight gain) in psychiatric patients using antipsychotics (8-10).

Third, some variables contributing to a patient's risk of the metabolic syndrome, for example, smoking behavior, exercise, and diet, were not taken into account. Fourth, in most of the patients, no values for fasting plasma glucose were available in this study. Therefore, we used HbA1c instead, with a cutoff value of greater than 6.1%. Using HbA1c instead of fasting glucose possibly has some effect on the diagnosis of the metabolic syndrome, thereby affecting our results. However, the review study by Bennett et al (15) showed that a recommended HbA1c cutoff point of greater than 6.1% had similar accuracy as fasting plasma glucose 6.0 mmol/L for predicting type 2 diabetes (sensitivity, 72.7%; specificity, 94.7%). Based on these results, and the fact that we did not even see a trend toward a positive association between the *ADRA2A* polymorphism and HbA1c, we believe that using HbA1c instead of fasting plasma glucose did not influence our results. Fifth, although predominantly white, our

sample was of mixed ethnic origin. Ethnicity could be an important confounder in data analysis because the studies in Asians and whites have shown opposite results. However, we corrected for ethnicity in our multivariate analysis, and moreover, using only the data from white patients (n = 440) did not affect our results.

Waist circumference is one of the most relevant determinants for insulin resistance and cardiovascular morbidity in the ATPIIIa definition of the metabolic syndrome (17, 18). Because the adrenergic α-2a receptor (ADRA2A) has an important function in lipolysis and therefore in waist circumference, as was shown by Garenc et al (6), one would expect an association between the *ADRA2A* 1291-C/G polymorphism and waist circumference and the metabolic syndrome. In this study, we could not find these associations, although the mean waist circumference did decrease with an increasing number of variant G alleles in the genotype (CC: 101 cm, CG: 100.0 cm, GG: 98.0 cm). However, because the average SD was around 14 cm, these differences were not significant. The trend we found for an association between the 1291-C/G polymorphism and triglyceride levels is suggestive for an association with lipolysis nevertheless (Table 2).

It is possible that drugs with an antagonistic action for ADRA2As, such as antipsychotics, mask the effects of the 1291-C/G genotype (protective effect of the 1291-G allele) on waist circumference and prevalence of the metabolic syndrome. This would explain why we found no association in the group of patients currently using antipsychotics, but did find an association in the group of patients not currently using antipsychotics.

Following this lead, we divided our study sample in a group of patients using antipsychotics with a high affinity for the α-2 receptors (clozapine, quetiapine, and risperidone) and a group of patients using antipsychotics with a lower affinity for the α-2 receptors based on the study by Matsui et al (19). The resulting analysis showed no association between the 1291-C/G polymorphism and prevalence of the metabolic syndrome (results not shown).

It is possible that even antipsychotics with a lower affinity for α-2a receptors still mask the protective effect of the 1291-G allele, but given the fact that the Bantipsychotic-naive[group of patients was small, the results found in this group could also be a type I error. To further explore the impact of the 1291-C/G polymorphism on lipolysis and prevalence of the metabolic syndrome, we are currently investigating this association in a larger antipsychotic naive population.

This is the first study in which the association between the *ADRA2A* 1291-C/G polymorphism and prevalence of the metabolic syndrome was investigated. Insight in the factors responsible for the metabolic syndrome can have implications for daily clinical psychiatric practice, because there is a strong association between the

metabolic syndrome and cardiovascular morbidity and mortality. A pharmacogenetic tool to predict a patient's chance of developing the metabolic syndrome would be helpful in psychiatric practice because this could identify patients at risk, offering an opportunity to choose an alternative treatment.

In conclusion, this study shows that the *ADRA2A* 1291-C/G polymorphism does not seem to be a strong predictor for longterm occurrence of the metabolic syndrome in patients using antipsychotics. However, the use of antipsychotics with antagonistic *ADRA2A* activity may mask the possible protective effect of the 1291-G allele as shown in patients not currently using antipsychotics.

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Chapter 3.3

Association between HTR2C Gene Polymorphisms and the Metabolic Syndrome in Patients using Antipsychotics: a Replication Study

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ABSTRACT

In two previous studies we found an association between *HTR2C* polymorphisms and the prevalence of the metabolic syndrome in patients using antipsychotics. In this study, we set out to replicate our findings in a third separate sample of patients. Data for this cross sectional study came from the ongoing Pharmacotherapy Monitoring and Outcome survey study, investigating the association between schizophrenia and metabolic or cardiovascular risk factors. Primary end point was the prevalence of the metabolic syndrome. Primary determinants were two polymorphisms in the *HTR2C* gene: rs3813929 (-759 C/T) and rs1414334:C > G. Carriership of the variant rs1414334 C-allele was significantly associated with an increase prevalence of the metabolic syndrome (odds ratio (OR) 3.73; 95% confidence interval (CI) 1.29–10.79, P = 0.015). No association was found between the *HTR2C*-759 C/T polymorphism and the metabolic syndrome. This study confirms previous findings that the variant C-allele of the rs1414334 polymorphism is associated with the metabolic syndrome.

INTRODUCTION

It has been shown that the prevalence of the metabolic syndrome is increased in patients with schizophrenia compared with the general population (1). In our own schizophrenic patient population the prevalence of the metabolic syndrome is 36%, compared with 15.5% in the general population (2, 3). The mechanism behind the metabolic abnormalities is not entirely clear (4). The high interindividual differences suggest that genetic make-up is a modulating factor. One of the potential genetic determinants is genetic variation in the X chromosomal gene coding for the serotonergic 2C-receptor *(HTR2C)*, as studies have shown that *HTR2C* knockout-mice become hyperphagic and *HTR2C* agonists reduce appetite in humans (5, 6). Furthermore, several studies found a significant association between *HTR2C* polymorphisms and metabolic abnormalities, including antipsychotic-induced weight gain. Most studies investigated the *HTR2C* rs3813929 (-759 C/T) polymorphism, although other *HTR2C* polymorphisms were studied as well (7).

We have investigated the association between several polymorphisms in the *HTR2C* gene (*HTR2C*:c.1-142948$(GT)_n$, rs3813929 (-759 C/T), rs518147 (-697 G/C)) in the promoter region and one polymorphism in intron 5 (rs1414334:C4G) and the metabolic syndrome in patients using antipsychotics in two previously reported studies (8, 9).

In a cross-sectional study with 112 schizophrenic inpatients using antipsychotic drugs, we found an association between *HTR2C* polymorphisms and the metabolic syndrome. This association looked particularly strong in patients carrying the variant C-allele of the rs1414334 polymorphism (odds ratio (OR) 4.09; 95% confidence interval (CI) 1.41–11.89).

In a cross-sectional replication study with 164 in-patients using antipsychotics, we could not confirm the association between the variant C-allele of the rs1414334 polymorphism and prevalence of the metabolic syndrome (OR 2.35; 95% CI 0.96–5.77), although the association showed a trend towards significance. A pooled analysis of both study populations, making a total of 276 patients, did show a significant association with the metabolic syndrome (OR 2.35; 95% CI 1.19–4.62).

A further analysis of individual antipsychotics showed that the variant rs1414334 C-allele was specifically associated with the metabolic syndrome in patients using clozapine (OR 9.20; 95% CI 1.95–43.45) or risperidone (OR 5.35; 95% CI 1.26–22.83). In both studies we did not find an association between the *HTR2C* 759C/T polymorphism and prevalence of the metabolic syndrome.

The primary objective of this study was to attempt a second replication of the association between *HTR2C* polymorphisms and the metabolic syndrome in an

independent sample of patients using antipsychotics. Secondary objectives were possible associations between *HTR2C* polymorphisms and individual parameters contributing to the metabolic syndrome.

MATERIALS AND METHODS

Setting

Patients were included from an ongoing 'Pharmacotherapy Monitoring and Outcome survey' (PHAMOUS). PHAMOUS is an initiative from the Rob Giel research centre, a number of Mental Healthcare institutions and the Department of Pharmacotherapy and Pharmaceutical Care from the University of Groningen. PHAMOUS combines a yearly somatic screening with routine outcome assessment in patients using antipsychotics. Risk factors for cardiovascular and metabolic complications are monitored and effectiveness of antipsychotic treatment is evaluated in this survey. Patients included in this study originated from the northern part of the Netherlands. A detailed description of the study design can be found on http://www.phamous.eu.

Design and patients

A cross-sectional design was used to investigate the association between HTR2C variants and the metabolic syndrome in patients diagnosed with schizophrenia, schizoaffective or schizophreniform disorder or psychotic disorder. Diagnosis was performed by the treating psychiatrists according to the DSM-IV criteria. Patients were eligible for inclusion in this study if they used one or more antipsychotic drugs, were 18 years or older and diagnosed with the above-mentioned disorders. After complete description of the study to the patients, informed consent was obtained and blood was drawn for genotyping.

Outcome measures

Primary end point of the study was the presence of the metabolic syndrome. Diagnosis of the metabolic syndrome was based on the definition by the National Cholesterol Education Program's Adult Treatment panel III (NCEP:ATP IIIa) (10). The metabolic syndrome was diagnosed in all patients when three or more of the following five metabolic criteria were met: waist circumference ≥102cm (male) or ≥88cm (female), triglycerides ≥1.7mmol l^{-1} or use of a fibrate, high-density lipoprotein (HDL) cholesterol <1.0mmol l^{-1} (male) or <1.3mmol l^{-1} (female) or use of a statin, blood pressure ≥130/85mm Hg or use of an antihypertensive drug, and finally fasting glucose ≥5.6mmol l^{-1}, or HbA1 > 6.1% or use of an antidiabetic drug. HbA1c was used when a fasting glucose was not available. The cutoff value used for HbA1c is based on a review by Bennett et al (11). With respect to triglyceridelowering therapy or HDL-increasing therapy, it was decided to allocate fibrates specifically to the

triglyceride category and statins to the HDL category. Allocating both fibrates and statines to both triglyceride and HDL categories would have led to an overestimation of the metabolic syndrome, as treatment with a statin or a fibrate would have led to a diagnosis of the metabolic syndrome almost immediately. Secondary end points were the separate metabolic parameters as mentioned above.

Determinants

Primary determinants were genotypes of polymorphisms flanking, or within, the X-linked *HTR2C* gene. The following two polymorphisms were investigated: the rs3813 929:C>T (-759 C/T) polymorphism located in the promoter region and the rs1414334:C>G polymorphism in intron 5 of the *HTR2C* gene close to the 3' UTR. The *HTR2c* rs1414334 polymorphism was chosen because of its association with the metabolic syndrome in our previous two studies, and the rs3813929 (759 C/T) polymorphism was chosen because of the multiple studies associating this polymorphism with antipsychotic-induced weight gain. It has been shown that the 759 C/T polymorphism affects the *HTR2c* transcription rate, with the 759 T-allele leading to a higher expression of the $5HT_{2c}$-receptor (12). Therefore, patients carrying the 759 T-allele will likely be protected against weight gain caused by *HTR2c* inhibition by antipsychotics. The intronic position of the rs1414334 polymorphism suggests that this polymorphism is nonfunctional. It is possible, however, that this polymorphism is in linkage with another polymorphism that is associated with the metabolic syndrome and therefore serves as a marker, or possibly changes transcriptional regulation (13).

It should be noted with regard to *HTR2C* polymorphism nomenclature that for reasons of clarity, we use the nomenclature and nucleotide numbering at the genomic level according to the guidelines of the Human Genome Variation Society (http://www.hgvs.org) as well as the 'traditional' nomenclature and numbering used in previous publications. The rs1414334 polymorphism allele C is described as the ancestral allele (dbSNP database; http:// www.ncbi.nlm.nih.gov/SNP). However, in western and northern Europeans, allele G appears to be the major allele, which is confirmed in our earlier research (8, 9). In the analysis we therefore considered the C-allele as the variant allele. For the Asians and Africans in our study, the variant rs1414334 allele would actually be the G-allele.

DNA isolation and genotyping

Genomic DNA of patients was isolated from EDTA blood using the X-tractor Gene (Corbett Robotics, Corbett Life Science, Westburg, Leusden, The Netherlands) with X-tractor Gene Liquid Sample Reagent Pack (XTR1, Sigma-Aldrich, Westburg, Leusden, The Netherlands).

The polymorphisms rs3813929 C/T and rs1414334 C/G were determined with allelic discrimination using predeveloped assays (C_27488117_10 and C_7455701_10, respectively, obtained from Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands) according to the protocol supplied by Applied Biosystems. The reaction was carried out in TaqMan universal PCR master mix (Applied Biosystems) in a Taqman 7500 apparatus. The genotyping of these assays was conducted blind to the clinical status of the patients.

Data analyses and statistics

The association between the metabolic syndrome or the individual metabolic parameters and *HTR2C* genotypes (presence or absence of the variant *HTR2C* alleles) was investigated with logistic regression. Data were investigated for potential confounding effects of age, ethnicity, DSM-IV diagnosis, gender, duration of illness, weight-increasing co-medication, weight-reducing co-medication and currently used antipsychotic drugs. We included these variables in the multivariate model if they were univariately associated with the primary end point metabolic syndrome at a significance level of $P < 0.20$ (14). Unless stated otherwise, results are expressed as adjusted OR. Data were investigated for interaction between carriership of variant alleles and gender.
We did not include a stratified analysis for individual antipsychotic drugs used at the moment of evaluation because of the cross-sectional design. The presence of the metabolic syndrome could have been caused by previously used antipsychotics, and therefore would not necessarily reflect the metabolic risk for the currently used antipsychotic.

A P-value < 0.05 or less was considered as significant. We did not adjust the P-value to the number of tests due to power considerations, because this could increase the type II error rate too much in this hypothesis-driven study (15). Data were analysed using SPSS 17.0 (Chicago, IL, USA).

RESULTS

In total, 186 patients were recruited for this replication study. Of these patients, 93% were of Caucasian origin. The remaining patients were of Asian, African or mixed origin. The obtained results did not change by leaving out the Asian, African and mixed ethnicities, and therefore we did not exclude these patients from the analysis. Other patient characteristics of this replication study are summarized in Table 1.

Table 1. Patient characteristics.

Characteristic	Sample n=186
Age, mean (SD)	37 (11)
Gender (%)	
• Male	127 (68%)
• Female	59 (32%)
Diagnosis (%)	
• Schizophrenia	146 (79%)
• Schizoaffective disorder	23 (12%)
• Psychotic disorder	17 (9%)
Prevalence of the metabolic syndrome (%)	56/162 (35%)[a]
Patients carrying variant alleles (%)	
• rs3813929 (-759) T	41 (22%)
• rs1414334 C	35 (19%)

BMI, body mass index.

[a] Diagnosis of metabolic syndrome not possible in 24 patients.

Olanzapine (n = 43 (23%)), risperidone (n = 40 (22%)) and clozapine (n = 31 (17%)) were the most frequently used antipsychotic drugs. The remaining 38% of the patients used aripiprazole (n = 11), quetiapine (n = 15), typical antipsychotics (n = 17) or a combination of antipsychotics (n = 29). Treatment with aripiprazole could influence the analysis because of its favourable metabolic risk profile. However, analysis without patients using aripirazole did not influence our results.

Genotype distribution of the polymorphisms did not deviate significantly from Hardy Weinberg equilibrium (calculated in females) (rs3813929 (-759 C/T) (P = 0.14) and rs1414334:C > G (P = 0.15)). There was no linkage disequilibrium between both polymorphisms ($r^2 = 0.04$, $D' = 0.17$).

Age, gender, duration of illness, currently used antipsychotic drug, weight-increasing co medication, weight-reducing co-medication and DSM-IV diagnosis were associated with the metabolic syndrome (P < 0.2) and corrected for in the multivariate analysis. The interaction term for *HTR2C* genotype and gender was not significant (P = 0.72).

Table 2 shows that carriership of the *HTR2C* rs1414334 C-allele is significantly associated with an increased risk for the metabolic syndrome (OR 3.73; 95% CI 1.29–10.79, P = 0.015).

Table 3 shows a trend for an association between carriership of the variant rs1414334 C allele and an increased risk for reaching the cutoff points for lowered HDL (OR 2.59; 95% CI 0.96–7.05) and elevated triglyceride levels (OR 2.39; 95% CI 0.98–5.79), respectively). Further analysis showed a significant association for carriership of the variant rs1414334 C allele and elevated triglyceride concentrations (2.4 vs 1.7 mmol l^{-1}, P = 0.014), but no association with HDL concentrations was found (1.32 vs 1.28 mmol l^{-1}, P = 0.72).

Table 2. HTR2C polymorphisms and metabolic syndrome.

Genotype	Patients[a]	Metabolic Syndrome (%)	Crude OR[b] (95%CI)	Adjusted[b,c] OR (95%CI)
Carriership of variant alleles	162	56 (35)		
rs3813929 T	34	12 (35)	1.04 (0.47-2.30)	1.13 (0.44-2.86)
rs1414334 C	30	16 (53)	2.63 (1.17-5.90)	3.99(1.40-11.33)

CI, confidence interval; OR, odds ratio.

[a] Diagnoses of the metabolic syndrome could not be made in 24 patients because of missing variables.

[b] Data were analysed with the common genotype as the reference.

[c] Data were adjusted for age, gender, antipsychotic drug, use of SSRI's, and DSM-IV diagnosis.

Table 3. HTR2C polymorphisms and individual parameters.

Determinant[a]	Patients	Rs3813929 (759) T OR (95% CI) [b,c]	Rs1414334 C OR (95% CI) [b,c]
HDL	170	1.21 (0.48-3.06)	2.47 (0.95-6.42)
TG	172	1.71 (0.74-3.95)	2.21 (0.94-5.18)
Waist	179	1.19 (0.51-2.79)	0.97 (0.38-2.44)
Hypertension	184	1.09 (0.50-2.38)	1.99 (0.86-4.58)
Glucose	139	2.06 (0.66-6.36)	1.36 (0.43-4.36)

CI, confidence interval; HDL, high-density lipoprotein; OR, odds ratio; TG, triglycerides.

[a] HDL, HDL-cholesterol < 1.0 mmol l^{-1} (male) or < 1.3 mmol l^{-1} (female) or use of a statin. TG, triglycerides ≥1.7 mmol l^{-1} or use of a fibrate. Waist, waist circumference ≥ 102 cm (male) or ≥ 88 cm (female). Hypertension, blood pressure ≥ 130/85 mmHg or use of an antihypertensive drug. Glucose, fasting glucose ≥ 5.6 mmol l^{-1}, or HbA1c > 6.1% or use of an antidiabetic.

[b] Data were adjusted for age, gender, duration of illness, antipsychotic drug, weight-increasing co-medication, weight-reducing co-medication and DSM-IV diagnosis.

[c] Data were analysed with the common genotype as the reference for all polymorphisms.

DISCUSSION

In this second replication study, we extend the evidence for the association between the *HTR2C* rs1414334 polymorphism and the prevalence of the metabolic syndrome. Patients carrying the C-allele of the *HTR2C* rs1414334 polymorphism are at an increased risk for the metabolic syndrome while taking antipsychotic drugs compared with patients not carrying the *HTR2C* rs1414334 C-allele (OR 3.73 95% CI 1.29–10.79). Again, in concordance with the other two studies no association was found between the *HTR2C*-759 C/T polymorphism and the metabolic syndrome.

There are some limitations to these results. First, we recognize that the cross-sectional design is an important limitation, because data on metabolic parameters of the patients at the initiation of antipsychotic drug treatment were not available to us. Therefore, we were unable to analyse data for changes in metabolic parameters over time related to the use of antipsychotic drugs, or correct our data for possible confounders that originated in the period before the inclusion period. This limitation makes it difficult to compare our results with prospective follow-up studies investigating the association between the *HTR2C* rs3813929 (-759 C/T) polymorphism and metabolic disturbances (weight gain) in psychiatric patients using antipsychotics (16, 17). It is possible that we did not find significant results for the *HTR2C* rs3813929:C > T (-759 C/T) polymorphism due to this limitation. Furthermore, the length of antipsychotic treatment was not always known, which could implicate that there was not enough time for the metabolic syndrome to develop in some patients. However, the average duration of illness was around 10 years in our population, with only a few patients (n = 5) being diagnosed less than a year before inclusion in this study. As treatment with antipsychotics is initiated almost immediately after the diagnosis, we believe that the treatment duration of the patients in our population was long enough for the metabolic syndrome to develop.

Second, the sample size of this replication study population was relatively small. The small sample size may have limited the power to detect differences between groups that are only moderate in size resulting in nonsignificant trends. However, this is the third time we found an association between the variant rs1414334 C-allele and the metabolic syndrome in a cross-sectional study with comparable sample sizes. This makes it less likely that the association found is the result of a type I error, but most likely represents a true association finding.

Third, some variables contributing to a patient's risk of the metabolic syndrome, for example, smoking behaviour, exercise and diet, were not taken into account. Fourth, in most of the patients no values for fasting plasma glucose were available, and therefore we used HbA1c instead, with a cutoff point of 46.1%. Using HbA1c instead of fasting glucose possibly has some effect on diagnosis of the metabolic syndrome, thereby affecting our results. However, the review study by Bennett et al (11).

showed that a recommended HbA1c cutoff point of 46.1% had similar accuracy as fasting plasma glucose 6.0mmol l^{-1} for predicting type 2 diabetes (sensitivity 72.7, specificity 94.7%). Neither in the current study nor in the first replication study did we find a trend towards a positive association between *HTR2C* polymorphisms and glucose measurements or HbA1c. Therefore, we believe that measurements of glucose or HbA1c do not influence the obtained results.

The main question regarding our current findings is: did we replicate the results of our previous two studies? Using the new ATPIIIa criteria for diagnosis of the metabolic syndrome, combined with HbA1c, we found a significant association between carriership of the variant rs1414334 C-allele and the metabolic syndrome. In our previous two studies, we used a slightly different set of criteria to diagnose the metabolic syndrome (8, 9). In those two studies, the metabolic syndrome was diagnosed when three or more of the following four metabolic criteria were met: waist circumference > 102 cm (male) or > 88 cm (female), triglycerides ≥ 1.7 mmol l^{-1}, HDL cholesterol < 1.0 mmol l^{-1} (male) or < 1.3 mmol l^{-1} (female) and blood pressure ≥ 135/85 mmHg. However, in these two studies we also corrected for potential confounding effects of drugs with an influence on glucose and lipid homeostasis. As these corrections are similar to the new ATPIIIa criteria, we believe that the results from the presented multivariate data analysis for an association between *HTR2C* genotype and the metabolic syndrome are comparable with the results of the other two studies and represent a true association.

We did not find an association between the 759 C/T genotype and prevalence of the metabolic syndrome. This was unexpected as the 759 C/T polymorphism has been repeatedly associated with antipsychotic-induced weight gain, and weight gain is an important predictor for meeting the criteria for the metabolic syndrome (12, 16–24). The fact that this is the third study in which we found an association between prevalence of the metabolic syndrome and *HTR2C* rs1414334 genotype, but not 759C/T genotype, requires an explanation. We suggest that we are dealing with two different phenotypes in two different phases of the disease with weight gain at the initiation of treatment and the presence (and prevalence) of the metabolic syndrome, after a longer period of treatment with antipsychotic drugs. The studies that found an association between 759 C/T genotype and antipsychotic-induced weight gain were almost exclusively carried out prospectively in first episode schizophrenic populations (16–19), whereas the studies that failed to find this association were most often carried out in populations with treatment-resistant schizophrenia (21–23). Studies are warranted to investigate whether the rs1414334 polymorphism also has an impact on antipsychotic-induced weight gain in the populations of the studies that reported a positive association between antipsychotic-induced weight gain and 759 C/T genotype (16–19). It would also be warranted to investigate the association between the rs1414334 polymorphism and prevalence of the metabolic syndrome or weight gain in the studies that used a treatment-resistant population, and failed to

find an association between weight gain and 759 C/T genotype (21–23). Investigating these associations could provide further evidence for the possible impact of the *HTR2C* rs1414334 genotype on short-term and longterm metabolic complications caused by treatment with antipsychotic drugs.

It is interesting to hypothesize that two polymorphisms located on the same gene coding for the $5HT_{2c}$ receptor result in different phenotypes. This could implicate that both polymorphisms have a different effect on receptor functioning or a different interaction with other metabolicregulating systems. One of the explanations could be a different interaction with the leptine system as reported by Templeman et al. (19), Yevtushenko et al. (24), and Gregoor et al. (25) (data submitted).

In conclusion, this study provides further evidence for the association between the *HTR2C* rs1414334 polymorphism and the metabolic syndrome, confirming previous findings. Studies investigating the possible association between the rs1414334 polymorphism and antipsychotic-induced weight gain are warranted, as well as studies investigating the interaction and genetic linkage between *HTR2C* genotypes (rs 1414334 and -759 C/T) and other metabolicregulating systems. These studies may explain the observed differences in results between studies investigating the -759 C/T genotype and antipsychotic-induced weight gain and our studies investigating the rs1414334 genotype and the metabolic syndrome.

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Chapter 3.4

Association between the ROBO1 Gene and Body Mass Index in Patients using Antipsychotics

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ABSTRACT

Background: Weight gain is one of the major problems in patients using antipsychotic medication, leading to relevant morbidities and reduced compliance to pharmacotherapy. Recently, an association has been reported between a single nucleotide polymorphism (rs1455832) of the roundabout axon guidance receptor, homolog 1 *(ROBO1)* gene and body mass index (BMI) in persons younger than 30 years. The aim of this study is to investigate the association between BMI and rs1455832 in patients with a psychotic disorder using antipsychotics.
Methods: A cross-sectional design was used in a pooled sample of Caucasian psychiatric patients obtained from three comparable Dutch psychiatric populations. Patients were eligible for inclusion in this study if they met the Diagnostic and Statistical Manual of Mental Disorders-IV criteria for a nonaffective psychotic disorder, were 18 years or older, and used one or more antipsychotics. Genotyping was performed according to standard protocols. Linear (for BMI) and logistic (for obesity, defined as BMI >30) regression analyses, corrected for age and sex, were applied in the statistical analyses.
Results: A total of 435 patients were included in this association analyses. The rs1455832 polymorphism studied was significantly associated with BMI and obesity in female patients. Female patients had a statistically significant ($P = 0.025$) decrease of 1.76kg/m^2 in BMI values per C allele. In contrast to female patients, this association was not exhibited in male patients.
Conclusion: The rs1455832 polymorphism may play a role in inducing obesity in female patients using antipsychotics.

INTRODUCTION

Weight gain is one of the major problems in patients using antipsychotic medication (1, 2). Antipsychotic-induced weight gain and obesity are associated with other relevant morbidities, such as type II diabetes mellitus, hypertension, and other cardiovascular diseases (2, 3). Furthermore, it greatly contributes to a reduced compliance and is a serious threat to successful pharmacotherapy (4). Particularly, the atypical antipsychotics, olanzapine and clozapine, may induce weight gain, although almost none of the atypical and typical antipsychotics are completely free of this side effect (5-7).

It has been suggested that certain genetic risk factors may be important to predict weight gain, and therefore enable individualized treatment in patients receiving antipsychotics (8, 9). The contribution of heredity is supported by the substantial interindividual and inter-racial differences in antipsychoticinduced weight gain (9). The pharmacogenetics of antipsychotic-induced weight gain has already been studied. To date, few targets have been identified, both at the level of the receptors, such as gene variants of the serotonin 2C receptor (10-13) and more downstream the metabolic pathway such as the leptin hormone (13, 14). However, the mechanisms underlying antipsychoticinduced obesity remain to be elucidated.

One gene possibly involved in the multifaceted development of antipsychotic-induced obesity is the roundabout axon guidance receptor, homolog 1 *(ROBO1)* gene. In a recent reanalysis of a scan of 86,604 single nucleotide polymorphisms (SNPs) among 1322 individuals in the Framingham Heart study offspring cohort [for the original analysis, see Herbert et al. (15)], Lasky-Su et al. (16) have identified an age-varying association between a SNP (rs1455832) of the *ROBO1* gene and obesity. Homozygosity for the minor allele (CC) was associated with an increased body mass index (BMI) in persons younger than 30 years, but this association diminished after the age of 30 years. Consequently, Lasky-Su et al. have hypothesized an age–gene interaction. More importantly, this finding was replicated by them in the same study in five of eight additional cohorts comprising in total 13,584 individuals. These replication samples differed in demographic properties, ascertainment conditions, and study designs. The combined P value from all replication samples showed significant (one-sided combined P value = 3.92×10^{-9}, combined P value from pediatric cohorts = 2.21×10^{-8}, and combined P value from adult cohorts = 4.22×10^{-3}) age-dependent relationships between rs1455832 and BMI as surrogate parameter of obesity. The *ROBO1* gene, which maps on chromosome 3p12, is expressed in adipose tissue, though its function in this tissue is to date unknown (16). The *ROBO1* gene contains 30 exons; SNP rs1455832 is located in its first intron. The gene is supposedly involved in the axonal connectivity of the dorsolateral prefrontal cortex (17), which plays an important role in the pathogenesis of schizophrenia. *ROBO1* is associated

with dyslexia (18, 19), and more recently identified as a possible risk gene for schizophrenia as well (20, 21).

Given the heterogeneity of the samples and the high reproducibility of the findings reported by Lasky-Su et al. (16), we have assumed that their findings are of good generalizability. As the onset of psychotic symptoms is mostly in the early twenties, we have hypothesized that the rs1455832 polymorphism of the *ROBO1* gene may, therefore, play a role in the mechanisms underpinning obesity in patients with a psychotic disorder as well. In this study, we have investigated the role of the rs1455832 polymorphism as a risk factor for obesity in patients with a psychotic disorder on antipsychotic medication. As previous studies suggest a higher sensitivity for antipsychotic-induced weight gain in women than in men (22), we carried out additional analyses while stratifying for sex.

MATERIALS AND METHODS

Setting

For this study, three similar psychiatric patient populations from the Netherlands were pooled. The majority of patients were from an ongoing 'Pharmacotherapy Monitoring and Outcome Survey' (PHAMOUS). PHAMOUS is an initiative from the Rob Giel Research Centre, a number of Mental Health Care institutions, and the Pharmaceutical Care Department from the University of Groningen. It combines a yearly somatic screening with routine outcome assessment in patients using antipsychotics. Risk factors for cardiovascular and metabolic complications are monitored and effectiveness of antipsychotic treatment is evaluated in this survey with the goal of improving healthcare for patients with a psychotic disorder. Patients included in this study originated from the northern part of the Netherlands. The two other study populations that were pooled with the PHAMOUS population have been described in detail elsewhere (23-25). In brief, these populations consisted of patients from a Department of Psychiatric Disorders of a general hospital in the north of the Netherlands (Mulder et al. (23, 24)) and patients from a Mental Health Care Organization in the West of the Netherlands (Cohen et al. (25)), respectively.

Design and patients

A cross-sectional design was used to assess the association between rs1455832 polymorphisms and BMI. Caucasian patients (European, North-African, or Middle-Eastern race, following the Food and Drug Administration guidance for Industry: collection of race and ethnicity data in clinical trials) were eligible for inclusion in this study when they met the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria for a non-affective psychotic disorder (schizophrenia, schizophreniform disorder, schizoaffective disorder, delusional

disorder, and psychotic disorder not otherwise specified), were 18 years or older, and used one or more antipsychotics.

Outcome measures

The primary endpoint of the study was BMI, calculated as body weight (kg) divided by the square of the height (m^2). Secondary endpoint was obesity. Following the World Health Organization, a BMI of more than 30 kg/m^2 is considered as obesity. A BMI between 25 and 30 kg/m^2 is considered as overweight.

Determinants

The primary determinant was the genotype (TT, TC, or CC) of the rs1455832 polymorphism located in the *ROBO1* gene. Other clinical and demographic (co)variables that were measured in the study were sex, age, cohort, DSM-IV diagnosis, and the type of antipsychotic medication used at the day of assessment.

Genotyping

The study protocol was approved by the local university hospital medical ethics committee and all participants gave their written informed consent. Genomic DNA was extracted from EDTA whole blood according to standard protocols. Genotyping of *ROBO1* rs1455832 was performed blinded to the clinical status of the patients. Fluorogenic 50-exonuclease TaqMan assay (Made To Order kit; C___7500712_10, obtained from Applied Biosystems (Nieuwerkerk a/d IJssel, the Netherlands) was applied for the genotyping.

Statistical analysis

Departure from the Hardy–Weinberg equilibrium was calculated for this polymorphism by a χ-test with 1 degree of freedom. To compare BMI values among various users of antipsychotics (e.g. BMI in users of clozapine vs. olanzapine vs. risperidon vs. aripiprazole vs. quetiapine vs. users of more than one antipsychotic) and between patients using typical versus atypical antipsychotics, we applied analysis of variance (ANOVA) and the Student's *t*-test, respectively. We used linear regression to explore the relationship of BMI with the independent variables cohort, age, and sex. We compared demographic and clinical characteristics between the genotypes of rs1455832 as well.

Our primary hypothesis concerned the main effect of polymorphism rs1455832 on BMI and obesity. This was tested in the total population and stratified by sex. As we did not have information on the mode of inheritance in this population, e.g. dominant, recessive, or additive, we initially considered an additive model. Simply

testing all models is inefficient because of the multiple testing penalties. When significant, other modes (dominant and recessive) were tested for better fit. We utilized the minor allele (C allele) for model specification in the following manner. For the additive model, we examined the number of C alleles ('C allele dose', which is 0, 1, and 2 for the homozygous, heterozygous, and wild-type patients, respectively). For the dominant and recessive models we compared CC/TC versus TT and CC versus TC/TT, respectively.

We assessed unadjusted differences in BMI between genotypes by ANOVA. Linear regression was used to adjust for potential confounding effects of age, sex, cohort, DSM-IV-diagnosis, and the type of prescribed antipsychotic drug. The associations between genotype and obesity (defined as BMI >30 kg/m2) were determined through binary logistic regression analyses, adjusting for potential confounders. The strength of the association with obesity was expressed as an odds ratio (OR) with a 95% confidence interval (CI).

Our secondary hypothesis concerned the age-varying effect of genotype on BMI like in Lasky-Su et al. (16). The same linear regression model as they used in their replication studies was run, with the CC genotype and genotype-by-age interactions as the primary predictors of interest and BMI as the response variable. The linear model used for these analyses was BMI = β_0 + β_1 genotype + β_2 age + β_3 sex + β_4 age x genotype + ε. Thus, we used a recessive mode in this linear model, like Lasky-Su et al. did. In addition, we used the more general additive model.

To test whether associations of the main genotype effect on BMI differed between men and women, we ran a linear regression analysis in the total group adjusting for potential confounders, with the predictors genotype and genotype-by-sex interaction. Post hoc, similar analyses were carried out for atypical and typical users. All of the analyses were carried out using the standard software (SPSS 16.0 for Windows, SPSS Inc., Chicago, Illinois, USA). The level of significance was set at a P value of 0.05 (two sided).

RESULTS

Patients

A total of 435 Caucasian patients met the inclusion criteria and all were genotyped successfully [cohort PHAMOUS (n = 168), cohort Cohen et al. (n = 155), and cohort Mulder et al. (n = 112)]. The genotype distribution was found to be consistent with the Hardy–Weinberg equilibrium (P value = 0.831). Table 1 presents basic genetic, demographic, and clinical data of the sample studied. Approximately 95% of the patients had a diagnosis in the schizophrenic spectrum [schizophrenia (n = 335),

schizo-affective disorder (n = 77), schizophreniform disorder (n = 2)]. The other 21 patients had a psychotic disorder not otherwise specified.

Medication

Most patients used clozapine (21.6%), olanzapine (22.5%), or risperidon (21.8%), followed by aripiprazole (2.3%), quetiapine (4.4%), typical antipsychotics (14.5%), and a combination of more than one antipsychotic (12.9%) on the day of assessment. No significant differences in mean BMI values were found between users of the various antipsychotics (ANOVA: P = 0.495), or between users of typical and atypical antipsychotics (Student's *t*-test: P = 0.977).

Association analyses

In the linear regression model with BMI as a dependent variable and cohort, age, and sex as independent variables, cohort did not turn out to be a significant predictor for BMI. Age (increase of 0.058 kg/m^2 per year, P = 0.015) and sex (increase of 2.89 kg/m^2 if female, P = 0.001) were significant predictors of BMI.

The results of the unadjusted analyses (Table 1) show a small dose effect between the rs1455832 genotype and BMI in the total population, which is not significant (ANOVA: P = 0.533; P value of C allele dose in linear regression is 0.261; OR for having a BMI > 30 following an additive model for the C allele 0.87, 95% CI: 0.62–1.21). Correction for age, cohort, and sex or changing the mode of inheritance did not change these outcomes. DSM-IV diagnosis and type of antipsychotic were not associated with BMI and adjusting for it in the linear regression model did not lead to different outcomes (data not shown).

However, after stratification by sex (Table 2), women, but not men, exhibited a significant association between BMI and rs1455832 genotype. The T allele is a risk factor for higher BMI values (BMI values TT>TC>CC, Fig. 1).
C allele dose linear regression analysis in women, adjusted for age and cohort, showed a mean decrease in BMI of 1.76 kg/m^2 per C allele with a P value of 0.025. Changing the mode of inheritance did not lead to better fit of the model. The stronger association of minor allele dose with BMI in women compared with men was statistically significant (P value of interaction term genotype by sex is 0.014). In addition, the risk of having obesity significantly decreased for female patients for each additional C allele (additive model, OR 0.54; 95% CI: 0.30–0.95). In any of the linear regression analyses conducted, we could not find evidence for the age–gene interaction reported by Lasky-Su et al. [estimate genotype-by-age interaction (β_4) in the total population using a recessive mode 0.046, P = 0.544]. Changing the mode of

inheritance in the linear model did not lead to an age-varying effect either (data not shown).

When we carried out a post-hoc linear regression analysis, adjusting for cohort and age, in female patients using atypical antipsychotics (n = 119), the magnitude of the association also increased and became more statistically significant [mean decrease in BMI per C allele (2.15 kg/m^2), P = 0.011]. In female patients, using typical antipsychotics only (n = 22), an opposite, nonsignificant, association between rs1455832 and BMI was found [mean increase in BMI per C allele (1.98 kg/m^2), P = 0.422]. An interaction term atypical antipsychotics by-genotype did not reach statistical significance in linear regression.

Table 1. Demographic and unadjusted clinical variables of patients in the total study sample and per TT-, TC-, and CC-genotype of rs1455832.

Characteristic	Total study sample (n=435)	Per genotype		
		TT (n=239)	TC (n=162)	CC (n=34)
Age (yrs)[a]	38.4 (10.6)	38.4 (10.3)	38.2 (10.5)	38.6 (12.3)
Gender				
• Male	294 (68%)	161 (67%)	111 (68%)	23 (68%)
• Female	141 (32%)	79 (34%)	51 (32%)	11 (32%)
DSM-IV Diagnosis				
• Schizophrenia/ schizophreniform disorder	337 (78%)	180 (75%)	127 (78%)	30 (88%)
• Schizoaffective disorder	77 (18%)	47 (20%)	28 (17%)	2 (6%)
• Psychotic disorder NOS	21 (5%)	12 (5%)	7 (4%)	2 (6%)
Antipsychotic medication				
• Typical	69 (16%)	32 (13%)	31 (19%)	6 (18%)
• Atypical	366 (84%)	207 (87%)	131 (81%)	28 (82%)
BMI (kg/m^2)[a]	28.0 (5.3)	28.3 (5.6)	27.8 (4.9)	27.5 (5.1)
Weight category[b]				
• Non-obese (BMI <25)	139 (32%)	80 (33%)	47 (29%)	12 (35%)
• Overweight (BMI 25-30)	159 (37%)	79 (33%)	69 (43%)	11 (32%)
• Obesity (BMI >30)	137 (31%)	80 (33%)	46 (28%)	11 (32%)

BMI, body mass index; DSM-IV, Diagnostic and Statistical Manual of Mental Disorders, IV edition; NOS, not otherwise specified.

[a] Values are presented as means (standard deviation).

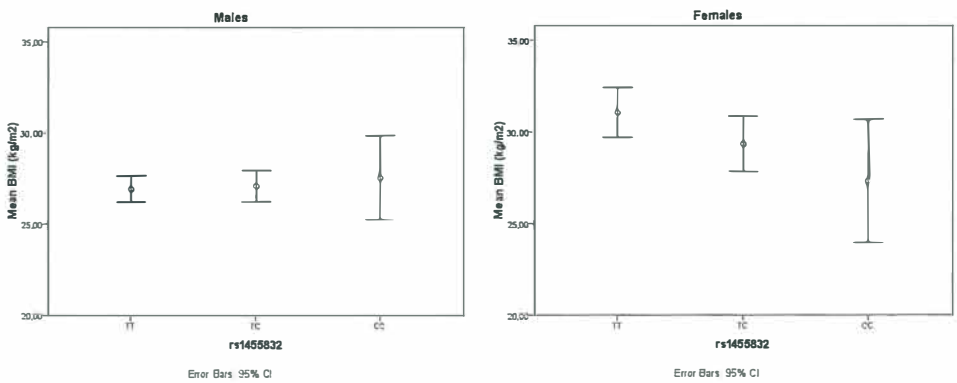
[b] Frequencies given are per genotype.

Table 2. Demographic and unadjusted clinical variables of patients with the CC-, TC-, and TT-genotype of rs1455832, presented for males and females separately.

	Males (n=294)			Females (n=141)		
Genotype rs1455832	**TT (n=160)**	**TC (n=111)**	**CC (n=23)**	**TT (n=79)**	**TC (n=51)**	**CC (n=11)**
Frequency[a]	54.4%	37.8%	7.8%	56.3%	35.9%	7.7%
Age (yrs)[b]	37.4 (9.8)	37.4 (10.0)	37.8 (9.0)	40.5 (11.0)	40.2 (11.5)	40.3 (17.9)
BMI (kg/m²)[b]	26.9 (4.6)	27.1 (4.6)	27.5 (5.3)	31.2 (6.2)	29.3 (5.4)	27.3 (5.0)
Obesity (BMI>30)	24.4%	23.4%	34.8%	55.7%	39.2%	27.3%

[a] Frequencies of genotype are given per gender.
[b] Values are presented as means (standard deviation).

Figure 1. Mean body mass index (BMI) with 95% confidence interval (CI) per genotype of rs1455832, in male and female patients. In female patients, a significant trend of decreasing BMI per C allele can be seen (mean decrease per C-allele 1.76 kg/m², P = 0.025, corrected for age and cohort).



DISCUSSION

This is the first study to examine the relationship between rs1455832 and BMI in psychotic patients, all of them receiving antipsychotics. We showed an association between the rs1455832 genotype and BMI in Caucasian female patients with a psychotic disorder on antipsychotic drugs. We observed no association in men and no modification by age.

Our study results are in agreement with and extend findings from other studies (16) in apparently healthy individuals that have shown that the rs1455832 SNP (*ROBO1* gene) is associated with obesity. However, whereas according to Lasky-Su et al. (16), the minor C allele confers risk for obesity, we found the T allele to be associated with higher BMI and the minor C allele with lower BMI. Differences in the linkage disequilibrium may be a possible explanation for the observed discrepancy. We cannot exclude the possibility that differences in linkage disequilibrium, if existent, are caused by the fact that all of our patients are psychotic and the samples of Lasky-Su et al. (16) consist of nonpsychotic, mostly healthy persons. In addition, we could not replicate the age–gene interaction effect reported by Lasky-Su et al. (16), although our population's age distribution was suited to find such an effect. A possible explanation for not detecting an age–gene interaction could be, next to the smaller sample size, that psychotic patients exhibit physical premature ageing. It is known that schizophrenic patients have a higher prevalence of age-related diseases as diabetes mellitus (26), and on average die 20 years earlier than those do in the general population. Next to suicide and other unnatural cases, roughly 60% of premature deaths are from natural causes such as cardiovascular and pulmonary disease (27). Hypothetically, it is also possible that psychotic patients receiving antipsychotics gain weight in their younger years to such an extent that this age–gene interaction is weakened by this ceiling effect.

When we carried out the analysis in female patients using atypical antipsychotics, the magnitude of the association increased and became more statistically significant. We found an opposite magnitude of association in female patients using typical antipsychotics, which did not reach the statistical significance value. Owing to the small number in this group with typical antipsychotics we had limited power. However, these findings might indicate that the association between rs1455832 and BMI is only present in patients using atypical antipsychotics. Atypical antipsychotics are known to cause more weight gain than typical antipsychotics, and different mechanisms may be involved (5-7).

Gregoor et al. (14) showed in their study on polymorphisms of leptine genes and obesity in patients using antipsychotic medication that the *LEPR* Q223R polymorphism may be associated with obesity in women, but not in men, with a psychotic disorder. They stress the importance of stratification for sex when

investigating the role of variations of the *LEP* and *LEPR* genes on the metabolic side effects of antipsychotic medications. Although the function of the *ROBO1* gene is unknown, it is known to be expressed in adipose tissue. Possibly similar pathways as influenced by the *LEPR* Q223R polymorphisms play a role in our association. In contrast to the analyses of Gregoor et al. (14), we did not adjust for the use of antidiabetic drugs, because it is not a confounding variable of BMI, but rather a consequence of the outcome. Therefore, including it in our multivariable regression analyses would lead, in our opinion, to an overadjustment. However, when we made an adjustment for antidiabetic drugs in the linear regression analysis in the female patients, the magnitude of the association became slightly more statistical significant [mean decrease in BMI per minor allele (1.81 kg/m^2), P = 0.017].

This study has a number of limitations. First, as this study is cross-sectional we did not have information on weight before antipsychotic treatment was started. The association we found between the *ROBO1* polymorphism and obesity may have been existent before treatment. In addition, longitudinal measures of BMI provide more power to detect modest age-related effects. Second, we did not have quantitative information on the cumulative exposure to antipsychotics (currently and previously used). Therefore, we cannot establish the relationship between BMI and *ROBO1* according to the type of antipsychotic used. However, because schizophrenia is a chronic disease starting at adulthood, it is reasonable to assume that the majority of our patients had been using antipsychotics for years. Third, weight gain and obesity are multifactorial conditions. Although genetics may be involved in the development of obesity from antipsychotics, additional factors need to be taken into consideration such as diet, exercise, and symptom severity. However, we do not assume that these covariates differ between genotypes. Finally, our results should be interpreted with caution. As in any pharmacogenetic study, replication is required to validate our results.

Despite its limitations, this study was able to show a significant association between the T allele of the rs1455832 SNP of the *ROBO1* gene and obesity in female patients receiving antipsychotic drugs. Although our findings are opposite in direction to those of Lasky-Su et al. (16), that is, whereas they report the C allele to predispose to obesity, we find that this allele confers protection against obesity, our findings underline the importance of this locus in relation to obesity in antipsychotic users. Further studies are needed to confirm and completely explain the potential role of the *ROBO1* gene in weight gain of patients with a psychotic disorder using antipsychotics.

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Chapter 4

Association of Two DRD2 Gene Polymorphisms with Acute and Tardive Antipsychotic Induced Movement Disorders in Young Caucasian Patients

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ABSTRACT

Background: Pharmacogenetic studies on antipsychotic-induced movement disorders (MD) have focused mainly on tardive dyskinesia. Only a few studies examined the more acute antipsychotic induced MD, such as Parkinsonism and akathisia. Notably, acute antipsychotic-induced MD have shown to be a risk factor for later tardive dyskinesia and all are related to dysregulation of the dopamine system. The aim of this study is to replicate previously reported associations of candidate genes with acute and tardive antipsychotic-induced MD in a young Caucasian sample.
Methods: In 402 patients (median age 26 years) a total of 13 polymorphisms were genotyped in 8 dopamine related candidate genes selected a priori from the literature (regarding dopamine and serotonin receptors, dopamine degradation, and free radicals scavenging enzymes pathways).
Results: Patients with MD used on average a higher haloperidol dose equivalent, when compared to those without MD. Prevalence of MD was high and did not differ between first generation antipsychotics and second generation antipsychotics. Significant associations were found between (i) the *DRD2* TaqI_D polymorphism and akathisia (OR=2.3, p=0.001 for each extra C- allele) and (ii) the *DRD2* -141C polymorphism and tardive dyskinesia (OR=0.20 for each extra *Del* allele, p = 0.001). The other polymorphisms were not significantly associated with an MD.
Conclusion: Two associations were found between genetic variation TaqI_D and the -141C polymorphisms in the *DRD2* gene and antipsychotic induced MD: one with acute akathisia and one with tardive dyskinesia. These were previously reported to be associated with tardive dyskinesia and acute Parkinsonism, respectively. These results suggest that the contribution of these *DRD2* gene variants in the vulnerability of antipsychotic-induced MD takes place in a more general or pleiotropic way.

INTRODUCTION

Antipsychotic induced movement disorders (MD), *i.e.* tardive dyskinesia, Parkinsonism, akathisia and dystonia, remain a major concern in the treatment of schizophrenia. They are associated with social stigmatization, physical disabilities, and poorer quality of life and may intervene with treatment adherence (1, 2). Lack of compliance is particularly of interest in relatively young patients diagnosed with schizophrenia as it may lead to more relapses, higher admission rate and poorer prognosis (3, 4).

Despite the introduction of the second generation antipsychotics with generally a lower propensity for motor side effects, the prevalence of antipsychotic-induced MD in patients with first episode schizophrenia, is still substantial with a frequency up to 19% (5).

It is therefore of clinical importance to detect patients who are prone to antipsychotic-induced MD. Well known risk factors include the use of first generation antipsychotics, higher dosages and duration of antipsychotic use, drug abuse, male gender in first episode schizophrenia, older age and ethnicity (6-17). The presence of early antipsychotic-induced MD is also a risk factor for development of later tardive dyskinesia (18, 19). In addition, genetic variations may in part explain the large inter-individual differences in the development of antipsychotic induced MD among patients with schizophrenia using similar antipsychotics (20).

We hypothesize that genes related to the dopamine system are candidate genes for antipsychotic induced MD in schizophrenia (21). Dopamine 2 and 3 receptors (*DRD2* and *DRD3*) are relevant being the primary targets for antipsychotic drugs (22, 23). In addition, the *DRD2* is densely expressed in the striatum (20, 24, 25), and even more so in schizophrenia (26). The *DRD3* is also selectively expressed in brain regions associated with schizophrenia including the striatum and pallidum, each implicated in motor function (27, 28). Several associations with mainly tardive dyskinesia have been reported for *DRD2* and *DRD3* variants (29-33).

In addition, serotonin receptors 2_a and 2_c (*HTR2A* and *HTR2C*, respectively) are involved because many antipsychotics, in particularly second generation antipsychotics, have a high affinity to these receptors. They are strongly expressed in the striatum (34, 35). Moreover, the serotonergic system interacts with the dopaminergic system and may be responsible for some of the dyskinetic effects of antipsychotics (36, 37). Several candidate studies reported significant associations for *HTR2A* and *HTR2C* (29, 38-42).

Furthermore, the Catechol-O-methyltransferase (*COMT*) gene is of interest as it encodes the central dopamine catabolic enzyme (COMT) that degrades dopamine

and noradrenaline. As COMT is mainly located in the frontal cortex, the relation with MD most likely results from secondary changes or upregulation in the frontal-striatal circuit (29). One significant association study with tardive dyskinesia has been published (43).

Additionally, oxidative stress may also contribute to the development of antipsychotic-induced MD and schizophrenia as free radicals may damage the dopamine receptor (44, 45). Indeed, several genetic variants in free radial scavenging enzymes have reported to be associated with tardive dyskinesia; NADPH Quinone Oxidoreductase 1 (*NQO1*), Glutathione S-transferases (*GSTP1*), Regulator of G protein signaling 2 (*RGS2*) and Mangase superoxide dismutase (*MnSOD*) (46, 47, 48, 49, 29, 50).

In the present study we aim to replicate reported associations in candidate genes for acute and tardive antipsychotic-induced MD in a young Caucasian sample with psychotic disorders.

METHODS

Study population

A sample of 402 in- and outpatients using antipsychotic medication was collected from the ongoing longitudinal Genetic Risk and Outcome of Psychosis study (GROUP) (51). In GROUP, patients were identified in selected representative geographical areas in the Netherlands and Belgium. Inclusion criteria for GROUP were: (i) age range 16 to 50 years, (ii) diagnosis of non-affective psychotic disorder and (iii) good command of Dutch language. For the present analysis the following extra inclusion criteria were applied: (iv) use of antipsychotic medication at the time of assessment for at least one month and (v) Caucasian ethnicity of Northern European ancestry. The study was approved by the Ethics Committee of the University Medical Center Utrecht and by the institutional review boards of all other participating hospitals. All subjects gave written informed consent in accordance with the committee's guidelines. Clinical variables included DSM-IV diagnosis, duration of illness, antipsychotic medication and dose.

Phenotyping

Trained raters evaluated all participants for MD using standardized clinical instruments. As part of the GROUP study protocol, yearly training sessions were held to maintain reliability on the assessment of movement disorders. Acute antipsychotic induced MD were measured by the Unified Parkinson Disease Rating Scale (UPDRS) (52), the Barnes Akathisia Rating Scale (BARS) (53), and one extra item for dystonia. Parkinsonism was considered present when there was 'mild' (≥ 2)

involvement on one of the items tremor or rigidity, or at least one 'moderate' (≥ 3) or two 'mild' scores on any of the other items of the UPDRS (52). Akathisia was considered present when there was a 'mild' (≥ 2) involvement on the global item of the BARS (53) and dystonia was considered present when there was a 'mild' (≥ 2) involvement. Tardive dyskinesia (TD) was evaluated with the Abnormal Involuntary Movement Scale (AIMS) (54). The aim of this study was to identify early genetic markers of vulnerability to all antipsychotic induced MD, including tardive dyskinesia. However, the included patients of the GROUP population had a relatively short duration of illness (median of 3.1 years). Therefore TD was considered present when any of the AIMS items scored at least 'minimal' (≥ 1) following the research criteria for TD of the GWAS in the CATIE-trial (55).

Genotyping

On the basis of significantly associated SNPs, reported in the literature we genotyped 14 SNPs in the following 9 candidate genes. For *DRD2*: (i) rs1800497 (TaqI_A), (ii) rs6277 (C957T), (iii) rs1799732 (-141CIns/Del) and (iv) rs1800498 (TaqI_D). For *DRD3*: (i) rs6280 (Ser9Gly). For *HTR2A*: (i) rs6313 (T102C>T), (ii) rs6314 (His452Tyr). For *HTR2C*: (i) rs6318 (Cys23Ser), (ii) rs3813929 (-759C_T). For *COMT*: (i) rs4680 (Val158Met). For oxidative stress enzymes: *NQO1* (i) rs1800566 (C609T), *GSTP1* (ii) rs1695 (Ile105Va), *RGS2* gene (iii) rs4606, *MnSOD* (iv) rs4880 (Ala-9Val). These *a priori* selected polymorphisms were genotyped by Sequenom (Hamburg, Germany) using the Sequenom MassARRAY iPLEX platform at the facilities of the manufacturer. Quality check for genotyping was performed in the total GROUP study, which encompassed exclusion of polymorphisms based on departure from Hardy-Weinberg equilibrium in a sample of 398 unaffected controls without a psychotic disorder.

Statistical analysis

Differences in prevalence of MD between users of fist generation antipsychotics and second generation antipsychotics and between men and women were evaluated and tested for statistical significance using Fisher's exact tests. Study inter-rater agreements of ratings were calculated with the multi-rater *kappas (56, 57)*, using 8 videotaped examinations of movement disorders. Kappa is a summary measure, ranging between -1 and +1, of the level of agreement beyond chance. According to Landis and Koch (58), kappa values below 0.40 should be considered poor, between 0.41 to 0.60 should be considered moderate, 0.61 to 0.80 should be considered substantial, and above 0.81 should be considered almost perfect. Haloperidol dose equivalents were subsequently calculated using power formulas (59). Differences in age, duration of illness and haloperidol dose equivalents between patients with and without a MD were evaluated and tested for statistical significance using a Mann-Whitney test. Logistic regression was used to test the association between genotyped

polymorphisms and Parkinsonism, akathisia, and tardive dyskinesia. Acute dystonia was not tested separately, because of its low prevalence. Two polymorphisms positioned on the X-chromosome were tested separately by gender, and in a dominant model for the total sample. Covariables corrected for in our regression model were age and gender.

Pairwise linkage disequilibrium (LD) between polymorphisms was calculated by D' and r^2. The haplotype trend regression (HTR) approach, as outlined by Zaykin et al. (60), was used to test the associations of statistically inferred haplotypes with extrapyramidal side-effects. The HTR tests for the contribution of individual haplotypes taking into account the uncertainty of haplotype estimation by PHASE 2.0 software (61, 62). The most frequent haplotype was used as the reference haplotype with which effects of the other haplotypes were contrasted. This was performed for the genes *DRD2*, *HTR2A*, and *HTR2C*, where multiple polymorphisms were genotyped. Similarly, correction for age and gender was performed in these regression analyses.

All statistical analyses, other than those involving haplotype estimation, were performed using (SPSS 16.0 for Windows). Since polymorphisms and MD variables are both partly correlated and the choice of the polymorphisms was based on earlier positive association studies, application of Bonferroni's procedure for correcting alpha for multiple testing was considered too conservative. In adjusting the significance level to account for multiple testing we follow the recommendations of Van den Oord and Sullivan (63, 64). The adjustment depends on p_0, the number of markers for which there is no true effect (i.e. the null hypothesis is not true), which is generally unknown in candidate gene studies. For a range of plausible p_0 values for candidate gene studies, a significance level of P=0.01 will, on average, control the false discovery rate at 0.10. Lower false discovery rates generally resulted in sharp increase in sample size, i.e., loss of power. Thus, the significant level of this study was pragmatically set at 0.01, two-sided.

RESULTS

Descriptive statistics of our study sample are presented in Table 1, shown stratified by patients with and without a MD present. The interrater agreement for MD using multi-rater kappas varied between 0.56 and 0.98. The prevalence of an MD was 46.8% (n = 188). The most frequent MD was Parkinsonism (n = 122, 30.3%), followed by tardive dyskinesia (n = 88, 21.9%), akathisia (n = 37, 9.2%), and dystonia (n = 7, 1.7%). Patients with an MD used on average a significantly higher haloperidol dose equivalents (Mann Whitney P value 0.009) than patients without an MD. Age and duration of illness were higher in patients with an MD but were not significantly associated (Mann Whitney P value 0.06 and 0.44, respectively). Prevalence of an MD did not significantly differ between users of first generation and second generation

antipsychotics or between men and women (Fisher's exact test P value 0.28 and 0.09, respectively).

Genotyping failed for the rs4880 polymorphism in the *MnSOD* gene, yielding the remaining set of 13 SNPs in 8 genes. All other polymorphisms were validated and had a missing genotype rate below 10% in our sample. No polymorphism deviated from Hardy-Weinberg equilibrium (data not shown). The allele and genotype frequencies are shown in Tables 2. LD patterns of the *DRD2* gene can be seen in Table 3. D' and r^2 between the two SNPs in *HTR2A* were 0.28 and 0.01, respectively, and in *HTR2C* 1.00 and 0.04, respectively.

In Table 4 the results of the genetic association tests of all MD (except dystonia) are depicted, corrected for age and gender. The TaqI_D polymorphism in the *DRD2* gene was significantly associated with akathisia (p=0.001). For each extra C-allele a 2.3 (95% CI 1.43-3.82) times higher risk of having akathisia was found. Also, -141C of the *DRD2* gene was significantly associated with TD (P= 0.001). Each extra *Del* allele decreased the risk of having TD by 0.20 (95% CI 0.08-0.50). None of the other polymorphisms showed a significant association with any of the MD. Haplotype analysis on genes *DRD2*, *HTR2A*, and *HTR2C* did not lead to significant results with any of the MD (data not shown).

Table 1. Descriptive statistics of 402 Caucasian patients using antipsychotics, stratified by presence of a movement disorder (MD).

	MD present (n=188)	No MD present (n=214)
Age (years), median (range)	28 (16-47)	26 (16-48)
Gender (male)	153 (81%)	159 (74%)
Duration of illness (years), median (range)	3.9 (0.1-21.7)	3.2 (0.1-20.6)
Diagnosis		
Schizophrenia	137 (73%)	40 (65%)
Schizo-affective disorder	26 (14%)	29 (14%)
Schizophreniphorm disorder	2 (1%)	8 (4%)
Delusional disorder	3 (2%)	7 (3%)
Psychotic disorders NOS	19 (10%)	23 (11%)
Other	1 (1%)	7 (3%)
Antipsychotic use		
First generation antipsychotics (FGA)	14 (7%)	23 (11%)
Second generation antipsychotics (SGA)	144 (77%)	159 (74%)
FGA and SGA	10 (5%)	5 (2%)
Missing	20 (11%)	27 (13%)
Current Haloperidol equivalents (mg), median (range)	6.7 (0.7-21.0)	4.7 (0.6-20.4)

Table 2. Frequencies of alleles and genotypes of polymorphisms in dopamine related candidate genes in 402 Caucasian patients with schizophrenia.

Gene	rs-id	Chromosome position	Variant	Alleles*	MAF	Genotypes 11	12	22
DRD2	rs1800497	Chr11:113270828	TaqI_A	C/T	0.19	273	109	20
	rs6277	Chr11:113283459	C957T	T/C	0.47	115	189	95
	rs1800498	Chr11:113291588	TaqI_D	T/C	0.39	151	185	63
	rs1799732	Chr11:113346252	-141C	C/Del	0.11	321	73	6
DRD3	rs6280	Chr3:113890815	Ser9Gly	T/C	0.30	193	165	38
HTR2A	rs6313	Chr13:47469940	T102C	C/T	0.45	115	211	74
	rs6314	Chr13:47409034	His452Tyr	C/T	0.09	315	66	3
HTR2C	rs3813929	X:113818520	-759C_T	C/T	0.18	319	82**	
	rs6318	X:113965735	Cys23Ser	G/C	0.16	327	73**	
COMT	rs4680	Chr22:19951271	Val158Met	A/G	0.47	109	208	84
NQO1	rs1800566	Chr16:69745145	C609T	C/T	0.18	276	107	18
RGS2	rs4606	Chr1:192781172	-	C/G	0.26	222	154	26
GSTP1	rs1695	Chr11:67352689	Ile105Va	A/G	0.41	140	195	67

MAF = minor allele frequency.
*Major allele is given first.
** Number of patients who is carrier of the minor allele.

Table 3. LD patterns of polymorphisms in *DRD2*, D' (lower triangle) and r^2 (upper triangle) between polymorphisms are given.

DRD2	rs1800497	rs6277	rs1800498	rs1799732
rs1800497	-	0.08	0.13	0.01
rs6277	0.56	-	0.58	0.04
rs1800498	0.59	0.91	-	0.13
rs1799732	0.59	0.56	0.83	-

Table 4. Association of dopamine related polymorphisms with antipsychotic induced movement disorders in 402 Caucasian patients with schizophrenia.

Gene	rs-id	Variant	Alleles*	Parkinsonism		Akathisia		Tardive dyskinesia	
				OR (95% C.I.)	P	OR (95% C.I.)	P	OR (95% C.I.)	P
DRD2	rs1800497	TaqI_A	C/T	1.25 (0.87-1.79)	0.23	1.76 (1.05-2.96)	0.03	1.09 (0.73-1.63)	0.68
	rs6277	C957T	T/C	1.11 (0.82-1.49)	0.51	1.58 (0.98-2.55)	0.06	0.88 (0.64-1.23)	0.47
	rs1800498	TaqI_D	T/C	1.19 (0.88-1.62)	0.27	**2.33 (1.43-3.82)**	**0.001**	0.89 (0.63-1.26)	0.51
	rs1799732	-141C	C/Del	1.01 (0.62-1.64)	0.97	1.48 (0.75-2.89)	0.26	**0.20 (0.08-0.50)**	**0.001**
DRD3	rs6280	SerGly	T/C	0.92 (0.66-1.28)	0.61	0.90 (0.53-1.54)	0.70	1.23 (0.86-1.76)	0.27
HTR2A	rs6313	T102C	C/T	1.21 (0.88-1.66)	0.24	1.37 (0.83-2.26)	0.22	0.85 (0.60-1.21)	0.37
	rs6314	His452Tyr	C/T	1.35 (0.81-2.27)	0.25	0.86 (0.36-208)	0.74	0.66 (0.34-1.29)	0.22
HTR2C	rs3813929**	-759C_T	C/T	0.58 (0.33-1.04)	0.07	0.91 (0.38-2.16)	0.82	0.80 (0.43-1.50)	0.50
	rs6318**	Cys23Ser	G/C	1.20 (0.69-2.09)	0.52	1.11 (0.46-2.67)	0.83	1.36 (0.74-2.50)	0.32
COMT	rs4680	Val158Met	A/G	0.84 (0.61-1.15)	0.27	1.01 (0.62-1.65)	0.96	0.91 (0.64-1.29)	0.60
NQO1	rs1800566	C609T	C/T	0.79 (0.53-1.17)	0.24	1.28 (0.73-2.24)	0.38	1.20 (0.80-1.81)	0.37
RGS2	rs4606	-	C/G	0.80 (0.56-1.14)	0.22	0.87 (0.49-1.54)	0.63	1.01 (0.69-1.49)	0.96
GSTP1	rs1695	Ile105Va	A/G	0.93 (0.68-1.27)	0.65	1.03 (0.63-1.68)	0.90	0.96 (0.68-1.35)	0.80

OR = Odds ratio, 95% C.I. = 95% Confidence Interval, *P* = p-value.

* Major allele is given first.

** Tested in a dominant model, because of position on X-chromosome.

DISCUSSION

This study aimed to replicate previously reported associations in candidate genes for acute and tardive antipsychotic-induced MD in a young Caucasian sample. Of the previously reported polymorphisms two showed significant associations with MD: the *DRD2* gene polymorphisms TaqI_D and -141C were associated to akathisia and tardive dyskinesia, respectively. The MD-prevalence *per se* did relate to the dosage of the prescribed antipsychotics as expressed in haloperidol equivalents, but not to the type of antipsychotics or to the duration of illness.

The reported association between functional *DRD2* promoter allele -141C *Del* and tardive dyskinesia was not found previously (32, 65-67), but an association was found between this promoter allele and antipsychotic induced parkinsonism (33). This may be of clinical interest as antipsychotic induced Parkinsonism has been shown to be a risk factor for the development of tardive dyskinesia (9, 18, 19). It could be argued that the blockade of the postsynaptic D2 receptors by antipsychotics induces hypersensitivity of the D2 receptor, leading to tardive dyskinesia over time, as has been demonstrated in rodent models (68-70). The -141CIns/Del polymorphism -although debated by some (71, 72)- has been suggested to be functional (73) or in linkage disequilibrium with another functional polymorphism (74). Thus, involvement of this *DRD2* allele in antipsychotic induced MD is in line with findings in healthy volunteers, where striatal receptor density is related to this *DRD2* promoter allele (-141C Del) (75).

This study also reports an association between TaqI_D and akathisia. This intronic SNP has previously only been investigated for tardive dyskinesia (32, 67, 76) and was significantly associated in a two marker haplotype with C939T (32). Although the pathophysiology of akathisia is still largely unknown, there is clinical evidence that medication interfering with the dopamine system and leading to a low dopaminergic tone is associated with the insistent feeling of restlessness and the urge to move (77, 78).

Taken together these results suggest a more pleiotropic effect, where involvement of genetic variants in the *DRD2* gene may lead to multiple phenotypic traits of antipsychotic-induced MD, which are pathophysiologically related to each other, albeit with differential clinical expression. This expression is directed additionally by contextual genetic and environmental factors such as population characteristics (i.e. age, ethnicity, duration and type of AP use)

The present study did not replicate other previously reported significant associations with either tardive or acute antipsychotic induced MD. Non-replication is a common problem in pharmacogenetic research and is attributable to several factors (*e.g.* cross-sectional design, inter-rater variation, non-compliance, sample size issues, false

positive versus true negative findings, differences in LD patterns, specific environmental factors and publication bias). Shorter duration of illness and use of second generation antipsychotics are both associated with lower prevalence and less severity of tardive dyskinesia (8). The majority of the candidate gene studies focus on chronic MD (tardive dyskinesia) and older patients, many of them using first generation antipsychotics. Here in contrast, the patients are relatively young and the majority of them use second generation antipsychotics. Five studies so far have reported associations with acute antipsychotic-induced MD, without specifying the MD under study (39-41, 48, 49). To our knowledge, only one candidate study has reported significant results with akathisia specifically (79) and only one with Parkinsonism (33) both in an older sample with a mean age of 40 and 49 years, respectively. The affinities for multiple receptors of second generation antipsychotics, other than the prevailing affinity for the D2 receptor of the first generation antipsychotics, may be responsible for the differing pharmacogenetic associations found in our group of patients. To explore heterogeneity of different side effect profiles, we repeated our analyses in two subgroups of patients, using the most frequently prescribed antipsychotics, risperidone (22%) and olanzapine (26%). This did not change the results. Finally, it is well established that susceptibility and risk factors differ among ethnic groups (16, 17). We studied Caucasian patients only, whereas the -141 C *Del* association with antipsychotic induced parkinsonism was reported in African Caribbean patients (33). The pharmacogenetic differences observed in our study may therefore reflect differential vulnerability for the observed MD in this specific population.

Of note, the prevalence of a MD in this study did not significantly differ between users of first and second generation antipsychotics, despite that second generation antipsychotics are associated with lower risk for MD as compared to first generation antipsychotics (8). However, the finding that patients with an MD used on average a significant higher haloperidol dose equivalent is in line with the clinical notion that the emergence of antipsychotic-induced MD relates first of all to the degree of dopamine blockade. This may pertain even more for the current population, being young and having a relatively short treatment history.

There are some caveats when interpreting these data. The design of this study was cross-sectional. Therefore definite conclusions about the predictive value of the reported associations cannot be made yet. Nonetheless, a reverse association from MD to polymorphism can be excluded. More importantly, the similar prevalence of MD for all prescribed antipsychotics may be the result of confounding by indication. Information on type and dose of antipsychotic medication was additionally provided by the treating physician but may nevertheless be insufficient, as non-compliance is prevalent among patients with schizophrenia (80). Non-compliance is not accounted for by most candidate studies (81). Future pharmacogenetic studies could increase their reliability by including blood levels of antipsychotic medication. Finally,

training and ascertaining of interrater-relaibility is uncommon in pharmacogenetic studies. Here the raters were trained yearly in the recognition of movement disorders and the degree of agreement varied from moderate (kappa 0.56) to good (kappa 0.98). Nonetheless, a certain degree of misclassification of MD cannot be ruled out.

The present study has several strengths. We focussed on previously reported significant associations in candidate genes, taking into account both acute and tardive MD. Our sample is relatively large, consisting of a homogeneous group of Caucasian patients, all diagnosed with a non-affective psychosis. The young age of the included population makes it unlikely that primarily neurological co-morbidity is interfering with the results.

In conclusion, this study did not replicate previously reported polymorphisms. However, we found two novel SNPs associations in the *DRD2* gene. The TaqI_D variant was associated with acute akathisia and the -141C variant with tardive dyskinesia. These polymorphisms were previously reported in tardive dyskinesia and acute Parkinsonism, respectively. These results suggest involvement of genetic variants in the *DRD2* gene for susceptibility of MD in a more general or pleiotropic way. Findings of associated polymorphisms in patients with a relatively short duration of illness are clinically relevant as they could further help to identify early markers of vulnerability for MD. Follow-up studies in similar samples with young patients and atypical antipsychotics are warranted to support our findings.

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Chapter 5

Clinical Response to Antipsychotic Drug Treatment: Association Study of Polymorphisms in Six Candidate Genes

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ABSTRACT

Introduction: The large variation in individual clinical responses to antipsychotic treatment hampers efficient treatment of psychotic disorders. Genetic factors are considered a main cause of this variation. Pharmacogenetic studies have demonstrated significant associations between several candidate genes (*DRD2*, *DRD3*, *HTR2A* and *HTR2C*, *COMT* and *MTHFR*) and antipsychotic drug response. The present study investigates the effect of eight polymorphisms in these genes for an association with antipsychotic treatment response.
Methods: 329 Caucasian patients with a non-affective psychotic disorder using antipsychotics were included. All patients participated in the longitudinal GROUP-study in The Netherlands. 8 SNPs in 6 candidate genes were genotyped (*DRD2* Taq1A, -141C Ins/Del; *DRD3* Ser9Gly; *HTR2A* T102C, His452Tyr; *HTR2C* Cys23Ser; *COMT* Val108Met; *MTHFR* 677-C/T) using standard protocols. Polymorphisms were based on previous studies showing associations with positive or global symptom treatment response. The Clinical Global Impression- Improvement (CGI-I) scale was used to assess improvement in positive psychotic symptoms since the start of current antipsychotic treatment. Ordinal regression was used for association analyses.
Results: Ninety percent of the patients used second generation antipsychotics, most frequently olanzapine (28%) and risperidone (29%). Ser9Gly of the dopamine D3 receptor gene (P value 0.034) and 677-C/T of *MTHFR* (P value 0.019) were associated with response in that Gly-carriers and T-carriers, respectively, showed more clinical improvement on the CGI-I. The other polymorphisms did not show a statistically significant association (P values >0.10).
Conclusion: Two out of eight previously reported associations between genetic variation and antipsychotic treatment response were replicated. The direction and magnitude of the associations presented in *DRD3* (Ser9Gly) and *MTHFR* (677-C/T) are in line with previous studies in Caucasian patients. These polymorphisms may be of value for predicting clinical response.

INTRODUCTION

Since their introduction in the 1950s antipsychotic drugs play a key role in the treatment of psychotic disorders. However, almost half of schizophrenic patients display insufficient response to antipsychotic treatment (1-3). Factors that influence the variation in response to antipsychotic drug treatment have not been well-elucidated. Inter-individual and inter-racial variability in response to drug treatment may possibly reflect genetic heterogeneity and the presence of modifier genes. Several genetic association studies have been performed showing positive associations between response and polymorphisms in genes coding for the dopaminergic, serotonergic, and several other systems. In this study, the focus was on polymorphisms associated with antipsychotic drug response (positive or global symptoms) in Caucasian patients only.

Dopamine D2 receptor blockade is a property of all antipsychotics. The Taq1A and -141C Ins/Del polymorphisms are variants of the dopamine receptor D2 gene (*DRD2*) which are linked to D2 receptor density at the level of the striatum (4, 5). Several studies have associated TaqI_A and -141C Ins/Del with response to various antipsychotics (6-9). The affinity of certain antipsychotics for the D3 receptor may reflect a part of the action mechanism (10). The Ser9Gly polymorphism of the dopamine receptor D3 gene (*DRD3*) is an amino-acid substitution in the N-terminal extracellular part of the receptor and might influence dopamine binding affinity (11). An association between treatment response and the Gly allele was found in several studies (12-15) and suggested by meta-analyses (16, 17).

Alterations in the serotonergic system have been implicated in the mechanisms of action of antipsychotics (particularly SGA), having antagonistic properties on serotonergic receptors, especially the serotonin 2A and 2C receptor. Two SNPs (His452Tyr and T102C) of the 5-hydroxytryptamine (serotonin) receptor 2A gene (*HTR2A*) were the subject of several association studies on the response to clozapine. Results were conflicting (18-24, 24-26) but suggest a possible role of these SNPs in treatment response. The T102C SNP does not provoke an amino acid substitution, but in Caucasians it is in complete linkage disequilibirum with variant -1438-G/A, which is located in the gene promoter. The A allele of this polymorphism shows higher activity of the promoter (27). The His452Tyr polymorphism affects the function of the receptor; the Tyr variant is associated with reduced ability to activate C and D phospholipases (28). The Cys23Ser polymorphism of the 5-hydroxytryptamine (serotonin) receptor 2C gene (*HTR2C*) was associated in one study with global response on clozapine (29). Five later studies failed to replicate, but a meta-analysis underpinned the association (30). The consequences of the involved amino acid substitution are unknown.

COMT (catechol-O-methyltransferase) is involved in the degradation of dopamine in the prefrontal cortex. The *COMT* gene has a relevant Val108Met polymorphism. This polymorphism was associated with response in several studies (31-33), but not in all (34). The MTHFR (methylenetetrahydrofolate reductase) enzyme is required for the synthesis of 5-MTHF, a cosubstrate for the conversion of homocysteine into methionine. High plasma levels of homocysteine have been suggested as a risk factor for schizophrenia (35). The *MTHFR* gene has a missense mutation 677-C/T. Patients possessing a copy of the T allele showed better treatment response (36).

In the present study, the above mentioned eight polymorphisms were tested for their association with response on positive symptoms in a sample of Caucasian patients with a psychotic disorder. The focus was on polymorphisms associated with response in terms of positive symptoms because antipsychotics are mainly effective for treating this symptoms domain.

PATIENTS AND METHODS

Sample

A sample of 329 in- and outpatients using antipsychotic medication was drawn from the participants in the ongoing longitudinal Genetic Risk and Outcome of Psychosis (GROUP) study. In GROUP, patients were identified in selected representative geographical areas in the Netherlands and Belgium. Inclusion criteria for GROUP were: (i) age range 16 to 50 years, (ii) diagnosis of non-affective psychotic disorder and (iii) good command of Dutch language. For the present analysis the following extra inclusion criteria were applied: (iv) use of antipsychotic medication at the time of assessment for at least one month and (v) Caucasian ethnicity of Northern European ancestry. The study was approved by the Ethics Committee of the University Medical Center Utrecht and by the institutional review boards of all other participating hospitals. All subjects gave written informed consent in accordance with the committee's guidelines.

Genotyping

A total of 8 polymorphisms in 6 candidate genes were selected for the current study: *DRD2* Taq1A and -141C Ins/Del; *DRD3* Ser9Gly; *HTR2A* T102C and His452Tyr; *HTR2C* Cys23Ser; *COMT* Val108Met; *MTHFR* 677-C/T. These polymorphisms were chosen *a priori* based on findings in other association studies and determined by Sequenom (Hamburg, Germany) using the Sequenom MassARRAY iPLEX platform at the facilities of the manufacturer. Quality check for genotyping was performed in the overall GROUP study, which encompassed exclusion of polymorphisms and individuals based on missingness and based on departure from Hardy-Weinberg equilibrium in a sample of 398 controls without a psychotic disorder.

Phenotyping

All patients were evaluated by their attending psychiatrist with the Clinical Global Impression - Improvement (CGI-I) scale at one point in time. This instrument was used to score the change in positive symptoms since the start of the current antipsychotic medication on a seven point scale (1, very much improved; 2, much improved; 3, minimally improved; 4, no change; 5, minimally worse; 6, much worse; or 7, very much worse). Response was dichotomized for some of the analyses: improvement was defined as an CGI-I score of very much improved or much improved. Attending psychiatrists were blinded to patient's genotype. Other clinical variables that were measured in this study were DSM-IV diagnosis, duration of illness, and antipsychotic medication and dose. Haloperidol dose equivalents were subsequently calculated as outlined by (37).

Statistical analysis

Age, duration of illness and haloperidol dose equivalents were compared between patients with and without response and were tested for statistical significance using a Mann-Whitney test or *t*-test when appropriate. Response between the most frequently prescribed antipsychotics and between diagnoses was compared and tested for statistical significance using a χ^2 test. Ordinal regression (38), with the logit link function, was used to quantify the association between the highly skewed CGI-I score on positive symptoms and polymorphisms, while adjusting for age and gender. Sequential low prevalent outcome groups were pooled to fulfill the assumptions of ordinal regression, i.e. high enough cell counts in each genotype/outcome group. The null hypothesis of parallel lines was tested for each polymorphism using χ^2 tests. An additive model with minor allele dose as independent variable was initially considered for all polymorphisms. When genotype/outcome group cell counts were too low, a dominant model for the polymorphism was considered. Polymorphism rs6318, positioned on the X-chromosome, was tested in a dominant model only. All statistical analyses were performed using SPSS 16.0 for Windows. Since all eight polymorphisms were based on earlier positive association studies in Caucasian patients, adjustment for multiple testing was not performed. The significance level of this study was set at 0.05, two-sided.

RESULTS

Descriptive statistics of the study sample are presented in Table 1 according to response on positive symptoms. Patients with response were on average two years older (Mann Whitney P value 0.031) than patients without response. Haloperidol equivalents and duration of illness were not significantly different between response groups. Improvement differed significantly between users of the six most prevalent

Table 1. Descriptive statistics of 329 Caucasian patients using antipsychotics, stratified by improvement on positive symptoms.

	Improvement (n=247)	No improvement (n=82)	P-value
Age (years), median (range)	27 (16-47)	25 (16-42)	**0.031**
Gender (male)	80%	77%	0.52
Duration of illness (years), median (range)	3.4 (0.2-21.4)	3.2 (0.2-12.0)	0.99
Current dose (haloperidol equivalents[1]), median (range)	4.8 (0.6-18.2)	6.6 (0.6-21.0)	0.33
Diagnosis			
Schizophrenia	163 (66%)	57 (70%)	
Schizo-affective disorder	37 (15%)	9 (11%)	
Schizophreniphorm disorder	6 (2%)	3 (4%)	
Psychotic disorder NOS	28 (11%)	9 (11%)	
Delusional disorder	6 (2%)	4 (5%)	
Other	7 (3%)	0 (0%)	0.45
Antipsychotic use			
Risperidone	68 (28%)	28 (34%)	
Olanzapine	81 (33%)	12 (15%)	
Quetiapine	16 (7%)	7 (9%)	
Aripiprazole	25 (10%)	11 (13%)	
Clozapine	28 (11%)	12 (15%)	
Haloperidol	5 (2%)	5 (6%)	**0.024**
Other	24 (10%)	7 (9%)	

[1] Calculated as outlined by Andreasen *et al* (2010).

Table 2. Frequencies of alleles and genotypes of polymorphisms in 329 Caucasian patients using antipsychotics.

Gene	rs-id	Chromosome position	Variant name	Alleles[1]	MAF[2]	Genotypes		
						11	12	22
DRD2	rs1800497	Chr11:113270827	Taq1A	C/T	0.17	228	89	12
	rs1799732	Chr11:113346252	-141C Ins/Del	C/Del	0.11	262	60	5
DRD3	rs6280	Chr3:113890814	Ser9Gly	T/C	0.32	150	139	36
HTR2A	rs6314	Chr13:47409033	His452Tyr	C/T	0.10	255	59	3
	rs6313	Chr13:47469940	T102C	C/T	0.45	92	177	59
HTR2C	rs6318	X:113965734	Cys23Ser	G/C	0.16	249	48[3]	
COMT	rs4680	Chr22:19951270	Val108Met	A/G	0.46	91	169	68
MTHFR	rs1801133	Chr1:11856378	677-C/T	C/T	0.32	153	141	35

[1]Major allele given first.
[2]MAF= Minor allele frequency.
[3]Carriers of variant allele.

Table 3. Results of association analyses in 329 Caucasian patients using antipsychotics between polymorphisms and clinical improvement on positive symptoms.

				Ordinal regression[1]		
Gene	rs-id	Variant name	Alleles[2]	Beta	Odds Ratio	P-value
DRD2	rs1800497	Taq1A	C/T	-0.24	0.79 (0.54-1.14)	0.21
	rs1799732[3]	-141C Ins/Del	C/Del	-0.36	0.70 (0.50-1.23)	0.17
DRD3	rs6280	Ser9Gly	T/C	**-0.33**	**0.72 (0.53-0.97)**	**0.034**
HTR2A	rs6314[3]	His452Tyr	C/T	-0.14	0.87 (0.53-1.40)	0.53
	rs6313	T102C	C/T	0.10	1.11 (0.81-1.49)	0.53
HTR2C	rs6318[3]	Cys23Ser	G/C	-0.47	0.63 (0.34-1.15)	0.13
COMT	rs4680	Val108Met	A/G	0.08	1.08 (0.80-1.45)	0.62
MTHFR	rs1801133	677-C/T	C/T	**-0.37**	**0.69 (0.51-0.94)**	**0.019**

[1]Ordinal regression analysis with CGI-I improvement as dependent variable, and minor allele frequency of the polymorphism as independent variable (additive model), corrected for age and gender. A lower beta or lower odds ratio means more improvement (lower CGI-I score) per extra minor allele.
[2] Major allele given first.
[3]Polymorphisms tested in a dominant model.

antipsychotics (χ^2 test, 5 d.f., P value 0.024), olanzapine having the highest response rate (87%) and haloperidol the lowest (50%).

All polymorphisms were validated and had a missing genotype rate below 10% in the total sample. No polymorphism deviated from Hardy-Weinberg equilibrium. The allele and genotype frequencies are shown in table 2. Due to the low prevalence of scores 4, 5, 6 and 7 on the CGI-I, three ordinal groups of response were chosen: a CGI-I score of 1, 2, and 3 or higher. His542Tyr and -141C Ins/Del showed too low cell counts when tested additive, and were subsequently tested dominantly. The null hypothesis of parallel lines was not rejected for any of the polymorphisms tested.

In table 3 the associations with response are depicted. Two of the eight polymorphisms showed a significant association with response on positive symptoms. The Gly (C) allele of Ser9Gly of the *DRD3* gene was associated with more improvement on positive symptoms (P value 0.034). The T-allele of 677-C/T of the *MTHFR* gene also showed more improvement (P value 0.019). The Gly-allele and T-allele showed odds ratios of 1.39 (95% CI 1.03-1.90) and 1.45 (95% CI 1.06-1.98) for being in a better response category, respectively.

DISCUSSION

This study aimed to replicate previously reported significant findings from candidate gene studies on positive symptoms improvement in patients with a psychotic disorder treated with antipsychotics. Of the eight tested polymorphisms two showed significant association with response: Ser9Gly of the *DRD3* gene and 677-C/T of the *MTHFR* gene. Both results were in the same direction as the previous positive association studies reporting on these polymorphisms (12-15, 36).
Previous positive association studies with Ser9Gly in Caucasian patients were performed in prospective studies with patients using clozapine (14, 15), olanzapine (12), and several SGA (13). In all four studies the Gly allele was associated with better response. All these studies used different symptom scales and treatment duration varied between 12 weeks and 6 months. Three other studies (two clozapine and one FGA) with Caucasian patients showed no significant association (16, 39, 40). Binding experiments showed that homozygotes for the Gly variant have an increased binding affinity for dopamine (11). However, the biological function is hard to interpret since the Gly variant was predominantly associated with poor response in Chinese populations (41, 42). The opposite direction of association between ethnicities may be explained by a linkage disequilibrium status with another functional polymorphism (43).

Only one study has been performed regarding 677-C/T of *MTHFR* and antipsychotic response (36). The T allele was found more often in responders compared to non-responders, all having FGA. The 677-C/T polymorphism is functional (44) and is

possibly associated with risk of schizophrenia itself (45, 46). MTHFR is required for the synthesis of 5-methyltetrahydrofolate, the primary circulatory form of folate and the carbon donor for homocysteine remethylation to methionine. Homozygous individuals (TT) have around one third of the expected MTHFR enzyme activity, and heterozygotes (CT) have around two third activity, compared to the most common genotype CC (44). Some patients with homocysteinuria, which can be caused by mutations in the MTHFR gene, have demonstrated schizophrenia-like symptoms (47). Furthermore, high levels of homocysteine have been observed in schizophrenia patients (35, 48). Several reports link a high plasma level of homocysteine to various neurological conditions also, such as pregnancies complicated by neural tube defects (49) and migraine (50), suggesting a possible role in the development of the nervous system. Taken together, these results support an interaction between *MTHFR* and antipsychotic medication.

However, six of the previously associated polymorphisms with response on positive symptoms were not replicated. A likely explanation for the variation in results from pharmacogenetic studies is the heterogeneity in the characterization of the phenotype and patient sample as well as in the characterization of the genetic variability. This concerns the present study as well. An important difference of this study with other studies is the time between start of the medication and assessment of improvement. The period for which response is assessed varied in the present study. However, since most patients show response in the first month after the start of an antipsychotic (51) this method seems valid for pharmacogenetic purposes. This study represents a relatively large group of patients showing improvement, what can be expected when response is cross-sectionally measured. Patients who do not respond well are less likely to continue an antipsychotic and will eventually switch to another antipsychotic. This might have underestimated the magnitude of our results, because of the lower variability in response outcome. Another difference between pharmacogenetic studies on antipsychotics (including ours) is the inclusion of all antipsychotics as opposed to focussing on a single antipsychotic. To do a true replication study, all methodologies and patient characteristics should be identical. This is however not possible when testing different polymorphisms at once, all tested before in different studies with different approaches. Publication bias might be an important contributing factor as well, i.e. positive results have a better chance of being published than negative results. A combination of above mentioned aspects is probably the reason why most pharmacogenetic association results regarding antipsychotic response are conflicting: positive associations are often not replicated (52, 53). Apart from the possible limitations of the cross-sectional design used, the present study has also several strengths. Compared to most other candidate studies a relatively large sample size of more than 300 patients using antipsychotics was achieved. This study does not suffer from heterogeneity with regard to ethnicity and diagnosis. A homogeneous group of Caucasian patients of Northern European ancestry, all diagnosed with a non-affective psychosis, was included.

In conclusion, two of the previously reported associations between polymorphisms and treatment response were replicated in the present study. Heterogeneity in patient samples and outcome variables as well as publication bias may all play a role in lack of replication, in the present and other studies. The direction of the associations presented here in *DRD3* (Ser9Gly) and *MTHFR* (677-C/T) are in line with previous association studies in Caucasian patients. These polymorphisms may be of clinical value if their added value to other clinical predictors of response can be demonstrated in future research.

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Chapter 6

General Discussion

Personalized pharmacotherapy

Pharmacotherapy of psychosis is still far from optimal. Lack of compliance, limited efficacy and side-effects are major problems (1, 2). Personalized pharmacotherapy may circumvent some of these problems or at least pare them down. Personalized pharmacotherapy implies the prediction of drug-response phenotype in the individual patients with the purpose to prescribe the optimal drug in the optimal dose. Two examples of personalized pharmacotherapy are (1) the use of clinical characteristics to identify patients who benefit most from depot drug administration and (2) the use of pharmacogenetics in selecting an appropriate antipsychotic for an individual patient. Depot antipsychotics are aimed at improving compliance in non-compliant patients, by administering a long-working antipsychotic every one to four weeks (3). Depots are widely available but the actual use has been limited in many places of the world (4), while advantages have been shown (5). The goal of pharmacogenetics is to predict the optimal drug and dose for a patient based on his genetic profile (6). Pharmacogenetics of antipsychotics is not widely applied due to limited evidence of firm associations between genetic variants and response. Nevertheless genotyping the cytochrome P450 status of a patient has already shown to be worthwhile in optimizing antipsychotic dose (7) and this is actually practiced, albeit limited.

In the present thesis clinical aspects of the prescription of oral versus depot medication and first generation antipsychotic (FGA) depot versus second generation antipsychotic (SGA) depot have been investigated (chapter 2). The larger part comprised pharmacogenetic studies on antipsychotic efficacy (chapter 5), antipsychotic-induced metabolic disturbances (chapter 3) and movement disorders (chapter 4). In this general discussion the results of the studies are briefly summarized, with some notes on the current status of depots in antipsychotic therapy added. Then, issues concerning the validity and precision in the field of pharmacogenetics of antipsychotics are addressed, illustrated by examples from this thesis and other studies. Finally, the clinical value of the results described in this thesis is evaluated and directions for future research are discussed.

Depot studies: results and current status

In chapter 2.1 it was shown that patients who switched to a depot showed signs of less compliance and more movement disorders compared to switchers to oral antipsychotics. This is in line with the Dutch guidelines. It was also found that switchers to depot had significantly less often used psychotropic comedication before. This might be a reflection of the tendency of most clinicians to believe that depots are less acceptable to patients than tablets are (8). Our results showed that

risperidone long-acting injectable (RLAI), the first SGA depot, is often used as a last resort to psychotic patients (chapter 2.1 and 2.2). This is a good example of the *channeling* of a drug. From the research perspective this is relevant because this could have important consequences for interpreting observational comparisons between groups of antipsychotics, possibly leading to an underestimated effect of depots by clinicians (9).

Compliance is a major problem in antipsychotic therapy (10, 11). Studies have shown that more than one third of the patients do not take the recommended dose after approximately one month of treatment. After two years of treatment the proportion of non-compliant patients increases to approximately 75% (2, 12, 13). Systematic reviews indicate that depots could have advantages over oral antipsychotics, with reduced risk of relapse and rehospitalization, better global outcomes, and less variable plasma levels (3, 5, 14). With the introduction of SGA depots mid 2000s there is a larger pharmacological spectrum, making the use of depot more attractive. However, sound studies comparing different depot antipsychotics have not been performed yet. Prospective studies investigating the optimal place of depots in current pharmacotherapy are also needed. In The Netherlands it was recently recommended to measure plasma levels when two antipsychotics have proven to be non-efficient, and to consider a depot when signs of non-compliance are present (15). In our studies (chapter 2) it was shown that relatively few antipsychotic drug users are switched to depots, which is in line with conclusions of others (16, 17). Also, in the GROUP studies (chapters 4 and 5) less than 10% of the patients used a depot antipsychotic at the time of the study. To conclude, the prescription of depot medication seems to be an underutilized strategy in suboptimal outcomes of treatment of psychosis. Therefore, depot formulations have the potential to achieve improvement in treatment results.

PHAMOUS studies: results on weight gain and metabolic parameters

In chapters 3.1-3.4 metabolic parameters were examined for an association with several genetic variants. We are the first to find histamine H1 variants to be associated with obesity in antipsychotic users (chapter 3.1). Histamine H1 receptor affinity of an antipsychotic has been correlated with weight gain data in previous studies and is often suggested as one of the primary mechanisms of antipsychotic-induced weight gain. However, few pharmacogenetic studies on this receptor have been performed and those who have did not show an association with antipsychotic-induced weight gain. The importance of taking into account the interaction between receptor affinity and genotype in a sample with users of different antipsychotics is accentuated in this study and will be discussed in more detail later.

An association was shown, in females only, between obesity and a variant in the *ROBO1* gene (chapter 3.4). This gene was previously associated in GWAS studies

with risk of schizophrenia (18, 19) and with BMI in non-schizophrenic populations (20). Thus, *ROBO1* might be a candidate gene for antipsychotic-induced weight gain. Further studies are warranted to unravel the possible role of this gene in the mechanisms of action of antipsychotics. Together with similar gender stratified pharmacogenetic results in leptin genes, this finding also emphasizes that antipsychotic-induced weight gain might involve different pathways in men and women.

No association between alpha2a adrenergic and muscarinic acetylcholine M3 receptor variants and metabolic parameters was found (chapter 3.2). Affinity for these receptors is suggested as possible mechanism for metabolic disturbances in antipsychotic medication (21), but these receptors were under-investigated in pharmacogenetic studies. Future studies including more genetic coverage of these receptor genes may still be valuable. A replication study (chapter 3.3) was performed and added more evidence that serotonin 2c receptor variant -759C/T is associated with the metabolic syndrome. This variant is often associated with antipsychotic-induced weight gain (7, 21). However, in our population the association with the metabolic syndrome is primarily the effect of dyslipidemia, suggesting the pleiotropic effects of this variant.

GROUP studies: results on movement disorders and response

In chapter 4 of this thesis strong significant associations were found of two variants in the dopamine D2 receptor with akathisia and tardive dyskinesia, which had not been observed previously. However, the main finding of this study was that all other eleven variants, which were chosen based on previous positive association studies, were not associated with any movement disorder in our population. Chapter 5 investigated previously associated variants with antipsychotic efficacy and showed that two out of eight investigated variants were significantly associated. Both associations were in the same direction as in earlier positive studies. These variants in the dopamine D3 receptor (*DRD3*) and methylenetetrahyrofolate reductase (*MTHFR*) gene could be helpful in predicting antipsychotic treatment response. However, *a priori*, in these two studies more significant associations were expected.

Potential and problems of pharmacogenetics

Negative results and lack of replication are a common finding in pharmacogenetic studies of antipsychotics (7, 22). Indeed, genetic association studies in general suffer from non-replication. Ioannidis *et al* (23) investigated 55 meta-analyses of various genetic associations, and only nine associations were successfully replicated. Others have confirmed that most candidate gene association studies cannot be replicated (24). Nevertheless, the implementation of pharmacogenetics into clinical practice has proven valuable in some fields of medicine (25). Thiopurine methyltransferase

(TPMT) is the drug-metabolizing enzyme that catalyzes the S-methylation of azathioprine and its initial product 6-mercaptopurine. Both drugs suppress the immune system and are used in cancer chemotherapy. Clinical studies have demonstrated that genotyping three variants of the TMPT gene can predict toxicity of 6-mercaptopurine therapy with high accuracy. These three variants account for the majority of persons with an intermediate (10% prevalence) or low (0.3-0.6% prevalence) TPMT activity (26, 27). Children with acute lymphocytic leukemia with intermediate or low TPMT activity are at increased risk of myelosuppression (28) and secondary cancers (29, 30), when prescribed standard doses. Other drugs for which there is a reasonable evidence base supporting genotyping include muscle relaxants suxamethonium and mivacurium (butyrylcholinesterase) (25). Nevertheless, the evidence for genetic testing for other drugs, including antipsychotics, is weak (25). Pharmacogenetic studies are impeded by multiple problems, especially in the case of complex disorders as psychiatric disorders, e.g. schizophrenia (31). Below we give an overview of the studies of the most examined variant in antipsychotic response, Taq1A, to illustrate which problems are encountered (in interpreting results) in the pharmacogenetics of antipsychotics.

Taq1A is located downstream of the dopamine receptor D2 gene, and the A1 allele is associated with reduced *DRD2* gene expression (32-34). To date, 13 pharmacogenetic studies have been performed, of which 8 found a significant association (35). Of these, 4 studies (25 Japanese patients for 3 weeks on nemonapride, 166 Japanese patients for 8 weeks on risperidone, 128 Chinese patients for 4 weeks on aripiprazole, 90 Korean patients for 26 weeks on aripiprazole) found the A1 allele to be associated with better response. Three studies (18 Caucasians patients for 6 weeks on amisulpiride/flupentixol, 183 Caucasians patients for 6 months on clozapine, 213 Asian Indian patients for 1 year on clozapine/haloperidol/risperidone) found, on the contrary, the A2 allele to be associated with better response. One study (57 Caucasians patients for 4 weeks on haloperidol) found heterozygotes to be associated with better response compared to both homozygote groups (35). A meta-analysis, not taking into account the different ethnicities used, with pooled response rates including eight of these studies showed no association with genotype (36). This is a good example of how inconsistent results in the pharmacogenetics of antipsychotics can be and how heterogeneous studies are. The critical reader already noticed the variability in ethnicities, antipsychotics and genetic models, which alone could be a sufficient explanation of the variability of results. In chapter 5 no association between this variant and antipsychotic response was found, decreasing the likelihood that this variant is important. Generalizability and implication into clinical practice of this variant is extremely problematic with such a range of results and studies. However, not all pharmacogenetic results are that inconsistent.

Ten out of seventeen studies, with mainly clozapine and olanzapine users, showed that the C-allele of the serotonin receptor 2C variant -759C/T was associated with

increased weight gain (35). The other 7 studies found no association. A meta-analysis showed that each extra C-allele showed a more than two-fold increased risk for clinically significant weight gain (37). This association was confirmed and extended in our population (chapters 4 and 5) with an increased risk of this allele for obesity and the metabolic syndrome. This polymorphism has however not proven its value in a good quality randomized controlled trial yet, which might be the barrier to general acceptance of implementation into clinical practice (38).

The reason for negative and inconsistent results in the pharmacogenetics of antipsychotics and the slow implementation into clinical practice may have several reasons. Pharmacogenetic studies in general are threatened by several validity problems, such as confounding by population stratification, ethnicity, and covariables that interfere in the association, and genotyping problems (31). Pharmacogenetic studies of antipsychotics in particular have additional validity problems, such as the large heterogeneity in antipsychotics and patient characteristics, and the definition of outcome measures. In addition, there are problems with precision, such as poor measurement quality, low sample sizes and multiple testing issues. These problems will be discussed in the following paragraphs, including the impact on this thesis and their possible solutions.

Problems of validity in the pharmacogenetics of antipsychotics

Population stratification

It has been suggested that population stratification is a major reason for the lack of replication in genetic association studies (31). It can lead to spurious associations when both outcome and genotype distributions differ in subgroups of the sample under investigation. It is often difficult to control for population stratification, especially when there is no rationale for possible genetic subgroups and genome wide data are not available. It is unlikely, however, that population stratification had much impact on the results of the GROUP studies performed in chapters 4 and 5. Only Caucasian patients were included from a limited geographical area in The Netherlands and Belgium. The three cohorts in chapter 3 had different recruitment procedures (e.g. inclusion of patients with overweight only in one population and all weights in another population), leading to substantial outcome differences between subsamples in different clearly defined geographical regions. Therefore we chose to correct for differences between cohorts by including a covariate 'cohort' in the analyses aiming to negate possible population stratification. However, this did not influence the results we found. In many studies the possibility of population stratification is not addressed, and this is hazardous, especially when different ethnicities are included or when patients are included from large geographical areas. Family based studies are a general solution for population stratification, taking into account the genotype and outcome of the parents of a person. The GROUP study is a

good example of a family based study with up to 1000 families of patients with a psychotic disorder. However this design is not appropriate for pharmacogenetic purposes. Family members mostly have no psychotic disorder and if so, pharmacotherapy virtually always differs between family members.

Ethnicity

Ethnicity is also an important determinant of genetic associations. Genetic associations are frequently only found in one or a few ethnicities. Opposite directions of genotype effect have also been found between ethnic groups. Combining different ethnicities in one pharmacogenetic study is therefore hazardous. However, a small number of patients with a non-Caucasian ethnicity were included in the study samples of chapters 3.2 and 3.3 to increase sample size. It was reasoned that this was valid since the genotype effect of these ethnicities was the same as in the Caucasian population. So, exclusion of these non-Caucasian ethnicities did not lead to a different genotype effect size. This approach may be questioned since it may induce false-positive results. The best approach is to include one ethnicity only. However, many pharmacogenetic studies on antipsychotics have included several ethnicities or do not address ethnicity in a clear way. Differences in minor allele frequency between ethnicities are also important. For example, the minor allele frequency of the -759C/T variant ranges from 3.3% in African subjects to 33.3% in Asians (35). This indicates that this receptor will be of little clinical value in Africans, but might be important in Asian populations, where more than half of the people will have at least one minor allele. Negative findings in samples with low minor allele frequency are only informative if the study has sufficient statistical power. Likewise, the variants important for genotyping CYP2D6 status differ between Caucasians and Asians. In addition, 6-10 % of Caucasian patients have a slow CYP2D6 status, in contrast to only 1-2% of Asians (39).

Role of covariables

Next to ethnicity, numerous covariables are capable of inducing bias in pharmacogenetic studies. For example, differences in compliance between patients could dramatically weaken or alter results, especially when compliance affects genotype groups differently. When compliance is influenced by similar brain structures as antipsychotics act on, for example dopamine pathways, false positive pharmacodynamic associations can be induced when the mediating role of compliance is not taken into account. In our studies compliance was unfortunately not (well) documented. In an ideal situation plasma levels of the antipsychotic should be measured, to control for in analyses. However, few pharmacogenetic studies have done this. Moreover, the exact relationship between antipsychotic plasma levels and outcomes (response, side-effects) is not always clear, so how to correct for plasma levels is another difficult issue.

Numerous other factors have been shown to influence the outcomes of antipsychotics (40). All those factors could weaken results, especially in cross-sectional studies. Some examples are psychiatric comorbidity, previous use of antipsychotics, and concomitant medication such as antidepressants and benzodiazepines, but also alcohol, caffeine and nicotine. Medication that directly influences the outcome under investigation can also disturb study results. This can be illustrated by the pharmacogenetic study on HbA1c (chapters 3.1) in which some patients used antidiabetics, which lower HbA1c values. Inadequate correction for this covariable may very well lead to extra bias as well. In general, only patients with a high HbA1c value will use antidiabetics. Consequently, correction for the use of antidiabetics when testing the association between genotype and HbA1c in a regression model, leads to a positive estimate of the use of antidiabetics on HbA1c (instead of the true negative effect). This leads to an even larger underestimation of the true genotype effect than when not controlling for antidiabetic use. Sadly, a good solution for this problem of medication influencing the outcome parameter is not present. For example, stratifying on the use of diabetics and then pooling the results will still underestimate a true genotype effect. The problem of adjustment for treatment effect in studies of quantitative traits have been described before, and the suggested most optimal solution is to decrease or increase the outcome parameter with a value based on the mean effect the medication shows in clinical trials (41, 42). In addition, it might be that the genotype under investigation also influences the effect of the antidiabetic medication on HbA1c levels, which could again disturb the true association under investigation between genotype and antipsychotic induced hyperglycaemia.

Genotyping errors

Genotyping errors may have several origins, such as low quantity or quality of DNA, biochemical artefacts and human errors (43). A rate of incorrect genotyping up to around 1% is common in many laboratories (44, 45). Genotyping errors could dramatically decrease the power for detecting associations, especially in the case of rare alleles (44, 46). If there is too low confidence in an allocated genotype it will be reported as missing. Self-evident, missing genotypes also lead to decreased power. More importantly, a high missingness rate might indicate that the non-missing genotypes of that SNP are of low quality. In the GROUP study an extensive quality check was performed on the genotype data. Missing genotype rates in the studied variants in chapters 4 and 5 are up to 10% in one variant of the *HTR2A* gene. In general, imputation of missing genotypes is advisable to restore power. Since a limited number of variants were genotyped, imputation of missing genotypes based on observed genotypes was not possible. Although several distributions of missingness that could indicate low quality genotyping were checked and ruled out, it was impossible to exclude the possibility that some of the remaining genotypes are

not correct. In pharmacogenetic papers, the description of genotype errors and missingness is often neglected, while this affects most data and can markedly influence conclusions of a study (31, 43).

Heterogeneity of antipsychotics

Antipsychotics are a structurally diverse group of drugs. Genetic effects may very well differ between antipsychotics. However, since antipsychotic mechanisms are not fully understood, it is for practical reasons often assumed that genetic associations are similar between the different types of antipsychotic. An example where this was assumed was the association study of the *ROBO1* gene and BMI (chapter 3.4) where patients with eight types of antipsychotics were pooled. On the contrary, in chapter 3.1 of this thesis it was shown that in pharmacodynamic association studies the affinity of a drug to a certain receptor may be very important. The haplotype of two variants of the *HRH1* gene had an opposite effect on BMI and obesity (p=0.015 and p=0.005, respectively) in low H1 versus high H1 affinity antipsychotics users. Moreover, in chapters 3, 4 and 5 it was also assumed that the pharmacogenetic outcomes of the tested variants are similar between ways of administration, i.e. oral or depot. However, the most precise approach is to include only one type of antipsychotic in a study, but this dramatically decreases sample sizes in most (observational) studies, since there are several used antipsychotics. Careful consideration of combinations of antipsychotics in analyses is an important, possibly underestimated, step in pharmacogenetic studies.

Heterogeneity of patient characteristics

Some studies only include patients with a DSM-IV diagnosis of schizophrenia, whereas other studies such as ours, also include patients with other psychotic disorders. Some studies do not clearly mention how a diagnosis is reached or just include patients using antipsychotics when investigating side-effects. It is important to realize that antipsychotics are used for many other psychiatric disorders, such as bipolar disorder, attention deficit hyperactivity disorder, conduct disorders, and Tourrette's disorder. In addition, different pathophysiological mechanisms might be involved in different psychotic disorders, and similarly different antipsychotic drug response mechanisms might be involved. Even in an apparently homogeneous group of schizophrenia patients heterogeneous subgroups may exist. Indeed, the classification and definition of schizophrenia is still fiercely debated (47-49). In addition, antipsychotic history might be an important patient characteristic. Patients that have been treated with antipsychotics for a long time may have important alterations in brain structures that are important in drug mechanisms. Moreover, inter-individual variation in the amount of alteration is also possible, which could lead to differential pharmacogenetic outcomes between patients with a long treatment duration as well. Inter- and intra-study variability in treatment duration

could also partly explain the lack of replication in pharmacogenetic studies, like in chapter 5. Other important characteristics that may need to be controlled for are a history of prior response and demographic factors such as age and gender (as shown in the *ROBO1* study in chapter 3.4).

Heterogeneity of outcome parameters

Outcome parameters in pharmacogenetic studies vary widely. Numerous scales on antipsychotic efficacy (BPRS, CGI, GAS, PANSS, WCST, GAF, clinical rating, SADS, SANS, SAPS) have been used in pharmacogenetic studies (50). The cut-off used to define response also varies between studies with the same scale. Others use the score or the relative change in score as a continuous measure. In addition, the different symptom complexes of schizophrenia (positive symptoms, negative symptoms, disorganisation, and altered affect) are measured globally or separately (as in chapter 5) (51). Duration to determine response varies between 14 days to 1 year in studies. It is hard to determine the duration of treatment required to adequately determine response. It has for example been shown that some patients have a delayed response to clozapine (52-54). In chapter 5 improvement since the start of antipsychotic therapy was cross-sectionally measured. The period for which response was assessed varied between persons. Since most patients show response in the first month after the start of an antipsychotic (55) this method seemed valid for pharmacogenetic purposes. However, generalizability of the study results is difficult. In conclusion, the lack of a good definition of response makes it difficult to determine significancy of results and comparability between studies. More objective phenotypes than interview-based rating scales, like neurohormone plasma levels, neurocognitive tests, and structural and functional brain imaging measures are upcoming and may increase the power to detect possible delicate effects of variants on the complex phenotype of antipsychotic response. Adverse effects show less variability as outcome parameters but are still far from definite. For antipsychotic induced weight gain different outcome parameters are used, such as obesity, overweight, BMI, waist circumference and absolute and relative weight gain. For antipsychotic induced movement disorders also different measurement scales are available, and cut-off thresholds for case definitions differ between studies.

Study design

The designs of pharmacogenetic studies on antipsychotics vary widely, from prospective cohort studies to case-control and retrospective cohort studies. Retrospective studies are attractive from a practical and economical point of view, but could lead to biased ascertainment of outcome. This may be the case in chapter 5, where psychiatrists were asked for response since the start of medication. In addition, retrospective studies have shown to underestimate environmental effects and overestimate genetic effects (56). In this thesis, only cross-sectional data were

used, what could be a major drawback of the results found. It is for example unsure whether the effect of genotypes on metabolic parameters such as BMI and HbA1c is truly induced by antipsychotics rather than antipsychotic independent. In the study on histamine H1 receptor variants and BMI the history of antipsychotic use was not known. Previous use of antipsychotics with a different H1 affinity could have weakened the true genotype effect. Not only the study design, but also statistical approaches such as statistical tests and correction for covariables vary between studies. This may contribute to the inconsistent results found as well.

Problems relating to precision in the pharmacogenetics of antipsychotics

Sample size and multiple testing

Lack of adequate sample sizes probably plays an important role in non-replication. Many pharmacogenetic studies do not have enough power to detect a significant gene effect. Taking into account minor allele frequencies and the modest effects of most variants, sample sizes of hundreds of patients are often minimally needed, especially when several variants are tested (57). Achieving large sample sizes in pharmacogenetic studies is a substantial problem (31). The most optimally designed studies on antipsychotic efficacy yet are substudies of randomized clinical trials, and sample sizes of these studies are all relatively low. Prospective and cross-sectional studies on e.g. movement disorders suffer from the fact that prevalence is relatively low. In chapter 4, the study on movement disorders in a relatively young patient population suffered from this problem. Only Parkinsonism had a reasonable prevalence (around 30%). Clinical tardive dyskinesia, akathisia and dystonia was present in less than 10% of patients. We, and others in this field, decided to make a more sensitive case definition of tardive dyskinesia to increase its prevalence, with the drawback of possible misclassification. Continuous outcome parameters like weight gain, BMI or response rate have the advantage of increased power to detect genotype effects. However, continuous outcomes such as response rate or movement disorder scores are often difficult to analyse due to the skewed distribution and still have low power. For example, in chapter 5, sequential classes of response scores were taken together as outcome to meet the assumptions of ordinal regression, leading to loss of information and power. Sample size and power calculations should ideally be reported in pharmacogenetic studies, but this is often omitted (31), as in our studies. One reason is that it is difficult to obtain accurate power estimations when multiple SNPs are tested, all with different allele frequencies and often unknown effect sizes.

The best genetic model of the effect of the polymorphisms on the outcome is mostly unknown. Several modes of inheritances are possible, with the dominant, recessive and additive model being the most investigated. Other models are the co-dominant and semi-dominant model (31). As shown in the example of variant *Taq1A*

heterozygous versus homozygous patients is sometimes considered as a model as well. Considering several models means multiple testing, with an increase in false-positive results and consequently lack of replication. In the studies of this thesis, a general additive model was chosen when the mode of inheritance was not known *a priori*. Another option to start with is the co-dominant model (58). When the initial model is significant, post hoc tests are permitted to find the best fitting mode of inheritance. Other approaches have been described (59, 60). Combining several SNPs in haplotype analysis is a way of optimizing the use of the genetic information and may increase the power of analyses (58). Often, the genetic models that are tested are not explained or convincingly justified and results of those studies should be interpreted with caution.

Another important source of false-positive results is within-study selective reporting (61). This means the reporting of only a subset of the set of variants that was primarily examined, often those with the highest statistical significance. It is essential that all performed analyses are reported (31). However, in practice, this is not always as easy as it sounds. When a dataset is collected it takes little time to run all sorts of tests, in different strata, with different covariables, and with different outcome definitions. It is hard to ignore significant results one is initially not really interested in. It is however easy to forget non-significant results. Publication bias, the phenomenon that positive results are more likely to be published than negative results, is an important problem in every scientific field. This problem might even be more important in genetic studies, because of the lack of interest in a negative finding of a particular SNP in a particular gene. In addition, numerous other SNPs in the same gene might still be associated with the outcome. This is in contrast with clinical, demographic and environmental characteristics, where negative findings are more striking.

Conclusions and suggestions for future studies

In conclusion, pharmacogenetic studies on outcomes of antipsychotics have been more challenging for researchers than promising for patients. Although it is very likely that genetic variation plays an important role in inter-individual differences in antipsychotic response and occurrence of side-effects, virtually no variations have shown to be of much value for the patient in spite of numerous studies, whether directly by predicting outcome or indirectly by detecting new molecular substrates. The pharmacogenetics of antipsychotics is hampered by numerous aspects, and lack of knowledge on this complex phenotype thwarts the optimization of pharmacogenetic studies. Next to the methodological issues of a pharmacogenetic study described above, several other obstacles in the development of personalized pharmacotherapy have been described (62-65). These include the lack of interest among funding agencies and pharmaceutical companies for pharmacogenetic studies in clinical practice. Pharmaceutical companies are more interested in developing a

new valuable blockbuster drug, compared to a pharmacogenetic test that may have limited benefit. In general, there is also a lack of psychiatrists education in the use of the new pharmacogenetic tests.

At the moment, pharmacogenetic tests in the pharmacotherapy of psychotic disorders virtually only show potential in optimizing dose for efficacy and tolerability (via CYP450 genotyping), especially in FGAs and risperidone. These antipsychotics are metabolized by CYP2D6, the cytochrome that showed the most evident pharmacogenetic results. FGAs have a narrow therapeutic range and thus personalized dosing becomes important. Since FGAs may be as efficacious as SGAs but much cheaper, marketing the use of FGAs together with personalized dosing might be useful (64). However, the current marketing strategy of pharmaceutical companies is to promote SGAs (64). Choosing the best drug or excluding some drugs for a patient based on his genetic profile is much more controversial and difficult. There is no evidence for clinical implementation of such tests yet. Only small steps towards personalized drug selection were made the last decades and only small steps appear possible the coming years. To date, the clinical field is slowly moving towards the application of pharmacogenetics regarding pharmacokinetics. Thus, the new Dutch Guidelines for Schizophrenia advocate the use of pharmacokinetic tests for all those patient who show either insufficient respons on high dosage (ultrarapid metabolizers) or high rates of side-effects at a low dosage of antipsychotic medication (poor metabolizers) (15).

This thesis has added some results to personalized pharmacotherapy regarding personalized drug selection, that might have potential. Several known and unknown variants in candidate genes were tested for an association with antipsychotic-induced weight-gain, and showed some promising results in the histamine H1 receptor and *ROBO1* genes, that warrant further research. Evidence was added that the 5HT2C polymorphism 759 C/T is involved in antipsychotic-induced metabolic disturbances. The pharmacogenetic study on movement disorders has underlined the complexity of the involved mechanism and the possible role of the dopamine D2 receptor, and has called previously observed significant associations into question. With regard to antipsychotic response, it was underscored that variants of the *DRD3* and *MTHFR* gene are possible valuable predictors and substrates in antipsychotic response. As said before, the positive results in this thesis are still far removed from implementation in clinical practice, as more studies are needed to replicate and extend our findings.

In the future, it is important that consensus is reached on how to perform the most optimal pharmacogenetic study to get more homogeneous studies. Attempts have been made (51, 66) but have seemingly not been acted upon. Large multicenter studies that take into account abovementioned aspects as good as possible may well contribute to increased knowledge. It is essential for clinical application that

genotyping shows its added value over clinical parameters with well-designed, large, prospective studies. All information important for prescribing antipsychotics should ideally be collected, such as environmental variables (e.g. co-medication, diet and smoking) and personal factors (e.g. age, gender, and concomittant disease). Such studies have not been performed yet. Most optimally from the clinical viewpoint, pharmacogenetic studies should be designed as a pragmatic RCT contrasting the use of pharmacogenetics in addition to conventional predictors of response to no use of pharmacogenetics. They preferably should not be part of or add-on to another study. More coverage of genetic variation in both gene- and genome-wide association studies and research of epigenetics, and ultimately studies that are able to identify gene-gene and gene-environment interactions, may also help to understand the inter-individual variation in treatment response and molecular substrates involved in antipsychotic action.

At present, the concept that pharmacogenetics can be used to predict responses and side-effects is still far from being implemented. Till then, shared-decision-making by psychiatrist and patient together will be the next best way for optimizing personalized pharmacotherapy for people with psychoses.

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Summary

The current pharmacotherapy of psychosis is still far from optimal. Lack of compliance, limited efficacy and side-effects are major problems. Personalized pharmacotherapy, i.e. prescribing the optimal drug in the optimal dose based on the individual characteristics of a patient, may circumvent some of these problems. This thesis aimed to contribute to a better personalized pharmacotherapy of psychosis, integrating two subjects: 1) clinical factors in the prescription of oral versus depot antipsychotic therapy and 2) the pharmacogenetics of antipsychotic response and antipsychotic-induced side-effects. The background and outline of this thesis is discussed in chapter 1.

Section 2 reports on studies investigating predictors of the prescription of oral versus depot medication and first versus second generation depot antipsychotics in clinical practice. For these studies the IADb was used, a large database that contains prescription data with information on users and prescribers with a catchment population of approximately 500,000 people in the north of the Netherlands. It was shown in chapter 2.1 that patients who switched to a depot showed signs of less compliance and more movement disorders compared to switchers to oral antipsychotics. This is in line with the Dutch guidelines. It was also found that switchers to depot had significantly less often used psychotropic comedication before. This might be a reflection of the tendency of most clinicians to believe that depots are less acceptable to patients than tablets are. Our results in chapters 2.1 and 2.2 showed that risperidone long acting injectable (RLAI), the first depot of a second generation antipsychotic, is being reserved for more difficult-to-treat patients, which is a good example of *channeling* of a drug. This channelling effect may also be the cause of our finding that patients on RLAI seemed less persistent on their antipsychotic medication compared with patients prescribed first generation antipsychotic depot drugs (chapter 2.2).

In section 3 four studies were performed investigating the pharmacogenetics of metabolic side-effects of antipsychotics. Three populations of patients with a psychotic disorder in the Netherlands were pooled. The major part consisted of patients from the PHAMOUS cohort, a longitudinal cohort study in which patients undergo a yearly somatic screening combined with Routine Outcome Assessments with the goal to optimize treatment and care. In chapter 3.1 we found an association between a haplotype in the histamine H1 receptor gene and obesity, when comparing users with a high versus low H1 receptor affinity antipsychotic. Histamine H1 receptor affinity of an antipsychotic has been correlated with weight gain data in previous studies and is often suggested as one of the primary mechanisms of antipsychotic-induced weight gain. However, in earlier pharmacogenetic studies no markers have been found indicating a genetic effect. In chapter 3.2 no association was found between an alpha2a adrenergic receptor variant and the metabolic syndrome. Affinity for this receptor is suggested as a possible mechanism for metabolic disturbances in antipsychotic medication. In chapter 3.3 a

replication study was performed, which added more evidence that serotonin 2c receptor variant -759C/T is associated with the metabolic syndrome. This variant is often associated with antipsychotic-induced weight gain. However, in our population the association with the metabolic syndrome is primarily the effect of dyslipidemia, suggesting the pleiotropic effect of this variant. In chapter 3.4 an association was found between a polymorphism in the *ROBO1* gene and BMI, in female patients only. This gene was previously associated with risk of schizophrenia and with BMI in non-schizophrenic populations, making it a good candidate gene for antipsychotic-induced weight gain. Gender specific pharmacogenetic results in leptin genes have been found in other studies, emphasizing antipsychotic-induced weight gain might involve different pathways in men and women.

Chapters 4 and 5 include pharmacogenetic studies investigating several candidate polymorphisms on antipsychotic induced movement disorders and response to antipsychotics, respectively. These two studies were performed as part of the Genetic Risk and Outcome of Psychosis (GROUP) study, a longitudinal cohort study from a consortium of four academic psychiatric centres in the Netherlands with their affiliated mental health care institutions, including approximately 1000 patients with a recently developed non-affective psychotic disorder. In chapter 4 strong significant associations were found of two variants in the dopamine D2 receptor with akathisia and tardive dyskinesia, which had not been observed previously. However, eleven other variants, which were chosen based on previous positive association studies, were not associated with any movement disorder in our population. Chapter 5 showed that only two out of eight previously associated variants were significantly associated with antipsychotic response in our population. The directions of these associations (in the dopamine D3 receptor (*DRD3*) and methylenetetrahydrofolate reductase (*MTHFR*) gene) were in the same direction as in earlier positive studies. These polymorphisms could be helpful in predicting antipsychotic treatment response.

Negative results and lack of replication are common findings in pharmacogenetic studies of antipsychotics. Pharmacogenetic studies on outcomes of antipsychotics have been more challenging for researchers than promising for patients. In the general discussion, the studies reported in this thesis are put in a broader perspective and the difficulties and pitfalls in performing and interpreting pharmacogenetic studies are discussed. Although it is very likely that genetic variation plays an important role in inter-individual differences in antipsychotic response and occurrence of side-effects, virtually no variations have shown to be of much value for the patient in spite of numerous studies. In future, it is important that consensus is reached on how to perform the most optimal pharmacogenetic study to get more consistent results. Large multicenter studies are essential to improve knowledge in the field of pharmacogenetics of antipsychotics. At present, the concept that pharmacogenetics can be used to predict responses and side-effects is still far from

being implemented. Till then, shared-decision-making by psychiatrist and patient together will be the next best way for optimizing personalized pharmacotherapy for people with psychoses.

Nederlandse Samenvatting

(Summary in Dutch)

De farmacotherapie van psychosen is nog steeds verre van optimaal. Gebrek aan therapietrouw, beperkte effectiviteit en bijwerkingen als metabole stoornissen en bewegingsstoornissen zijn grote problemen. *Personalized* farmacotherapie -het voorschrijven van het meest optimale medicijn in de optimale dosis gebaseerd op de individuele karakteristieken van een patiënt- kan deze problemen verminderen. Dit proefschrift probeert bij te dragen aan betere *personalized* farmacotherapie van psychosen. Twee onderwerpen zijn bestudeerd, namelijk 1) klinische factoren die het voorschrijven van de verschillende antipsychotica bepalen en 2) de farmacogenetica van antipsychotica geïnduceerde bijwerkingen en respons op antipsychotica. Doel en achtergrond van dit proefschrift worden beschreven in hoofdstuk 1.

Sectie 2 beschrijft twee studies die klinische factoren onderzochten op het voorschrijven van orale versus depot antipsychotica en eerste versus tweede generatie depot antipsychotica. Voor deze studies is gebruik gemaakt van *de InterAction Database* (IADb), een grote database met voorschrijfdata met informatie over gebruikers en voorschrijvers van een totale populatie van ongeveer 500.000 mensen in het Noorden van Nederland. In hoofdstuk 2.1 werd aangetoond dat patiënten die switchen naar een depot antipsychoticum meer tekenen van therapie-ontrouw en bewegingsstoornissen hadden, in vergelijking met switchers naar een oraal antipsychoticum. Dit is lijn met de Nederlandse richtlijnen. Ook werd aangetoond dat switchers naar depot antipsychotica significant minder psychotrope comedicatie hadden gebruikt. Dit zou verklaard kunnen worden door het idee bij artsen dat depot antipsychotica voor een patiënt niet zo acceptabel zijn als orale antipsychotica. De resultaten in hoofdstukken 2.1 en 2.2 duiden erop dat risperidon depot (RLAI), het eerste tweede generatie antipsychotica depot, wordt gereserveerd voor patiënten die moeilijk te behandelen zijn. Deze zogenaamde *channeling* van RLAI kan ook de oorzaak zijn van de verlaagde therapieduur die gevonden werd in hoofdstuk 2.2 bij patiënten die RLAI gebruiken, in vergelijking met gebruikers van eerste generatie antipsychotica depots.

In sectie 3 worden vier studies beschreven die de farmacogenetica van metabole bijwerkingen van antipsychotica hebben onderzocht. Drie populaties van patiënten met een psychotische stoornis in Nederland zijn voor deze studies samengevoegd. Het grootste gedeelte bestond uit patiënten van het *PHAMOUS* cohort, een longitudinale cohort studie waarin patiënten een jaarlijkse somatische screening krijgen gecombineerd met een Routine Outcome Assessment. In hoofdstuk 3.1 werd een associatie gevonden tussen een haplotype in het histamine H1 receptor gen (*HRH1*) en obesitas, bij het vergelijken van gebruikers van een antipsychoticum met een hoge versus een lage H1 receptor affiniteit. Histamine H1 receptor affiniteit van een antipsychoticum is in voorgaande studies gecorreleerd met gewichtstoename en is vaak voorgesteld als een van de primaire mechanismen van antipsychotica geïnduceerde gewichtstoename. Er zijn echter nooit markers gevonden die een farmacogenetisch effect hiervan ondersteunen. In hoofdstuk 3.2 werd geen associatie

gevonden tussen een alpha2a adrenerge receptor variant en het metabool syndroom. Affiniteit voor deze receptor is ook voorgesteld als een mogelijk mechanisme voor de metabole stoornissen in antipsychotische medicatie. In hoofdstuk 3.3 werd een replicatiestudie uitgevoerd welke bijdraagt aan het bewijs dat serotonine 2c receptor variant -759C/T geassocieerd is met het metabole syndroom. Deze variant is al vaak geassocieerd met gewichtstoename. In onze populatie werd de genetische associatie met het metabool syndroom vooral verklaard door dyslipidemie. Dit suggereert een mogelijk pleiotroop metabool effect van het serotonine 2c receptor (HTR2C) gen. In hoofdstuk 3.4 werd een associatie gevonden tussen een polymorfisme in het *ROBO1* gen en BMI, echter alleen in vrouwelijke patiënten. Dit gen was eerder geassocieerd met het risico op schizofrenie en met BMI in een niet-schizofrene populatie, waardoor het *a priori* een geschikt kandidaatgen leek. Geslachtsspecifieke farmacogenetische resultaten zijn ook in soortgelijke studies gevonden bij leptine genen, wat zou kunnen wijzen op verschillende mechanismen van antipsychotica geïnduceerde gewichtstoename tussen mannen en vrouwen.

Hoofdstukken 4 en 5 betreffen farmacogenetische studies die talloze kandidaatpolymofismen voor respectievelijk antipsychotica geïnduceerde bewegingsstoornissen en respons op antipsychotica onderzoeken. Deze twee studies zijn uitgevoerd als deel van de *Genetic Risk and Outcome of Psychosis* (GROUP) studie, een longitudinale cohort studie van een consortium van vier academische psychiatrische centra in Nederland met de aan hen geaffilieerde geestelijke gezondheidszorg instellingen. Deze studie omvat een totaal van 1000 patiënten met een recent ontwikkelde niet-affectieve psychotische stoornis. In hoofdstuk 4 worden twee sterk significante associaties gevonden tussen varianten van het dopamine D2 receptor gen (*DRD2*) en akathisie en tardieve dyskinesie, die niet eerder beschreven zijn in de literatuur. Echter, elf andere varianten toonden geen enkele associatie met bewegingsstoornissen, terwijl deze varianten uitgekozen waren op basis van eerdere positieve associatie studies. Hoofdstuk 5 toont dat slechts twee van de acht voorheen geassocieerde varianten significant geassocieerd waren met respons op antipsychotica in onze populatie. De richtingen van deze associaties (in het dopamine D3 receptor (*DRD3*) en methylenetetrahydrofolaat reductase (*MTHFR*) gen) waren in dezelfde richting als voorgaande positieve studies. Deze polymorfismen zouden nuttig kunnen zijn in het voorspellen van de respons op antipsychotica.

Negatieve bevindingen en gebrek aan replicatie zijn veelvoorkomende bevindingen in farmacogenetische studies van antipsychotica. De farmacogenetica van antipsychotica lijkt vooralsnog meer een uitdaging voor onderzoekers dan een belofte voor patiënten. In de discussie in hoofdstuk 6 worden de studies in een breder daglicht gesteld en worden moeilijkheden en valkuilen van het uitvoeren en interpreteren van farmacogenetisch onderzoek besproken. Al lijkt het vrij logisch dat genetische variaties een belangrijke rol in de inter-individuele verschillen in

antipsychotica respons en bijwerkingen spelen, toch is er van vrijwel geen enkele variant onomstreden aangetoond dat het een voorspellende therapeutische waarde heeft voor de patiënt. In de toekomst is het belangrijk dat consensus gevormd wordt hoe een farmacogenetische studie uitgevoerd moet worden, zodat meer consistente resultaten kunnen ontstaan. Grote multicenter studies zijn waarschijnlijk onvermijdelijk om de kennis te vergroten in het veld van de farmacogenetica van antipsychotica. Momenteel is het concept dat de farmacogenetica respons en bijwerkingen kan voorspellen verre van geïmplementeerd in de praktijk. Tot dan is het gezamenlijk beslissen door psychiater en patiënt waarschijnlijk de beste manier om tot een optimale *personalized* farmacotherapie van mensen met een psychose te komen.

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About the Author

Jelle Vehof was born in 1982 (July 31th) in Apeldoorn. He grew up in Beekbergen, a small village on the forest-rich ridge of hills The Veluwe, in the middle of The Netherlands. He received primary education at the Prinses Julianaschool in Lieren (1986-1991) and the Openbare Basisschool in Beekbergen (1991-1994). He subsequently got his pre-university secondary education (VWO) degree at the Gymnasium Apeldoorn (1994-2000), with an A+ for mathematics.

Because of his interest in probability theory he moved to Amsterdam in 2000 to study Econometrics at the University of Amsterdam. In that year he won the first National IQ Test on Dutch television. In 2001 he moved to Groningen, where he finished his master Econometrics in 2004 at the University of Groningen. Because of his fascination for the human body and mind he started to study Medicine in 2003. In 2006 he began doing research at the department of epidemiology (chapter 2 of this thesis). In 2008 he joined an MD/PhD program of the Junior Scientific Masterclass, combining his medical internships at the University Medical Center Groningen (UMCG) and Martini Ziekenhuis Groningen and his pharmacogenetic research (chapters 3 to 5 of this thesis) at the departments of epidemiology and psychiatry (both UMCG). In 2009 he received his Doctor of Medicine (MD) degree. With the completion of this PhD thesis and several courses he will also be registered as epidemiologist ('epidemioloog B' in Dutch).

In February 2011 he started an ophthalmology residency at the Department of Ophthalmology (UMCG). He will stay affiliated to the Unit of Genetic Epidemiology and Bioinformatics within the Department of Epidemiology, and will continue doing research in collaboration with the Department of Twin Research and Genetic Epidemiology, King's College, London, investigating clinical and genetic markers of dry eye disease and glaucoma. In his free time he likes to visit places around the world and to play sports like soccer, running, and squash.

List of publications

Vehof J, Postma MJ, Bruggeman R et al. Predictors for starting depot administration of risperidone in chronic users of antipsychotics. J.Clin.Psychopharmacol. 2008;28:625-30.

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Hysi P, **Vehof J**, Carbonaro F et al. Identification of candidate genetic loci altering susceptibility to glaucoma.

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