Genetic determinants for metabolic abnormalities

Arne Risselada

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(met een samenvatting in het Nederlands)

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geboren op 24 december 1976 te Kollum

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Contents

Chapter 1

Introduction

The basis of our understanding of the genetics of heredity was formed by Mendel who conducted his phenotyping experiments with pea plants in the middle of the 19th century. More than 140 years later, following the discovery of the DNA structure by Watson and Crick in 1953, the Human Genome Project was started in 1990. This international collaborative research project had the primary goal to determine the sequence of the 3 billion chemical base pairs that make up the human DNA and to identify and map the approximately 20.000-25.000 genes of the human genome from both a physical and functional standpoint. With the completion of this Human Genome Project in 2003, in conjunction with innovations on chemical analytics and bio-informatics, the field of genetics received an enormous boost and the number of studies investigating associations between genetic determinants and etiology or prognosis of diseases and response to medication increased enormously.

Obesity and metabolic syndrome

Obesity is one of the medical conditions that has frequently been investigated in studies searching for genetic determinants, because there is a clear heritability of obesity within families. First-degree relatives of obese patients having an up to nine-fold increased risk for developing obesity, suggesting that genetic make-up plays a role in the etiology of obesity.[1] Over the past few decades, public and medical awareness for obesity has grown, because obesity has become a serious public health problem. Obesity is not a disease itself but a risk factor and is clearly associated with an increased incidence of cardiovascular disease, type 2 diabetes mellitus (DM), hypertension, obstructive sleep apnoea syndrome, polycystic ovarian syndrome, stroke, dyslipidemia, osteoarthritis and some forms of cancer.[2,3] Obesity is often classified by using a cutoff value (> 30 kg/m^2) for the Body Mass Index (BMI), which is calculated by dividing the body weight in kilograms by the square of the height in meters. Other means of estimating obesity that are often used are the waist circumference or waist/hip ratio. These measurements provide information about the amount of abdominal fat, making it possible to diagnose so-called 'central-obesity', which has an even better predictive value for associated health risks like cardiovascular disease than the BMI. Since 1981, the prevalence of obesity in the general population has almost tripled in the Netherlands (5% vs. 12%).[4] It was estimated in 2004 that if the prevalence of obesity in the Dutch population would reach the current prevalence of obesity in the United States (39.5%), the total population mortality would increase by 3% because of the associated co-morbidities.[5]

Although there is great variability between subpopulations, regions, countries and continents, the increase in prevalence of obesity appears to be a worldwide phenomenon. The reason for this steep increase during the last few decades might originate from an unbalance in our weight-regulating system that, from an evolutionary point of view, aims at keeping a positive energy balance. This so-called "thrifty gene hypothesis", which was suggested in 1962 by the geneticist James Neel, would have been advantageous for hunter-gatherer populations, especially

child-bearing women, because it would allow them to fatten more quickly during times of abundance and thus increase chances of survival during times of food scarcity. However, in our modern Western society with a constant abundance of food, this genotype efficiently prepares individuals for a famine that never comes, resulting in widespread obesity and obesity-related health problems like diabetes. Other factors that explain the steep increase in obesity the last few decades include a global shift in diet towards an increased intake of energy-dense foods that are high in fat and sugars (fast food) and a trend towards decreased physical activity due to the increasingly sedentary nature of many forms of work (e.g. automation, computer use), changing modes of transportation and increasing urbanization.

Obesity can also be part of a larger whole of metabolic risk factors, which can ultimately lead to a syndrome called "the metabolic syndrome". This metabolic syndrome was propounded in 1988 by an American endocrinologist, Gerald Reavan, who stated that the co-morbidities central-obesity, diabetes and hypertension share a common cause in insulin resistance and impaired glucose tolerance. The metabolic syndrome is a strong predictor for cardiovascular disease (CVD) and diabetes with around 70% of CVD cases explained by the metabolic syndrome and 80% of new onset diabetes.[6] However, classification of the metabolic syndrome has been debated by the American Diabetes Organisation that states that the metabolic syndrome is not a discrete syndrome itself, but merely a clustering of cardiovascular risk factors. They prefer to use the term "pre-diabetic" for patients formally diagnosed with the metabolic syndrome. When physicians do adopt the idea of a metabolic syndrome, its presence is often diagnosed by using the definition from the National Cholesterol Education Program's Adult Treatment panel III (NCEP:ATP IIIa) in which three or more of the following five metabolic criteria have to be met:[7]

- Waist circumference ≥ 102 cm (male) or ≥ 88 cm (female) (≈ BMI > 30 kg/m^2)
- Triglycerides ≥1.7 mmol/l or use of a fibrate
- HDL cholesterol <1.0 mmol/l (male) or <1.3 mmol/l (female) or use of a statin
- Blood pressure ≥ 130/85 Hg or use of an antihypertensive drug
- Fasting glucose ≥ 5.6 mmol/l or use of an antidiabetic drug

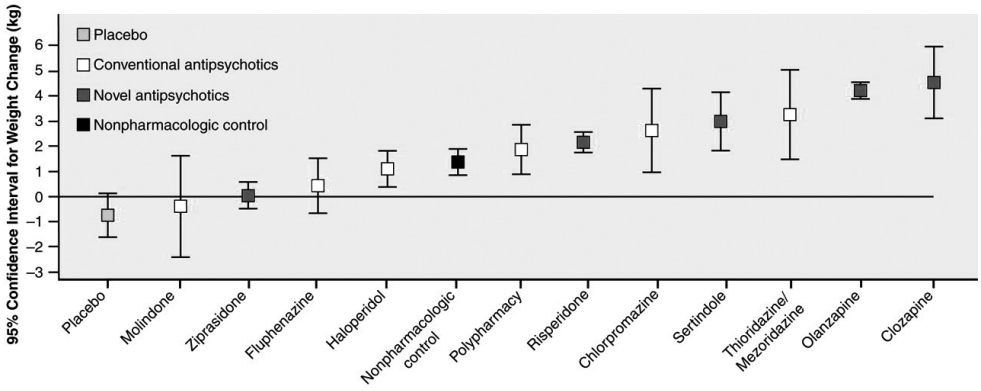
Prevalence of the metabolic syndrome using the ATPIIIa criteria is around 15% in the general Dutch population, with a higher prevalence in men (19%) than in women (10%).[8] However, prevalence of the metabolic syndrome is much higher in some subgroups of the population.

Metabolic syndrome and obesity in schizophrenic patients

Schizophrenic patients form one of the subgroups within the general population with a much higher prevalence of the metabolic syndrome and obesity. Studies have shown that 50% of the patients in a Dutch schizophrenic population are obese and 35% of these patients can be diagnosed with the metabolic syndrome.[9,10] This higher prevalence of obesity and the

metabolic syndrome in schizophrenic patients can partly be explained by an unhealthy lifestyle that includes more smoking and drinking and a poor diet. However, a large part can also be explained by the antipsychotic drugs that are being used by these patients, because these drugs are known to frequently cause metabolic adverse effects of which weight gain is the most prominent one.[11] Weight gain can occur during use of any antipsychotic, but is more severe with atypical antipsychotics, especially clozapine and olanzapine (Figure 1), since they have a higher affinity for receptors associated with weight gain (e.g. muscarin, histamine-1, $5HT_{1a}$ and $5HT_{2c}$).[11,12]

Figure 1. Antipsychotic-induced weight gain in first 10 weeks of treatment.[11]



Weight gain is most clearly visible during the first three months of treatment with antipsychotics, after which a new plateau is reached. However, weight gain can endure for years during treatment with olanzapine or clozapine. Apart from the increased risk of co-morbidities associated with weight gain, psychiatric patients also consider weight gain as one of the most disturbing adverse events and an important reason for non-adherence to therapy.[13] Increased awareness for weight gain, and other metabolic adverse effects caused by antipsychotic drugs, lead in 2008 to the development of a protocol for routine somatic screening and treatment procedures in schizophrenic patients in order to prevent long term complications like cardiovascular disease and diabetes.[14]

Pharmacogenetics

However, not all patients experience antipsychotic-induced weight gain and other metabolic adverse effects to the same extent; some patients don't gain weight at all or even lose weight. This high interindividual variability in the risk of metabolic adverse effects suggests that genetic variability plays an important role in a person's susceptibility to weight gain and the metabolic syndrome, making it a target for pharmacogenetic studies. The past few years, numerous

studies have been published investigating the association between genetic determinants and antipsychotic-induced weight gain and/or the metabolic syndrome. These genetic determinants include genes that are associated with the weight-regulating system (e.g. leptin gene (*LEP*), adiponectin gene (*ADIPOQ*), insulin related genes (*INSIG2*), G-protein ß3 subunit (*GNB3*)) as well as genes associated with the pharmacological action of the psychotropic drugs (e.g. serotonergic (*HTR*), histaminergic (*HRH*), dopaminergic (*DRD*) and adrenergic receptor genes (*ADR*)). This variety in selected genes illustrates the complexity of choosing the best genetic targets when studying antipsychotic-induced metabolic adverse effects, owing to the complex pharmacologic profile of antipsychotics and the complex etiology of weight gain and the metabolic syndrome. Furthermore, most of these genetic determinants were studied only once or twice with negative results, or have been studied more frequently with conflicting results. However, some of the genetic determinants have repeatedly been associated with antipsychotic-induced weight gain or the metabolic syndrome.

The genetic determinant most frequently studied in this context is genetic variation in the X-chromosomal *HTR2C* gene coding for the serotonergic 2C-receptor ($5HT_{2C}$). This is based on the fact that studies have shown that *HTR2C* knockout-mice become hyperphagic and $5HT_{2C}$ agonists reduce appetite in humans.[15,16] Owing to the strong $5HT_{2C}$ antagonistic properties of atypical antipsychotic drugs, the *HTR2C* gene is a likely target to be associated with antipsychotic-induced metabolic dysregulation, which has indeed been confirmed in several studies including earlier studies from our group and two meta-analyses.[17-23] This makes the *HTR2C* gene the most likely candidate gene to be studied when investigating antipsychotic-induced metabolic adverse effects. Apart from the *HTR2C* gene, the *ADRA2A* gene coding for the adrenergic α_{2A} receptor has also repeatedly been associated with antipsychotic-induced metabolic adverse effects. All three studies investigating the association between genetic variation within the *ADRA2A* gene and antipsychotic-induced weight gain found significant results, making the *ADRA2A* gene another likely candidate gene.[24-26] The rationale for investigating the *ADRA2A* gene lie in its effects on lipolysis and the α_{2A}-antagonistic properties of most (atypical) antipsychotics. Studies have shown that stimulation of the G-protein coupled α_{2A} receptor inhibits lipolysis and weight loss during very-low-calorie diets was associated with decreased α_{2A} sensitivity.[27,28] Remarkably, α_{2A} antagonism caused by antipsychotics would therefore actually be expected to protect against weight gain because of less inhibition of lipolysis. However, this protective effect could vary between individuals when genetic variation leads to changes in receptor function. Individuals with a more active α_{2A} -receptor would possibly benefit more from antagonism than individuals with a less active α_{2A} -receptor.

When the *HTR2C* and *ADRA2A* genes are associated with antipsychotic-induced weight gain or the metabolic syndrome, it would also be interesting to investigate whether these associations can be found in individuals without antipsychotic drugs. If the associations found in psychiatric patients truly reflect a pharmacologic influence of polymorphisms in *HTR2C* and *ADRA2A*

genes on a patient's metabolic state, it can be expected that this influence might also be visible in patients without psychotropic drugs. This could explain why some people have a higher tendency to gain weight or develop the metabolic syndrome. Moreover, comparing the effects of the genetic determinants between populations that use psychotropic drugs and populations that don't use psychotropic drugs could help clarify to which extent these drugs modify outcome. Ultimately, knowledge of a patient's metabolic risk profile increases the possibility to tailor pharmacotherapy, thereby preventing drug-induced side effects and improving adherence to therapy and therapy outcome.

Objective of the thesis

The objective of this thesis is to investigate whether polymorphisms in the *HTR2C* and *ADRA2A* genes play a role in explaining the interindividual variability in metabolic abnormalities in patients that use psychotropic drugs and patients that don't use psychotropic drugs.

Outline of the thesis

This thesis consists of two parts. The first part describes the association between polymorphisms in the *HTR2C* and *ADRA2A* genes and metabolic abnormalities in psychiatric patients using psychotropic drugs. In chapter 2.1, a systematic review article is presented in which the results of all pharmacogenetic studies until April 2011, investigating the association between genetic determinants and antipsychotic- induced weight gain, are summarized. This review article focuses on differences in study methodology as a possible explanation for conflicting study results, discusses which criteria have to be met before implementing pharmacogenetic testing into daily clinical practice and shows our current position in that process. In chapter 2.2, the association between *HTR2C* polymorphisms and mirtazapine-induced weight gain is investigated. The antidepressant mirtazapine is a strong antagonist for the $5HT_{2C}$ receptor, and use of mirtazapine frequently results in weight gain. Therefore it would not be unlikely that the associations between *HTR2C* polymorphisms and weight gain found in atypical antipsychotics also apply to mirtazapine. Chapters 2.3 and 2.4 describe cross-sectional studies investigating associations between *HTR2C* and *ADRA2A* polymorphisms and the metabolic syndrome, respectively.

The second part of this thesis describes the association between polymorphisms in the *HTR2C* and *ADRA2A* genes and metabolic abnormalities in patients that don't use psychotropic drugs. In chapter 3.1, the association between *HTR2C* polymorphisms and prevalence of obesity is investigated by comparing patients with a BMI < 25 kg/m^2 with obese patients (BMI > 30 kg/m^2) in a cross-sectional study. Chapter 3.2 describes a study in which the association between the *HTR2C* rs1414334 and *ADRA2A* 1291 C/G polymorphisms and lipid values was investigated in obese patients. In chapter 3.3, a follow up study for the study in chapter 3.1 is presented in which polymorphisms from the genes coding for leptin (*LEP*) and the leptin receptor (*LEPR*)

have been added to investigate the presence of potential gene-gene interactions between these polymorphisms and the *HTR2C* polymorphisms. Chapter 3.4 describes an ongoing prospective follow-up study in obese patients entering the weight loss program of the obesity clinic. The results presented reflect an interim analysis. The goal of this study is to investigate whether the *ADRA2A* and *HTR2C* polymorphisms can be predictive for success or failure of the anti-obesity program. Finally, the main findings of this thesis, as well as the extent to which these findings are applicable in clinical practice, will be discussed in chapter 4. Suggestions for future studies are also given in this chapter.

References

1. Price RA, Lee JH. Risk ratios for obesity in families of obese African-American and Caucasian women. Hum Hered 2001;51:35-40.
2. Field AE, Coakley EH, Must A, Spadano JL, Laird N, Dietz WH, et al. Impact of overweight on the risk of developing common chronic diseases during a 10-year period. Arch Intern Med 2001;161:1581-1586.
3. Must A, Spadano J, Coakley EH, Field AE, Colditz G, Dietz WH. The disease burden associated with overweight and obesity. JAMA 1999;282:1523-1529.
4. Visscher TLS, Kromhout D, Seidell JC. Long-term and recent time trends in the prevalence of obesity among Dutch men and women. Int J Obes 2002;26:1218-24.
5. Bemelmans WJE, Hoogenveen RT, Visscher TLS, Verschuren WMM, Schuit AJ. RIVM rapport 260301003/2004. Toekomstige ontwikkelingen in overgewicht. Inschatting effecten op de volksgezondheid.
6. Pajunen P, Rissanen H, Härkänen T, Jula A, Reunanen A, Salomaa V. The metabolic syndrome as a predictor of incident diabetes and cardiovascular events in the Health 2000 study. Diabetes & Metabolism 2010;36:395-401.
7. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, et al. Diagnosis and management of the metabolic syndrome: an american heart association/national heart,lung, and blood institute scientific statement. Circulation 2005;112:2735-2752.
8. Bos MB, de Vries JH, Wolffenbuttel BH, Verhagen H, Hillege JL, Feskens EJ. The prevalence of the metabolic syndrome in the Netherlands: increased risk of cardiovascular diseases and diabetes mellitus type 2 in one quarter of persons under 60. NTvG 2007;151:2382-8.
9. Schorr SG, Slooff CJ, Bruggeman R, Taxis K. The prevalence of metabolic syndrome in patients with psychotic disorders in the Netherlands. J Clin Psychopharmacol 2009;29:399-402.
10. De Hert M, Schreurs V, Vancampfort D, van Winkel R. Metabolic syndrome in people with schizophrenia: a review. World Psychiatry 2009;8:15-22.
11. Allison DB, Mentore JL, Heo M, Chandler LP, Cappelleri JC, Infante MC, et al. Antipsychotic-induced weight gain: a comprehensive research synthesis. Am J Psychiatry 1999;156(11):1686-1696.
12. Matsui-Sakata A, Ohtani H, Sawada Y. Receptor occupancy-based analysis of the contributions of various receptors to antipsychotics-induced weight gain and diabetes mellitus. Drug Metab Pharmacokinet 2005;20(5):368-378.
13. Hugenholtz GW, Heerdink ER, Meijer WE, Stolker JJ, Egberts AC, Nolen WA. Reasons for switching between antipsychotics in daily clinical practice. Pharmacotherapy 2005;38:122-124.

14. Cahn W, Ramlal D, Bruggeman R, de Haan L, Scheepers FE, van Soest MM, et al. Preventie en behandeling van somatische complicaties bij antipsychoticagebruik. Tijdschrift voor psychiatrie 2008;50:579-591.
15. Tecott L, Sun L, et al. Eating disorder and epilepsy in mice lacking 5HT2C-serotonin receptors. Nature 1995;374:542-546.
16. Gallant DM, Bishop MP, Bishop G, O'Meallie L. U-22,394A: a controlled evaluation in chronic schizophrenic patiënts. Curr Ther Res Clin Exp 1976:9:579-581.
17. Mulder H, Franke B, van der Beek-van der AA, Arends J, Wilmink FW, Egberts AC, et al. The association between HTR2c polymorphisms and obesity in psychiatric patients using antipsychotics: a cross-sectional study. Pharmacogenomics J 2007;7(5):318-324.
18. Mulder H, Franke B, van der-Beek van der Aart A, Arends J, Wilmink FW, Scheffer H, et al. The association between HTR2C gene polymorphisms and the metabolic syndrome in patients with schizophrenia. J Clin Psychopharmacology 2007;27:338-343.
19. Mulder H, Cohen D, Scheffer H, Gispen-de Wied C, Arends J, Wilmink FW, et al. HTR2C gene polymorphisms and the metabolic syndrome in patients with schizophrenia: a replication study. J Clin Psychopharmacology 2009;29:16-20.
20. Gregoor JG, Mulder H, Cohen D, van Megen HJ, Egberts AC, Heerdink ER, et al. Combined HTR2C-LEP genotype as a determinant of obesity in patients using antipsychotic medication. J Clin Psychopharmacol 2010:30:702-705.
21. Reynolds GP, Zhang ZJ, Zhang XB. Association of antipsychotic drug-induced weight gain with a 5-HT2c receptor gene polymorphism. Lancet 2002;359: 2086-87 (letter).
22. De Luca V, Mueller DJ, de Bartolomeis A, Kennedy JL. Association of the HTR2C gene and antipsychotic-induced weight gain: a meta-analysis. Int J Neuropsychopharmacol 2007;10(5): 697-704.
23. Sicard MN, Zai CC, Tiwari AK, Souza RP, Meltzer HY, Lieberman JA, et al. Polymorphisms of the HTR2C gene and antipsychotic-induced weight gain: an update and meta-analysis. Pharmacogenomics 2010;11:1561-1571.
24. Wang YC, Bai YM, Chen JY, Lin CC, Lai IC, Liou YJ. Polymorphism of the adrenergic receptor alpha 2a-1291C>G genetic variation and clozapine-induced weight gain. J Neural Transm 2005;112:1463-1468.
25. Park YM, Chung YC, Lee SH, Lee KJ, Kim H, Byun YC, et al. Weight gain associated with the alpha2a-adrenergic receptor 1291C/G polymorphism and olanzapine treatment. Am J Med Gen Part B (Neuropsychiatric genetics) 2006;141B:394-397.
26. Sickert L, Müller DJ, Tiwari AK, Shaikh S, Zai C, De Souza R. Association of the alpha 2A adrenergic receptor -1291 C/G polymorphism and antipsychotic-induced weight gain in European-Americans. Pharmacogenomics 2009;10:1169-1176.
27. Hellstrom L, Reynisdottir S, Langin D, Rössner S, Arner P. Regulation of lipolysis in fat cells of obese women during long-term hypocaloric diet. Int J Obes Relat Metab Disord 1996;20:745-752.
28. Hellstrom L, Rossner S, Hagström-Toft E, Reynisdottir S. Lipolytic catecholamine resistance linked to alpha 2-adrenoceptor sensitivity – a metabolic predictor of weight loss in obese subjects. Int J Obes Relat Metab Disord 1997;21:314-320.

2

Polymorphisms in the HTR2C and ADRA2A genes and metabolic abnormalities in patients using psychotropic drugs

Chapter 2.1

Pharmacogenetic testing to predict antipsychotic-induced weight gain: a systematic review

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Abstract

Background

Weight gain is an important side effect of antipsychotic drugs. Since the high interindividual difference in weight gain suggests that genetic factors play a role in this weight gain, studies have tried to identify these factors. Most of these studies were carried out in the past few years and focussed largely on receptor polymorphisms, although some tried to explain the variation in weight gain with differences in pharmacokinetics. Unfortunately, the results of these association studies are often conflicting, which makes it hard to apply this genetic knowledge in daily clinical practice.

Objective

To summarize the findings of studies investigating the association between genetic determinants and antipsychotic-induced weight gain up to April 2011 and discuss the feasibility of genetic testing for antipsychotic-induced weight gain in clinical practice.

Methods

A systematic approach was used to screen PubMed, Embase, Web of Science and the HuGE database for eligible articles. Original articles collected until April 2011, published in English, describing a longitudinal association study in humans on genetic determinants for antipsychotic-induced weight gain were included.

Results

In total, 57 publications were included for data extraction. The majority of these studies had been published in the past 5 years and focussed on receptor polymorphisms. Polymorphisms in the adrenergic, histaminergic, dopaminergic and serotonergic system were most frequently studied. The study results were often conflicting, which is largely caused by variation in study methodology. For example, sample sizes are often small, and the co-variables corrected for in the analyses varies. Studies in antipsychotic-naive populations probably provide most information about genetic associations. The clinical utility of genetic testing for antipsychotic-induced weight gain is unknown, because current studies are still focussed on clinical validity.

Conclusions

It is too early to implement genetic testing for antipsychotic-induced weight gain in daily clinical practice because clinical utility is unknown. Future studies should combine polymorphisms that have been repeatedly associated with weight gain to generate algorithms with a high predictive value and form the basis for clinical utility studies.

Introduction

Weight gain is an important side effect of treatment with antipsychotic drugs and an important cause of non-adherence to therapy.[1] Weight gain is also associated with an increased risk of cardiovascular disease (e.g. hypertension and diabetes) and subsequently a higher risk of mortality.[2] Weight gain can occur during use of any antipsychotic, but is most prominent with atypical antipsychotics, especially clozapine and olanzapine (Table 1). Weight gain occurs mainly during the first three months of treatment with antipsychotics after which a new plateau is reached. However, during treatment with olanzapine and clozapine weight gain can endure for years.[3]

Table 1. Antipsychotic-induced weight gain

Antipsychotic	Weight gain (kg)	Type and duration of study	Reference
Clozapine	4.45	Meta-analysis, 10 weeks	4
Olanzapine	4.15	Meta-analysis, 10 weeks	4
Thioridazine	3.19	Meta-analysis, 10 weeks	4
Sertindole	2.92	Meta-analysis, 10 weeks	4
Chloorpromazine	2.58	Meta-analysis, 10 weeks	4
Risperidone	2.10	Meta-analysis, 10 weeks	4
Quetiapine	2.18	Meta-analysis, 6 weeks	4
Sulpiride	1.90	Retrospective, Cohort, 4-5 weeks	86
Haloperidol	1.08	Meta-analysis, 10 weeks	4
Aripiprazol	0.71	Meta-analysis, 4-6 weeks	85
Broomperidol	0.50	Retrospective, Cohort, 4-5 weeks	86
Fluphenazine	0.43	Meta-analysis, 10 weeks	4
Ziprasidone	0.04	Meta-analysis, 10 weeks	4
Placebo Pimozide	-0.74	Meta-analysis, 10 weeks	4
	-2.69 – 5.4	Meta-analysis, 10 weeks / RCT 9 months	4/87

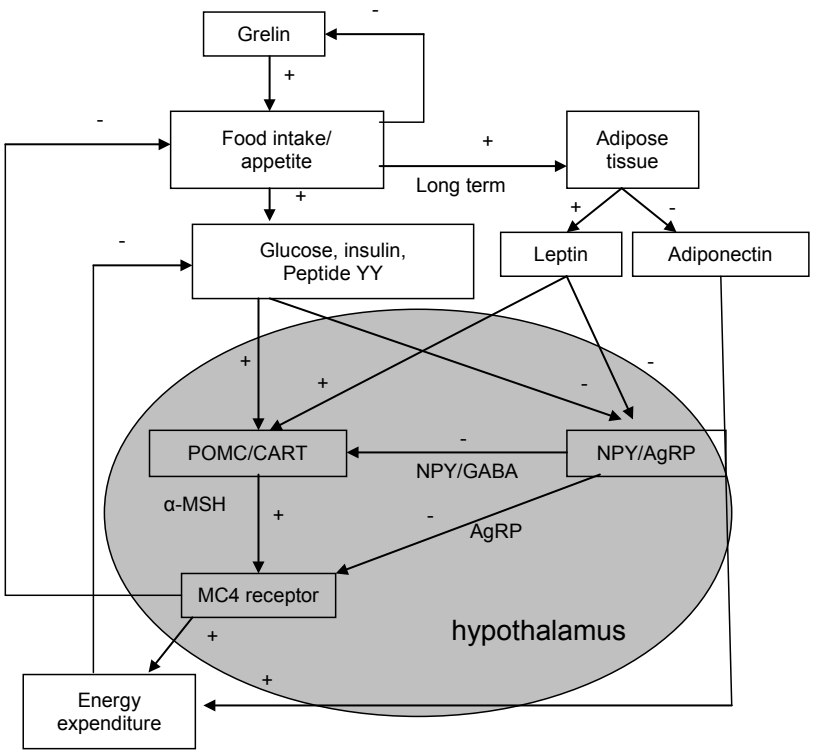
Adapted from ref [8]

Antipsychotic-induced weight gain is most likely to be caused by deregulating the weight-regulating system responsible for energy homeostasis. A simplified overview of this system is shown in Figure 1. Starting from a position of energy homeostasis, antipsychotic-induced weight gain could be the result of either an influence on mechanisms involved in regulating appetite, satiety and/or energy expenditure. Differences in the extent of weight gain between individual antipsychotics is most likely caused by differences in receptor-binding profiles, since antagonism of some receptors (e.g., muscarin, histamine-1, $5HT_{1a}$ and $5HT_{2c}$) has been associated with weight gain.[4-7] Furthermore, the high interindividual patient variability in the risk of antipsychotic-induced weight suggests that genetically determined variability in these receptors is a modulating factor.

Numerous studies have been published investigating the association between genetic constitution and antipsychotic-induced weight gain in an effort to identify patients at risk. Since

2006 no review articles concerning this subject have been published.[8,9] The objective of the current systematic review was to summarize the findings of studies investigating the association between genetic determinants and antipsychotic-induced weight gain up to April 2011. In addition, we will also discuss the feasibility of genetic testing for antipsychotic-induced weight gain in clinical practice.

Figure 1. Simplefied schematic for the weight-regulating system.[88-91]



(+): Stimulation; (-): Inhibition; α-MSH: Melanocyt-stimulating hormone. AgRP; Agouti-related peptide; CART:Cocaine-amfetamin-regulated transcription; MC: Melanocortin; NPY; Neuropeptide Y; POMC: Pro-opiomelanocortin.

Method

Data sources and search strategy

PubMed was searched using the medical subject headings (MeSH®) `Body weight changes' or 'Obesity' combined with 'antipsychotic agents' and 'genetics'. A combination of the keywords 'pharmacogenetic', 'pharmacogenomic', 'polymorphism*', 'gene', '*genetic*', '*genomic*', 'schizophrenia', 'antipsychotic', 'allele*', 'weight' and 'genotype' was also used. To identify articles not indexed in PubMed, a search in Embase was performed with the Emtree headings 'neuroleptic agents' combined with 'weight gain' and 'genetics'. In addition, all references in the selected articles were screened and included in this review when relevant. Finally, a cited reference search in Web of Science was performed in order to identify missing articles and the Human Genome Epidemiology (HuGE) database was checked for additional articles.[96]

Study selection

The full texts of potentially eligible articles were examined in terms of the methodology used. Original articles collected until April 2011, published in English, describing longitudinal association studies in humans on genetic determinants for antipsychotic-induced weight gain were included. Studies with a cross-sectional design were excluded because the studied clinical phenotype antipsychotic-induced obesity or metabolic syndrome cannot be generalized to the main clinical phenotype (antipsychotic-induced weight gain) as studied in this article. No criteria for the quality of the selected articles were applied, since there is no validated checklist for the quality of pharmacogenetic studies and the available checklists for the quality of studies in general are not dissimilar enough for quality differences between genetic studies.

Data extraction

The following data were extracted from the included publications: study characteristics (year of publication, design, sample size, investigated antipsychotic drug, follow-up duration, genetic target, covariates adjusted for); patient characteristics (ethnicity, first episode population yes/no); and primary study results on differences in weight gain.

Results

In April 2011, the electronic search identified 694 publications that were potentially eligible for inclusion. A total of 641 publications were excluded, most of which investigated an association between a genetic determinant and schizophrenia or the side effects of antipsychotic drugs other than weight gain. The remaining 53 publications included 1942 references which, combined with a search in the HuGE navigator and Web of Science, resulted in the inclusion of 4 additional publications, making a total of 57 publications included for data extraction (Figure 2).

Characteristics of the included publications

The grouped characteristics of the 57 included studies are described in Table 2. No genome-wide association studies were found. All studies were association studies looking at one or more SNPs. The published evidence is presented in two separate groups: studies investigating the effect of pharmacokinetic genetic variability and studies investigating the effect of pharmacodynamic genetic variability. The characteristics and results of these studies are summarized in Table 3.

Figure 2. Literature search results

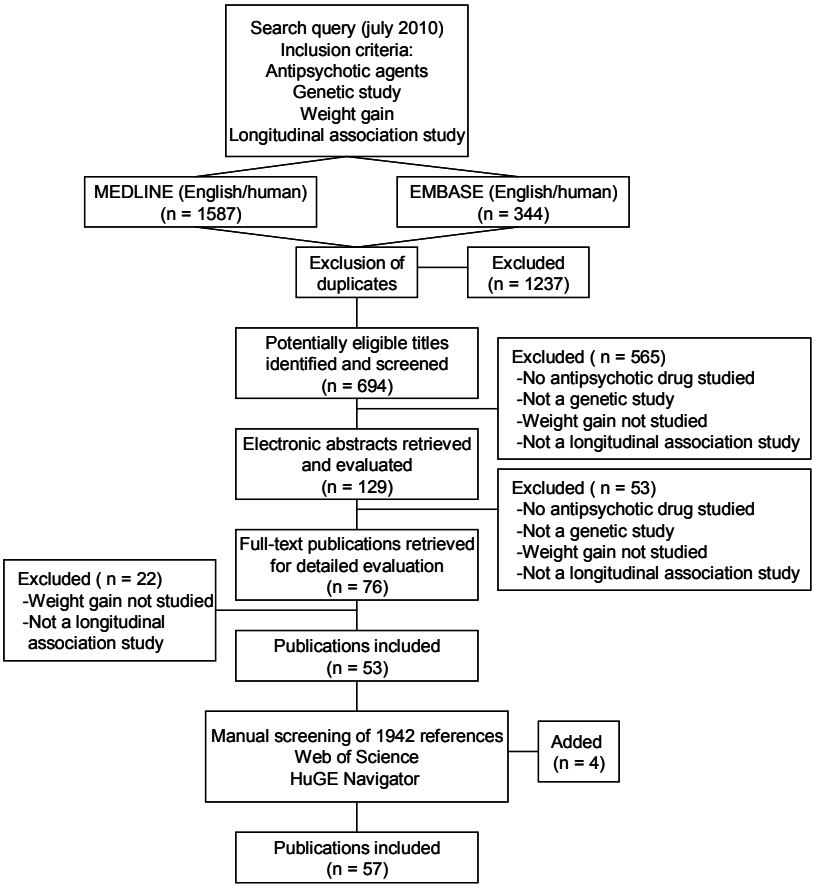


Table 2. Grouped characteristics of the included publications

Characteristic	Number of studies (% of total [n=57])
Study characteristics	
Year of publication	
1995 -< 2000	2 (4)
2000 -< 2005	11 (19)
2005 -< April 2011	45 (79)
Design	
Prospective follow-up	48 (84)
Retrospective follow-up	9 (16)
Follow-up	
< 2 months	21 (37)
2 months -< 6 months	23 (40)
6 months -< 1 year	4 (7)
≥ 1 year	9 (16)
Sample size	
< 50	7 (12)
50 -< 100	21 (37)
100 -< 150	13 (23)
≥ 150	16 (28)
Antipsychotic used	
Clozapine	17 (30)
Olanzapine	16 (28)
Risperidone	3 (5)
Other/various	21 (37)
Target of study	
Pharmacokinetic subject	5 (9)
Pharmacodynamic subject	52 (91)
Patient characteristics	
Ethnicity	
Caucasian	20 (35)
Asian	22 (39)
African	0 (0)
Mixed	15 (26)
Population with first antipsychotic treatment	10 (18)

Pharmacokinetic genetic variability

Table 3 demonstrates that five studies focused on pharmacokinetic polymorphisms to explain the interindividual differences in antipsychotic-induced weight gain, compared with 44 studies on pharmacodynamic polymorphisms. Most pharmacokinetic studies focused on the cytochrome P450 enzymatic complex.

Cytochrome P450 enzymatic complex

Ellingrod et al. found an association between carriers of the *1/*3 or *1/*4 genotype of the CYP2D6 enzyme and olanzapine-induced weight gain in a small population (n=11).[10] This result is surprising since the CYP2D6 iso-enzyme only plays a limited role in olanzapine metabolism, contrary to CYP1A2.[11] Lane et al. also found an association between CYP2D6

Table 3: Studies on pharmacogenetics of antipsychotic-induced weight gain

Gene	SNP	N Follow Up** Ethnicity Population Disease state	Anti-psychotic	Results	Corrected for/tested*	Ref
Pharmacokinetic targets						
CYP1A2	*1F	70 6 wks Cauc /Afr.Am. Unclear popul. Unclear state	Clozapine	No association found with weight gain (p 0.22)	C, I, J, T	19
CYP2D6	*3,*4	11 47 wks Caucasian Unclear popul. Unclear state	Olanzapine	Carriers *3 and *4 allele experience higher increase in BMI than wildtype (+28% vs. +13%, p < 0.0097)).	A, C, F, H, L, V	10
CYP2D6	*10	123 6 wks Asian Inpatients Acutely ill	Risperidone	Carriers *10/wt and *10/10 experience more weight gain than wt/wt (+1.14 kg (p 0.004) vs. +0,8 kg (p 0.04) respectively)	A, C, E, F, G, H, J, K, N, O, Q, T , U, V, Y, Z	12
MDR1	G2677T (rs2032582) C3435T (rs045642)	108 4 months Caucasian Inpatients Acutely ill	Olanzapine Risperidone	Carriers G2677/C3435 haplotype less often in group with ≥ 7% weight gain (OR = 0.54; 95%CI 0.31-0.93), in overall sample. Carriers 3435 C-allele less often in group with ≥ 7% weight gain (OR = 0.35; 95%CI 0.15-0.80), in risperidone group. Carriers 2677 G-allele less often in group with ≥ 7% weight gain (OR 0.36; 95%CI 0.15-0.87), in risperidone group.	A, C, F, G, K, N, M, U, Y	13
	C1236T (rs1128503) G2677T (rs2032582) C3435T (rs045642)	41 6 wks >90% Cauc. In- + Outpat. Chronic state	Olanzapine	No associations found with weight gain. (p 0.3 – 0.4)	A,C, J, K, R, U	14
Pharmacodynamic targets						
ACLY	Rs2304497	154 6 wks Asian (Indian) Inpatients Acute+Chron.	Olanzapine	No associations found with weight gain. Data not shown.	F, K, M, X	68

ADIPOQ	G276T (rs1501299)	164 8-24 wks Asian Inpatients Unclear state	Olanzapine	No association found with weight gain. Data not shown.	A, C, D, F, H, V	25
	Multiple (12)	160 12 wks + retrospective Caucasian Unclear popul. Chronic	Clozapine (various in retrospective analysis)	Rs86265, rs17300539, rs1501299, rs3821799, rs6773957, rs17373414 associated with ΔBMI. Strongest association for rs17300539 (0.0009 < p < 0.034 at various timepoints)	A, C, J, K	71
ADRA1A	Arg347Cys (rs1048101)	60 6 wks Cauc/Afr.Am. Unclear popul. Unclear state	Clozapine	No significant association found with weight gain. Trend towards less weight gain with Cys/Cys (0,8 kg) vs Arg/Cys (4,9 kg) and Arg/Arg (3,2 kg). p 0.22	C, I, J, T	19
	Arg492Cys (rs1048101)	123 6 wks Asian Inpatients Acutely ill	Risperidone	No association found with weight gain. Data not shown.	A, C, E, F, G, H, J, K, N, O, Q, T , U, V, Y, Z	12
ADRA2A	1291 C/G (rs1800544)	93 14 ± 6 months Asian Inpatients Chronic state	Clozapine	Carriers G/G genotype experienced more weight gain than C/C genotype (8.45 vs 2.79 kg, p 0.023). G-allele associated with >7% increase in body weight. OR 3.45 (95%CI 1.87 – 6.35)	A, C, F, H, J, N, Z	20
	1291 C/G (rs1800544)	62 Min 3 months Asian Inpatients Chronic state	Olanzapine	Carriers G-allele more frequently >10% increase in body weight. OR 2.58 (95%CI 1.21-5.51)	A, C, J, H, F, S, X	21
	1291 C/G (rs1800544)	129 6-14 wks Cauc/Afr.Am Inpatients Chronic state	Olanzapine, Clozapine	Carriers C-allele more weight gain compared to GG genotype in Caucasians. 3.73 kg vs. 0.23 kg, p 0.013. No association in African Americans found.	A,B,C,H,I,J,K	22
ADRB3	Trp64Arg (rs4994)	73 6 wks Cauc/Afr.Am. Unclear popul. Unclear state	Clozapine	No association found with weight gain. Trend towards less weight gain in carriers Trp/Trp (3.4 kg) vs Trp/Arg (7.1) and Arg/Arg (7.4) (p 0.1)	C, I, J, T	19

Table 3: Studies on pharmacogenetics of antipsychotic-induced weight gain (*continued*)

Gene	SNP	N Follow Up** Ethnicity Population Disease state	Anti-psychotic	Results	Corrected for/tested*	Ref
	Trp64Arg (rs4994)	164 8-24 wks Asian Inpatients Unclear state	Olanzapine	Arg/Arg genotype associated with olanzapine induced weight gain (p 0.024)	A, C, D, F, H, V	25
	Trp64Arg (rs4994)	87 4 months Asian Unclear popul. Unclear state	Clozapine	No association found with body weight change (p 0.62). Only 1 patiënt with Arg/Arg genotype.	A, C, F, J, P	36
BDNF	66-Val/Met (Rs6265)	123 6 wks Asian Inpatients Acutely ill	Risperidone	Carriers Met/Met-genotype experienced less weight gain than carriers Val/Val-genotype (-0,81 kg, p 0.02).	A, C, E, F, G, H, J, K, N, O, Q, T , U, V, Y, Z	12
	66-Val/Met (Rs6265)	196 > 2 years Retrospective Asian Inpatients Chronic state	Various	Met-allele associated with more weight gain in overall population (p 0.009) and men (p 0.004), but not in women (p 0.13)	A, F, G, J, K, Q, Z	26
CCK	Rs747455 Rs1868522	215 6 wks Mixed Inpatients Chronic state	Olanzapine, Clozapine	No associations found with weight gain.	A, H, K, M, U, Z	92
CCKAR	Rs2968720 Rs915890 Rs1573596	215 6 wks Mixed Inpatients Chronic state	Olanzapine, Clozapine	No associations found with weight gain.	A, H, K, M, U, Z	92

CCKBR	Rs2929183 Rs11040826 Rs1042048 Rs10769675 (CT)n	215 6 wks Mixed Inpatients Chronic state	Olanzapine, Clozapine	Trend for SNP rs2929183 in European ancestry patients. Carriers of genotype AA (3.23% ± 4.8) gained less weight than AG and GG genotypes (6.5% ± 6.5; p 0.035). Similar trend for CTn repeat; carriers LL genotype gained less weight (3.73% ± 5.41, than carriers S-allele (6.29%±6.2, p=0.05).	A, H, K, M, U, Z	92
CNR1	Multiple (20)	183 14 wks Mixed Inpatients Chronic state	Clozapine, Olanzapine, haloperidol, risperidone	Carriers of the rs806378 T-allele experienced more weight gain than carriers of the rs806378 C/C genotype (4.33 ± 3.89 kg vs. 2.21± 4.51 kg, p 0.022)	A, H, J, K, M, N, U	77
	Rs1049353 (1359 G/A)	163 6 months Caucasian Outpatients Chronic state	Various	No association found with weight gain.	A, C, F, G, J, K, Z	78
DRD1	800 T/C (rs265981) 48 A/G (rs4532)	123 6 wks Asian Inpatients Acutely ill	Risperidone	No associations found with weight gain (data not shown)	A, C, E, F, G, H, J, K, N, O, Q, T , U, V, Y, Z	12
	Rs4532 Rs686 Rs265976	206 6 wks Cauc./Afr.Am. Unclear popul. Chronic state	Clozapine, Olanzapine, Risperidone, Haloperidol	No associations found with weight gain.	B, I, K, M, N, AB	32
DRD2	311-Ser/Cys (rs1801028) 141C-Ins/Del (rs1799732) TaqI A (rs1800497)	123 6 wks Asian Inpatients Acutely ill	Risperidone	Non-significant association. Trend for carriers Ser/Cys genotype to experience less weight gain than carriers Cys/Cys genotype (-0,45 kg, p 0.09)	A, C, E, F, G, H, J, K, N, O, Q, T, U, V, Y, Z	12
	Multiple (13)	479 4 ± 2 yrs retrospective Asian Inpatients Chronic state	Clozapine, Olanzapine, Risperidone	Rs4436578-C/C genotype associated with increased risk of 7% weight gain (OR 3.36; 95%CI 1.62-7.00). Rs4436578-C-allele haplotype more frequent in patients with 7% weight gain (p = 0.01-0.00019) .	A, B, C, H, J, K, M, N, Z	30

Table 3: Studies on pharmacogenetics of antipsychotic-induced weight gain (*continued*)

Gene	SNP	N Follow Up** Ethnicity Population Disease state	Anti-psychotic	Results	Corrected for/tested*	Ref
	TagI A (rs1800497)	164 8-24 wks Asian Inpatients Unclear state	Olanzapine	No association found with weight gain (data not shown)	A, C, D, F, H, V	25
	TaqI A (rs1800497)	117 10 wks Asian Inpatients Acutely ill	Chloroprom. (66), Risperidone (43),Other(12)	No association found with weight gain for allelic frequencies (c2=0.65, v1, P>0.05) and genotype (χ_2=1.47, v2, p>0.05).	A, C, F, G, J, K, Q, Z	82
	Rs1799732 (141C-Ins/ Del)	58 16 wks Mixed Unclear popul. Acutely ill	Olanzapine, Risperidone	Carriers of the deletion allele gained more weight than carriers of the Ins/Ins genotype after 6 weeks (F (10.242) = 2.11, p =0.024	B, F, I, J, Z, AA, AB, AC	31
	Multiple (14)	206 6 wks Cauc./Afr.Am. Unclear popul. Chronic state	Clozapine, Olanzapine, Risperidone, Haloperidol	Nominal significant association in total sample for rs1079598 marker and >7% weight gain. In stratified sample according to ethnicity and antipsychotics with high weight gain risk; rs6277 (C957T), rs1079598, rs1800497 (TaqIA) associated with weight gain.	B, I, K, M, N, AB	32
DRD3	205 A/G 9-Ser/Gly (rs6280)	123 6 wks Asian Inpatients Acutely ill	Risperidone	No associations found with weight gain (data not shown)	A, C, E, F, G, H, J, K, N, O, Q, T , U, V, Y, Z	12
	Multiple (14)	206 6 wks Cauc./Afr.Am. Unclear popul. Chronic state	Clozapine, Olanzapine, Risperidone, Haloperidol	No associations found with weight gain.	B, I, K, M, N, AB	32

DRD4	Rs3758653 Exon3-VNTR Rs11246226	206 6 wks Cauc./Afr.Am. Unclear popul. Chronic state	Clozapine, Olanzapine, Risperidone, Haloperidol	No associations found with weight gain.	B, I, K, M, N, AB	32
	48 bp VNTR (exon 1)	102 4 wks Caucasian Inpatients Acutely ill	Various	Less weight gain in patients with VNTR < 7 repeats compared to patients with VNTR > 7 repeats (+0.38 kg/m^2 vs. +0.89 kg/m^2 respectively, p 0.003) in overall population. Difference remained significant in men (+0.39 kg/m^2 vs. 1.30 kg/m^2, p 0.005) but not in women (+0.38 kg/m^2 vs. 0.58 kg/m^2, p 0.13)	A, C, D, J, T, V, Z	33
	48 bp VNTR 12 bp VNT 235Del13 Gly11Arg	149 min. 4 wks retrospective Caucasian Inpatients Unclear state	Clozapine	No associations found between genotypes and frequency of reporting weight gain as a side-effect by the patient.	H, N	34
DRD5	Rs10033951 Rs6283 Rs10001006	206 6 wks Cauc./Afr.Am. Unclear popul. Chronic state	Clozapine, Olanzapine, Risperidone, Haloperidol	No associations found with weight gain.	B, I, K, M, N, AB	32
FAAH	Pro129Thr (Rs324420)	164 8-24 wks Asian Inpatients Unclear state	Olanzapine	No association found with weight gain. Data not shown.	A, C, D, F, H, V	25
	Rs324420 (385C/A)	163 6 months Caucasian Outpatients Chronic state	Various	Carriership of A allele more frequent in patients who gained more than 7% of their baseline weight. OR 3.11 (95% CI 1.42-6.77)	A, C, F, G, J, K, Z	78
FABP3	Multiple (7) Not specified	160 12 wks + retrospective Caucasian Unclear popul. Chronic	Clozapine (various in retrospective analysis)	No associations found with weight gain.	A, C, J, K	71

Table 3: Studies on pharmacogenetics of antipsychotic-induced weight gain (*continued*)

Gene	SNP	N Follow Up** Ethnicity Population Disease state	Anti-psychotic	Results	Corrected for/tested*	Ref
FTO	Rs9939609	239 1 year Mixed Outpatients Acutely ill	Various	No association found with weight gain.	A, C, J, K	63
	Rs9939609	160 12 wks + retrospective Caucasian Unclear popul. Chronic	Clozapine (various in retrospective analysis)	No association found with weight gain.	A, C, J, K	71
GHRL	Leu72Met (rs696217)	164 8-24 wks Asian Inpatients Unclear state	Olanzapine	No association found with weight gain. Data not shown.	A, C, D, F, H, V	25
GNB3	C825T (rs5443)	164 8-24 wks Asian Inpatients Unclear state	Olanzapine	T-allele associated with olanzapine induced weight gain (p 0.043)	A, C, D, F, H, V	25
	C825T (rs5443)	134 +/- 13 months Asian Inpatients Chronic state	Clozapine	Carriers T/T-genotype experienced more weight gain (16,2%) than carriers C/T (9,3%) and C/C-genotype (5,5%). (p=0,003)	A, C, F, H, K, Z	35
	C825T (rs5443)	87 4 months Asian Unclear popul. Unclear state	Clozapine	No association found with weight gain. Trend for carriers C/C-genotype to experience more weight gain than carriers T- or T/T genotype . (p 0.112)	A, F, J	36

	C825T (rs5443)	42 6 wks Caucasian Unclear popul. Acute+Chron.	Olanzapine	No association found with weight gain. Trend towards more weight gain in carriers T/T-genotype (16.6%) vs C/C en C/T genotype (9,4%). (p 0.43)	F, J, K, U, Z	37
	C825T (rs5443)	104 3 months Asian Inpatients Chronic state	Olanzapine	No association found with weight gain.	A, C, F, H, J, K, X	38
GSTM1 GSTT1 GSTP1	Wild/null (M1) Wild/null (T1) Ile105Val (P1) (rs1695)	78 ± 1.5 yrs Asian Inpatients Chronic state	Olanzapine	No associations found with weight gain.	A, C, F, J, K, X	93
HRH1	Unclear	73 6 wks Cauc/Afr.Am. Unclear popul. Unclear state	Clozapine	No association found with weight gain. (p 0.55)	C, I, J, T	19
	Leu449Ser (rs2067470)	164 8-24 wks Asian Inpatients Unclear state	Olanzapine	No association found with weight gain. Data not shown.	A, C, D, F, H, V	25
	Glu349Asp (rs2067467)	88 4 months Asian Unclear popul. Unclear state	Clozapine	No association found with weight gain. (p 0.3)	A, F, J, N	42
HRH2	1018GA (rs2979099)	50 6 wks Cauc/Afr.Am. Unclear popul. Unclear state	Clozapine	No association found with weight gain. Trend towards more weight gain in carriers A-allele. (GG: 3.1kg, AG 4.6kg, AA: 5.4kg. (p 0.34))	C, I, J, T	19
	1018GA (rs2979099)	164 8-24 wks Asian Inpatients Unclear state	Olanzapine	No association found with weight gain. Data not shown.	A, C, D, F, H, V	25

Table 3: Studies on pharmacogenetics of antipsychotic-induced weight gain (*continued*)

Gene	SNP	N Follow Up** Ethnicity Population Disease state	Anti-psychotic	Results	Corrected for/ tested*	Ref
HRH3	Ser332Ser (rs3787429)	164 8-24 wks Asian Inpatients Unclear state	Olanzapine	No association found with weight gain. Data not shown.	A, C, D, F, H, V	25
HTR1A	Gly272Asp (rs1800042)	123 6 wks Asian Inpatients Acutely ill	Risperidone	No association found with weight gain (data not shown)	A, C, E, F, G, H, J, K, N, O, Q, T , U, V, Y, Z	12
	CAn-repeat	73 6 wks Cauc/Afr. Am. Unclear popul. Unclear state	Clozapine	No significant association found (p 0.23). Trend towards less weight gain with increasing number of repeats (22,23,33 vs. 11 and 12 repeats).	C, I, J, T	19
HTR2A	102 T/C (rs6313) His452Tyr (rs6314)	123 6 wks Asian Inpatients Acutely ill	Risperidone	Carriers 102-C/C genotype experience less weight gain than carriers 102-T/T genotype (-1.4 kg, $p < 0.0001$). No association found for His452Tyr.	A, C, E, F, G, H, J, K, N, O, Q, T , U, V, Y, Z	12
	102 T/C (rs6313) His452Tyr (rs6314)	78 6 wks Cauc/Afr.Am. Unclear popul. Unclear state	Clozapine	No associations found with weight gain (102 T/C; p 0.75, His452Tyr; p 0.95).	C, I, J, T	19
	102 T/C (rs6313)	164 8-24 wks Asian Inpatients Unclear state	Olanzapine	T-allele associated with weight gain (p 0.0078).	A, C, D, F, H, V	25

	102 T/C (rs6313)	93 4 months Asian Unclear popul. Unclear state	Clozapine	No association found with weight gain (p 0.59).	A, C, F, J	94
HTR2C	Cys23Ser (rs6318)	102 4 wks Caucasian Inpatients Acutely ill	Various	No association found with weight gain (p 0.7).	A, C, J, T, V,	33
	Cys23Ser (rs6318)	77 6 wks Cauc/Afr.Am Unclear popul. Unclear state	Clozapine	No association found with weight gain (p 0.54).	C, I, J, T	19
	Cys23Ser (rs6318)	93 4 months Asian Unclear popul. Unclear state	Clozapine	No association found with weight gain (p 0.66).	A, C, F, J	94
	Cys23Ser (rs6318)	152 Min 4 wks retrospective Caucasian Inpatients Unclear state	Clozapine	No association between genotypes and frequency of reporting weight gain as a side-effect (data not shown).	H, J, N, T	95
	759 C/T (rs3813929)	123 6 wks Asian Inpatients Acutely ill	Risperidone	Carriers T-allele experienced less weight gain vs. Carriers C- or C/C-genotype (-0,64 kg, p 0.04)	A, C, E, F, G, H, J, K, N, O, Q, T , U, V, Y, Z	12
	759 C/T (rs3813929)	108 4 months Caucasian Inpatients Acutely ill	Olanzapine, Risperidone	No significant difference found in prevalence of variant T-allele in groups with ≥ 7% or < 7% weight gain.	A, C, F, G, K, M, N, U, Y	13

Table 3: Studies on pharmacogenetics of antipsychotic-induced weight gain (*continued*)

Gene	SNP	N Follow Up** Ethnicity Population Disease state	Anti-psychotic	Results	Corrected for/tested*	Ref
	Cys23Ser (rs6318) 759 C/ (rs3813929)	164 8-24 wks Asian Inpatients Unclear state	Olanzapine	23 Cys-allele predicted olanzapine induced weight gain. Mean ± Sd coefficient: 9.1 ± 3.4	A, C, D, F, H, V	25
	759C/T (rs3813929)	123 10 wks Asian Inpatients Acutely ill	Chloroprom.(69), Risperidone (46), Other (8)	Carriers 759T-allele experience less weight gain after 6 wks (p <0,0001, -0,01 BMI vs +0,85BMI) and after 10 wks (p =0,003, +0,41BMI vs +1.38BMI) than patients with C/C genotype.	A, C, F, G, Q	45
	759C/T (rs3813929)	32 6 wks Asian Inpatients Acutely ill	Clozapine	Carriers 759-T-allele (n=10) experienced less weight gain than carriers 759 C- or C/C-genotype (0.32 kg/m^2 vs. + 1.12 kg/m^2 respectively, p<0,02). Strongest association was found in men (+ 0.03 kg/m^2 vs. +1.26 kg/m^2 , p 0.008). Findings in women were non-significant.	A, C, F, H, J, Q	46
	759C/T (rs3813929)	42 6 wks Caucasian Inpatients Unclear state	Olanzapine	Carriers T-allele were significantly less often in the group with > 10% weight gain compared to carriers C- or C/C-genotype (0% vs. 40.7% respectively, p 0.0035)	F, J,	47
	759C/T (rs3813929)	73 9 months Caucasian Outpatients Acutely ill	Risp (26), Olan.(19), Quet (11), Halo (10), Zipra (6), Amisul.(1)	Carriers T-allele experience less weight gain compared to carriers C- and C/C-genotype after 6 wks (0.94 vs 1.88 kg/m^2,p 0.003), 3 months (1.4 vs. 2.53 kg/m^2, p 0.018) and 9 months (2.35 vs. 4.53 kg/ m^2 p =0,031)	A, C, Z	48
	759C/T (rs3813929)	41 6 months Mixed Unclear popul. Chronic state	Clozapine	No significant association with weight gain found. Trend for patients carrying T-allele to experience less weight gain compared to patients carrying C- or C/C-genotype (+2. kg vs. 4.44 kg, p 0.071). Prevalence T-allele significantly higher in group with less than 7% gain in body weight. (p=0,002)	A, C, I, J, K, R,	49

	759C/T (rs3813929)	84 4 wks Asian Inpatients Acutely ill	Risp. (53), Olan (12), Amisul (5), Quet (4), typical (10)	Patients carrying variant T-allele less likely to have substantial (>5%) weight gain (p=0.03).	A, B, C, J, Q, W, X	50
	759 C/T (rs3813929) 697 G/C (rs518147)	107 6 wks Caucasian Inpatients Acutely ill	Olanzapine	759T-allele and 697C-allele were protective for weight gain. Significantly less patients with 697 C (3/51) and no patient with 759T (0.28) alleles experienced a BMI increase ≥ 10% (p 0.0006 vs 0.002 respectively)	A, C, G, H, J, K, M, Q, U, Z	51
	759 C/T (rs3813929) Cys23Ser (rs6318) 997G/A (rs3813928) 1165 A/G (rs498207)	128 6 wks Retrospective Caucasian In- + Outpat. Acute+Chron.	Various	1165 A/AA-genotype associated with > 7% weight gain (OR 1.65; 95%CI 1.09-2.51). 759 C-alleles and 997 G-alleles also associated with > 7% weight gain (p 0.04 in both cases, no regression analysis).	A, D, G, J, K, M, N, X, Z	52
	759 C/T (rs3813929) Rs1414334	32 8 wks Caucasian Outpatients Acutely ill	Risperidone	Carriers 759 T-allele gained less weight than non-T-allele carriers (1,84 ± 1.51 kg vs. 3.23 ± 1.47 kg, p < 0.001). No association found for rs1414334 polymorphism.	A, C, F, J, X	53
	759C/T (rs3813929)	80 4 months Asian Unclear popul. Unclear state	Clozapine	No association found with weight gain in overall population (p 0.67), men (p 0.62) and women (p 0.94).	A, C, F, H, J	54
	759C/T (rs3813929)	97 12 wks Caucasian Unclear popul. Unclear state	Clozapine	No significant association found with weight gain. Carriers 759 C- or C/C-genotype: 2.11 kg vs. carriers 759 T-allele: 1.97 kg, p 0.77).	A, C, F, G, J, K	55
	759 C/T (rs3813929)	79 3 months(min) Asian Inpatients Unclear state	Olanzapine	No significant difference in prevalence of T-allele in groups with ≥ 7% or < 7% weight gain (p 0.445)	A, C, F, H, J, S, X	56

Table 3: Studies on pharmacogenetics of antipsychotic-induced weight gain (*continued*)

Gene	SNP	N Follow Up** Ethnicity Population Disease state	Anti-psychotic	Results	Corrected for/ tested*	Ref
	759 C/T (rs3813929)	216 203 days Mixed Inpatients Acutely ill	Iloperidone	No association found with weight gain (p > 0.5).	C, I, J	57
	759C/T (rs3813929)	73 6 wks Cauc/Afr.am. Unclear popul. Unclear state	Clozapine	Male carriers of T-allele experienced more weight gain than carriers of C-genotype (7,2 kg vs 3.6 kg, p=0,047). No association found in women and total population.	A, C, I, J, T	58
	759 C/T (rs3813929) Cys23Ser (rs6318) GTn (c.1142948)	139 6-14 wks Mixed etnicity Inpatients Chronic state	Cloz (92), Halo (13), Olan (21), Risp (13)	Long-C-Ser haplotype protective for weight gain (p 0.042)	A, B, I, J, K, T, W,	59
HTR6	267 C/T (rs1805054)	123 6 wks Asian Inpatients Acutely ill	Risperidone	Carriers T/C and C/C-genotype experienced more weight gain than carriers T/T-genotype (resp. 1,4 en 1,2 kg, p 0.02)	A, C, E, F, G, H, J, K, N, O, Q, T , U, V, Y, Z	12
	267 C/T (rs1805054)	164 8-24 wks Asian Inpatients Unclear state	Olanzapine	No association found with olanzapine induced weight gain. Data not shown.	A, C, D, F, H, V	25
	267C/T (rs1805054)	92 4 months Asian Unclear popul. Unclear state	Clozapine	No association found with change in body weight (p 0.777).	A, C, F, J	94

INSIG2	Rs17587100 Rs10490624 Rs17047764 Rs7566605	128 6 wks Retrospective Caucasian In- + Outpat. Acute+Chron.	Various	No associations found with weight gain.	A, D, G, J, K, M, N, X, Z	52
	Multiple (44)	160 12 wks retrospective Caucasian Unclear popul. Chronic state	Clozapine	Significant association between three markers within INSIG2 gene (p = 0.0003–0.00007), rs17587100, rs10490624 and rs17047764 and antipsychotic-related weight gain.	A, C, J, K, N	73
	Rs17587100 Rs10490624 Rs17047764 Rs7566605	154 14 wks Cauc/Afr.Am. Inpatients Chronic state	Clozapine, Olanzapine, Haloperidol, Risperidone	No associations found with weight gain in patients with either European or African ancestry.	A, I, J, K, M, N	74
LEP	2548A/G (rs7799039)	73 9 months Caucasian Outpatients Acutely ill	Risp (26),Olan (19) Quet (11), Halo (10), Zipra (6), Amisu (1)	Carriers G/G-genotype experienced more weight gain than carriers A/G- and A/A-genotype after 9 months (p =0,03), but not after 6 weeks and 3 months. (delta BMI 7 kg/m^2 vs 3 kg/m^2 after 9 months)	A, C, Z	48
	2548 A/G (rs7799039)	128 6 wks Retrospective Caucasian In- + Outpat. Acute+Chron.	Various	No association found with weight gain.	A, D, G, J, K, M, N, X, Z	52
	2548 A/G (rs7799039)	74 3 months Asian Inpatients Chronic state	Olanzapine	More weight gain in patients with AG-genotype compared tot AA-genotype (8.6 vs 4.4 kg, p 0.046)	H, J, K, X	62
	Rs7799039 (2548 A/G)	239 1 year Mixed Outpatients Acutely ill	Various	No association found with weight gain.	A, C, J, K	63

Table 3: Studies on pharmacogenetics of antipsychotic-induced weight gain (*continued*)

Gene	SNP	N Follow Up** Ethnicity Population Disease state	Anti-psychotic	Results	Corrected for/ tested*	Ref
	2548 A/G (rs7799039)	102 > 6 yrs Retrospective Asian Inpatients Chronic state	Clozapine	Variant 2548 G-allele associated with weight gain (BMI). Patients carrying G-allele gained more weight than patients carrying C/C-genotype (+4.4 vs. +2.6 kg/m^2, p 0.014). Association only present in men.	A, C, F, G, H, J, K, Q, X, Z	64
	2548A/G (rs7799039)	73 6 wks Prim. Cauc. Unclear popul. Unclear state	Olanzapine	No significant association with weight gain found. Change in BMI for carriers G-allele +2.34 kg/m^2 vs +1.47 kg/m^2 for carriers AA-genotype. (p 0.33) More weight gain in higher plasma level group (p 0.049).	A, C, J, K, U	65
	2548 A/G (rs7799039)	74 2 years max. Mixed Outpatients Chronic state	Risperidone	A-allele associated with higher leptin concentration at low weight and BMI-Z scores before treatment with risperidone. After start of treatment carriers A-allele steeper increase in weight and BMI-Z scores and 2.5 times more often obese/overweight.	A,C,I,J,K	66
	Rs10487506 Rs3828942 Rs17151922 Rs4731426 Rs2278815 Rs28954113	154 6 wks Asian (Indian) Inpatients Acute+Chron.	Olanzapine	Rs4731426 C/G moderately associated with weight gain; more than mean compared to less than mean: (OR 2.2;95%CI 0.99-4.90, p 0.05).	F, K, M, X	68
LEPR	Rs1137101	239 1 year Mixed Outpatients Acutely ill	Various	No association found with weight gain.	A, C, J, K	63
LPL	Rs320GT Rs285CT Rs4922115 Rs328CG Rs326AG	154 6 wks Asian (Indian) Inpatients Acute+Chron.	Olanzapine	No associations found with weight gain. Data not shown.	F, K, M, X	68

MTHFR	Rs1801133 (C677T) Rs1801131 (A1298C)	104 3 months Mixed Unclear popul. Acutely ill	Various	MTHFR A1298C, but not C677T, genotype predicted pos-baseline increases in weight (β =2,5, SE = 0.92, p=0.006). 1298C allele was predictive for weight gain.	A, B, J, K,	79
PPARG	Pro12Ala (rs1801282)	164 8-24 wks Asian Inpatients Unclear state	Olanzapine	No association found with weight gain. Data not shown.	A, C, D, F, H, V	25
	Pro12Ala (rs1801282)	95 6 wks Caucasian Unclear popul. Acutely ill	Olanzapine	Carriers Ala-allele experienced more weight gain; 8.42 kg vs 3.99 kg (p 0.016).	A, C , F, G, Z	75
PRKAA1	Multiple (5)	160 12 wks + retrospective Caucasian Unclear popul. Chronic	Clozapine (various in retrospective analysis)	Rs3792823, rs11740266, rs10074991 associated with ΔBMI. Strongest association for rs10074991 (0.0009 < p < 0.011 at various time points)	A, C, J, K	71
PRKAA2	Multiple (9) Not specified	160 12 wks + retrospective Caucasian Unclear popul. Chronic	Clozapine (various in retrospective analysis)	Rs7519509, rs4912411, rs10489617 associated with ΔBMI. At various time points.	A, C, J, K	71
PRKAB1	Multiple (3) Not specified	160 12 wks + retrospective Caucasian Unclear popul. Chronic	Clozapine (various in retrospective analysis)	No associations with weight gain found.	A, C, J, K	71
PRKAG1	Multiple (4) Not specified	160 12 wks + retrospective Caucasian Unclear popul. Chronic	Clozapine (various in retrospective analysis)	No associations with weight gain found.	A, C, J, K	71

Table 3: Studies on pharmacogenetics of antipsychotic-induced weight gain (*continued*)

Gene	SNP	N Follow Up** Ethnicity Population Disease state	Anti-psychotic	Results	Corrected for/ tested*	Ref
PRKAG2	Multiple (33) Not specified	160 12 wks + retrospective Caucasian Unclear popul. Chronic	Clozapine (various in retrospective analysis)	Rs17714947, rs7800069 associated with ΔBMI at various time points. Strongest association for rs7800069 (p = 0.0008)	A, C, J, K	71
PRKAG3	Not specified (2)	160 12 wks + retrospective Caucasian Unclear popul. Chronic	Clozapine (various in retrospective analysis)	No associations found with weight gain.	A, C, J, K	71
SH2B1	Rs7498665	239 1 year Mixed Outpatients Acutely ill	Various	No association found with weight gain.	A, C, J, K	63
SLC6A4	HTTLPR L/S (rs4795541)	94 3 months Caucasian Inpatients Acutely ill	Olanzapine	Carriers S-allele experienced more weight gain than carriers LL-genotype (+1.34 vs. +0.32 kg/m^2, p 0.021), in group patients with BMI < 27.3 kg/m^2.	A, C, F, G, K, M, N, U, X, Y	72
	HTTLPR L/S (rs4795541)	94 4 months Asian Unclear popul. Unclear state	Clozapine	No association found between weight gain and serotonin transporter gene (p 0.814)	A, C, F, J	94
SNAP25	401C/T (rs8636) 1065T/G (rs3746544) 1069T/C (rs1051312)	164 8-24 wks Asian Inpatients Unclear state	Olanzapine	No association found with weight gain. Data not shown.	A, C, D, F, H, V	25

	DdeI (rs1051312) MnlI (rs3746544) TaiI (rs8636)	59 11 wks Mixed Inpatients Chronic state	Cloz. / Risp, Halo / Olanz.	MnlI and TaiI associated with weight gain (p 0,01 and 0,04 resp.). No association found after correction for co-variables.	A, B, H, I, U,	69
	DdeI (rs1051312) MnlI (rs3746544) TaiI (rs8636)	162 5 wks min. Caucasian Unclear popul. Unclear state	Risp, Olan, Quet, Amisul, Sert	Significant association between DdeI polymorphism and baseline weight and weight gain. Carriers DdeI TT-genotype higher baseline BMI (25.9 kg/m^2) than TC + CC (23.4 kg/m^2), p 0.035. Less weight gain in carriers DdeI TT-genotype (+0.55 kg/m^2) than in carriers CC or CT genotype (+1.1kg/m^2), p 0.038	A, B, J, T, U, Z	70
TAG	Rs2915748 Rs2234972	154 6 wks Asian (Indian) Inpatients Acute+Chron.	Olanzapine	No associations found with weight gain. Data not shown.	F, K, M, X	68
TNFA	308G/A (rs1800629)	74 6 wks Cauc/Afr.Am. Unclear popul. Unclear state	Clozapine	No association found with weight gain. (p 0.15)	C, I, J, T	19
	308G/A (rs1800629) 857C/T (rs1799724)	164 8-24 wks Asian Inpatients Unclear state	Olanzapine	No association found with weight gain. Data not shown.	A, C, D, F, H, V	25
	308 G/A (rs1800629)	55 8 yrs Retrospective Asian Inpatients Chronic state	Clozapine	Carriers 308 A-allele experienced less weight gain than carriers of the -308 G/G genotype (coeff: -1.24; 95%CI -2.17 – - 0.32, p 0.0084)	A, C, D, F, H, J, K, Q, T, X, Z	76
TNFRS-F1A	36A/G (rs767455)	164 8-24 wks Asian Inpatients Unclear state	Olanzapine	No association found with weight gain. Data not shown.	A, C, D, F, H, V	25

Table 3: Studies on pharmacogenetics of antipsychotic-induced weight gain (*continued*)

Gene	SNP	N Follow Up** Ethnicity Population Disease state	Anti-psychotic	Results	Corrected for/ tested*	Ref
TNFRS-F1B	196Met/Arg (rs1061622)	164 8-24 wks Asian Inpatients Unclear state	Olanzapine	No association found with weight gain. Data not shown.	A, C, D, F, H, V	25

Abbreviations:
ACLY: Gene coding for ATP Citrate Lyase. **ADIPOQ**: Adiponectin gene. **ADRA1A-ADRB3**: Genes coding for the Adrenergic Receptor 1alfa – Adrenergic Receptor Beta3. **BDNF**: brain derived neurotrophic factor gene. **CCK**: cholecystokinin gene. **CCKAR**: Cholecystokinin A receptor. **CCKBR**: Cholecystokinin B receptor. CNR1: cannabinoid receptor I gene. **DRD1-DRD5**: Genes coding for the Dopamine Receptor 1 – Dopamine Receptor 5. **FAAH**: fatty acid amide hydrolase. **FABP3**: fatty acid-binding protein 3. **FTO**: Fat mass and obesity associated gene. **GHRL:** Growth Hormone secretagogue Receptor Ligand, Ghrelin. **GST-M1/T1/P1:** Glutathione S-transferase Mu1/Theta1/Pi 1. **HRH1-HRH3**: Genes coding for Histamine 1-Histamine 3 Receptor. **HTR1A-HTR6:** Genes coding for the Serotonergic 1A-Serotonergic 6 Receptors. **INSIG2:** insulin induced gene protein 2. **LEP:** Leptin gene. **LEPR**: Leptin receptor gene. **LPL**: lipoprotein lipase. **MDR1**: Multi Drug Resistance Gene 1, coding for P-glycoprotein. **MTHFR**: 5,10 methylenetetraydrofolate reductase. **PPARG**: peroxisome proliferator-activated receptor gamma. **PRKAA1-G3**: Protein Kinase, AMP activated, alpha 1- gamma 3 catalytic subunit. **SH2B1**: SH2B adaptor protein 1. **SLC6A4**: Solute Carrier Family 6, member 4; coding for Serotonin Transporter. **SNAP-25**: synaptosomal-associated protein of 25 kDa **TAG**: tri-acyl glycerol lipase. **TNFA**: Gene coding for TNF-alfa. **TNFR1a-b**: Tumor Necrosis Factor receptor 1A – B gene.
* **A**; age. **B**; Antipsychotic agent. **C**; Baseline weight/BMI. **D**; Combination of antipsychotic drugs allowed. **E**; diagnosis subtype. **F**; Dose. **G**; duration of illness. **H**; duration of treatment. **I**; ethnicity. **J**; gender. **K**; Followed Hardy-Weinberg Equilibrium. **L**; Drug Interactions. **M**; Linkage disequilibrium. **N**; Multiple testing. **O**; number of prior hospitalizations. **P**; other polymorphisms. **Q**; Controlled diet. **R**; plasma concentration. **S**; previous antipsychotic treatment. **T**; response status. **U**; severity of disease. **V**; Smoking behaviour. **W**; time of LOCF. **X**; weight influencing drugs. **Y**; education. **Z**; Substance abuse excluded. **AA**; Compliance monitored, **AB**; treatment site. **AC**; concomitant

genotype and antipsychotic-induced weight gain.[12] Patients carrying the less functional CYP2D6 *10 allele experienced more risperidone-induced weight gain. Surprisingly they found a stronger association for heterozygotes then homozygous *10 carriers.

The patients mentioned in the studies by Ellingrod et al. and Lane et al. would all be classified as intermediate metabolisers. Most intermediate metabolisers are able to metabolise CYP2D6 substrates normally; however, there is a large variance in phenotype activity within this group with some intermediate metabolisers showing a marked decrease in CYP2D6 metabolic capacity. This could explain why even for intermediate metabolisers an association was found. However, since CYP2D6 only plays a minor role in olanzapine metabolism and risperidone has an active metabolite (9-OH risperidone) with similar properties, it cannot be ruled out that these results reflect type I errors.

P-glycoprotein efflux transporter

Kuzman et al. found an association between the G2677T (rs2032582, exon 21) and C3435T (rs045642, exon 26) polymorphisms in the *MDR1* gene encoding the P-glycoprotein efflux transporter (PGP) and risperidone-induced weight gain in female schizophrenic patients.[13] Female patients carrying the 3435T allele or 2677T allele, resulting in a lower PGP function, gained more weight with risperidone, but not with olanzapine, compared with patients with the 3435-CC or 2677-GG genotype, respectively. Lin et al. could not find an association between these polymorphisms in the gene encoding the PGP and olanzapine induced weight gain.[14]

Pharmacokinetic polymorphisms can only be relevant for antipsychotic-induced weight gain when the occurrence of weight gain is dose-dependent. The above mentioned studies suggest this association. This hypothesis of dose-dependent antipsychotic-induced weight gain is confirmed in two studies investigating weight gain on olanzapine and risperidone.[15,16] However, in the studies by Dunayevich et al. and Lane et al. this association was not found.[17,18] Based on current evidence, it is premature to suggest an association between pharmacokinetic polymorphisms and antipsychotic-induced weight gain, especially since patients most likely to experience this side effect, e.g. CYP2D6 poor metabolisers, were not included in the studies.

Pharmacodynamic genetic variability

Since pharmacodynamic polymorphisms influence receptor affinity or receptor activity, studies regarding this subject have been categorized based on their receptor system.

Adrenergic system

Adrenergic receptors such as α_1, α_2 and $ß_3$-receptors are important for the mitochondrial uncoupling proteins pathway (UCP 1-3), which is involved in the maintenance of basic metabolic rate, thermogenesis and the efficiency of energy utilization.[19] Stimulation of the UCP-pathway by α_1, α_2 and $ß_3$-receptor agonists, results in a greater basic metabolic rate,

increased lipolysis, and less weight gain. Studies investigating the adrenergic system focused on polymorphisms in the genes encoding the α_{1A} (*ADRA1A*), α_{2A} (*ADRA2A*) and $ß_3$ receptor (*ADRB3*). The most promising receptor in this aspect is the α_{2A}-receptor.

The studies by Wang et al., Park et al. and Sickert et al. found an association between the 1291C/G polymorphism (rs1800544) in the promoter region of the *ADRA2A* gene and clozapine- or olanzapine-induced weight gain.[20-22] In both studies in Asians the G allele was associated with increased weight gain expressed as a >7% (Wang et al.; 8.45 vs 2.79 kg; p=0.023) or 10% (Park et al.; odds ratio [OR]: 2.58 [95% CI 1.21-5.51]) increase in body weight. Remarkably, in the study by Sickert et al. an association in the opposite direction was found for Caucasians. Caucasian patients carrying the C allele experienced more weight gain than patients with the G/G genotype (3.73 kg vs 0.23 kg; p=0.013), demonstrating the potential impact of ethnicity on the association.

The $ß_3$ receptor is also of interest since this receptor is involved in the phenotype of obesity and agonism of $ß_3$ receptors increases lipolysis.[23] $ß_3$ agonists are even being developed as drugs to treat patients with obesity.[24] The Trp64Arg polymorphism (rs4994) in the *ADRB3* gene encoding the $ß_3$ receptor could possibly be a candidate for an association with antipsychotic-induced weight gain, although only one study found a significant association. Ujike et al. found a significant (p=0.024) association between carriers of the Arg/Arg genotype and weight gain in patients treated with olanzapine.[25] A trend for this association was also found in the study by Basile et al.[19] A complicating factor in the study of the Trp64Arg polymorphism is the low prevalence of the Arg allele.

Brain-derived neurotrophic factor

Brain-derived neurotrophic factor (BDNF) and its tyrosine kinase receptor, TrkB, play a role in weight-regulation and are associated with eating behaviour, food consumption and control of body weight. Administration of BDNF decreases food intake, increases energy expenditure and reduces weight in diabetic db/db mice.[26] The two studies investigating the association between the 66Val/Met polymorphism (Rs6265) in the *BDNF* gene and antipsychotic-induced weight gain found conflicting results. In the study by Lane et al.,[12] the Met/Met genotype was associated with less weight gain (-0.81 kg; p=0.02), whereas in the study of Zhang et al.[26] the Met-allele was associated with greater weight gain (p=0.009).

Dopaminergic system

Dopaminergic mechanisms are believed to be involved in modulating rewarding properties of food intake. A study in mice has demonstrated that dopamine receptor agonists (D_1/D_2) normalize hyperphagia, body weight gain, hyperglycemia and hyperlipidemia in genetically obese, ob/ob mice.[27] A study in humans demonstrated a decreased availability of dopamine (D_2) receptors in obese individuals in proportion to their BMI.[28] The D_4 receptor has also been associated with obesity in humans.[29]

Relatively few studies focused on polymorphisms in the dopaminergic system as a potential cause for interindividual differences in antipsychotic-induced weight gain. Most studies focusing on the dopaminergic system (D_1-D_5 receptors) did not find a significant association between the polymorphisms studied and antipsychotic-induced weight gain. However, recently, three studies found associations between polymorphisms in the *DRD2* gene encoding the D2-receptor and antipsychotic-induced weight gain. The study by Hong et al. demonstrated that patients carrying the *DRD2* rs4436578-C/C genotype had a higher risk of >7% weight gain than patients with the C/T or T/T genotype (OR 3.36; 95%CI 1.62-7).[30] Furthermore, the study by Lencz et al. demonstrated that patients carrying the Del allele of the 141C Ins/Del polymorphism (rs1799732) in the *DRD2* gene gained more weight than carriers of the Ins/Ins genotype after 6 weeks of treatment with either olanzapine or risperidone ($p=0.024$).[31] Finally, in the study by Müller et al. an association was found for the *DRD2* rs1079598 marker and ≥7% weight gain in the total sample, and an association between the rs6277, rs1079598 and rs1800497 polymorphisms and weight gain in a sample stratified for ethnicity and antipsychotics.[32] Popp et al. also found an association between the 48-bp variable number of tandem repeats (VNTR) polymorphism in exon 1 of the *DRD4* gene, encoding the D_4-receptor, and antipsychotic-induced weight gain.[33] Patients with < 7 repeats experienced less weight gain during antipsychotic treatment than patients with > 7 repeats (+0.38 kg/m^2 vs +0.89 kg/m^2 respectively; $p = 0.003$). This association was particularly strong in men. Rietschel et al. could not confirm this association between polymorphisms in the *DRD4* gene and clozapine-induced weight gain.[34] However, the study by Rietschel et al. was not primarily designed for the association between these polymorphisms and clozapine-induced weight gain. Therefore, the study might have been underpowered to find a significant association.

G-protein ß3 subunit (GNB3)

The mechanism by which G-protein ß3 subunit (*GNB3*) influences weight gain is not entirely clear, but the C825T polymorphism (rs5443) in the *GNB3* gene has been associated with an increased BMI and weight gain during pregnancy and was a good predictive marker for weight reduction caused by sibutramine.[35] Five studies focussed on the association between the C825T polymorphism in the *GNB3* gene and antipsychotic-induced weight gain. The studies by Wang et al. and Ujike et al. found a significant association between the T allele (Ujike et al.; $p=0.043$) or T/T genotype (Wang et al.; $p=0.003$) and increased weight gain in patients treated with olanzapine and clozapine, respectively.[25,35] The study by Bishop et al. demonstrated a similar trend; however, the studies by Tsai et al. and Park et al. did not confirm this association.[36-38] A meta-analysis by Souza et al. also demonstrated a trend for the T allele being associated with weight gain ($p=0.072$); however, this trend was lost when the more appropriate random-effects model was used.[39]

Histaminergic system

Antagonism for the histamine H_1 receptor is known to increase feeding behaviour and weight gain.[19] It has also been noted by Wirshing et al. that antipsychotics with the maximum weight gain liabilities (ie, clozapine and olanzapine) had the greatest affinities for the histamine H_1 receptor, while those with the least amount of weight gain (ie, haloperidol and sertindole) have the weakest affinity.[40] The histamine H_2 receptors may also be involved in weight-regulation, but there is less evidence to support this assumption. Despite studies linking the histaminergic system to antipsychotic-induced weight gain (e.g. Matsui et al.[41]), surprisingly few studies (n=3) investigated the association between polymorphisms in the genes encoding histamine receptors and antipsychotic-induced weight gain. None of the studies found a significant association between polymorphisms in the *HRH1*, *HRH2* and *HRH3*- genes and antipsychotic-induced weight gain. Definite conclusions are not possible however, because the studies by Basile et al., Hong et al. and Ujike et al. had limited power to detect differences between genotypes owing to small genotype groups.[19,25,42]

Serotonergic system

There is strong evidence for a role of the serotonergic (5-HT) system in regulating feeding behaviour. It has been demonstrated that agonists for the 5-HT_{1A} receptors cause hyperphagia, and agonists for the $5HT_{2A/2C}$ receptors cause hypophagia.[19] In particular, the $5HT_{2C}$ receptor encoded by the *HTR2C* gene has been studied extensively, as *HTR2C* knockout mice become hyperphagic and $5HT_{2C}$ agonists reduce appetite in humans resulting in weight loss.[43,44] Therefore, it is not surprising that most genetic studies on antipsychotic-induced weight gain focussed on polymorphisms in the serotonergic system. A total of 18 studies focused on the *HTR2C* 759C/T polymorphism (rs3813929). In 10 of these studies, the variant T allele was associated with less antipsychotic-induced weight gain,[12,45-53] whereas in 6 other studies no association was found.[13,25,54-57] Two studies even found an association in the opposite direction.[58,59] A meta-analysis by De Luca et al. confirmed the association between the T allele and less antipsychotic-induced weight gain in a fixed-effect model, although significance of the association was lost owing to heterogeneity of the studies if the more appropriate random-effects model was chosen for analysis (OR 2.29; 95%CI 0.98-5.36).[60] Recently, the meta-analysis by De Luca et al. was updated by Sicard et al. who confirmed an increased risk for antipsychotic-induced weight gain in patients carrying the 759C allele (OR 2.42; 95%CI 1.15-5.09) in all samples; and OR 3.20; 95%CI 1.4-7.28) in European samples).[61] Apart from the 759 C/T polymorphism, the study by Sicard et al. also investigated the rs498207, 697GC and Ser23Cys polymorphisms, and demonstrated a significant over-representation of the 759C-697G-Cys23 haplotype in patients with weight gain (OR 1.93; 95%CI 1.04-3.56, p=0.0015). A complicating factor when comparing the results of both meta-analyses is the fact that De Luca et al. presented an odds ratio for carriership of the 759T allele, whereas Sicard et al. presented an odds ratio for carriership of the 759C allele. This makes the interpretation difficult for female patients carrying

the 759 CT genotype, since they would be protected against weight gain according to the study by De Luca et al., but more vulnerable to weight gain according to the study by Sicard et al. Since most studies demonstrated a protective effect of carriership of the 759T allele however, it is likely that these female patients are protected against antipsychotic-induced weight gain.

The disease state of the schizophrenic patients can be an important factor in the studies investigating antipsychotic-induced weight gain, since all studies investigating the 759 C/T polymorphism in a first-episode schizophrenic population found a significant result.[45,47,48,50] The genetic association with antipsychotic-induced weight gain is probably most profound in first-treatment populations because there is no interference with antipsychotic-induced weight gain of previous treatments. Furthermore, the association seems to be particularly strong in male patients, which is not surprising since the *HTR2C* gene is X-linked. Some other polymorphisms located in the *HTR2C* gene, such as the Cys23Ser (rs6318), 697 G/C (rs518147), 997 G/A (rs3813928) and 1165 A/G (rs498207) polymorphisms, have only once been associated with antipsychotic-induced weight gain and require replication studies.[25,51,52]

Besides *HTR2C* polymorphisms, the 102 T/C polymorphism in the *HTR2A* gene encoding the $5HT_{2A}$-receptor and the 267 C/T polymorphism in the *HTR6* gene encoding the $5HT_{6}$-receptor, were also investigated for associations with antipsychotic-induced weight gain. Lane et al. and Ujike et al. found an association between the 102T allele in the *HTR2A* gene and weight gain.[12,25] Furthermore, Lane et al. found an association between the 267T/C and 267C/C genotype of the *HTR6* gene and weight gain as well.[12]

Leptin

Leptin is a 16-kDa protein hormone that plays an important role in regulating body weight. Leptin is stored in adipocytes and serum leptin concentration is positively correlated with the amount of adipose tissue. After being secreted by the adipocytes, leptin affects the hypothalamus, resulting in a reduced food intake and fat storage and promoting energy expenditure.[62] The number of studies investigating the association between polymorphisms in the leptin system and antipsychotic-induced weight gain is increasing. To date, 8 studies investigating this association have been published. Most studies focused on the 2548A/G polymorphism (rs7799039) in the *LEP* gene encoding leptin; however, one study also investigated the rs1137101 polymorphism in the *LEPR* gene encoding the leptin receptor.[63]

The G allele of this *LEP* 2548A/G polymorphism appears to be linked to an increased susceptibility to antipsychotic-induced weight gain as is further supported by the results of the studies by Templeman et al., Kang et al., and Zhang et al.[48,62,64] The studies by Ellingrod et al., Opgen-Rhein et al. and Perez-Iglesias et al. could not confirm this association, although the study by Ellingrod et al. demonstrated a similar trend.[52,63,65] Remarkably, the study by Calarge et al. found a protective effect of the GG genotype for weight gain and obesity in children and adolescents.[66] Furthermore, the study by Yevtushenko et al. displayed a strong interaction

between the *LEP* 2548 A/G and *HTR2C* 759 C/T genotypes in their effects on antipsychotic-induced weight gain.[67] This demonstrates that haplotype analysis of multiple polymorphisms could contribute to the understanding of the mechanisms involved. Srivastava et al. focussed on other polymorphisms in the *LEP* gene and found the rs4731426 C/G polymorphism to be moderately associated with median weight gain and significantly associated with extreme weight gain.[68] The rs1137101 polymorphism in the *LEPR* gene was not found to be associated with antipsychotic-induced weight gain.[63]

Synaptosomal associated protein of 25 kDa

Synaptosomal associated protein of 25 kDa (SNAP25) interacts with calcium and potassium channels in pancreatic islet cells and thereby mediates insulin release which affects energy or body weight homeostasis.[69] Three studies investigated whether polymorphisms in the *SNAP25* gene were associated with antipsychotic-induced weight gain; the results of these studies are conflicting. Ujike et al. did not find an association between the 3 studied polymorphisms and weight gain,[25] Müller et al. found an association between the MnlI (rs3746544) and TaiI (rs8636) polymorphisms and weight gain, which was lost after correcting for co-variables,[69] and Musil et al. found a significant association between the DdeI polymorphism (rs1051312) and weight gain.[70]

Miscellaneous

Other genes studied for a possible association with antipsychotic-induced weight gain have predominantly been single-target studies, with most being studied in hypothesis-generating studies investigating a large number of SNPs [e.g.[19,25,68,71]].

Bozina et al. found a significant association between the long/short polymorphism (rs4795541) in the promoter region of the *SLC6A4* gene, encoding the serotonin transporter, and antipsychotic-induced weight gain.[72] Patients with a BMI <27.3 kg/m^2 carrying the short allele experienced more weight gain than patients with the L/L genotype (1.34±1.39 vs 0.32±1.721 kg/m^2, $p=0.021$), after correction for multiple testing. Variation in the activity of the serotonin transporter could have an impact on weight gain since it modifies the extent to which the postsynaptic serotonergic receptors (e.g. $5HT_{2C}$) are activated by the serotonin released in the synaptic cleft.

Le Hellard et al. found significant associations between 3 polymorphisms within the Insulin-induced gene-2 (*INSIG2*) and clozapine induced weight gain;[73] however, these associations could not be replicated in two other studies.[52,74] *INSIG2* is present in the endoplasmatic reticulum and blocks the processing of sterol regulatory element binding proteins (SREBPs) by binding to SREBP cleavage-activating protein (SCAP), and thus prevents SCAP from escorting SREBPs to the Golgi. This impact on lipogenesis by *INSIG2* might influence antipsychotic-induced weight gain.

The study by Herken et al.[75] demonstrated an association between carriership of the 12Ala allele in the peroxisome proliferator-activated receptor y gene (*PPARG*) and increased weight gain; however, this association could not be confirmed in the study by Ujike et al..[25] The PPAR-G2 receptor is a transcription factor belonging to the same family of nuclear receptors as steroid and thyroid hormone receptors. PPAR-G2 is specific to adipose tissue, where it plays a key role in regulating adipogenic differentiation.

The study by Wang et al. demonstrated that patients carrying the -308A allele in the gene encoding tumor necrosis factor α (*TNFA*) experienced less weight gain than carriers of the -308 G/G genotype (coefficient: -1.24; 95%CI -2.17 – -0.32; p=0.0084).[76] TNFα might play a role in antipsychotic-induced weight gain because it affects adipogenesis, insulin resistance and lipid metabolism, and has been suggested to play an important role in various metabolic and appetite behaviours.

The study by Tiwari et al. demonstrated that patients carrying the rs806378 T allele in the *CNB1* gene encoding the cannabinoid receptor I experienced more weight weight gain than carriers of the rs806378 C/C genotype (4.33 ± 3.89 kg vs 2.21± 4.51 kg; p=0.022).[77] The study by Monteleone et al. also investigated the endocannabinoid system, and found a higher prevalence of carriership of the *FAAH* 385A allele (rs324420) in patient who gained >7% weight (OR 3.11; 95%CI 1.42-6.77).[78] This association was not found in the study by Ujike et al. however.[25] The endocannabinoid system is involved in the modulation of energy balance by regulating satiety and levels and/or action of other orexigenic and anorectic mediators in the hypothalamus. Furthermore, the endocannabinoids are a crucial component of the leptin system and are therefore likely to be involved in antipsychotic-induced weight gain.

The study by van Winkel et al. highlighted that the 1298C allele of the A1298C polymorphism in the 5,10-methylenetetraydrofolate reductase gene (*MTHFR*) predicted post-baseline increases in weight (p=0.006).[79] *MTHFR* is involved in the pathway for the donation of methyl groups and therefore the regulation of gene transcription through DNA methylation; however, this methylation is also relevant for hormones, neurotransmitters, receptors and signal transducers. *MTHFR* might therefore have some impact on weight gain.

Finally, the study by Jassim et al. investigated the association between 76 tagSNPs and antipsychotic-induced weight gain in the first treatment period, during clozapine therapy, and during the whole duration of therapy.[71] In this study, several associations were found between antipsychotic-induced weight gain on the different evaluation moments and polymorphisms in the genes encoding adiponectin (*ADIPOQ*) and protein kinase (*PRKA*) (p-values 0.0008 – 0.011); however, no correction for multiple testing was performed, and it is doubtful that any of these associations would survive this correction. Adiponectin and protein kinases might both be involved in antipsychotic-induced weight gain. Adiponectin has insulin-sensitizing abilities and stimulates fatty acid oxidation, increases glucose uptake and reduces the activity of factors

involved in gluconeogenesis. The protein kinases regulate fatty acid biosynthesis and stimulate orexigenic processes leading to increased food intake and weight gain. No other significant associations between genetic determinants and antipsychotic-induced weight gain have been found.

Discussion

The 57 studies identified by the literature search investigated various genetic targets for an association with antipsychotic-induced weight gain; however, the results of these studies were often inconsistent, possibly owing to differences in methodology used and power issues. Polymorphisms in the genes encoding the α_{2a}-receptor, $5HT_{2c}$-receptor, G-protein ß3 subunit and leptin have been associated with antipsychotic-induced weight gain most consistently. The strength of this review is its focus on weight gain, excluding studies regarding other phenotypes such as obesity and the metabolic syndrome, and its focus on differences in methodology of the identified studies as an explanation for variation in results obtained. Compared with the reviews from 2006, this review lists more potentially relevant genetic determinants for antipsychotic-induced weight gain, and adds more evidence for polymorphisms investigated prior to 2007. Unfortunately, the majority of the results from the pharmacogenetic studies are contradictory or negative, as can be seen in Table 3, which makes the relevance for most genetic determinants uncertain.

Pharmacogenetic testing will only be valuable for daily clinical practice when it is possible to predict accurately to what drug or which dosage regimen a patient will respond in genotyped patients compared with non-genotyped patients. In this discussion we will focus on criteria that influence the question whether the presented results are applicable in daily clinical practice. To evaluate whether a genetic test is usable in clinical practice the ACCE model can be used.[80] The ACCE model consists of four components; Analytical validity, Clinical validity, Clinical utility and Ethical, legal and social issues, of which the fourth component will not be discussed in this article.

Analytical validity; prediction of genotype

The analytical validity defines a test's ability to accurately and reliably measure the genotype of interest. It is hard to assess whether the analytical tests used for the genotyping procedures in the studies from Table 3 are valid, as only a few studies mentioned whether the genotyping procedure was carried out in duplo or what the call rates and error rates for their genotyping method were. This is not in accordance with the recent guidelines concerning 'Strengthening the reporting of genetic associations' (STREGA), which state that these aspects of laboratory testing should be reported.[81]

The resultant genotype of a genetic test is also dependent on which mutations are screened for in the target. Looking at CYP2D6 as an example, there are a multitude of mutations leading

to a non- or less functional allele. Deciding only to screen for the most prevalent *3 and *4 mutations will result in a misclassification of the genotype and corresponding phenotype in a small percentage of the population. Using our own laboratory data, which includes analysis for the *5,*6, *10, *17 and *41 variant alleles, we would "miss" 10 percent of the intermediate metabolisers (30% of total) and 2% of the poor metabolisers (40% of total) in our population.

Clinical validity; prediction of phenotype

Besides analytical validity, the clinical validity is also an important component of the ACCE model. Clinical validity defines a test's ability to predict the associated phenotype. This prediction of a phenotype, in this case antipsychotic-induced weight gain, is the main subject and goal of the current studies, but proves to be difficult. At present, only polymorphisms in the genes encoding the α_{2a}-receptor, $5HT_{2c}$-receptor, G-protein ß3 subunit and leptin have repeatedly been associated with antipsychotic-induced weight gain. The other investigated candidate genes and their polymorphisms, as specified in Table 3, have not, or only once, been associated with antipsychotic-induced weight gain, or the results from studies investigating these genes and polymorphisms were contradictory. However, before these candidate genes can be dismissed for an association with antipsychotic-induced weight gain, apart from the need for replication studies, it will be necessary to study tagSNPs across each of these genes to cover variations within the genome. With regard to the lack of replication, a few possible explanations can be given.

First of all, most studies have been conducted in relatively small populations with an increased risk of a type I error with false positive findings. Second, the ethnicity of the patient populations varies between studies, and some study populations even consisted of mixed ethnic groups. Variation in ethnicity can result in different associations due to different linkage profiles with other genes, different gene-environment interactions and different frequencies of variant alleles. Rare alleles are often regarded as the variant alleles; however, these rare alleles in one ethnic group can be the predominant alleles in another ethnic group. This can affect study results. Moreover, in situations where multiple loci act in concert to cause a condition, as is likely the case in antipsychotic-induced weight gain, this can even result in flip-flop phenomena. In a flip-flop association SNPs can appear to be protective in one ethnic group, but causative in another ethnic group owing to a lack of consideration of other weight gain influencing genetic loci or environmental factors correlated with the target susceptibility locus. The results from the studies investigating the *ADRA2A* 1291 C/G polymorphism could possibly serve as an example for this phenomenon, since the 1291G allele was a risk allele in Asians but protective in Caucasians, although a false positive finding in one or several of those studies could also be the reason for this flip-flop. Third, the studies used different antipsychotic drugs. Several studies even included multiple antipsychotic drugs or allowed combinations of antipsychotic drugs. Since the (atypical) antipsychotic drugs have different pharmacologic profiles, there is bound

to be a different modification of the gene-gene interactions that play a part in the etiology of weight gain resulting in ambiguous findings. Fourth, in some studies the follow-up duration was short (4-6 weeks). It is possible that differences in weight gain between genotype groups are not maximal after 4-6 weeks, since the period in which most antipsychotic-induced weight gain occures endures for 3 months. This could partly explain non-significant results in studies using a follow-up duration of 4-6 weeks. This is especially relevant for the studies that choose a cutoff point for their weight gain (e.g., ≥ 7 or 10% increase in weight), because reaching these clinically relevant cutoff points is likely to take a longer follow-up duration than 4-6 weeks. Moreover, choosing different endpoints for weight gain also partly explains the variation between study results, because the proportion of the population reaching clinically significant cutoff points is smaller than the proportion of the population with any increase in BMI or body weight. This makes it more difficult to find significant results between genotype groups when using cutoff points, especially when the impact of the genetic marker on weight gain is small. Fifth, there is a lot of variation in methodology like data analysis and measurement and adjustment for co-variables. Co-variables corrected for most often are age, baseline weight or BMI, gender and dose, in respectively 47, 39, 44 and 29 of the 57 studies as shown in Table 3. Hardy-Weinberg Equilibrium was tested in around 60% of the studies and duration of treatment was also taken into account in 21 studies. Multiple testing was corrected for in 13 studies, but would have been applicable in 27 studies in which multiple SNPs were tested. Correcting for multiple testing reduces the chance of finding statistically significant results, thereby increasing the possibility that a relevant association is missed (type II error) but reduces false positive results (type I error). Co-variables relevant for antipsychotic-induced weight gain which were seldom included in the data analysis are smoking behaviour, polypharmacy, weight-influencing drugs and physical activity. It is clear that these methodological issues can influence the results of a study, thereby possibly explaining the contradictory results often found. Therefore, further replication of significant association studies are essential in order to confirm associations found.

A final important difference in study design of the presented studies that has to be mentioned separately is the fact that most studies failed to investigate the association between genetic determinants and antipsychotic-induced weight gain in an antipsychotic-naive first-episode schizophrenic population. The studies only including first episode schizophrenics all found an association between their chosen genetic determinant and antipsychotic-induced weight gain,[10,31,45-48,50,64] except for Zhang et al.[63] who investigated the TaqI A polymorphism (rs1800497) in the *DRD2* gene and Perez-Iglesias et al.[82] who investigated several polymorphisms in several genes. It can be expected that associations between genetic determinants and antipsychotic-induced weight gain are more significant in antipsychotic-naive patients compared with patients that were treated with several other antipsychotics or one antipsychotic for a longer period of time. It is possible that these patients already gained weight as a result of earlier treatments and

therefore biased results obtained. Especially in meta-analyses we believe authors should stratify their analyses for drug-naive patients and patients that are not drug-naive.

Some studies in Table 3 mentioned which part of the total variability in weight gain could be explained by the studied polymorphism. Studies by Reynolds et al.46 and Templeman et al.48 reported that the *HTR2C* 759 C/T genotype (rs3813929) explained around 20% of the total variability in weight gain in the overall population, with 32% in men and none in women. The study by Wang et al. reported that the *GNB3* C825T polymorphism (rs5443) explained around 7% of the weight gain, and the study by Zhang et al. reported that the *LEP* 2548 A/G (rs7799039) and *BDNF* 66-Val/Met (Rs6265) polymorphisms explained 16% and 14% of the variability in weight gain in men, respectively, but not women.[26,35,64] These differences between men and women appears odd, but can be explained by the fact that more men were included in the analysis and some polymorphisms (e.g. *HTR2C*) were X-linked. Given the complexity of the weight-regulating system, a predictive value of 7-32% for antipsychotic-induced weight gain after testing for one SNP seems surprisingly high and has to be replicated in other studies. The predictive value of genetic testing for antipsychotic-induced weight could possibly increase further when the results of multiple genetic determinants are taken into account. At this moment there are only hypothesis-driven studies based on candidate genes. It is unclear whether the studied genetic determinants in Table 3 fully reflect the pharmacodynamics of antipsychotics and resultant weight gain. To investigate the presence of other relevant genetic determinants, a genome-wide-association study (GWAS) is warranted. Such a GWAS would serve as a hypothesis-generating study, possibly resulting in the discovery of new genetic determinants for antipsychotic-induced weight gain. Resultant hypothesis-driven studies investigating the association of these determinants in prospective studies in antipsychotic-naive patients could further uncover the origin of antipsychotic-induced weight gain. Together with non-genetic determinants such as social economic status, disease state, treatment response, smoking behaviour, drug abuse and environmental factors, a clinical decision support-algorithm might be suggested with a predictive value of >50%. At present however, the clinical validity of genetic tests for predicting weight gain is low.

Clinical utility; added value of genotyping

Clinical utility defines the risks and benefits associated with a test's introduction into practice. Since clinical validity is lacking, clinical utility is unknown. It can be expected that knowing a patient's risk profile for weight gain could influence prescribing patterns of antipsychotics, thereby possibly improving compliance and treatment success. As mentioned above, it might be possible in the future to generate an algorithm for antipsychotic-induced weight gain based on a combination of genetic and non-genetic determinants. Studies would be warranted to test these algorithms in prospective designs in order to investigate whether genetic testing before

the start of pharmacotherapy can increase the treatment success compared with an algorithm without genetic testing. At this moment however, no such algorithms exist.

Conclusion

To date, the criteria from the ACCE model have not been met. It would therefore be premature to use genotypes from targets in Table 3 to predict a patient's chance of antipsychotic-induced weight gain in daily clinical practice. More replication studies are needed for the majority of the targets in Table 3, preferably in large antipsychotic-naive populations in order to elucidate which genetic targets are primarily associated with antipsychotic-induced weight gain. These targets should be combined in large studies, compliant with the STREGA guidelines, and investigate gene-gene interactions that might be present to increase the predictive value of genetic testing and form a solid basis for future studies investigating the clinical utility of these tests.

Future perspective

With the current state-of-the-art in genotyping equipment and ready-to-use genotyping kits, genotyping has become cheaper, quicker and more accessible to researchers over the years, which is reflected in the rapidly increasing number of genetic studies. Achieving an acceptable analytical validity should therefore not really be an issue for most research laboratories anymore. However, it will probably still take a few years before we are able to accurately predict a patients risk for antipsychotic-induced weight gain and thereby achieve acceptable clinical validity. In order to take this next step, we need studies combining the genotypes of relevant polymorphisms. Given the current evidence, this would mean combining the *HTR2C* 759 C/T genotype, *ADRA2A* 1291 C/G genotype, *LEP* 2548 A/G genotype, and *GNB3* C825T genotype, since these polymorphisms have most consistently been associated with antipsychotic-induced weight gain and are therefore most likely to contribute to increasing the predictive value of a genetic test. Apart from being compliant with the STREGA-guidelines, using a specific antipsychotic drug, and taking other relevant co-variables into account, a study combining these 4 polymorphisms would need a large number of patients because of the multiple genotype groups.

In order to make such a study possible in the next 5 years, several research centres should combine their efforts in their inclusion of patients since it would be very hard, if not impossible, for a single research centre to include enough patients in such a time period. A quicker alternative would be to combine study populations from studies in Table 3, although it would still take some effort to collect a comparable dataset from the different study populations. Therefore, a meta-analytic approach could be preferable. Ideally, this study would result in a genotype combination that could explain a high percentage of the variation caused in weight gain. Combined with other relevant variables such as gender, age, duration of illness and so on,

this could result in an algorithm with a high predictive value that can subsequently be tested in prospective trials to assess clinical utility compared with standard care.

By comparison, studies regarding warfarin dosing have reached the stage at which the clinical utility of pharmacogenetics is being tested by comparing genotype-guided dosing with standard care.[83] The first studies investigating the impact of genetic variation on warfarin dose date back to 1997,[84] which means that it took more than 10 years to reach this stage. Since both the pharmacologic profile of antipsychotics and the weight-regulating system are more complicated than the pharmacologic profile of anticoagulants and the coagulation system it can be expected that genotype-guided therapy to prevent weight-gain will take considerably more time.

Given the increasing frequency of studies reporting associations between genetic determinants and antipsychotic-induced weight gain, and therefore also the increasing possibility to carry out meta-analyses to confirm potential associations, it is possible that in the next 5 to 10 years, steps can be taken towards developing an algorithm to predict antipsychotic-induced weight gain.

References

1. Hugenholtz GW, Heerdink ER, Meijer WE, Stolker JJ, Egberts ACG, Nolen WA. Reasons for switching between antipsychotics in daily clinical practice. Pharmacotherapy 2005;38:122-124.
2. Kurtzthaler I, Fleischhacker WW. The clinical implications of weight gain in schizophrenia. J Clin Psychiatry 2001;62(S):32-37.
3. Zipursky RB, Gu H, Green AI, et al. Course and predictors of weight gain in people with first-episode psychosis treated with olanzapine or haloperidol. Br J Psychiatry 2005;187:537-543.
4. Allison DB, Mentore JL, Heo M, et al. Antipsychotic-induced weight gain: a comprehensive research synthesis. Am J Psychiatry 1999;156(11):1686-1696.
5. Matsui-Sakata A, Ohtani H, Sawada Y. Receptor occupancy-based analysis of the contributions of various receptors to antipsychotics-induced weight gain and diabetes mellitus. Drug Metab Pharmacokinetics 2005;20(5):368-378.
6. Roth BL, Sheffler DJ, Kroeze WK. Magic shotguns versus magic bullets: selectively non-selective drugs for mood disorders and schizophrenia. Nature reviews 2004;3:353-359.
7. Sussman N. The implications of weight changes with antipsychotic treatment. J Clin Psychopharmacol 2003;21:s21-s26.
8. Müller DJ, Kennedy JL. Genetics of antipsychotic treatment emergent weight gain in schizophrenia. Pharmacogenomics 206;7:863-887.
9. Chagnon YC. Susceptibility genes for the side effect of antipsychotics on body weight and obesity. Current Drug Targets 2006;7:1681-1695.
10. Ellingrod VL, Miller D, Schultz SK, Wehring H, Arndt S. CYP2D6 polymorphisms and atypical antipsychotic weight gain. Psychiatric Genetics 2002;12:55-58.
11. Urichuk L, Prior TV, Dursun S, Baker G. Metabolism of atypical antipsychotics: involvement of cytochrome P450 enzymes and relevance for drug-drug interactions. Current Drug Metabolism 2008;9:410-418.

12. Lane HY. Liu YC, Huang CL. Risperidone-related weight gain. Genetic and nongenetic predictors. J Clin Psychopharmacol 2006;26:128-134.
13. Kuzman MR, Medved V, Bozina N, Hotujac L, Sain I, Bilusic H. The influence of 5-HT2c and MDR1 genetic polymorphisms on antipsychotic-induced weight gain in female schizophrenic patients. Psychiatry Research 2008;160:308-315.
14. Lin YC, Ellingrod VL, Bishop JR, Miller DD. The relationship between P-glycoprotein (PGP) polymorphisms and response to olanzapine treatment in schizophrenia. Ther Drug Monit 2006;28:668-672.
15. Perry PJ, Argo TR, Carnahan RM, et al. The association of weight gain and olanzapine plasma concentrations. J Clin Psychopharmacol 2005;25:250-254.
16. Lane HY, Chang YC, Cheng YC, et al. Effects of patient demographics, risperidone dosage, and clinical outcome on body weight in acutely exacerbated schizophrenia. J Clin Psychiatry 2003;64:316-320.
17. Dunayevich E, Sethuraman G, Wei H. Olanzapine dose and weight gain. Biol Psychiatry 2005;57:107S.
18. Lane HY, Liu YC, Huang CL, et al. Risperidone-related weight gain, genetic and nongenetic predictors. J Clin Psychopharmacol 2006;26:128-134.
19. Basile VS, Masellis M, McIntyre RS, Meltzer HY, Lieberman JA, Kennedy JL. Genetic dissection of atypical antipsychotic-induced weight gain: novel preliminary data on the pharmacogenetic puzzle. J Clin Psychiatry 2001;62(S23):45-66.
20. Wang YC, Bai YM, Chen JY, Lin CC, Lai IC, Liou YJ. Polymorphism of the adrenergic receptor alpha 2a-1291C>G genetic variation and clozapine-induced weight gain. J Neural Transm 2005;112:1463-1468.
21. Park YM, Chung YC, Lee SH, Lee KJ, Kim H, Byun YC, et al. Weight gain associated with the alpha2a-adrenergic receptor 1291C/G polymorphism and olanzapine treatment. Am J Med Gen Part B (Neuropsychiatric genetics) 2006;141B:394-397.
22. Sickert L, Müller DJ, Tiwari AK, Shaikh S, Zai C, De Souza R. Association of the alpha 2A adrenergic receptor -1291 C/G polymorphism and antipsychotic-induced weight gain in European-Americans. Pharmacogenomics 2009;10:1169-1176.
23. Ferrer-Lorente R, Cabot C, Fernandez-Lopez JA, Alemany M. Comparative effects of oleoyl-estrone and a specific beta3-adrenergic agonist (CL316, 243) on the expression of genes involved in energy metabolism of rat white adipose tissue. Nutrition & Metabolism 2010;7:15.
24. Nisoli E, Tonello C, Carruba M. Beta3-adrenergic receptors: really relevant targets for anti-obesity drugs? Curr Med Chem - Central Nervous System Agents 2003;3(3):257-273.
25. Ujike H, Nomura A, Morita Y, Morio A, Okahisa Y, Kotaka T, et al. Multiple genetic factors in olanzapine-induced weight gain in schizophrenia patients: a cohort study. J Clin Psychiatry 2008;69:1416-1422.
26. Zhang XY, Zhou DF, Wu GY, Cao LY, Tan YL, Haile CN, et al. BDNF levels and genotype are associated with antipsychotic-induced weight gain in patients with chronic schizophrenia. Neuropsychopharmacology 2008;33:2200-2205.
27. Bina KG. Cincotta AH. Dopaminergic agonists normalise elevated hypothalamic neuropeptide Y and corticotropinreleasing hormone, body weight gain, and hyperglycemia in ob/ob mice. Neuroendocrinology 2000;71:68-78.
28. Wang GJ, Volkow ND, Logan J, Pappas NR, Wong CT, Zhu W, et al.Brain dopamine and obesity. Lancet 2001;357:354-357.

29. Nöthen MM, Cichon S, Hemmer S,Hebebrand J, Remschmidt H, Lehmkuhl G, et al. Human dopamine D4 receptor gene: frequent occurrence of a null allele and observation of homozygosity. Hum Mol Genet 1994;3:2207-2212.
30. Hong CJ, Liou YJ, Bai YM, Chen TT, Wang YC, Tsai SJ. Dopamine receptor D2 gene is associated with weight gain in schizophrenic patients under long-term atypical antipsychotic treatment. Pharmacogenetics and Genomics 2010;20:359-366.
31. Lencz T, Robinson DG, Napolitano, Sevy S, Kane JM, Goldman D, et al. DRD2 promoter region variation predicts antipsychotic-induced weight gain in first episode schizophrenia. Pharmacogenetics and genomics 2010;20:569-572.
32. Müller DJ, Zai CC, Sicard M, Remington E, Souza P, Tiwari AK, et al. Systematic analysis of dopamine receptor genes (DRD1-DRD5) in antipsychotic-induced weight gain. The Pharmacogenomics J 2010;1-9.
33. Popp J, Leucht S, Heres S, Steimer W. DRD4 48 bp VNTR but not 5-HT2c Cys23Ser receptor polymorphism is related to antipsychotic-induced weight gain. The Pharmacogenomics J 2008;1-7.
34. Rietschel M, Naber D, Oberländer H, Holzbach R, Fimmers R, Eggermann K, et al. Efficacy and side-effects of clozapine:testing for association with allelic variation in the dopamine D4 receptor gene. Neuropsychopharmacology 1996;15:491-496.
35. Wang YC, Bai YM, Chen JY, Lin CC, Lai IC, Liou YJ: C825T polymorphism in the human G protein beta3 subunit gene is associated with long-term clozapine treatment-induced body weight change in the chinese population. Pharmacogenetics and genomics 2005;15:743-748.
36. Tsai SJ, Yu YWY, Lin CH, Wang YC, Chen JY, Hong CJ. Association study of adrenergic beta3 receptor (Trp64Arg) and G-protein beta3 subunit gene (C825T) polymorphisms and weight change during clozapine treatment. Neuropsychobiology 2004;50:37-40.
37. Bishop JR, Ellingrod VL, Moline J, Miller Del. Pilot study of the G-protein beta3 subunit gene (c825t) polymorphism and clinical response to olanzapine or olanzapine-related weight gain in persons with schizophrenia. Med Sci Monit 2006;12(2):BR47-50.
38. Park YM, Chung YC, Lee SH, Lee KJ, Kim H, Choi JE, et al. G-protein ß3 subunit gene 825 C/T polymorphism is not associated with olanzapine-induced weight gain in Korean schizophrenic patients. Psychiatry Invest 2009;6:39-43.
39. Souza RP, De Luca V, Muscettola G, Rosa DV, de Bartolomeis A, Romano Silva M, et al. Association of antipsychotic-induced weight gain and body mass index with GNB3 gene: a meta-analysis. Progress in Neuro-Psychopharmacol & Biol Psych 2008;32:1848-1853.
40. Wirshing DA, Wirshing WC, Kysar L, Berisford MA, Goldstein D, Pashdag J, et al. Novel antipsychotics: comparison of weight gain liabilities. J Clin Psychiatry 1999;60:358-363.
41. Matsui-Sakata A, Ohtani H, Sawada Y. Receptor occupancy-based analysis of the contributions of various receptors to antipsychotics-induced weight gain and diabetes mellitus. Drug Metab Pharmacokinet 2005;20(5):368-378.
42. Hong CJ, Lin CH, Yu YWY, Chang SC, Wang SY, Tsai SJ. Genetic variant of the histamine-1 receptor (glu349asp) and body weight change during clozapine treatment. Psychiatric genetics 2002;12:169-171.
43. Tecott L, Sun L, Akana SF, Strack AM, Lowenstein DH, Dallman MF, et al. Eating disorder and epilepsy in mice lacking 5HT2C-serotonin receptors. Nature 1995;374:542-546.

44. Gallant DM, Bishop MP, Bishop G, O'Meallie L. U-22,394A: a controlled evaluation in chronic schizophrenic patiënts. Curr Ther Res Clin Exp 1976;9:579-581.
45. Reynolds GP, Zhang ZJ, Zhang XB. Association of antipsychotic drug-induced weight gain with a 5-HT2c receptor gene polymorphism. Lancet 2002;359:2086-2087 (letter).
46. Reynolds GP, Zhang ZJ, Zhang XB. Polymorphism of the promoter region of the serotonin 5HT2c receptor gene and clozapine-induced weight gain. Am J Psychiatry 2003;160:677-679.
47. Ellingrod VL, Perry PJ, Ringold JC, Lund BC, Bever-Stille K, Fleming F, et al. Weight gain associated with the 759C/T polymorphism of the 5HT2c receptor and olanzapine. Am J Med Gen Part B (Neuropsychiatric Genetics) 2005;134B:76-78.
48. Templeman LA, Reynolds GP, Arranz B, San L. Polymorphisms of the 5HT2c receptor and leptin genes are associated with antipsychotic drug-induced weight gain in Caucasian subjects with a first-episode psychosis. Pharmacogenetics and Genomics 2005;15:195-200.
49. Miller DD, Ellingrod VL, Holman TL, Buckley PF, Arndt S. Clozapine-induced weight gain associated with the 5HT2c receptor -759C/T polymorphism. Am J Med Gen Part B (Neuropsychiatric Genetics) 2005;133B:97-100.
50. Ryu S, Cho EY, Park T, Oh S, Jang WS, Kim SK, et al. 759 C/T polymorphism of 5-HT2C receptor gene and early phase weight gain associated with antipsychotic drug treatment. Progress in Neuro-Psychopharmacol & Biol Psychiatry 2007;31:673-677.
51. Godlewska BR, Olajossy-Hilkesberger L, Ciwoniuk M, Olajossy M, Marmurowska-Michalowska H, Limon J, et al. Olanzepine-induced weight gain is associated with the -759 C/T and -697 G/C polymorphisms of the HTR2C gene. The Pharmacogenomics J 2009;9:234-241.
52. Opgen-Rhein C, Brandl EJ, Müller DJ, Tiwari AK, Sander T, Dettling M. Association of HTR2C, but not LEP or INSIG2. genes with antipsychotic-induced weight gain in a German sample. Pharmacogenomics 2010;11:773-780.
53. Hoekstra PJ, Troost PW, Lahuis BE, Mulder H, Mulder EJ, Franke B, et al. Risperidone-induced weight gain in referred children with autism spectrum disorders is associated with a common polymorphism in the 5-hydroxytryptamine 2C receptor gene. J Child & Adolescent Psychopharmacol 2010;20:473-477.
54. Tsai SJ, Hong CJ, Yu YWY, Lin CH. -759C/T genetic variation of 5HT2c receptor and clozapine-induced weight gain. Lancet 2002;360(30):1790 (letter).
55. Theisen FM, Hinney A, Bromel T, Heinzel-Gutenbrunner M, Martin M, Krieg JC, et al. Lack of association between the -759C/T polymorphism of the 5HT2c receptor gene and clozapine-induced weight gain among German schizophrenic individuals. Psychiatric genetics 2004;14:139-142.
56. Park YM, Cho JH, Kang SG, Choi JE, Lee SH, Kim L, et al. Lack of association between the 759C/T polymorphism of the 5-HT2c receptor gene and olanzapine-induced weight gain among Korean schizophrenic patients. J Clin Pharmacy and Therapeutics 2008;33:55-60.
57. Thompson A, Lavedan C, Volpi S. Absence of weight gain association with the HTR2C- 759 C/T polymorphism in patients with schizophrenia treated with iloperidone. Psychiatry Research 2010;175:271-273.
58. Basile VS, Masellis M, de Luca V, Meltzer HY, Kennedy JL. -759C/T genetic variation of 5HT2c receptor and clozapine-induced weight gain. Lancet 2002;360(30):1791 (letter).

59. De Luca V, Müller DJ, Hwang R, Lieberman JA, Volavka J, Meltzer HY, et al. HTR2C haplotypes and antipsychotics-induced weight gain: X-linked multimarker analysis. Hum Psychopharmacol Clin Exp 2007;22:463-467.
60. De Luca V, Mueller DJ, de Bartolomeis A, Kennedy JL. Association of the HTR2C gene and antipsychotic-induced weight gain: a meta-analysis. Int J Neuropsychopharmacol 2007;10(5):697-704.
61. Sicard MN, Zai CC, Tiwari AK, Souza RP, Meltzer HY, Lieberman JA, et al. Polymorphisms of the HTR2C gene and antipsychotic-induced weight gain: an update and meta-analysis. Pharmacogenomics 2010;11:1561-1571.
62. Kang SG, Lee HJ, Park YM, Choi JE, Han C, Kim YK, et al. Possible association between the -2548A/G polymorphism of the leptin gene and olanzapine-induced weight gain. Progress in Neuro-Psychopharmacol & Biol Psych 2008;32:160-163.
63. Perez-Iglesias R, Mata I, Amado JA, Berja A, Garcia-Unzueta MT, Martinez Garcia O, et al. Effect of FTO, SH2B1, LEP, and LEPR polymorphisms on weight gain associated with antipsychotic treatment. J Clin Psychopharmacol 2010;30:661-666.
64. Zhang XY, Tan YL, Zhou DF, Haile CN, Cao LY, Xu Q, et al. Association of clozapine-induced weight gain with a polymorphism in the leptin promoter region in patients with chronic schizophrenia in a chinese population. J Clin Psychopharmacol 2007;27:246-251.
65. Ellingrod VL, Bishop JR, Moline J, Lin YC, Miller DD. Leptin and leptin receptor gene polymorphisms and increases in body mass index (BMI) from olanzapine treatment in persons with schizophrenia. Psychopharmacol Bulletin 2007;40(1):57-62.
66. Calarge CA, Ellingrod VL, Zimmerman B, Acion L, Sivitz WI, Schlechte JA. Leptin Gene -2548G/A variants predict risperidone-associated weight gain in children and adolescents. Psychiatric Genetics 2009;19:320-327.
67. Yevtushenko OO, Cooper SJ, O'Neill R, Doherty JK, Woodside JV, Reynolds GP. Influence of 5-HT2c receptor and leptin gene polymorphisms, smoking and drug treatment on metabolic disturbances in patients with schizophrenia. Br J of Psychiatry 2008;192:424-428.
68. Srivastava V, Deshpande SN, Nimgoankar VL, Lerer B, Thelma BK. Genetic correlates of olanzapine-induced weight gain in schizophrenia subjects from north India: role of metabolic pathway genes. Pharmacogenomics 2008;9(8):1055-1068.
69. Müller DJ, Klempan TA, de Luca V, Sicard T, Volavka J, Czobor P, et al. The SNAP-25 gene may be associated with clinical response and weight gain in antipsychotic treatment of schizophrenia. Neuroscience letters 2005;378:81-89.
70. Musil R, Spellmann I, Riedel M, Dehning S, Douhet A, Maino K, et al. SNAP-25 gene polymorphisms and weight gain in schizophrenic patients. J Psych Research 2008;42:963-970.
71. Jassim G, Ferno J, Theisen FM, Haberhausen M, Christoforou A, Havik B, et al. Association study of energy homeostasis genes and antipsychotic-induced weight gain in patients with schizophrenia. Pharmacopsychiatry 2011;44:15-20.
72. Bozina N, Medved V, Kuzman MR, Sain I, Sertic J. Association study of olanzapine-induced weight gain and therapeutic response with SERT gene polymorphisms in female schizophrenic patients. J Psychopharmacol 2007;21:728-734.

73. Le Hellard S, Theisen FM, Haberhausen M, Raeder MB, Ferno J, Gebhardt S, et al. Association between the insulin-induced gene 2 (INSIG2) and weight gain in a German sample of antipsychotic-terated schizophrenic patients: perturbation of SREBP-controlled lipogenesis in drug-related metabolic adverse effects? Molecular Psychiatry 2009;14(3):308-317.
74. Tiwari AK, Zai CC, Meltzer HY, Lieberman JA, Müller DJ, Kennedy JL. Association study of polymorphisms in Insulin Induced Gene 2 (INSIG2) with antipsychotic-induced weight gain in European and African-American schizophrenia patients. Hum Psychopharmacol Clin Exp 2010;25:253-259.
75. Herken H, Erdal M, Aydin N, Sengul C, Karadaq F, Barlas O, et al. The association of olanzepine-induced weight gain with peroxisome proliferator-activated receptor- γ2 Pro12Ala polymorphism in patients with schizophrenia. DNA and Cell biology 2009;28:515-519.
76. Wang YC, Bai YM, Chen JY, Lin CC, Lai IC, Liou YJ. Genetic association between TNF-α -308 G>A polymorphism and longitudinal weight change during clozapine treatment. Hum Psychopharmacol Clin Exp 2010;25:303-309.
77. Tiware AK, Zai CC, Likhodi O, Lisker A, Singh D, Souza RP, et al. A common polymorphism in the cannabinoid receptor I (CNR I) gene is associated with antipsychotic-induced weight gain in schizophrenia. Neuropsychopharmacol 2010;35:1315-1324.
78. Monteleone P, Milano W, Petrella C, Canestrelli B, Maj M. Endocannabinoid Pro129Thr FAAH functional polymorphism but not 1359 G/A CNR1 polymorphism is associated with antipsychotic-induced weight gain. J Clin Psychopharmacol 2010;30:441-445.
79. Van Winkel R, Moons T, Peerbooms O, Rutten B, Peuskens J, Claes S, et al. MTHFR genotype and differential evolution of metabolic parameters after initiation of a second generation antipsychotic: an observational study. Int Clin Psychopharmacol 2010;25:270-276.
80. Haddow JE, Palomaki GE. ACCE: a model process for evaluating data on emerging genetic tests. In: Human genome epidemiology. Khoury MJ (Ed.), Little J (Ed.), Burke W (Ed.), Oxford University P, New York, USA, 217-233 (2004).
81. Little J, Higgins J, Loannidis J, Moher D, Gagnon F, von Elm E, et al. Strengthening the Reporting of Genetic Association Studies (STREGA): An Extension of the STROBE Statement. Ann Intern Med 2009;150:206-215.
82. Zhang ZJ, Yao ZJ, Zhang XB, Chen JF, Sun J, Yao H, et al. No association of antipsychotic agent-induced weight gain with a DA receptor gene polymorphism and therapeutic response. Acta Pharmacol Sin 2003;24(3):235-240.
83. Anderson JL, Horne BD, Stevens SM, Grove AS, Barton S, Nicholas ZP, et al. Randomized trial of genotype-guided versus standard warfarin dosing in patients initiating oral anticoagulation. Circulation 2007;116:2563-2570.
84. Crespi CL, Miller VP. The R144C change in CYP2C9*2 allele alters interaction of the cytochrome P450 with NADPH: cytochrome P450 oxidoreductase. Pharmacogenetics 1997;7:203-210.
85. Marder SR, McQuade RD, Stock E, Kaplita S, Marcus R, Safferman AZ, et al: Aripiprazole in the treatment of schizophrenia: safety and tolerability in short-term, placebo-controlled trials. Schizophrenia Research 2003;61:123-136..
86. Wetterling T, Mussigbrodt HE. Weight gain: side effect of atypical neuroleptics? J Clin Psychopharmacol 1999;19:316-321.

87. McCreadie R, Mackie M, Morrison D, Kidd J.: Once weekly pimozide versus fluphenazine decanoate as maintenance therapy in chronic schizophrenia. Br J Psychiatry 1982;140:280-286.
88. Zimmermann U, Kraus T, Himmerich H, Schuld A, Pollmächer T. Epidemiology, implications and mechanisms underlying drug-induced weight gain in psychiatric patiënts. J Psych Research 2003;37:193-220.
89. Woods SC, Seeley RJ, Porte D, Schwartz MW. Signals that regulate food intake and energy homeostatis. Science 1998;280:1378-1383.
90. Woods SC, Benoit SC, Clegg DJ, Seeley RJ. Regulation of energy homeostatis by peripheral signals. Best Pract & Res Clin Endocr & Metab 2004;18(4):497-515.
91. Seeley RJ, Woods SC. Monitoring of stored and available fuel by the CNS: implications for obesity. Neuroscience 2003;4:901-909.
92. Tiwari AK, Rodgers JB, Sicard M, Zai CC, Likhodi O, Freeman N, et al. Association study of polymorphisms in cholecystokinin gene and its receptors with antipsychotic-induced weight gain in schizophrenia patients. Progr Neuro-Psychopharm & Biol Psych 2010;34:1484-1490.
93. Park YM, Lee HJ, Kang SG, Choi JE, Cho JH, Kim L. Lack of association between glutathione S-transferase-M1, -TI and –PI polymorphisms and olanzapine-induced weight gain in Korean schizophrenic patients. Psychiatry Investig 2010;7:147-152.
94. Hong CJ, Lin CH, Yu YWY, Yang KH, Tsai SJ. Genetic variants of the serotonin system and weight change during clozapine treatment. Pharmacogenetics 2001;11:265-268.
95. Rietschel M, Naber D, Fimmers R, Möller HJ, Propping P, Nöthen MM. Efficacy and side-effects of clozapine not associated with variation in the 5HT2C-receptor. Neuroreport 1997;8:1999-2003.
96. Human Genome database. http://www.hugenavigator.net

Chapter 2.2

The association between serotonin 2C receptor polymorphisms and weight gain and eating behaviour in patients using mirtazapine: a prospective follow-up study

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Abstract

Background

The use of mirtazapine is often associated with weight gain. Patients consider weight gain as one of the most burdensome adverse events and an important reason for non-compliance. It is therefore important to search for determinants that can predict the risk for mirtazapine-induced weight gain.

Objective

The objective of this prospective follow-up study was to investigate the role of two polymorphisms in the *HTR2C* gene on mirtazapine-induced weight gain and changes in eating behaviour.

Methods

This prospective follow-up study consisted of 27 patients with a major depression who started treatment with mirtazapine. Primary endpoint was the percentage change in BMI (kg/m^2) after 6 and 12 weeks of treatment. Secondary endpoint was any change in eating behaviour after 6 and 12 weeks. Primary determinants were polymorphisms in the promoter region of the *HTR2C* gene (759 C/T) and an intragenic *HTR2C* polymorphism (rs1414334).

Results

Mean weight gain in the total sample after 6 weeks of treatment was 3.5 kg (95% CI 3.1-3.9) and 4.6 kg (95% CI 3.9-5.3) after 12 weeks. No associations were found between the *HTR2C* rs1414334 and 759C/T polymorphisms and mirtazapine induced weight gain. Analysis of eating behaviour showed a non-significant trend for patients carrying the variant rs1414334 C-allele to experience an increase in appetite more often (odds ratio, 7.7; 95% confidence interval 0.7 - 79,8).

Conclusions

The results of this study suggest that the *HTR2C* rs1414334 and 759 C/T polymorphisms are not significantly associated with mirtazapine induced weight gain. The trend seen in changes in eating behaviour for carriers of the variant rs1414334 C-allele requires further study.

To the Editors:

Weight gain is an important and well-known adverse effect of treatment with psychotropic drugs, especially antipsychotic agents. Patients with overweight are at an increased risk of developing diabetes and cardiovascular disease and subsequently have a higher risk of mortality.[1] In addition, psychiatric patients consider weight gain as one of the most disturbing adverse events and an important reason for noncompliance.[2] The high interindividual differences in the occurrence of weight gain suggests that genetic make-up is a modulating factor. Polymorphisms in the gene encoding the serotonin 2C receptor (*HTR2C*) have been studied extensively for an association with antipsychotic agent-induced weight gain. A recent meta-analysis showed an association between the *HTR2C* rs3813929 (759C/T) polymorphism and antipsychotic agent-induced weight gain.[3] In addition, our group reported an association between the *HTR2C* rs1414334 C>G polymorphism and obesity and the metabolic syndrome in patients using antipsychotic drugs.[4] Our association was particularly strong in patients using clozapine, an antipsychotic agent with a strong affinity for *HTR2C*. If the association between *HTR2C* polymorphisms and antipsychotic agent-induced weight gain presents a true association, this can possibly be generalized to weight gain observed in patients using other drugs with high affinity for the $5HT_{2C}$ receptor, which was also suggested by Reynolds.[5] The antidepressant mirtazapine is such an agent with an antagonistic activity for $5HT_2$ receptors. Studies have shown that more than 10% of patients experience substantial weight gain during mirtazapine therapy, especially during the first 4 weeks of treatment. An increased appetite has also been reported by more than 10% of patients.[6] To our knowledge, there are no studies available in which the association between *HTR2C* polymorphisms and mirtazapine-induced weight gain and changes in eating behaviour has been investigated. Hence, the objective of our study was to investigate whether polymorphisms in the *HTR2C* gene (759 C/T [rs3813929] and rs1414334) are associated with mirtazapine-induced weight gain and/or changes in eating behaviour.

Patients were recruited from Mental Healthcare Services Drenthe in the northern part of the Netherlands in accordance with the declaration of Helsinki. We used a prospective follow-up design to assess the association between *HTR2C* variants and weight gain and changes in eating behaviour in psychiatric patients who started using mirtazapine. Adults using antidepressants at the time of inclusion or using the weight-influencing drugs methylphenidate, orlistat, sibutramine, topiramate, corticosteroids, mood stabilizers, or antipsychotic agents during the 3 months preceding inclusion were excluded. Patients with eating disorders were also excluded. Severity of depression at the start of treatment and after 6 and 12 weeks was assessed using the Montgomery and Asberg Depression Rating scale (MADRS). Patients were included between June 2005 and November 2008.

Our primary endpoint was the percentage change in BMI (kg/m^2) after 6 and 12 weeks of treatment, measured by the treating psychiatrist at research visits. Our secondary endpoint was any change in eating behaviour after 6 and 12 weeks. To assess these changes in eating behaviour, patients were asked by their psychiatrist whether they noticed any changes in eating behaviour and if so, what the changes were (increased appetite, less appetite or unchanged). Our primary determinants were polymorphisms flanking, or within, the X-linked *HTR2C* gene. We investigated 2 polymorphisms: the rs3813929: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 untranslated region. We considered the rs1414334 C-allele to be the variant allele, based on our previous studies.

Genomic DNA was isolated from ethylenediaminetetraacetic acid-anticoagulated peripheral blood using standard methods. The *HTR2C* genotypes were determined using the 7300 Real-Time polymerase chain reaction system (TaqMan single-nucleotide polymorphism genotyping with allelic discrimination) from Applied Biosystems (Nieuwerkerk AD IJssel, the Netherlands). Detailed information on genotyping procedures, including primer sequences and reaction conditions, is available upon request. Both psychiatrist and researcher were blinded to genotyping results before data-analysis. The association between *HTR2C* genotypes (presence or absence of the variant *HTR2C* allele) and the change in BMI was investigated with linear regression. Results were expressed as percentage change in BMI with SD and range. Analysis of eating behaviour was investigated with logistic regression analysis. Data were investigated for potential confounding effects of severity of depression, dose, ethnicity and weight at the start of treatment. Data were investigated for interaction between carriership of variant alleles and gender. $p \leq 0.05$ was regarded as significant. Data were analysed using SPSS 17.0 (SPSS Inc, Chicago, Ill).

In total, 27 patients were included in this study, 67% of whom were female and 96% were white, with a mean (SD) age of 54 (18) years, a mean (SD) body weight of 70.8 (13.9) kg, a mean (SD) BMI of 24.0 (4.1) kg/m^2, and a mean (SD) MADRS score at study entry of 30 (7). The variant rs3813929 (759C/T) T-allele was present in 30% of the patients, whereas the variant rs1414334 C-allele was present in 26% of the patients. Haplotype frequencies for carriership of variant alleles (759C/T mentioned first) were 18.5% for C-C, 51.9% for C-G, 7.4% for T-C and 22.2% for T-G. No linkage disequilibrium between the 2 polymorphisms was observed ($D' = 0.04$, $r2 = 0.0002$). Genotype frequencies did not deviate from those expected under Hardy-Weinberg equilibrium (759 C/T [$p = 0.79$], rs1414334 [$p = 0.59$], calculated in women). There were no statistically significant differences in baseline characteristics between genotype or haplotype groups. One patient was of Asian origin. Analysis without this patient did not affect the obtained results.

We did not find an association between mirtazapine-induced weight gain and the covariates severity of depression, dose, ethnicity and BMI at the start of treatment. Therefore, we did not adjust obtained results for these covariates. The interaction term for *HTR2C* genotype and gender was not significant (rs1414334, p = 0.74; 759C/T; p = 0.47). We did not find a significant association between *HTR2C* polymorphisms or haplotypes (data not shown) and mirtazapine-induced weight gain or eating behaviour (Table 1). Association of *HTR2C* genotype with eating behaviour showed a non-significant trend for patients carrying the variant rs1414334 C-allele to experience increased appetite more often in the first 6 weeks of treatment with mirtazapine (odds ratio, 7.7; 95% confidence interval, 0.7 – 79,8; p = 0.09). This trend was not seen in the second half of the follow-up period (data not shown). No association or trend between genotype and eating behaviour was found for the 759 C/T polymorphism.

Table 1. Association between HTR2C polymorphisms and mirtazapine-induced weight gain and changes in eating behaviour.

	Δ start- 6 wk	Δ start-12 wk
759 C/T polymorphism		
Carriers of rs3813929 (759 C/T) T-allele		
Change in BMI (SD; range)	+ 4.1% (3.0; 0.0-8.7)[a]	+ 5.9% (4.9; -2.9-13.1)[b]
Increased appetite, %	57% (n=4)	
No change in appetite, %	43% (n=3)	
Reduced appetite, %	0%	
Wild type		
Change in BMI (SD; range)	+ 5.3% (5.2; 0.0-18.0)	+ 7.6% (7.3; -1.9-28.0)
Increased appetite, %	56% (n=9)	
No change in appetite, %	31% (n=5)	
Reduced appetite, %	13% (n=2)	
Rs1414334 polymorphism		
Carriers of rs1414334 C-allele		
Change in BMI (SD; range)	+ 5.2% (4.6; 0.0-12.5)[c]	+ 6.5% (4.2; 2.5-12.5)[d]
Increased appetite, %	86% (n=6)[e]	
No change in appetite, %	14% (n=1)	
Reduced appetite, %	0%	
Wild type		
Change in BMI (SD; range)	+ 4.9% (4.8; 0.0-18.0)	+ 7.2% (7.2; -2.9-28.0)
Increased appetite, %	31% (n=5)	
No change in appetite, %	56% (n=9)	
Reduced appetite, %	13% (n=2)	

Changes in eating behaviour were not recorded in 4 patients.
a p = 0.55 compared with wild type
b p = 0.57 compared with wild type
c p = 0.87 compared with wild type
d p = 0.84 compared with wild type
e p = 0.09 compared with wild type

Discussion

Although we did not find an association between the *HTR2C* polymorphisms and mirtazapine-induced weight gain, we cannot exclude a presence of this association owing to the small sample size of our study, especially where eating behaviour is concerned. An association between eating behaviour and receptor affinity or activity influencing polymorphisms is not unlikely because studies have shown that *HTR2C* knockout mice become hyperphagic and *HTR2C* agonists reduce appetite in humans.[7,8] The absence of an association or trend between the *HTR2C* 759 C/T polymorphism and changes in eating behaviour was surprising because this polymorphism has been most strongly and repeatedly linked to antipsychotic agent-induced weight gain.[3] An explanation for this, besides the small sample size, can be that there is no association between *HTR2C* genotype and eating behaviour but that the rs1414334 polymorphism is in linkage disequilibrium with another relevant polymorphism.

Our data analysis revealed a significant interaction between MADRS score at study entry and weight gain in the first 6 weeks of treatment for the rs1414334 polymorphism, but not for the 759C/T polymorphism ($p = 0.048$). Splitting the sample in 2 groups based on MADRS scores at study entry of 32 or less ($n = 20$) and 33 or more ($n = 6$) showed a significant association between carriership of the variant rs1414334 C-allele and weight gain in both groups (data not shown). Patients with a MADRS score at study entry of 32 or less gained more weight when they carried the variant rs1414334 C-allele ($n = 3$). Interestingly, patients with a MADRS at study entry of 33 or more gained less weight when they carried the variant rs1414334 C-allele ($n = 3$). It has been postulated that a hyperfunctional *HTR2C* activity attributes to depressive symptoms due to inhibitory effects on accumbal dopamine release.[9] If increasing *HTR2C* hyperfunctionality corresponds to increasing severity of depression, patients with a more severe depression would benefit more from reducing *HTR2C* activity. Perhaps the variant rs1414334 allele plays some sort of modulating role in mirtazapine-induced inhibition of *HTR2C*, thereby explaining the difference between both groups. However, this finding could also be an artifact given the small groups in which the interaction was studied and requires further study.

There are some other limitations to this study besides the small sample size. First, some patients used a selective serotonin reuptake inhibitor (SSRI) 1 week before the start of mirtazapine therapy. Because SSRIs are often associated with weight loss, it is possible that patients switching from an SSRI to mirtazapine will gain weight, thereby affecting our results. However, correcting for prior SSRI use in our data analysis did not influence our results. Second, smoking behaviour was not recorded during the follow-up period and can be a possible confounder in this study owing to its possible effects on eating behaviour. Third, we only studied the potential effects of *HTR2C* polymorphisms. It is likely that mirtazapine-induced weight gain has a multifactoral cause, which is possibly also partly explained by polymorphisms in genes coding for leptin, histamine, $5HT_{2A}$-receptor, α_1 receptor, and other proteins. It is possible that polymorphisms in

other receptor systems have clouded our results, explaining the lack of significance found. To predict mirtazapine-induced weight gain more accurately, haplotype studies in large samples are needed.

This is the first study in which the association between *HTR2C* polymorphisms and mirtazapine-induced weight gain was investigated. Insight in the factors responsible for this weight gain can have implications for daily clinical psychiatric practice because psychiatric patients consider weight gain as one of the most disturbing adverse events. A pharmacogenetic tool to predict weight gain on mirtazapine or other antidepressants would be helpful in psychiatric practice because this could identify patients at risk for weight gain, offering an opportunity to choose an alternative treatment, which in turn could have positive effects on compliance. Whether the association between the studied *HTR2C* polymorphisms and mirtazapine-induced weight gain or changes in eating behaviour is present or absent requires further study. By writing this letter, we hope to stimulate other researchers to investigate this association in a larger setting.

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References

1. Must A, Spadano J, Coakley EH, Field AE, Colditz G, Dietz WH. The disease burden associated with overweight and obesity. JAMA 1999;282:1523-1529.
2. Muller DJ, Muglia P, Fortune T, Kennedy JL. Pharmacogenetics of antipsychotic agent-induced weight gain. Pharmacol Res 2004;49:309-329.
3. De Luca V, Mueller DJ, de Bartolomeis A, Kennedy JL. Association of the HTR2C gene and antipsychotic-induced weight gain: a meta-analysis. Int J Neuropsychopharmacol 2007;10(5):697-704.
4. Mulder H, Franke B, van der Beek-van der AA, Arends J, Wilmink FW, Egberts AC, et al. The association between HTR2c polymorphisms and obesity in psychiatric patients using antipsychotics: a cross-sectional study. Pharmacogenomics J 2007;7(5):318-24.
5. Reynolds GP. Weight gain, antipsychotic drug treatment and pharmacogenomics. Pharmacogenomics 2002;3:567-570.
6. Anttila S.A.K, Leinonen E.V.J. A review of the pharmacological and clinical profile of mirtazapine. CNS Drug Rev 2001;7:249-264
7. Tecott L, Sun L, Akana SF, Strack AM, Lowenstein DH, Dallman MF, et al. Eating disorder and epilepsy in mice lacking 5HT2C-serotonin receptors. Nature 1995;374:542-546.
8. Gallant DM, Bishop MP, Bishop G, O'Meallie L. U-22,394A: a controlled evaluation in chronic schizophrenic patients. Curr Ther Res Clin Exp 1967:9:579-581.
9. Dremencov E, Weizmann Y, Kinor N, Gispan-Herman I, Yadid G, et al. Modulation of dopamine transmission by 5HT2c and 5HT3 receptors : a role in the antidepressant response. Curr Drug Targets 2006;7:165-175.

Chapter 2.3

Association between HTR2C gene polymorphisms and the metabolic syndrome in patients using antipsychotics: a replication study

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Abstract

Background

In two previous studies we found an association between *HTR2C* polymorphisms and the prevalence of the metabolic syndrome in patients using antipsychotics.

Objective

To investigate whether we can replicate the association between *HTR2C* polymorphisms and the metabolic syndrome in a third separate sample of patients using antipsychotics.

Methods

Data for this cross-sectional study came from the ongoing Phamous study, investigating the association between schizophrenia and metabolic or cardiovascular risk factors. Primary endpoint was the prevalence of the metabolic syndrome as classified by the National Cholesterol Education Program's Adult Treatment panel III (NCEP:ATP IIIa). Primary determinants were genotypes of two polymorphisms flanking, or within, the X-linked *HTR2C* gene: the rs3813929: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.

Results

Carriership of the variant rs1414334 C-allele was significantly associated with an increase prevalence of the metabolic syndrome (OR 3.73; 95%CI 1.29-10.79, p=0.015). No association was found between the *HTR2C* -759 C/T polymorphism and the metabolic syndrome.

Conclusions

This study confirms previous findings that the variant C-allele of the rs1414334 polymorphism is associated with an increased prevalence of the metabolic syndrome.

Introduction

It has been shown that the prevalence of the metabolic syndrome is increased in patients with schizophrenia compared to the general population.[1] In our own schizophrenic patient population the prevalence of the metabolic syndrome is 36%, compared to 15.5% in the general population.[2,3] The mechanism behind the metabolic abnormalities is not entirely clear.[4] The high interindividual differences suggests 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*), since 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:C>G) 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 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 endpoint 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 ≥ 102 cm (male) or ≥ 88 cm (female), triglycerides ≥ 1.7 mmol l^{-1} or use of a fibrate, high-density lipoprotein (HDL) cholesterol <1.0 mmol l^{-1} (male) or < 1.3 mmol l^{-1} (female) or use of a statin, blood pressure ≥ 130/85 mmHg or use of an antihypertensive drug, and finally fasting glucose ≥ 5.6 mmol l^{-1}, or HbA1c > 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 bij Bennett et al.[11] With respect to triglyceride-lowering 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 endpoints 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 rs3813929: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 $5HT_{2C}$ inhibiton by antipsychotics. The intronic position of the rs1414334 polymorphism suggests that this polymorphism is non-functional. 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 (HGVS; www.hgvs.org) as well as the 'traditional' nomenclature and numbering used in previous publications. The rs1414334 plymorphism allel C is described as the ancestral allele (dbSNP database; 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 pre-developed 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 polymerase chain reaction 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 analysis 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 endpoint 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 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. 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 women) (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 1. Patient characteristics

Characteristic	Sample (n = 186)
Age, mean (s.d.)	37 (11)
Gender (%)	
Male	127 (68)
Female	59 (32)
Somatic disorders (%)	
Diabetes	18
Hypertension	44
Hypercholesterolaemia	32
Hypertriglyceridaemia	47
Overweight (BMI > 25 kg m^2)	66
Obesity (BMI > 30 kg/m^2)	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)

Abbreviation: BMI, body mass index.
a Diagnosis of metabolic syndrome could not be carried out in 24 patients.

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 2. HTR2C polymorphisms and metabolic syndrome

Carriership of variant alleles	Patients [a]	Metabolic Syndrome (%)	Crude OR [b] (95%CI)	Adjusted [b,c] OR (95%CI)
	162	56 (35)		
rs3813929 T	34	12 (35)	1.04 (0.47-2.30)	1.39 (0.51-3.76)
rs1414334 C	30	16 (53)	2.63 (1.17-5.90)	3.73 (1.29-10.79)

Abbreviations: CI, confidence interval; OR, odds ratio.
a Diagnosis of the metabolic syndrome could not be carried out 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, duration of illness, antipsychotic drug, weight-increasing co-medication, weight-reducing co-medication and DSM-IV diagnosis.

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 mmol l^{-1} *vs* 1.7 mmol l^{-1}, p=0.014), but no association with HDL concentrations was found (1.32 mmol l^{-1} *vs* 1.28 mmol l^{-1}, p=0.72).

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	161	1.46 (0.56-3.80)	2.59 (0.96-7.05)
TG	163	2.01 (0.83-4.89)	2.39 (0.98-5.79)
Waist	170	1.24 (0.52-3.00)	0.97 (0.38-2.45)
Hypertension	175	1.14 (0.51-2.53)	1.74 (0.74-4.13)
Glucose	130	3.40 (0.94-12.35)	1.41 (0.42-4.79)

Abbreviations: 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 ≥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 to 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 to 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 non-significant 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 > 6.1%. 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 > 6.1% had similar accuracy as fasting plasma glucose 6.0 mmol 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 represents 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.[16-10] 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 long-term 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 metabolic-regulating 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]

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 (rs1414334 and -759 C/T) and other metabolic-regulating 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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References

1. McEvoy JP, Meyer JM, Goff DC, Nasrallah HA, Davis SM, Sullivan L, et al. Prevalence of the metabolic syndrome in patients with schizophrenia: Baseline results from the Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) schizophrenia trial and comparison with national estimates from NHANES III. Schizophr Res 2005;80:19-32.
2. Schorr SG, Sloof CJ, Bruggeman R, Taxis K. Prevalence of metabolic syndrome in patients with psychotic disorders in the Netherlands. J Clin Psychopharmacol 2009;29:399-401.
3. van den Donk M, Bobbink IW, Gorter KJ, Salomé PL, Rutten GE. Identifying people with metabolic syndrome in primary care by screening with a mailed tape measure. A survey in 14,000 people in the Netherlands. Prev Med 2009;48:345-350.
4. de Leon J. Beyond the "hype" on the association between metabolic syndrome and atypical antipsychotics: the confounding effects of cohort, typical antipsychotics, severe mental illness, comedications, and comorbid substance use. J Clin Psychopharmacol 2008;28:125-131.
5. Tecott L, Sun L, Akana S, Struch AM, Lowenstein DH, Dallman MF, et al. Eating disorder and epilepsy in mice lacking 5HT2C-serotonin receptors. Nature 1995;374:542-546.
6. Gallant DM, Bishop MP, Bishop G, O'Meallie L. U-22,394A: a controlled evaluation in chronic schizophrenic patients. Curr Ther Res Clin Exp 1967:9:579-581.
7. De Luca V, Mueller DJ, de Bartolomeis A, Kennedy JL. Association of the HTR2C gene and antipsychotic-induced weight gain: a meta-analysis. Int J Neuropsychopharmacol 2007;10(5):697-704.
8. Mulder H, Franke B, van der-Beek van der Aart A, Arends J, Wilmink FW, Scheffer H, et al. The association between HTR2C gene polymorphisms and the metabolic syndrome in patients with schizophrenia. J Clin Psychopharmacol 2007;27:338-343.
9. Mulder H, Cohen D, Scheffer H, Gispen-de Wied C, Arends J, Wilmink FW, et al. HTR2C gene polymorphisms and the metabolic syndrome in patients with schizophrenia: a replication study. J Clin Psychopharmacol 2009;29:16-20.
10. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, et al. Diagnosis and management of the metabolic syndrome: an american heart association/national heart,lung, and blood institute scientific statement. Circulation 2005;112:2735-2752.
11. Bennett CM, Guo M, Dharmage SC. HbA1c as a screening tool for detection of type 2 diabetes: a systematic review. Diabet Med 2007;24:333-343.
12. Godlewska BR, Olajossy-Hilkesberger L, Ciwoniuk M, Olajossy M, Marmurowska-Michalowska H, Limon J. Olanzapine-induced weight gain is associated with the -759 C/T and -697 G/C polymorphisms of the HTR2c gene. Pharmacogenomics J 2009;9:234-241.
13. Sandelin A, Wasserman W.W., Lenhard B. ConSite: web-based prediction of regulatory elements using cross-species comparison. Nucleic Acids Research 2004; W239-W252.
14. Maldonado G, Greenland S. Simulation study of confounder-selection strategies. Am J Epidemiology 1993;138:923-936.

15. Perneger TV. What's wrong with Bonferroni adjustments. Br Med J 1998;316:1236-1238.
16. Reynolds GP, Templeman LA, Zhang ZJ. The role of 5-HT2C receptor polymorphisms in the pharmacogenetics of antipsychotic drug treatment. Prog Neuropsychopharmacol Biol Psychiatry 2005;29(6):1021-1028.
17. Reynolds GP, Hill MJ, Kirk SL. The 5-HT2C receptor and antipsychoticinduced weight gain - mechanisms and genetics. J Psychopharmacol 2006; 20(4 Suppl):15-18.
18. Ellingrod VL, Perry PJ, Ringold JC, Lund BC, Bever-Stille K, Fleming F, et al. Weight gain associated with the 759C/T polymorphism of the 5HT2c receptor and olanzapine. Am J Med Gen Part B (Neuropsychiatric Genetics) 2005;134B:76-78.
19. Templeman LA, Reynolds GP, Arranz B, San L. Polymorphisms of the 5HT2c receptor and leptin genes are associated with antipsychotic drug-induced weight gain in Caucasian subjects with a first-episode psychosis. Pharmacogenetics and Genomics 2005;15:195-200.
20. Ryu S, Cho EY, Park T, Oh S, Jang WS, Kim SK. 759 C/T polymorphism of 5-HT2C receptor gene and early phase weight gain associated with antipsychotic drug treatment. Prog Neuropsychopharmacol Biol Psychiatry 2007;31:673-677.
21. Miller DD, Ellingrod VL, Holman TL, Buckley PF, Arndt S. Clozapine-induced weight gain associated with the 5HT2c receptor -759C/T polymorphism. Am J Med Gen Part B (Neuropsychiatric Genetics) 2005;133B:97-100.
22. Tsai SJ, Hong CJ, YU YWY, Lin CH. -759C/T genetic variation of 5HT2c receptor and clozapine-induced weight gain. Lancet 2002;360(30):1790 (letter).
23. Park YM, Cho JH, Kang SG, Choi JE, Lee SH, Kim L, et al. Lack of association between the 759C/T polymorphism of the 5-HT2c receptor gene and olanzapine-induced weight gain among Korean schizophrenic patients. J Clin Pharm Ther 2008;33:55-60.
24. Yevtushenko OO, Cooper SJ, O'Neill R, Doherty JK, Woodside JV, Reynolds GP. Influence of 5-HT2c receptor and leptin gene polymorphisms, smoking and drug treatment on metabolic disturbances in patients with schizophrenia. Br J Psychiatry 2008;192:424-428.
25. Gregoor JG, Mulder H, Cohen D, van Megen HJGM, Egberts ACG, Heerdink ER, et al. Combined HTR2C-LEP genotype as a determinant of obesity in patients using antipsychotic medication. J Clin Psychopharmacol 2010;30:702-705.

Chapter 2.4

Association between the 1291-C/G polymorphism in the adrenergic α-2a receptor and the metabolic syndrome

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Abstract

Background

The use of antipsychotics can result in developing the metabolic syndrome. The interindividual differences in subsceptability to developing this metabolic syndrome suggest that genetic make-up is a modulating factor.

Objective

To investigate whether the *ADRA2A* 1291-C/G polymorphism is associated with the metabolic syndrome in patients using antipsychotic drugs.

Methods

Data for this cross-sectional study came from three different samples. All three samples (n=114, n=170 and n=186) were previously used to investigate the association between *HTR2C* polymorphisms and the metabolic syndrome. Primary endpoint was the prevalence of the metabolic syndrome as classified by the National Cholesterol Education Program's Adult Treatment panel III (NCEP:ATP IIIa).

Primary determinant was the genotype of the 1291-C/G (rs1800544) polymorphism located in the *ADRA2A* gene.

Results

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).

Conclusions

This study shows that the *ADRA2A* 1291-C/G polymorphism does not appear to be a strong predictor for long term occurrence of the metabolic syndrome in antipsychotic using patients.

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 insuline 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 make-up 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 a 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 Familiy 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 antipsychotic-induced 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 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 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 were diagnosed with 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 endpoint 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 III (ATP IIIa).[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 A_{1C} (HbA_{1C}) greater than 6.1% or use of an antidiabetic. Hemoglobin A_{1C} was used when fasting glucose level data were not available. The cutoff value used for HbA_{1C} is based on a review bij Bennett et al.[15] With respect to triglyceride-lowering 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 statines 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 endpoints 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 analysis 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, gender, and antipsychotic drugs prescribed. We included these variables in the multivariate model if they were univariately associated with the primary endpoint 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. $p\leq0.05$ or less was regarded as significant. Data were analysed using SPSS 17.0 (SPSS Inc, Chicago, Ill).

Results

In total, 497 patients were recruited for this study. Twenty-seven 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 an 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, gender 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 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 (b = 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 of the variant allele.
d Data could be investigated only for an association between carriership of the variant allele and the metabolic syndrome because of sample size.
e Compared with 56% in group with 1291-CC genotype.

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 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-cholesterol < 1.0 mmol/L (male) or < 1.3 mmol/L (female) or use of a statin. Triglycerides ≥ 1.7 mmol/L or use of a fibrate. Waist circumference ≥ 102 cm (male) or ≥ 88 cm (female). Hypertension = blood pressure ≥ 30/85 mm Hg or use of an antihypertensive drug. Glucose = fasting glucose ≥ 5.6 mmol/L, or HbA_{1c} > 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.

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).

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

Antipsychotic	N	MS	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)	NA
Aripiprazole	10	30%	0.21 (0.01-3.13)	NA
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)

MS indicates metabolic syndrome; NA, not applicable; a.p.; antipsychotics.
a Data were adjusted for age, gender, carriership of variant *HTR2C* rs1414334 C-allele, ethnicity, and DSM-IV diagnosis.

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<0.2) co-variables, age and gender (OR 0.05; 95% CI 0.003 - 0.97, p=0.048; Table 1).

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 analyse 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 behaviour, 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 HbA_{1c} instead, with a cutoff value of greater than 6.1%. Using HbA_{1c} 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.[15] showed that a recommended HbA_{1c} 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 towards a positive association between the *ADRA2A* polymorphism and HbA_{1c}, we believe that using HbA_{1c} 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 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 adrenergic α_{2a}-receptors, 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_{2a}-receptors (clozapine, quetiapine and risperidone) and a group of patients using antipsychotics with a lower affinity for the α_{2a}-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 'antipsychotic-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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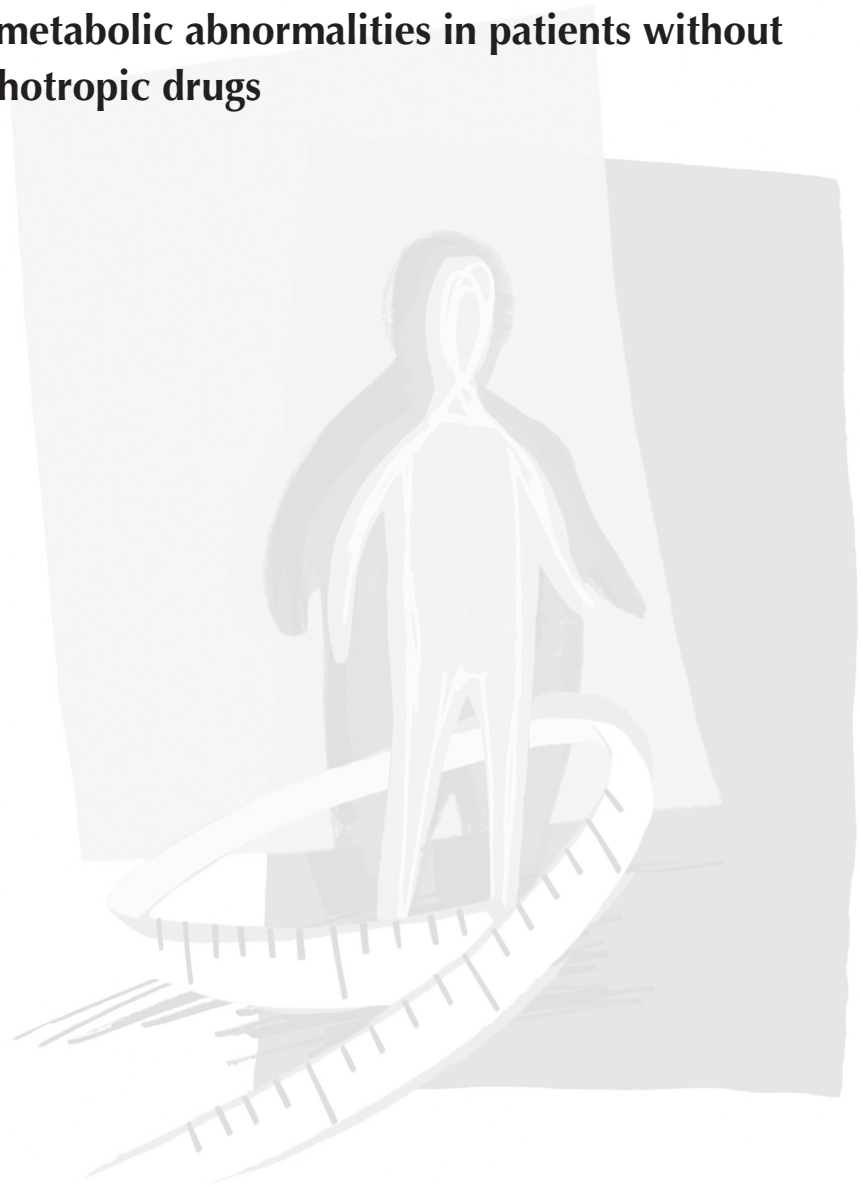
References

1. McEvoy JP, Meyer JM, Goff DC, Nasrallah HA, Davis SM, Sullivan L, et al. Prevalence of the metabolic syndrome in patients with schizophrenia: Baseline results from the Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) schizophrenia trial and comparison with national estimates from NHANES III. Schizophr Res 2005;80:19-32.
2. Arranz B, Rosel P, Ramirez N, Duenas R, Fernandez P, Sanchez JM, et al. Insulin resistance and increased leptin concentrations in noncompliant schizophrenia patients but not in antipsychotic-naïve first-episode schizophrenia patients. J Clin Psychiatry 2004;65:1335-1342.

3. Hellstrom L, Reynisdottir S, Langin D, Rössner S, Arner P. Regulation of lipolysis in fat cells of obese women during long-term hypocaloric diet. Int J Obes Relat Metab Disord 1996;20:745-752.
4. Hellstrom L, Rossner S, Hagstrom-Toft E, Reynisdottir S. Lipolytic catecholamine resistance linked to α2adrenoceptor sensitivity – a metabolic predictor of weight loss in obese subjects. Int J Obes Relat Metab Disord 1997;21:314-320.
5. Bylund DB. Subtypes of α2adrenoceptors: pharmacological and molecular biological evidence converge. Trends Pharmacol Sci 1988;9:356-361.
6. Garenc C, Perusse L, Chagnon YC, Rankinen T, Gagnon J, Borecki IB, et al. The α 2adrenergic receptor gene and body fat content and distribution : the HERITAGE family study. Mol Med 2002;8:88-94.
7. Rosengren AH, Jokubna R, Tojjar D, Granhall C, Hansson O, Li DQ, et al. Overexpression of α2a-adrenergic receptors contributes to type 2 diabetes. Science 2010;217-220.
8. Wang YC, Bai YM, Chen JY, Lin CC, Lai IC, Liou YJ: Polymorphism of the adrenergic receptor alpha 2a-1291C>G genetic variation and clozapine-induced weight gain. J Neural Transm 205;112:1463-1468.
9. Park YM, Chung YC, Lee SH, Lee KJ, Kim H, Byun YC, et al. Weight gain associated with the α2a-adrenergic receptor 1291C/G polymorphism and olanzapine treatment. Am J Med Gen Part B (Neuropsychiatric Genetics) 2006;141B:394-397.
10. Sickert L, Müller DJ, Tiwari AK, Shaikh S, Zai C, De Souza R. Association of the alpha 2A adrenergic receptor -1291 C/G polymorphism and antipsychotic-induced weight gain in European-Americans. Pharmacogenomics 2009;10:1169-1176.
11. Mulder H, Cohen D, Scheffer H, Gispen-de Wied C, Arends J, Wilmink FW, et al. HTR2C gene polymorphisms and the metabolic syndrome in patients with schizophrenia: a replication study. J Clin Psychopharmacol 2009;29:16-20.
12. Mulder H, Franke B, van der-Beek van der Aart A, Arends J, Wilmink FW, Scheffer H, et al. The association between HTR2C gene polymorphisms and the metabolic syndrome in patients with schizophrenia. J Clin Psychopharmacol 2007;27:338-343.
13. Cohen D, Stolk RP, Grobbee DE, Gispen-de Wied CC. Hyperglycaemia and diabetes mellitus in patients with schizophrenia or schizo-affective disorders. Diabetes Care 2006;29:786-791
14. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, et al. Diagnosis and management of the metabolic syndrome: an american heart association/national heart,lung, and blood institute scientific statement. Circulation 2005;112:2735-2752.
15. Bennett CM, Guo M, Dharmage SC. HbA1c as a screening tool for detection of type 2 diabetes: a systematic review. Diabet Med 2007;24:333-343.
16. Maldonado G, Greenland S. Simulation study of confounder-selection strategies. Am J Epidemiol 1993;138:923-936.
17. Wahrenberg H, Hertel K, Leijonhufvud BM, Persson LG, Toft E, Arner P. Use of waist circumference to predict insulin resistance: retrospective study. Br Med J 2005;330:1363-1364.
18. Han TS, van Leer EM, Seidell JC, Lean ME. Waist circumference action levels in the identification of cardiovascular risk factors: prevalence study in a random sample. Br Med J 1995;311:1401-1405.
19. Matsui-Sakata A, Ohtani H, Sawada Y. Receptor occupancy-based analysis of the contributions of various receptors to antipsychotics-induced weight gain and diabetes mellitus. Drug Metab Pharmacokinet 2005;20:368-378.

3

Polymorphisms in the HTR2C and ADRA2A genes and metabolic abnormalities in patients without psychotropic drugs



Chapter 3.1

Association between HTR2C polymorphisms and obesity in patients without antipsychotic drugs

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Abstract

Background

Changes in eating behaviour appear to be a major cause for the increasing prevalence of obesity. The serotonin $5HT_{2c}$-receptor plays an important role in modifying eating behaviour, and polymorphisms within the *HTR2C* gene have been associated with antipsychotic-induced weight gain and obesity. The question arose to what extent antipsychotic drugs modify the effects of these *HTR2C* polymorphisms and whether the associations found in schizophrenic patients are also present in patients without antipsychotic drugs.

Objective

To investigate whether prevalence of the *HTR2C* rs1414334 and 759 C/T genotypes differs between obese and non-obese patients without antipsychotic drugs and address the question whether the effects of these polymorphisms are independent of antipsychotic drugs.

Methods

A cross-sectional case-control design was used to assess the association between *HTR2C* genotypes and obesity. Adult Caucasian patients with obesity (BMI > 30 kg/m^2) were recruited from an obesity clinic located within the hospital. Adult Caucasian patients with a normal body weight (BMI < 25 kg/m^2) were recruited on the orthopaedic ward and among the employees of the hospital pharmacy. Primary endpoint was difference in *HTR2C* genotype frequency between cases and controles. Primary determinants were the rs3813929:C>T (-759 C/T) polymorphism located in the promoter region and the intronic rs1414334:C>G polymorphism in the *HTR2C* gene.

Results

In total, 216 patients were included consisting of 116 cases and 100 controls. The case-group was significantly younger than the control-group (45.7 (SD 12.5) vs. 65.1 (SD 15.0) years, respectively, $p < 0.0001$). Two-thirds of the patients in both groups were women. Prevalence of the 759T (males) or TT (women) genotype was significantly lower in patients with obesity (OR 0.18; 95%CI 0.04 - 0.78). Carriership of the variant 759T allele showed a protective point estimate but was not significant (OR 0.53; 95%CI 0.24 - 1.17). No other associations were found.

Conclusions

This study extends the evidence for a protective effect of the *HTR2C* 759T allele in developing obesity independent of antipsychotics drugs.

Introduction

Obesity is an important problem in modern society. Obesity has been associated with an increased incidence of cardiovascular disease, type 2 diabetes mellitus (DM), hypertension, obstructive sleep apnea syndrome, polycystic ovarian syndrome, stroke, dyslipidemia, osteoarthritis, some forms of cancer and subsequently an increase in overall mortality.[1,2] In addition, obesity can have psychosocial consequences since it can negatively influence self-image, social functioning and physical activity. Obesity is often classified by a cutoff value (usually $\geq$ 30 kg/m^2) for the Body Mass Index (BMI), which is calculated by dividing the body weight in kilograms by the square of the height in meters. The past few decades there has been a worldwide steep increase in the prevalence of obesity. Changes in eating behaviour, including a global shift in diet towards increased intake of energy-dense foods that are high in fat and sugar, are most likely the most important cause for this increase.

Several studies have shown that the serotonin $5HT_{2c}$-receptor might play an important role in the aetiology of eating behaviour, since $5HT_{2c}$ knock-out mice become hyperphagic and obese and $5HT_{2c}$-agonists reduce appetite in humans.[3,4] Currently, selective $5HT_{2c}$-agonistic drugs are being developed and tested as weight reducing agents in obese patients.[5] However, studies have shown that polymorphisms exist in the X-linked *HTR2C* gene coding for the $5HT_{2c}$-receptor that may affect receptor function and thereby influence $5HT_{2c}$ mediated eating behaviour. Most of these studies were studies in schizophrenic patients investigating the association between *HTR2C* polymorphisms and weight gain caused by antipsychotics with $5HT_{2c}$-antagonising properties. The majority of these studies focused on the *HTR2C* 759C/T polymorphism, which resulted in a meta-analysis by De Luca et al.[6] The meta-analysis confirmed the association between the T allele and less antipsychotic-induced weight gain in a fixed-effect model, but lost significance due to heterogeneity of the studies if the more appropriate random-effects model was chosen for analysis (OR 2.29; 95%CI 0.98-5.36). Recently, the meta-analysis by De Luca et al. was updated by Sicard et al. who confirmed an increased risk for antipsychotic-induced weight gain in patients carrying the 759C allele (OR 2.42; 95%CI 1.15-5.09) in all samples, and OR 3.20; 95%CI 1.4-7.28 in European samples).[7] The *HTR2C* rs1414334 polymorphism has also been associated with obesity in a previous study from our group.[8] Schizophrenic patients carrying the variant rs1414334 C allele were at risk for being obese (OR 2.8; 95%CI 1.03-7.62). However, the association could not be confirmed in two other studies in schizophrenic patients investigating the association between *HTR2C* polymorphisms and the metabolic syndrome (data not shown).[9,10] To our knowledge, the association between the rs1414334 polymorphism and obesity has not been investigated in non psychiatric patients.

A question that arose is to what extent antipsychotic drugs modify the effects of these *HTR2C* polymorphisms and whether the associations found in schizophrenic patients are also present in patients without antipsychotic drugs. A few studies have investigated the possible association

between *HTR2C* polymorphisms and obesity in populations without antipsychotic drugs, but the results are conflicting. In the study by Yuan et al., investigating the association between four *HTR2C* polymorphisms (759 C/T, 997 G/A, 697G/C, and 1027 (GT)n) and obesity and diabetes, a reduced prevalence of the variant 759 T allele was found in obese patients and patients with diabetes (OR 0.36; 95%CI 0.16 - 0.83 and OR 0.43; 95%CI 0.20 - 0.91 respectively).[11] Prevalence of the 697 C allele was also reduced in obese patients (OR 0.4; 95%CI 0.20 - 0.77). In the study by Pooley et al., investigating the association between the *HTR2C* 759 C/T polymorphism and obesity in women, the prevalence of the 759 C allele was higher in obese women (OR 1.72; 95%CI 1.13 - 2.64).[12] Moreover, patients with the *HTR2C* 759 C/T genotype lost less weight than patients with the 759 C/C and T/T genotype. However, the study by Kring et al. could not replicate the association between the *HTR2C* 759 C/T polymorphism and obesity in a large male Danish cohort.[13] Surveys at a mean age of 46 and 49 years showed no significant difference in genotype distribution between the obese and non-obese men ($p = 0.40$).

The primary objective of this study was to investigate whether prevalence of the *HTR2C* rs1414334 and 759 C/T genotypes differs between obese and non-obese patients and address the question whether the effects of these polymorphisms are independent of antipsychotic drugs.

Materials and Methods

Setting, design and study population

Patients were recruited in the Wilhelmina Hospital Assen in the Northern part of the Netherlands between July 2008 and January 2011. The Wilhelmina Hospital Assen is a medium-sized hospital with several wards and 300 beds, covering a total population of approximately 100,000 persons. A cross-sectional case control design was used to assess the association between *HTR2C* genotypes and obesity. Patients with obesity (cases) were recruited from the obesity clinic located within the hospital. The obesity clinic offers a nine month program for obese patients in which they are closely monitored and treated by a team consisting of an internist, psychologist, physiotherapist, dieticians and nurses. Exercise and changing eating behaviour are the keystones of the program. Cases were eligible for inclusion in this study if they had a BMI > 30 kg/m^2, were 18 years or older and were of Caucasian descent. Patients using weight increasing drugs or drugs with $5HT_{2c}$ agonistic or antagonistic properties were excluded. Patients with a normal body weight (controls) were primarily recruited on the orthopaedic ward. Some of the controls were recruited among the employees of the hospital pharmacy. Controls were eligible for inclusion in this study if they had a BMI < 25 kg/m^2, were also 18 years or older and were of Caucasian descent. Smoking, cancer, following a diet, use of weight reducing drugs, and excessive drug or alcohol use were considered as exclusion criteria for the controls. After complete description of the study to the patients, informed consent was obtained and blood

was drawn for genotyping. The study protocol was reviewed and approved by an independent medical ethics committee (Stichting BEBO, Assen, The Netherlands).

Determinants

Primary determinants were genotypes of polymorphisms flanking, or within, the X-linked *HTR2C* gene. The following two polymorphisms were investigated: the rs3813929: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. It should be noted with regard to *HTR2C* polymorphism nomenclature that for reasons of clarity, the nomenclature and nucleotide numbering at the genomic level according to the guidelines of the Human Genome Variation Society (HGVS; www.hgvs.org) as well as the ' traditional' nomenclature and numbering used in previous publications was used. The rs1414334 polymorphism allele C is described as the ancestral allele (dbSNP database; www.ncbi.nlm.nih.gov/SNP). However, in Western and Northern Europeans, allele G appears to be the major allele, which is confirmed in this study. Allele C was therefore considered as the variant allele.

DNA Isolation and genotyping

Genomic DNA was isolated from EDTA-anticoagulated peripheral blood using standard methods. The *HTR2C* genotypes were determined using the StepOnePlus Real Time polymerase chain reaction system from Applied Biosystems (TaqMan SNP genotyping with allelic discrimination). Detailed information on genotyping procedures, including primer sequences and reaction conditions is available upon request.

Data analysis and statistics

Patient characteristics were transferred to a database (MS Access 2003) for analysis. Differences between cases and controls regarding frequencies of *HTR2C* genotypes were investigated using logistic regression and expressed as odds ratios (OR) with a 95% confidence interval (95%CI). Data were investigated for potential confounding effects of age and gender. We included these variables in the multivariate model if they were univariately associated with the primary endpoint at a significance level of $p<0.20$.[14] Data were also investigated for interaction between genotype and gender. A p-value of 0.05 or less was regarded as significant. Data were analysed using SPSS 17.0 (SPSS Inc, Chicago, Ill).

Results

In total, 216 patients were included in this study, consisting of 116 cases (BMI >30 kg/m^2) and 100 controls (BMI <25 kg/m^2). Mean age in the case-group was significantly lower compared to the control-group (45.7 (SD 12.5) vs. 65.1 (SD 15.0) years, respectively, $p<0.0001$). Two-thirds of the patients in both groups were women. Prevalence of diabetes was 20% (n=23) in the case group. No patients with diabetes were present in the control group, because these

patients were excluded based on the use of insulin or metformin which were regarded as weight reducing agents. Smoking cigarettes was reported by 3% (n=3) of the case group, compared to 0% in the control group (exclusion criterium). Genotype distribution of the polymorphisms did not deviate significantly from Hardy-Weinberg-equilibrium (HWE, calculated in women) (rs3813929 (-759 C/T) [p=0.95] and rs1414334:C>G [p=0.83]). No linkage disequilibrium between both polymorphisms was found ($r^2<0.0001$, $D'=0.003$). Gender was not found to be a confounding factor (p=0.36) and no interaction between genotype and gender was found (p=0.72). The results of genotype frequencies in both groups are shown in Table 1.

Table 1. HTR2C allele and genotype frequencies in obese and normal weight patients.

	Cases (n=116)	Controls (n=100)	Adjusted [a,b] OR (95%CI)
Genotype rs3813929 (759 C/T)			
C/CC	93 (80%)	72 (72%)	1 (ref)
CT	19 (16%)	18 (18%)	0.76 (0.31-1.82)
T/TT	4 (3%)	10 (10%)	0.18 (0.04-0.78)
Carriership of variant 759 T allele (C/T+T/T compared to C/C genotype)	23 (20%)	28 (28%)	0.53 (0.24-1.17)
Genotype rs1414334 (C/G)			
G/GG	81 (70%)	80 (80%)	1 (ref)
CG	26 (22%)	12 (12%)	1.21 (0.28-5.22)
C/CC	9 (8%)	8 (8%)	0.72 (0.20-2.56)
Carriership of variant rs1414334 C allele (C/G + C/C compared to G/G genotype)	35 (30%)	20 (20%)	1.58 (0.72-3.43)

a Data were analysed with the common genotype as the reference.
b Data were adjusted for age.

Table 1 shows that prevalence of the 759T (males) or TT (women) genotype was significantly lower in patients with obesity (OR 0.18; 95%CI 0.04-0.78). An analysis for carriership of the variant 759T allele showed a protective point estimate, but this was not significant (OR 0.53; 95% CI 0.24-1.17). No associations were found for the rs1414334 polymorphims, and neither for a combined genotype analysis (data not shown).

Discussion

In accordance with the results found in the studies by Pooley et al. and Yuan et al., the prevalence of the *HTR2C* 759 T/T genotype was lower in the obese patients than in patients with a normal body weight (OR 0.18; 95%CI 0.04-0.78). Grouping the patients with the 759 C/T and T/T genotype and comparing them with carriers of the 759 C/C genotype (in analogy with the meta-analysis by De Luca et al.[6]) did not show significant prevalence differences between both study groups. Grouping the 759 C/C and C/T genotype and comparing them

with carriers of the 759 T/T genotype (in analogy with the meta-analysis by Sicard et al. and the study by Pooley et al.,[7,12]), showed an increased prevalence of the 759 C allele in patients with obesity (OR 5.4; 95%CI 1.23 - 23.65), suggesting the T allele to be a recessive allele.

However, most studies in schizophrenic patients, investigating the association between the *HTR2C* 759 C/T polymorphism and antipsychotic-induced weight gain, found a protective effect of carriership of the variant 759 T allele and not the 759 T/T genotype per se.[6] In this study, prevalence of the 759 C/T genotype was also slightly higher in the control group, but the difference between both groups was too small to be significantly different. Furthermore, contrary to the results by Mulder et al., no association between the rs1414334 genotype or carriership of the variant rs1414334 C allele and obesity was found. This is in accordance with two other studies in schizophrenic patients where no association between the variant rs1414334 C allele and obesity was found either (data not shown).[9,10] Therefore, the initial finding of this association might reflect a type I error.

We realize that the cross-sectional design of this study is an important limitation, because information about a person's body weight in the past is missing. Therefore it is possible that patients being obese in the past lost a lot of weight and were included in the control-group. However, this could only have had a negative influence on the results found, since differences between groups would become smaller. However, it is possible that this partly contributed to the fact that we did not find significant results for carriership of the variant 759 T allele. Another limitation of this study is that we were unable to correct for exercise. It is plausible that patients with a normal BMI exercise more than patients with obesity. However, there is no known association between *HTR2C* genotype and exercise, and therefore this doesn't explain the difference in prevalence of *HTR2C* genotypes between both groups. Also, the obese patients were significantly younger than the patients with a normal BMI. Since both physical activity and basal metabolic rate decrease with age, this might compensate for more exercise in the group of patients with a normal BMI. Moreover, most patients with a normal BMI were included on the orthopaedic ward, waiting for a knee or hip replacement. This makes it less likely that these patients were more physically active on the moment of inclusion, and the months before that, than the obese patients.

The results of this study confirm that the *HTR2C* 759 C/T polymorphism, and not the rs1414334 polymorphism, could be relevant for the development of obesity in patients without antipsychotic drugs. It is plausible however that antipsychotic drugs modify the impact of this polymorphism on body weight to some extent. Recently the study by Hill and Reynolds established the 759 C/T polymorphism as a functional polymorphism and suggested disruption of DNA-protein interactions as a mechanism by which *HTR2C* expression is perturbed in carriers of the 759 T allele.[15] It was proposed that decreased expression of the $5HT_{2C}$-receptor leads to adaptive changes in other systems that control feeding resulting in resistance to weight gain. This could

explain the difference in susceptibility to antipsychotic-induced weight gain between patients carrying the 759 T allele and patients carrying the 759 C/C genotype, since the impact of $5HT_{2C}$-antagonistic antipsychotics on $5HT_{2C}$-mediated processes is likely to be different in a situation where there are a large number of active $5HT_{2C}$-receptors compared to a situation in which the number of $5HT_{2C}$-receptors is small and other systems have already been adapted to compensate this. The rs1414334 polymorphism does not seem to play a role in weight gain or prevalence of obesity, but has been associated with prevalence of the metabolic syndrome, reduced HDL-cholesterol and elevated triglyceride levels in patients using antipsychotic drugs.[10] Therefore, these two polymorphisms located within the *HTR2C* gene appear to be predicting different phenotypes. Since the 759 C/T polymorphism has been shown to be functional, and the rs1414334 polymorphism has not, it is plausible that the rs1414334 polymorphism is in Linkage Disequilibrium with a causative SNP or group of SNPs that are involved in lipolysis, given the associations previously found with HDL-cholesterol and triglyceride levels.

In conclusion, this study extends the evidence for a protective effect of the *HTR2C* 759 T allele in developing obesity that is independent of the use of antipsychotic drugs.

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References

1. Field AE, Coakley EH, Must A, Spadano JL, Laird N, Dietz WH, et al. Impact of overweight on the risk of developing common chronic diseases during a 10-year period. Arch Intern Med 2001;161:1581-1586.
2. Must A, Spadano J, Coakley EH, Field AE, Colditz G, Dietz WH. The disease burden associated with overweight and obesity. JAMA 1999;282:1523-1529.
3. Tecott L, Sun L, Akana S, Struch AM, Lowenstein DH, Dallman MF, et al. Eating disorder and epilepsy in mice lacking 5HT2C-serotonin receptors. Nature 1995;374:542-546.
4. Gallant DM, Bishop MP, Bishop G, O'Meallie L. U-22,394A: a controlled evaluation in chronic schizophrenic patients. Curr Ther Res Clin Exp 1967:9:579-581.
5. Smith SR, Prosser WA, Donahue DJ, Morgan ME, Anderson CM, Shanahan WR, et al. Lorcaserin (APD356), a selective 5-HT2c agonist, reduces body weight in obese men and women. Obesity 2008;17:494-503.
6. De Luca V, Mueller DJ, de Bartolomeis A, Kennedy JL. Association of the HTR2C gene and antipsychotic-induced weight gain: a meta-analysis. Int J Neuropsychopharmacol 2007;10(5):697-704.
7. Sicard MN, Zai CC, Tiwari AK, Souza RP, Meltzer HY, Lieberman JA, et al. Polymorphisms of the HTR2C gene and antipsychotic-induced weight gain: an update and meta-analysis. Pharmacogenomics 2010;11:1561-1571.

8. Mulder H, Franke B, van der Beek-van der AA, Arends J, Wilmink FW, Egberts AC, et al. The association between HTR2c polymorphisms and obesity in psychiatric patients using antipsychotics: a cross-sectional study. Pharmacogenomics J 2007;7(5):318-324.
9. Mulder H, Cohen D, Scheffer H, Gispen-de Wied C, Arends J, Wilmink FW, et al. HTR2C gene polymorphisms and the metabolic syndrome in patients with schizophrenia: a replication study. J Clin Psychopharmacol 2009;29:16-20.
10. Risselada AJ, Vehof J, Bruggeman R, Wilffert B, Cohen D, Al Hadithy AF, et al. Association between HTR2c gene polymorphisms and the metabolic syndrome in patients using antipsychotics: a replication study. Pharmacogenomics J 2010; doi:10.1038/tpj.2010.66.
11. Yuan X, Yamada K, Ishiyama-Shigemoto S, Koyama W, Nonaka K. Identification of polymorphic loci in the promoter region of the serotonin 5HT2C receptor gene and their association with obesity and type II diabetes. Diabetologica 2000;43:373-376.
12. Pooley EC, Fairburg CG, Cooper Z, Sodhi MS, Cowen PJ, Harrison PJ. A 5-HT2c receptor promoter polymorphism (HTR2C-759C/T is associated with obesity in women, and with resistance to weight loss in heterozygotes. Am J Medical Genetics Part B (Neuropsychiatric Genetics) 2004;126B:124-127.
13. Kring SI, Werge T, Holst C, Toubro S, Astrup A, Hansen T, et al. Polymorphisms of serotonin receptor 2A and 2C genes and COMT in relation to obesity and type 2 diabetes. PloS ONE 2009;4(8):e6696.
14. Maldonado G, Greenland S. Simulation study of confounder-selection strategies. Am J Epidemiology 1993;138:923-936.
15. Hill MJ, Reynolds GP. Functional consequences of two HTR2C polymorphisms associated with antipsychotic-induced weight gain. Pharmacogenomics 2011;12(5): 727-734.

Chapter 3.2

Association between the HTR2C rs1414334 and ADRA2A 1291 C/G polymorphisms and lipid levels in obese patients

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Submitted

Abstract

Background

In previous studies associations were found between the *HTR2C* rs1414334 C/G and *ADRA2A* 1291 C/G polymorphisms and lipid levels in schizophrenic patients. The question arose to what extent antipsychotic drugs modify these associations.

Objective

To investigate if the *HTR2C* rs1414334 and *ADRA2A* 1291 C/G polymorphisms are associated with lipid levels in obese patients without antipsychotic drugs.

Methods

A cross-sectional design was used to investigate the association between carriership of variant alleles of the *HTR2C* rs1414334 and *ADRA2A* 1291 C/G polymorphisms and lipid levels in obese patients enrolled in an obesity clinic. Primary endpoint were concentrations (mmol/L) of HDL cholesterol, LDL cholesterol, total cholesterol and triglycerides in blood. Secondary endpoints were the HDL cholesterol and triglycerid ATPIIIa criteria. Primary determinants were genotypes of the rs1414334:C>G polymorphism within the X-linked *HTR2C* gene and the 1291 C/G (rs1800544) polymorphism located in the *ADRA2A* gene.

Results

In total, 130 obese patients were included. Mean age was 45 years (SD 12.3), and the majority (69%) was female. Patients carrying the variant *HTR2C* rs1414334 C-allele had higher triglyceride levels than patients without this allele ($\Delta_{triglyceride}$ +0.34 (95%CI 0.02 - 0.66) mmol/L). Furthermore, patients carrying the variant *ADRA2A* 1291 G-allele had lower levels of LDL cholesterol than patients without this allele (Δ_{LDL} -0.41 (95%CI -0.77 - -0.04**)** mmol/L). In a combined genotype analysis these associations became stronger when only one of the variant alleles was present. No other associations with lipid levels were found.

Conclusions

This study extends the evidence for an association between the *HTR2C* rs1414334 polymorphism and triglyceride levels independent of antipsychotic drugs. The association between *ADRA2A* and LDL cholesterol, and the interaction between the *HTR2C* and *ADRA2A* polymorphisms, requires further study.

Introduction

In previous studies we have found associations between the rs1414334 polymorphism in the gene coding for the $5HT_{2C}$-receptor (*HTR2C*) and the prevalence of metabolic syndrome in psychiatric patients using antipsychotic drugs. In a cross-sectional study with 112 schizophrenic inpatients using antipsychotic drugs, carriership of the variant C-allele of the rs1414334 polymorphism was associated with an increased prevalence of the metabolic syndrome (OR 4.09; 95%CI 1.41 - 11.89).[1] This result was replicated in a study with 186 inpatients using antipsychotics, in which carriership of the variant rs1414334 C-allele was also associated with an increased prevalence of the metabolic syndrome (OR 3.99; 95%CI 1.40 - 11.33).[2] The association between the rs1414334 polymorphism and the metabolic syndrome in the replication study appeared to be mainly caused by an association with lipid abnormalities, since there were trends for an increased risk of reaching the cutoff points for lowered High Density Lipoprotein (HDL) cholesterol (OR 2.47; 95%CI 0.95 - 6.42) and elevated triglyceride levels (OR 2.21; 95% CI 0.94 - 5.18) respectively, in patients carrying the variant rs1414334 C-allele.

The 1291 C/G polymorphism in the gene coding for the α_{2A} -receptor (*ADRA2A*) might also be relevant for metabolic effects, since several studies have found an association between this polymorphism and antipsychotic-induced weight gain.[3-5] Based on these studies, we investigated whether the 1291 C/G polymorphism was also associated with prevalence of the metabolic syndrome, since stimulation of the G-protein coupled α_{2A} adrenergic receptor has been associated with an inhibition of lipolysis. In a cross-sectional study with 470 psychiatric patients using antipsychotic drugs, we did not find a significant association between carriership of the variant 1291 G-allele and prevalence of the metabolic syndrome (OR 0.73; 95% CI 0.49 - 1.15).[6] However, carriership of the variant G-allele appeared to be protective for elevated triglyceride levels (>1.7 mmol/L)(OR 0.67; 95%CI 0.44 - 1.00, p=0.051).

If the reported trends and associations truly reflect a causative relation between the polymorphisms in the α_{2A} and $5HT_{2C}$ receptors and lipid values, these associations might also be present in patients without antipsychotic drugs. However, it is also possible that antipsychotic drugs or the underlying diseases are strong effect modifiers. Comparing the results found in an antipsychotic naïve population with the results found in antipsychotic-using populations might elucidate this potential modifying influence of antipsychotic drugs. The objective of this study was to investigate whether the *HTR2C* rs1414334 and *ADRA2A* 1291 C/G polymorphisms are associated with lipid values in a population without antipsychotic drugs.

Materials and Methods

Setting

Patients were recruited in the Wilhelmina Hospital Assen between July 2008 and March 2011. The Wilhelmina Hospital Assen is a medium-sized hospital in the Northern part of the Netherlands with several wards and 300 beds, covering a total population of approximately 100,000 persons. Patients were recruited from the obesity clinic located within the hospital. The study protocol was reviewed and approved by an independent medical ethics committee (Stichting BEBO, Assen, The Netherlands).

Design and patients

A cross-sectional design was used to assess the association between *HTR2C* and *ADRA2A* genotypes and lipid levels. These lipid levels were routinely measured in the application procedure for the obesity clinic. Patients were eligible for inclusion in this study if they participated in the obesity clinic, were 18 years or older and Caucasian. Patients using antipsychotic drugs or antidepressants at the time of inclusion were excluded from all analysis. Patients using statins were excluded from analyses regarding the association between *HTR2C* and *ADRA2A* polymorphisms and HDL and Low Density Lipoprotein (LDL) cholesterol levels. Patients using fibrates were excluded from analyses regarding the association between *HTR2C* and *ADRA2A* polymorphisms and triglyceride levels. After complete description of the study to the patients, informed consent was obtained and blood was drawn for genotyping.

Outcome measures

Primary endpoints of this study were concentrations (mmol/L) of HDL cholesterol, LDL cholesterol, total cholesterol and triglycerides in blood. Secondary endpoints were the HDL cholesterol and triglyceride criteria according to the Adult Treatment Panel IIIa (ATPIIIa), presented by the National Cholesterol Education Program (NCEP), as used to diagnose the metabolic syndrome: HDL cholesterol <1.0 mmol/L (male) or <1.3 mmol/L (female) or use of a statin, triglycerides ≥ 1.7 mmol/L or use of a fibrate.[7] Presence of the metabolic syndrome could not be chosen as a primary outcome because the group of patients not having the metabolic syndrome in the obesity clinic was too small.

Determinants

Primary determinants were genotypes of the rs1414334:C>G polymorphism within the X-linked *HTR2C* gene and 1291 C/G (rs1800544) polymorphism located in the *ADRA2A* gene. It should be noted with regard to *HTR2C* and *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 (HGVS; 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; www.ncbi.nlm.nih.gov/SNP). However, in Western and Northern Europeans, allele G appears to be the major allele, which is confirmed in this study. In the analysis we therefore considered the allele C as the variant allele.

DNA Isolation and genotyping

Genomic DNA was isolated from EDTA-anticoagulated peripheral blood using the TaqMan® Sample-to-SNP kit (article nr. 4403313, Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands). The *HTR2C* and *ADRA2A* genotypes were determined with allelic discrimination using the pre-developed assays C_7611979_10 (rs1800544) and C_7455701_10 (rs1414334), obtained from Applied Biosystems, according to the protocol supplied by the manufacturer. The reaction was carried out with TaqMan® universal polymerase chain reaction master mix in a StepOne Plus® real time polymerase chain reaction. Further information on genotyping procedures, including primer sequences and reaction conditions is available upon request.

Data analysis and statistics

Patient characteristics were transferred to a database (MS Access 2003) for analysis. The association between lipid values and *ADRA2A* and *HTR2C* genotype groups (presence or absence of the variant alleles) was investigated with linear regression. The results from these analyses are presented as differences (Δ) in lipid levels (mmol/L) between both genotype groups together with a 95% confidence interval (95%CI). The association between *ADRA2A* and *HTR2C* genotypes (presence or absence of the variant alleles) and the ATPIIIa criteria for HDL cholesterol and triglycerides was investigated with logistic regression. The strength of these associations was expressed as odds ratios (OR) together with a 95% confidence interval using the genotype with absence of the variant allele as a reference. Data were investigated for potential confounding effects of age and gender. These variables were included in the multivariate model if they were univariately associated with the primary endpoint at a significance level of $p < 0.20$.[8] Data were also investigated for interaction between genotype and gender for the X-linked *HTR2C* polymorphism and for interaction between the *ADRA2A* and *HTR2C* genotypes. A p-value of 0.05 or less was regarded as significant. The p-value was not adjusted 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.[9] Data were analysed using SPSS 17.0 (SPSS Inc, Chicago, Ill).

Results

In total, 130 obese patients were included in this study. Mean age was 45 years (SD 12.3), and 69% of these patients were female. Mean BMI was 41.6 kg/m^2 (SD 6.9) in the population and comparable between men (42.8 kg/m^2) and women (41.2 kg/m^2). Prevalence of the metabolic syndrome was 74%, based on the ATPIIIa criteria. Overall lipid values and the percentage of patients meeting the lipid ATPIIIa criteria are shown in Table 1.

Table 1. Lipid levels.

	Sample (n = 130) [a]	Concentration (mean (SD)) mmol/L
Lipid		
Total cholesterol	99	5.2 (1.0)
HDL-cholesterol	99	1.2 (0.3)
Males	31	1.0 (0.2)
Females	68	1.3 (0.3)
LDL-cholesterol	99	3.3 (0.9)
Triglycerides	118 [b]	1.7 (0.9)
Lipid ATPIIIa criteria	*Sample*	*Meeting criteria*
HDL [c]	121	57% (n = 69)
TG [d]	118	38% (n = 45)

a Number of patients may vary because of missing variables. Patients using statins were excluded.
b Including patients with statins.
c HDL-cholesterol < 1.0 mmol/L (male) or < 1.3 mmol/L (female) or use of a statin.
d Triglycerides ≥ 1.7 mmol/L or use of a fibrate.

Twenty-six patients used a statin and were excluded from linear regression on HDL and LDL lipid levels. Both gender and age were found to be confounding factors for lipid levels, and were therefore included as co-variables in the multivariate regression. Prevalence of carriership of variant alleles was 41% (n=53) for the *ADRA2A* 1291 C/G polymorphism (7.7% homozygous for variant G-allele), and 32% (n=41) for the *HTR2C* rs1414334 polymorphism (9.3% homozygous for variant C-allele). No interaction between genotype and gender was found (p = 0.76).

Genotype distribution of the polymorphisms did not deviate significantly from Hardy-Weinberg-equilibrium (HWE); *ADRA2A* 1291 C/G (p = 0.53) and rs1414334:C>G (calculated in women) (p = 1.0). There was no linkage disequilibrium between both polymorphisms ($r^2 = 0.02$, $D' = 0.11$).

The *HTR2C* rs1414334 polymorphism was significantly associated with triglyceride levels (Table 2). Patients carrying the variant rs1414334 C-allele had higher triglyceride levels than patients not carrying the variant rs1414334 C-allele ($\Delta_{triglyceride}$ +0.34 (95%CI 0.02 – 0.66) mmol/L). No associations with other lipid fractions were found. Table 2 also shows that the *ADRA2A* 1291 C/G polymorphism was associated with LDL cholesterol levels. Patients carrying the variant 1291 G-allele had lower levels of LDL cholesterol than patients not carrying the variant 1291 G-allele (Δ_{LDL} -0.41 (95%CI -0.77 - -0.04) mmol/L). No other associations with lipid levels were found.

Table 2. Association between the variant HTR2C rs1414334 C-allele and ADRA2A 1291 G-allele and lipid levels.

Determinant	Patients	*HTR2C* rs1414334 Carriership C-allele (mmol/L, 95%CI) [a,b]	*ADRA2A* 1291 Carriership G-allele (mmol/L, 95%CI) [a,b]
ΔTotal cholesterol	104	+0.17 (-0.27 - 0.60)	-0.39 (-0.80 – 0.02)
ΔHDL cholesterol	104	+0.05 (-0.06 - 0.17)	+0.00 (-0.11 - 0.11)
ΔLDL cholesterol	104	+0.02 (-0.37 - 0.41)	-0.41 (-0.77 - -0.04) [c]
ΔTG	130	+0.34 (0.02 – 0.66) [d]	+0.10 (-0.20 – 0.41)

a Data were analysed with the common genotype as the reference for both polymorphisms (rs1414334GG or 1291CC).
b Data were corrected for age and gender.
c p = 0.028, explaining 16% of the variation.
d p = 0.041, explaining 22% of the variation.

A combined genotype analysis showed that the association between the *ADRA2A* 1291 G-allele and LDL cholesterol became stronger in absence of the variant rs1414334 C-allele (ΔLDL cholesterol -0.77 (-1.21- -0.34) mmol/L, p = 0.001, explaining 17% of variation), whereas it was lost in the presence of the variant rs1414334 C-allele (ΔLDL cholesterol 0.24 (-0.4-0.88) mmol/L, p = 0.46). The same was seen for the association between the *HTR2C* rs1414334 C-allele and triglyceride levels, which became stronger in absence of the variant 1291 G-allele (ΔTG 0.44 (0.03-0.85) mmol/L, p = 0.034, explaining 24% of variation) and was lost in the presence of the variant 1291 G-allele (ΔTG 0.17 (-0.40 – 0.75) mmol/L, p = 0.54).

Table 3 shows that no associations were found between carriership of the variant *HTR2C* rs1414334 C-allele or *ADRA2A* 1291 G-allele and meeting the ATPIIIa criteria for HDL cholesterol and Triglyceride levels. A combined genotype analysis did not show other results.

Table 3. Association between the variant HTR2C rs1414334 C-allele and ADRA2A 1291 G-allele and the ATPIIIa criteria for HDL cholesterol and Triglycerides.

Determinant [a]	Patients	Crude OR (95% CI, p)	Adjusted [b] OR (95% CI, p)
HTR2C rs1414334 Carriership C-allele			
HDL	121	1.23 (0.57-2.69, 0.60)	1.22 (0.55-2.74, 0.62)
TG	118	0.92 (0.42-2.05, 0.84)	1.41 (0.58-3.44, 0.45)
ADRA2A 1291 C/G Carriership G-allele			
HDL	121	1.19 (0.57-2.50, 0.65)	1.20 (0.57-2.52, 0.63)
TG	118	1.07 (0.50-2.29, 0.86)	1.07 (0.47-2.45, 0.87)

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.
b Data were adjusted for age and gender, with the common genotype (rs1414334GG or 1291CC) as the reference for both polymorphisms.

Discussion

In this study we found an association between the *HTR2C* rs1414334 polymorphism and triglyceride levels in obese patients. Triglyceride levels were significantly higher (0.34 mmol/L (95% CI; 0.02 – 0.66)) in the group of patients carrying the variant rs1414334 C-allele. Furthermore, an association was found between the *ADRA2A* 1291 C/G polymorphism and LDL cholesterol levels. Patients carrying the variant 1291 G-allele had significantly lower levels of LDL cholesterol (Δ -0.41 mmol/L (95% CI; -0.77 - - 0.04)) than patients not carrying this allele. Moreover, the associations between the rs1414334 C-allele and triglyceride levels or 1291 G-allele and LDL cholesterol became stronger in absence of the other investigated variant allele (ΔTG 0.44 (0.03-0.85) mmol/L and ΔLDL -0.77 (-1.21- -0.34) mmol/L respectively) showing the presence of a potential gene-gene interaction. No other associations with lipid levels were found. The analysis for an association between carriership of the variant alleles of both polymorphisms and meeting the ATPIIIa criteria for HDL cholesterol and triglycerides yielded negative results as well.

There are some limitations to these results. First, the sample size of this study may have limited the power to detect differences between groups that are only moderate in size, explaining why some of the previously found trends were not replicated. Second, we recognize that the cross-sectional design is an important limitation. It is possible that measures have been taken to lower lipid levels prior to entry in this study. If carriership of the variant rs1414334 C-allele is truly associated with elevated lipid levels, patients carrying this allele could have been more prone to pre-inclusion measures to lower these values. The opposite could have been the case for patients carrying the (protective) variant 1291 G-allele. As a result, differences between genotype groups, and therefore also chances of finding significant associations, would become smaller. Third, some variables contributing to lipid levels, e.g. smoking behaviour, coffee, exercise and diet, were not taken into account and could have influenced the results. However, there are no known associations between *HTR2C* and *ADRA2A* genotypes and these variables so there is no reason to assume that these variables were not evenly distributed between the genotype groups.

Comparing the results from this study with the results from our studies in schizophrenic patients using antipsychotic drugs shows that the association between carriership of the variant rs1414334 C-allele and elevated triglyceride concentrations has been replicated but the association between carriership of the variant 1291 G-allele and reduced triglyceride levels has not. Therefore, the association between the rs1414334 polymorphism and triglyceride levels seems to be independent of antipsychotic drug use. The mechanism behind this association is unclear. A possible explanation for could be that the rs1414334 polymorphism is in LD with a polymorphism involved in the regulation of the enzymes responsible for triglyceride lipolysis, like hormone-sensitive lipase, adipose triglyceride lipase or triacylglyceride hydrolase.[10] Patients

carrying the rs1414334 C-allele could have less functional enzymes involved in triglyceride lipolysis, thereby explaining the higher triglyceride levels in these patients. Further studies are needed to investigate this hypothesis.

An important question regarding our current findings is: why are the trends previously seen in our schizophrenic patient population, between the *HTR2C* rs1414334 polymorphism and HDL cholesterol or *ADRA2A* 1291C/G polymorphism and triglyceride levels, not present in this study? One of the reasons for this discrepancy could be the use of (atypical) antipsychotic drugs, since these drugs are known to frequently elevate lipid levels. If the impact of a certain genotype on lipid levels in a situation without antipsychotic drugs is present but small, it could go undetected. However, these small differences could become bigger when the genotype also modifies to what extent antipsychotic drugs elevate the lipid levels, thereby increasing the differences between genotype groups and exposing the association. The reverse could also be true when the rise in lipid levels caused by antipsychotic drugs is large, and the genotype does not modify the extent of the rise in lipid levels caused by antipsychotic drugs. In such a scenario small differences could be overrun by a large rise in lipid levels. The association between *ADRA2A* genotype and LDL cholesterol found in this study could possibly serve as an example for this since reanalyzing our previous data in a schizophrenic population for this association yielded negative results (data not shown). Moreover, since gene-gene interactions are likely to be present, as has also been shown in this study, presence of antipsychotic drugs might modify the impact of these gene-gene interactions on study results by their complex pharmacologic profile. Another explanation could be the gender composition of the population. In our study in schizophrenic patients approximately two-thirds of the population consisted of men, whereas in the current study two-thirds of the population was female. Despite corrections for gender this could still lead to discrepancies in results because of smaller genotype groups in one gender. For instance, the association between carriership of the variant *ADRA2A* 1291 G-allele and lower levels of LDL cholesterol was mainly caused by the female patients (female Δ_{LDL} -0.50 mmol/L [95% CI -0.95 - -0.04, p=0.034] vs male Δ_{LDL} -0.24 mmol/L [95% CI -0.86 - 0.37, p=0.43]). This lack of association in men could be caused by the smaller group size but could also indicate that this association is mainly present in women. One of the possible explanations for this could be the fact that oestrogen is known to regulate the α_{2A}-receptor and its effects on lipolysis.[11] Since the group of female patients in our schizophrenic population was relatively small, a possible association could have remained undetected. A final explanation that cannot be ruled out at this point is that some of the previous associations or trends, or even the current results, are actually type I errors. Therefore more replication studies are needed to confirm the results found.

In conclusion, this study extends the evidence for an association between carriership of the variant *HTR2C* rs1414334 C-allele and elevated triglyceride levels, which seems to be independent of antipsychotic drugs. Studies are needed to test if this association is caused by

an impact on the enzymatic system involved in lipolysis. Furthermore, studies are also needed to confirm the association between the *ADRA2A* polymorphism and LDL cholesterol and other possible associations between the *HTR2C* and *ADRA2A* polymorphisms and lipid values as well as the interaction between these genes found in this study.

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References

1. Mulder H, Franke B, van der-Beek van der Aart A, Arends J, Wilmink FW, Scheffer H, et al. The association between HTR2C gene polymorphisms and the metabolic syndrome in patients with schizophrenia. J Clin Psychopharmacol 2007;27:338-343.
2. Risselada AJ, Vehof J, Bruggeman R, Wilffert B, Cohen D, Al Hadithy AF, et al. Association between HTR2c gene polymorphisms and the metabolic syndrome in patients using antipsychotics: a replication study. Pharmacogenomics J 2010; doi:10.1038/tpj.2010.66.
3. Sicard MN, Zai CC, Tiwari AK, Souza RP, Meltzer HY, Lieberman JA, et al. Polymorphisms of the HTR2C gene and antipsychotic-induced weight gain: an update and meta-analysis. Pharmacogenomics 2010;11:1561-1571.
4. Wang YC, Bai YM, Chen JY, Lin CC, Lai IC, Liou YJ: Polymorphism of the adrenergic receptor alpha 2a-1291C>G genetic variation and clozapine-induced weight gain. J Neural Transm 205;112:1463-1468.
5. Park YM, Chung YC, Lee SH, Lee KJ, Kim H, Byun YC, et al. Weight gain associated with the α2a-adrenergic receptor 1291C/G polymorphism and olanzapine treatment. Am J Med Gen Part B (Neuropsychiatric Genetics) 2006;141B:394-397.
6. Risselada AJ, Vehof J, Bruggeman R, Wilffert B, Cohen D, Al Hadithy AF, et al. Association between the 1291-C/G polymorphism in the adrenergic alfa-2a receptor and the metabolic syndrome. J Clin Psychopharmacol 2010;30:667-671.
7. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, et al. Diagnosis and management of the metabolic syndrome: an american heart association/national heart,lung, and blood institute scientific statement. Circulation 2005;112:2735-2752.
8. Maldonado G, Greenland S. Simulation study of confounder-selection strategies. Am J Epidemiol 1993;138:923-936.
9. Perneger TV. What's wrong with Bonferroni adjustments. Br Med J 1998;316:1236-1238.
10. Duncan RE, Ahmadian M, Jaworski K, Sarkadi-Nagy E, Sul HS. Regulation of lipolysis in adipocytes. Annu Rev Nutr 2007;27:79-101.
11. Pedersen SB, Kristensen K, Hermann PA, Katzenellenbogen JA, Richelsen B. Estrogen controls lipolysis by up-regulating alfa2A-adrenergic receptors directly in human adipose tissue through the estrogen receptor alfa. Implications for the female fat distribution. J Clin Endocrinol Metab 2004;89:1869-1878.

Chapter 3.3

Combined HTR2C-LEP and HTR2C-LEPR genotypes as a determinant for obesity

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Submitted

Abstract

Background

In previous studies interactions were found between the leptin and the serotonergic system regarding prevalence of obesity in schizophrenic patients. The question arose to what extent antipsychotic drugs modify the effects of this interaction and if this interaction is also visible in patients without antipsychotic drugs.

Objective

To investigate whether prevalence of combined *HTR2C-LEP* and *HTR2C-LEPR* genotypes differs between obese and non-obese patients and investigate if the interaction previously found in schizophrenic patients is also present in patients without psychotropic drugs.

Methods

A cross-sectional case-control design was used to assess the association between combinations of the *HTR2C-LEP* and *HTR2C-LEPR* genotypes and obesity. Adult Caucasian patients with obesity (BMI > 30 kg/m^2) were recruited from an obesity clinic located within the hospital. Adult Caucasian patients with a normal body weight (BMI < 25 kg/m^2) were recruited on the orthopaedic ward and among the employees of the hospital pharmacy. Primary endpoints were differences in frequencies of the investigated genotype-combinations between cases and controles. Primary determinants were the rs1137101 (Q223R, Gln223Arg or 668A/G) polymorphism in the *LEPR* gene, the rs7799039 (2548G/A) polymorphism in the *LEP* gene, and the rs3813929 (759C/T) and rs1414334 polymorphisms in the *HTR2C* gene.

Results

In total, 216 patients were included in this study, consisting of 116 cases (BMI > 30 kg/m^2) and 100 controls (BMI < 25 kg/m^2). Mean age in the case-group was significantly lower compared to the control-group (45.7 (SD 12.5) vs. 65.1 (SD 15.0) years, respectively, $p < 0.0001$). Two-thirds of the patients in both groups were women. There was an overall lower prevalence of the *HTR2C* 759T allele in obese patients compared to patients with a normal body weight regardless of *LEP* or *LEPR* genotypes. No significant interaction was found between the *HTR2C* polymorphisms and the *LEP* or *LEPR* polymorphisms regarding prevalence of obesity.

Conclusions

No significant interactions were found between the investigated polymorphisms within the serotonergic and leptin systems in patients without antipsychotic drugs.

Introduction

Obesity is an important problem in modern society because of its association with cardiovascular disease, type 2 diabetes mellitus (DM), and hypertension among others, resulting in an increase in overall mortality.[1,2] Obesity can also negatively influence self-image, social functioning and physical activity. Furthermore, there is a clear heritability of obesity within families, with first-degree relatives of obese patients having an up to nine-fold increased risk for developing obesity, which suggests that genetic make-up plays a role in the etiology of obesity.[3] Eleven different genes have already been associated with Mendelian forms of human obesity and more than 52 genes have been shown to cause obesity in knockout or transgenic mice.[4] Among these genes are the genes coding for leptin (*LEP*), the leptin receptor (*LEPR*) and the $5HT_{2C}$ receptor (*HTR2C*). Apart from genetic determinants there are also other factors (e.g. environmental factors) that explain an increased prevalence of obesity in some populations. One of these populations is formed by schizophrenic patients, because of the weight gain frequently caused by antipsychotic drugs. The *LEP*, *LEPR* and *HTR2C* genes that have been associated with obesity also appear to be relevant for these patients, since polymorphisms within these genes have been associated with antipsychotic-induced weight gain and obesity.

In particular the *HTR2C* 759 C/T polymorphism (rs3813929) has repeatedly been associated with antipsychotic-induced weight gain; summarized in two meta-analyses that showed a protective effect of the variant 759T allele.[5,6] Carriership of the variant rs1414334 C allele of the *HTR2C* rs1414334 polymorphism has been associated with an increased risk of obesity,[7] although this association could not be confirmed in two other studies.[8,9] Most studies investigating the association between polymorphisms in the leptin system and antipsychotic-induced weight gain focussed on the *LEP* 2548 A/G polymorphism (rs7799039). The G allele of this *LEP* 2548A/G polymorphism has been shown to increase susceptibility to weight gain in several studies,[10-12] although some studies could not confirm this association.[13-15] The Q223R polymorphism in the *LEPR* gene (rs1137101) has also been investigated for an association with antipsychotic induced weight gain, but no association was found.[14]

However, since weight gain and obesity have a multi factorial origin, it is likely that various gene-gene interactions play a role in their etiology and have to be taken into account. Both the studies by Yevtushenko et al. and Gregoor et al. found an interaction between the *HTR2C* 759 C/T and *LEP* 2548 A/G polymorphisms and body weight in schizophrenic patients.[16,17] Patients without the variant 759T allele carrying the 2548G allele had a higher BMI than patients without the 2548G allele carrying the 759T allele in the study by Yevtushenko et al. (29.89 kg/m^2 vs. 26.09 kg/m^2 respectively; $p=0.021$) and an increased risk for obesity in the study by Gregoor et al. (OR 2.88; 95%CI 1.05 - 7.95). The study by Gregoor et al. also included the *HTR2C* rs1414334 and *LEPR* Q223R polymorphisms, but no other interactions were found.

A question that arose is to what extent antipsychotic drugs modify the effects of this interaction between the leptin and serotonergic system and if this interaction is also visible in patients without antipsychotic drugs. Therefore, the primary objective of this study was to investigate whether prevalence of combined *HTR2C-LEP* and *HTR2C-LEPR* genotypes differs between obese and non-obese patients and investigate if the interaction found by Yevtushenko et al. and Gregoor et al. is also present in patients without psychotropic drugs.

Materials and Methods

Setting, design and study population

Patients were recruited in the Wilhelmina Hospital Assen in the Northern part of the Netherlands between July 2008 and January 2011. The Wilhelmina Hospital Assen is a medium-sized general hospital covering a total population of approximately 100,000 persons. In this study population we have previously investigated the association between the *HTR2C* polymorphisms and obesity.[18] A cross-sectional case control design was used to assess the association between *HTR2C-LEP* and *HTR2C-LEPR* genotype combinations and obesity. Obesity was classified by a cutoff value ($\geq$30 kg/m^2) for the Body Mass Index (BMI), which is calculated by dividing the body weight in kilograms by the square of the height in meters. Patients with obesity (cases) were recruited from the obesity clinic located within the hospital. Exercise and changing eating behaviour are the keystones of the program within this clinic. Cases were eligible for inclusion if they had a BMI$>$30 kg/m^2, were 18 years or older and of Caucasian descent. Patients using weight increasing drugs or drugs with $5HT_{2C}$ agonistic or antagonistic properties (antipsychotics and antidepressants) were excluded. Patients with a normal body weight (controls) were primarily recruited on the orthopaedic ward. Some of the controls were recruited among the employees of the hospital pharmacy. Controls were eligible for inclusion in this study if they had a BMI $<$25 kg/m^2, were also 18 years or older and were of Caucasian descent. Smoking, cancer, following a diet, use of weight reducing drugs, and excessive drug or alcohol use were considered as exclusion criteria for the controls. After complete description of the study to the patients, informed consent was obtained and blood was drawn for genotyping. The study protocol was reviewed and approved by an independent medical ethics committee (Stichting BEBO, Assen, The Netherlands).

Determinants

Primary determinants were combined *HTR2C-LEP* and *HTR2C-LEPR* genotypes. The following polymorphisms were investigated: rs1137101 (Q223R, Gln223Arg or 668A/G) in the *LEPR* gene on chromosome 1p31; rs7799039 (2548G/A) in the promoter region of the *LEP* gene on chromosome 7q31.3; rs3813929 (759C/T) in the promoter region of the X-linked *HTR2C* gene, and rs1414334, a polymorphism in intron 5 close to the '3 UTR of the *HTR2C* gene. In accordance with previous studies, the rs1414334 C allele was considered as the variant allele.

DNA Isolation and genotyping

Genomic DNA was isolated from EDTA-anticoagulated peripheral blood using the TaqMan® Sample-to-SNP kit (article nr. 4403313, Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands). The *HTR2C*, *LEP*, and *LEPR* genotypes were determined with allelic discrimination using the pre-developed assays C_27488117_10 (rs3813929), C_7455701_10 (rs1414334), C_8722581_10 (rs1137101) and C_1328079_10 (rs7799039) obtained from Applied Biosystems, according to the protocol supplied by the manufacturer. The reaction was carried out with TaqMan® universal polymerase chain reaction master mix in a StepOne Plus® real time polymerase chain reaction. Further information on genotyping procedures, including primer sequences and reaction conditions is available upon request.

Data analysis

Patient characteristics were transferred to a database (MS Access 2003) for analysis. Differences between cases and controls regarding frequencies of *HTR2C-LEP* and *HTR2C-LEPR* genotype combinations were investigated using logistic regression and expressed as odds ratios (OR) with a 95% confidence interval (95%CI). Data were investigated for potential confounding effects of age and gender. Variables were included in the multivariate model if they were univariately associated with the primary endpoint obesity at a significance level of $p<0.20$.[19] Data were also investigated for interaction between genotype and gender. A p-value of 0.05 or less was regarded as significant. The p-value was not adjusted 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.[20] Data were analysed using SPSS 17.0 (SPSS Inc, Chicago, Ill).

Results

In total, 216 patients were included in this study, consisting of 116 cases (BMI > 30 kg/m^2) and 100 controls (BMI < 25 kg/m^2). Mean age in the case-group was significantly lower compared to the control-group (45.7 (SD 12.5) vs. 65.1 (SD 15.0) years respectively, $p<0.0001$). Two-thirds of the patients in both groups were women. Prevalence of diabetes was 20% (n=23) in the case group. No patients with diabetes were present in the control group, because these patients often had a BMI > 25 kg/m^2 or used metformin, which was regarded as a weight reducing agent. Smoking was reported by 3% (n=3) of the case group, compared to 0% in the control group (exclusion criterion). Genotype distribution of the polymorphisms did not deviate significantly from Hardy-Weinberg-equilibrium (HWE, calculated in women) (rs3813929 (-759 C/T) ($p=0.95$) and rs1414334:C>G [$p=0.83$]). No significant linkage disequilibrium was found between the polymorphisms ($r^2<0.1$). Gender was not found to be a confounding factor ($p=0.36$) and no interaction between genotype and gender was found for the *HTR2C* polymorphisms ($p=0.72$). The frequencies of genotype combinations combined with prevalence of obesity are shown in Table 1.

Table 1. HTR2C-LEP and HTR2C-LEPR genotype combinations as a determinant of obesity.

		LEPR 223R allele		LEP 2548G allele	
		Absent	Present	Absent	Present
HTR2C-759 T allele					
Absent	% Obese	28/42 (67%)	65/123 (53%)	21/35 (60%)	72/130 (55%)
	OR	Ref	0.43 (0.17-1.06)	Ref	0.94 (0.38-2.33)
Present	% Obese	6/17 (35%)	17/34 (50%)	3/7 (43%)	20/44 (45%)
	OR	0.22 (0.05-0.97)	0.31 (0.1-0.998)	0.28 (0.04-2.0)	0.56 (0.19-1.68)
HTR2C-Rs1414334 C allele					
Absent	% Obese	26/48 (54%)	55/113 (49%)	18/31 (58%)	63/130 (48%)
	OR	Ref	0.67 (0.29-1.55)	Ref	0.68 (0.27-1.76)
Present	% Obese	8/11 (73%)	27/44 (61%)	6/11 (55%)	29/44 (66%)
	OR	2.26 (0.44-11.5)	0.97 (0.34-2.78)	0.44 (0.09-2.31)	1.54 (0.49-4.85)

* Data were corrected for age.

Table 1 shows that prevalence of obesity was lower in patients carrying the *HTR2C* 759 T allele regardless of the *LEPR* and *LEP* genotypes. Prevalence of obesity was lowest in the group of patients carrying the 759T allele without presence of the 223R allele (35%), which was significantly less compared to the reference combination (67%) (OR 0.22; 95%CI 0.05 - 0.97). Presence of the 223R allele in patients carrying the variant 759T allele non-significantly ($p=0.67$) increased the prevalence of obesity in this group to 50%, but this was still significantly less compared to the reference combination (OR 0.31; 95%CI 0.1 - 0.998). The interaction between the *HTR2C* 759 C/T and *LEP* 2548 A/G polymorphisms, previously found by Gregoor et al. and Yevtushenko et al., was not present in this study. There were also no associations found with any of the *HTR2C* rs1414334 polymorphism combinations.

Discussion

The results from this study showed an overall lower prevalence of the *HTR2C* 759T allele in obese patients compared to patients with a normal body weight regardless of *LEP* or *LEPR* genotypes. The *HTR2C* 759C/T polymorphism therefore appears to be dominating the effects of the other investigated polymorphisms regarding prevalence of obesity. Prevalence of obesity was significantly lower in patients carrying the variant *HTR2C* 759T allele in absence of the *LEPR* 223R allele compared to patients without the 759T allele and 223R allele (OR 0.22; 95%CI 0.05 - 0.97). Prevalence of obesity was also significantly lower in patients carrying the variant *HTR2C* 759T allele combined with the *LEPR* 223R allele compared to patients without the 759T allele and the 223R allele (OR 0.31; 95%CI 0.1 - 0.998). Presence of the *LEPR* 223R allele appeared to have some effect on prevalence of obesity in patients carrying the variant *HTR2C* 759T allele, but the difference in prevalence of obesity between the genotype groups was not significantly different (35% vs. 50%, $p=0.67$). Therefore no significant interaction

was found between the *HTR2C* 759C/T polymorphism and the *LEPR* 223QR polymorphism. Furthermore, there was also no visible influence of the *LEP* 2548 AG genotype on prevalence of obesity in patients with or without the variant 759T allele, contrary to the results from previous studies in schizophrenic patients.[16,17] An interaction between the *HTR2C* rs1414334 polymorphism and *LEP* or *LEPR* genotypes regarding prevalence of obesity was not found either. Therefore we were unable to find any interaction between the investigated polymorphisms within the serotonergic and leptin systems regarding prevalence of obesity in patients without antipsychotic drugs.

We realize that the cross-sectional design of this study is an important limitation because information about a person's body weight in the past is missing. Therefore it is possible that patients being obese in the past lost a lot of weight and were included in the control-group. However, this would only have had a negative influence on the results found, since differences between groups would become smaller. It is possible however that this partly contributed to the fact that we did not find significant results for the combinations with the *LEP* and LEPR genotypes. The power of this study is also a limitation due to relatively small genotype groups in some of the genotype combinations. This could explain why no significant interaction was found between the *HTR2C* 759 C/T and *LEPR* 223QR polymorphisms despite a 15% difference in prevalence of obesity between 223QR genotype groups in patients carrying the variant *HTR2C* 759T allele. Another limitation of this study is that we were unable to correct for exercise. It is plausible that patients with a normal BMI exercise more than patients with obesity. However, there is no known association between the studied genes and exercise, and therefore this doesn't explain the differences in prevalence of the genotype combinations between both groups. Also, the obese patients were significantly younger than the patients with a normal BMI. Since both physical activity and basal metabolic rate decrease with age, this might compensate for more exercise in the group of patients with a normal BMI. Moreover, most patients with a normal BMI were included on the orthopaedic ward, waiting for a knee or hip replacement. This makes it less likely that these patients were more physically active on the moment of inclusion, and the months before that, than the obese patients.

Comparing the results from this study with the results from our previous study in this population suggests that adding *LEP* or *LEPR* genotypes to the *HTR2C* genotypes does not add additional information in relation to identifying genotype associated risk groups for obesity. This is in contrast with the studies from Gregoor et al. and Yevtushenko et al. A big difference between the studies from Gregoor et al. and Yevtushenko et al. and this study is the type of patient population in which the effects of the genotype combinations were studied. Since obesity has a multiple factorial origin, differences in gene-gene interactions or gene-environment interactions may account for the discrepancies between those studies and this one. For example, the weight gain and obesity caused by antipsychotic drugs might have a different etiology than the obesity in patients without antipsychotic drugs. It is also possible that antipsychotic drugs

increase the effects of the investigated genotype combinations, possibly by interacting with the leptin or histamine system, making it easier to observe differences between genotype groups in schizophrenic populations.

In conclusion, no significant interactions were found between the investigated polymorphisms within the serotonergic and leptin systems in patients without antipsychotic drugs. Replication studies in larger populations are needed to confirm these results.

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References

1. Field AE, Coakley EH, Must A, Spadano JL, Laird N, Dietz WH, et al. Impact of overweight on the risk of developing common chronic diseases during a 10-year period. Arch Intern Med 2001;161:1581-1586.
2. Must A, Spadano J, Coakley EH, Field AE, Colditz G, Dietz WH. The disease burden associated with overweight and obesity. JAMA 1999;282:1523-1529.
3. Price RA, Lee JH. Risk ratios for obesity in families of obese African-American and Caucasian women. Hum Hered 2001;51:35-40.
4. Rankinen T, Zuberi A, Chagnon YC, Weisnagel SJ, Argyropoulos G, Walts B, et al. The human obesity gene map: the 2005 update. Obesity 2006;14:529-644.
5. De Luca V, Mueller DJ, de Bartolomeis A, Kennedy JL. Association of the HTR2C gene and antipsychotic-induced weight gain: a meta-analysis. Int J Neuropsychopharmacol 2007;10(5):697-704.
6. Sicard MN, Zai CC, Tiwari AK, Souza RP, Meltzer HY, Lieberman JA, et al. Polymorphisms of the HTR2C gene and antipsychotic-induced weight gain: an update and meta-analysis. Pharmacogenomics 2010;11:1561-1571.
7. Mulder H, Franke B, van der Beek-van der AA, Arends J, Wilmink FW, Egberts AC, et al. The association between HTR2c polymorphisms and obesity in psychiatric patients using antipsychotics: a cross-sectional study. Pharmacogenomics J 2007;7(5):318-324.
8. Mulder H, Cohen D, Scheffer H, Gispen-de Wied C, Arends J, Wilmink FW, et al. HTR2C gene polymorphisms and the metabolic syndrome in patients with schizophrenia: a replication study. J Clin Psychopharmacol 2009;29:16-20.
9. Risselada AJ, Vehof J, Bruggeman R, Wilffert B, Cohen D, Al Hadithy AF, et al. Association between HTR2c gene polymorphisms and the metabolic syndrome in patients using antipsychotics: a replication study. Pharmacogenomics J 2010; doi:10.1038/tpj.2010.66.
10. Templeman LA, Reynolds GP, Arranz B, San L. Polymorphisms of the 5HT2c receptor and leptin genes are associated with antipsychotic drug-induced weight gain in Caucasian subjects with a first-episode psychosis. Pharmacogenetics and Genomics 2005;15:195-200.
11. Kang SG, Lee HJ, Park YM, Choi JE, Han C, Kim YK, et al. Possible association between the -2548A/G polymorphism of the leptin gene and olanzapine-induced weight gain. Progress in Neuro-Psychopharmacol & Biol Psych 2008;32:160-163.

12. Zhang XY, Tan YL, Zhou DF, Haile CN, Cao LY, Xu Q, et al. Association of clozapine-induced weight gain with a polymorphism in the leptin promoter region in patients with chronic schizophrenia in a chinese population. J Clin Psychopharmacol 2007;27:246-251.
13. Opgen-Rhein C, Brandl EJ, Müller DJ, Tiwari AK, Sander T, Dettling M: Association of HTR2C, but not LEP or INSIG2. genes with antipsychotic-induced weight gain in a German sample. Pharmacogenomics 2010;11:773-780.
14. Perez-Iglesias R, Mata I, Amado JA, Berja A, Garcia-Unzueta MT, Martinez Garcia O, et al. Effect of FTO, SH2B1, LEP, and LEPR polymorphisms on weight gain associated with antipsychotic treatment. J Clin Psychopharmacol 2010;30:661-666.
15. Ellingrod VL, Bishop JR, Moline J, Lin YC, Miller DD. Leptin and leptin receptor gene polymorphisms and increases in body mass index (BMI) from olanzapine treatment in persons with schizophrenia. Psychopharmacol Bulletin 2007;40(1):57-62.
16. Yevtushenko OO, Cooper SJ, O'Neill R, Doherty JK, Woodside JV, Reynolds GP. Influence of 5-HT2c receptor and leptin gene polymorphisms, smoking and drug treatment on metabolic disturbances in patients with schizophrenia. Br J of Psychiatry 2008;192:424-428.
17. disturbances in patients with schizophrenia. Br J Psychiatry 2008;192:424-428.
18. Gregoor JG, Mulder H, Cohen D, van Megen HJGM, Egberts ACG, Heerdink ER, et al. Combined HTR2C-LEP genotype as a determinant of obesity in patients using antipsychotic medication. J Clin Psychopharmacol 2010;30:702-705.
19. Risselada AJ, Mulder H, Heerdink ER, Gonera RK, Egberts ACG. Association between HTR2C polymorphisms and obesity in non-psychiatric patients. J Clin Psychopharmacol 2011 (Submitted).
20. Maldonado G, Greenland S. Simulation study of confounder-selection strategies. Am J Epidemiology 1993;138:923-936.
21. Perneger TV. What's wrong with Bonferroni adjustments. Br Med J 1998;316:1236-1238.

Chapter 3.4

Association between ADRA2A and HTR2C polymorphisms and weight loss or lipid levels in obese patients: an interim analysis

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Rob K. Gonera
Toine C.G. Egberts

Ongoing study.

Abstract

Background

In previous studies, associations were found between the *HTR2C* 759 C/T polymorphism and obesity. An association between this polymorphism and weight loss during an anti obesity program has also been found. Furthermore, in a previous cross-sectional study an association was found between polymorphisms in the *ADRA2A* and *HTR2C* genes and lipid levels in obese patients.

Objective

To investigate whether the *HTR2C* 759 C/T polymorphism modifies weight loss in obese patients during the first three months of an anti-obesity program and whether the *HTR2C* rs1414334 and *ADRA2A* 1291 C/G polymorphisms modify changes in lipid values during that period.

Methods

A longitudinal follow-up study was used to assess the association between *HTR2C* and *ADRA2A* genotypes and weight loss or lipid levels. Adult Caucasian patients with obesity, participating in the anti obesity program, were eligible for inclusion in this study. Primary endpoints of this study were changes in body weight (change in BMI (%)/month) and lipid levels (HDL, LDL, total cholesterol and triglycerides (mmol/l)). Primary determinants were genotypes of the rs3813929:C>T (-759 C/T) and rs1414334:C>G polymorphisms within the X-linked *HTR2C* gene and the 1291 C/G (rs1800544) polymorphism within the *ADRA2A* gene.

Results

In total, 76 patients were included in this interim analysis, consisting of 15 men and 61 women. Mean age at inclusion was 45 years (SD 11), and mean BMI at inclusion was 41.1 kg/m^2 (SD 6.0) for women and 43 kg/m^2 (SD 10.2) for men. No associations were found in this interim analysis between carriership of the variant *HTR2C* 759 T-allele and weight loss during the anti obesity program or carriership of the variant rs1414334 C-allele or *ADRA2A* 1291 G-allele and changes in lipid values.

Conclusions

No evidence was found for an effect of the investigated *HTR2C* and *ADRA2A* polymorphisms on efficacy of an anti obesity program regarding weight loss and changes in lipid levels in obese patients in the first three months of that program.

Introduction

Obesity is an important problem in modern society. Obesity has been associated with an increased incidence and worse prognosis of cardiovascular disease (hypertension, dyslipidemia, stroke), type 2 diabetes mellitus (DM), obstructive sleep apnea syndrome, polycystic ovarian syndrome, osteoarthritis, some forms of cancer and subsequently an increase in overall mortality.[1,2] In addition, obesity can have psychosocial consequences since it can negatively influence self-image, social functioning and physical activity. Obesity is often classified by a cutoff value (usually ≥ 30 kg/m^2) for the Body Mass Index (BMI), which is calculated by dividing the body weight in kilograms by the square of the height in meters. During the past few decades there has been a worldwide steep increase in the prevalence of obesity. Changes in eating behaviour, including a global shift in diet towards increased intake of energy-dense foods high in fat and sugar, are an important cause for this increase. The increase in prevalence of obesity gave rise to the foundation of obesity clinics to address this growing problem and to help people lose weight. Unfortunately, treatment of obesity within these clinics is not always successful. Some patients are able to lose weight more easily in anti-obesity programs than others, possibly because they are better able to change eating behaviour.

Several studies have shown that the serotonin $5HT_{2c}$-receptor might play an important role in modifying this eating behaviour, because $5HT_{2c}$ knock-out mice become hyperphagic and obese and $5HT_{2c}$-agonists reduce appetite in humans.[3,4] Currently, selective $5HT_{2c}$-agonistic drugs are being developed and tested as weight reducing agents in obese patients.[5] Interestingly, the study by Pooley et al. showed that weight loss during an anti-obesity program might be modified by the 759 C/T polymorphism within the *HTR2C* gene coding for this $5HT_{2C}$-receptor. Patients with the *HTR2C* 759 C/T genotype showed less weight loss than patients with the 759 C/C and T/T genotype.[6] Moreover, prevalence of the variant 759 T-allele or 759 T/T genotype was lower in obese patients in the study by Yuan et al. and Risselada et al. respectively and was also associated with less antipsychotic-induced weight gain in psychiatric patients in two meta-analyses.[7-10]

Dyslipidemia which is often present in obese patients, also appears to be mediated by genetic variation. In a previous study we have found the *HTR2C* rs1414334 polymorphism to be associated with triglyceride levels, and the *ADRA2A* 1291 C/G polymorphism to be associated with LDL cholesterol levels in obese patients.[11] Carriership of the variant rs1414334 C-allele was associated with elevated triglyceride levels, whereas carriership of the variant 1291 G-allele was associated with reduced LDL-cholesterol levels. The objective of this study was to investigate whether the *HTR2C* 759 C/T polymorphism modifies weight loss in obese patients during the first three months of an anti-obesity program and whether the *HTR2C* rs1414334 and *ADRA2A* 1291 C/G polymorphisms modify changes in lipid values during the first three months of that program.

Materials and Methods

Setting

Patients were recruited in the Wilhelmina Hospital Assen in the Northern part of the Netherlands. Inclusion of patients for this interim analysis took place between July 2008 and March 2011, but is still ongoing. The Wilhelmina Hospital Assen is a medium-sized hospital, covering a total population of approximately 100,000 persons. Patients were recruited from the obesity clinic located within the hospital. The obesity clinic offers a nine-month program for obese patients in which they are closely monitored and treated by a team consisting of an internist, psychologist, physiotherapist, dieticians and nurses. Exercise and eating behaviour, including a very low calorie diet, are the keystones of the program. The study protocol was reviewed and approved by an independent medical ethics committee (Stichting BEBO, Assen, The Netherlands).

Design and patients

A longitudinal follow up study was used to assess the association between *HTR2C* and *ADRA2A* genotypes and weight loss or lipid levels. The majority of patients (>80%) was prospectively included, but 12 patients were retrospectively included (data from 2006-2008). Data was extracted from the patient medical records. The patients were part of a study population previously used for a cross-sectional analysis for an association between *HTR2C* and *ADRA2A* polymorphisms and lipid levels.[11] Patients were eligible for inclusion in this study if they participated in the obesity clinic, were 18 years or older and Caucasian. Patients using antipsychotic drugs or antidepressants at the time of inclusion were excluded from the data analysis. Patients using statins were excluded from analyses regarding the association between *HTR2C* and *ADRA2A* polymorphisms and HDL and Low Density Lipoprotein (LDL) cholesterol levels. Patients using fibrates were excluded from analyses regarding the association between *HTR2C* and *ADRA2A* polymorphisms and triglyceride levels. After complete description of the study to the patients, informed consent was obtained and blood was drawn for genotyping.

Outcome measures

Endpoints of this study were changes in body weight (change in BMI (%)/month) and lipid levels (HDL, LDL, total cholesterol and triglycerides (mmol/l)) during the first three months of the anti-obesity program. The three months cutoff point was chosen for this interim analysis to enable the inclusion of a larger number of patients, since follow up for a large proportion of patients is still ongoing. This interim analysis was not part of the original research protocol.

Determinants

Primary determinants were genotypes of the rs3813929:C>T (-759 C/T) and rs1414334:C>G polymorphisms within the X-linked *HTR2C* gene and the 1291 C/G (rs1800544) polymorphism within the *ADRA2A* gene. It should be noted with regard to *HTR2C* and *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 (HGVS; 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; www.ncbi.nlm.nih.gov/SNP). However, in Western and Northern Europeans, allele G appears to be the major allele, which is confirmed in this study. In the analysis we therefore considered the allele C as the variant allele.

DNA Isolation and genotyping

Genomic DNA was isolated from EDTA-anticoagulated peripheral blood using the TaqMan® Sample-to-SNP kit (article nr. 4403313, Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands). The *HTR2C* and *ADRA2A* genotypes were determined with allelic discrimination using the pre-developed assays C__7611979_10 (rs1800544), C__27488117_10 (rs3813929) and C___7455701_10 (rs1414334), obtained from Applied Biosystems, according to the protocol supplied by the manufacturer. The reaction was carried out with TaqMan® universal polymerase chain reaction master mix in a StepOne Plus® real time polymerase chain reaction. Further information on genotyping procedures, including primer sequences and reaction conditions is available upon request.

Data analysis and statistics

Patient characteristics were transferred to a database (MS Access 2003) for analysis. The associations between changes in body weight (BMI) and lipid values (mmol/l) and the *ADRA2A* and *HTR2C* genotype groups (presence or absence of the variant alleles) were investigated with linear regression. Change in body weight is presented as the change in BMI per month (ΔBMI/month in combination with a 95% confidence interval (95%CI). Changes in lipid levels are presented as percentages compared to the values at study entry (%) in combination with a 95% confidence interval. Data were investigated for potential confounding effects of age, gender, initial BMI, duration of the very low calory diet and initial lipid levels. These variables were included in the multivariate model if they were univariately associated with the primary endpoints at a significance level of $p < 0.20$.[12] Data were also investigated for interaction between genotype and gender for the X-linked *HTR2C* polymorphisms. A p-value of 0.05 or less was regarded as significant for the primary analyses, because adjusting the p-value for the number of tests could increase the type II error rate too much in this hypothesis driven study.[13] Data were analysed using SPSS 17.0.

Results

In total, 76 patients were included in this interim analysis, consisting of 15 men and 61 women. Baseline characteristics are shown in Table 1.

Table 1. Baseline characteristics

Characteristic	Population (n = 76)
Age, mean (SD)	
women	45.0 (11.6)
men	45.0 (10.5)
BMI, mean (SD)	
women	41.1 (6.0) kg/m^2
men	43.2 (10.2) kg/m^2
Somatic disorders (treated)	
diabetes	21%
hypertension	34%
dyslipidemia	18%
Lipid values	
Total cholesterol**, mean (SD)	5.1 (1.0) mmol/l
HDL-cholesterol**	
men, mean (SD)	1.0 (0.3) mmol/l
women, mean (SD)	1.3 (0.3) mmol/l
LDL-cholesterol**, mean (SD)	3.2 (0.9) mmol/l
Triglycerides, mean (SD)	1.6 (0.8) mmol/l

* Presence of the metabolic syndrome could not be assessed in 4 patients.
** In patients without cholesterol lowering drugs (n=60).
*** In patients without diabetes medication (n=58).

Prevalence of the variant alleles was 26.3% for the variant *HTR2C* 759T-allele (n = 20), 25% for the variant *HTR2C* rs1414334C-allele (n = 19), and 39.5% for the variant *ADRA2A* 1291G-allele (n = 30). Genotype distribution of the polymorphisms did not deviate significantly from Hardy-Weinberg-equilibrium (HWE, calculated in women for *HTR2C* polymorphisms) (rs3813929 (-759 C/T) (p = 0.88), rs1414334:C>G (p = 0.79), rs1800544 (p = 0.93)). No significant linkage disequilibrium between the polymorphisms was found ($r^2 << 0.01$), and no interaction between genotype and gender for the *HTR2C* polymorphisms was found either.

Weight loss during the 3 months program showed a non-linear curve, with weight loss during the first month being significantly higher than in the second and third month (Table 2). Therefore the decision was made to investigate the association with weight loss over two separate periods; weight reduction during the first month (first period), and weight reduction during the remainder of the 3 months follow up period (second period). Neither gender nor age were found to be confounders for weight reduction in the first month, but age was classified as a confounder for weight reduction in the second period and therefore included in the analysis. Regarding the associations with lipid levels, both age and gender were classified as confounders and included in the analyses.

No association was found between the *HTR2C* 759 C/T genotype and weight loss during the first or second period of the anti obesity program (Table 2). Table 2 shows that weight loss per

month was highest in the first month of the program with a mean weight reduction of around 3 kg/m^2 compared to the second period with a weight reduction of around 1.7 kg/m^2. However, weight loss between genotype groups was comparable.

Table 2. Association between the HTR2C 759 C/T genotype and weight loss.

Time period	N	Wildtype (C/CC genotype)	Carriers 759 T-allele (T(T)/TC genotypes)
First period (1st month)			
Mean ΔBMI/month (SD, range)	76	- 3.33 kg/m^2 (0.7, -1.8- -4.9)	- 3.18 kg/m^2 (1.1, -0.6- -5.2) [a]
Second period (2nd & 3rd month)			
Mean ΔBMI/month (SD, range)	70	- 1.68 kg/m^2 (0.7, -0.3- -3.4)	- 1.73 kg/m^2 (0.7, -0.5- -3.4) [b]

a p = 0.3, adjusted for age and BMI at start.
b p = 0.7, adjusted for age and BMI at start.

Furthermore, no associations were found between carriership of the variant rs1414334 C-allele or *ADRA2A* 1291 G-allele and changes in lipid values. After 3 months of treatment in the obesity program, reduction in lipid values was comparable between genotype groups (Table 3). A combined genotype analysis was not possible due to the small sample size.

Table 3. Association between the HTR2C rs1414334 and ADRA2A 1291 C/G polymorphisms and lipid levels after 3 months in the program.

Endpoint	N [a]	Wildtype	Carriers variant allele
HTR2C Rs1414334		*Rs1414334 G(G)*	*Rs1414334 C(C)/CG*
Δ HDL cholesterol (mean (SD))	34	0.01 (0.19) mmol/l	- 0.03 (0.12) mmol/l [b]
Δ LDL cholesterol (mean (SD))	34	- 0.36 (0.56) mmol/l	- 0.61 (0.75) mmol/l [c]
Δ Triglycerides (mean (SD))	39	- 0.27 (0.39) mmol/l	- 0.57 (0.83) mmol/l [d]
ADRA2A 1291 C/G		*1291 CC*	*1291 CG/GG*
Δ HDL cholesterol (mean (SD))	34	- 0.00 (0.19) mmol/l	0.02 (0.11) mmol/l [e]
Δ LDL cholesterol (mean (SD))	34	- 0.58 (0.64) mmol/l	- 0.04 (0.31) mmol/l [f]
Δ Triglycerides (mean (SD))	39	- 0.43 (0.59) mmol/l	- 0.16 (0.30) mmol/l [g]

a Patient number varies due to the in- or exclusion of patients using cholesterol lowering drugs.
b p = 0.32, corrected for HDL at start.
c p = 0.11, corrected for LDL at start.
d p = 0.94, corrected for triglycerides at start.
e p = 0.76, corrected for HDL at start.
f p =0.22, corrected for LDL at start.
g p = 0.30, corrected triglycerides at start. No other confounders found.

Discussion

Contrary to the results found in the study by Pooley et al., no association was found between the *HTR2C* 759 C/T genotype and weight loss during an anti obesity program. Weight loss during the first period (first month) and second period (2nd & 3rd month) was comparable between genotype groups. Furthermore, the associations previously found in a cross-sectional study between the *HTR2C* rs1414334 and *ADRA2A* 1291 C/G polymorphisms and triglyceride and LDL cholesterol levels respectively were also not present in this prospective follow up study.[11] Changes in mean lipid levels after 3 months of treatment were sometimes slightly different between genotype groups, but none of these differences were statistically significant.

We realize that the sample size of this study is an important limitation because of the small genotype groups. This could explain why no associations were found with the investigated genotypes, since the endpoints studied are likely to have a multi-factorial origin. Therefore the estimated effect size of one polymorphism is expected to be small, which requires a much larger sample size of a study if the null hypothesis is to be confirmed or rejected. It also prohibited a combined genotype analysis which has been shown to be of interest in a previous cross-sectional study.[11] Further continuation of patient recruitment is therefore a necessity. Another important limitation in this interim analysis is the short follow up period. The entire program lasts 9 months whereas the chosen cutoff point in this interim analysis was 3 months. It is possible that differences between genotype groups become larger when the follow up period is extended, revealing the impact of the investigated polymorphisms. It might also be possible that initial weight loss in the program between genotype groups remains comparable, but that patients with a less favourable genotype are more likely to relapse and regain weight, or lose less weight, in the remainder of the program, of even after the program has ended. The relatively short follow up period can also be relevant for the associations with lipid levels. For instance, HDL cholesterol levels have been shown to decrease or remain stable during a very low calorie diet (VLCD) over a course of 4 to 12 weeks, whereas these levels rose above baseline after a longer maintenance period without the VLCD over a period of 12 months.[14] However, studies investigating the impact of VLCD on LDL cholesterol and triglyceride levels have shown marked effects after 8 weeks of treatment which means that the 3 months follow up period could have been sufficient to observe an effect of genotype on these lipid levels, although the effects on LDL cholesterol have been inconsistent.[15] The question remains however whether the current setup of this study is sufficient to investigate secondary effects of an anti obesity program regarding lipid levels. A study investigating the impact of the *HTR2C* and *ADRA2A* polymorphisms on efficacy of cholesterol lowering drugs might be better suited.

It is possible that some effects of the investigated polymorphisms on weight loss and lipid levels that have been seen in patients using antipsychotic drugs are not seen in this study in patients without antipsychotic drugs because of the antagonistic properties of (atypical) antipsychotic

drugs on $5HT_{2C}$-receptors and α_{2A}-receptors. A $5HT_{2C}$- or α_{2A}-antagonistic action might modify weight loss and increase differences between the *HTR2C* and *ADRA2A* genotype groups.

In conclusion, the preliminary analysis of this study does not show evidence for an effect of the investigated *HTR2C* and *ADRA2A* polymorphism on efficacy of an anti obesity program regarding weight loss and changes in lipid levels in obese patients.

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References

1. Field AE, Coakley EH, Must A, Spadano JL, Laird N, Dietz WH, et al. Impact of overweight on the risk of developing common chronic diseases during a 10-year period. Arch Intern Med 2001;161:1581-1586.
2. Must A, Spadano J, Coakley EH, Field AE, Colditz G, Dietz WH. The disease burden associated with overweight and obesity. JAMA 1999;282:1523-1529.
3. Tecott L, Sun L, Akana S, Struch AM, Lowenstein DH, Dallman MF, et al. Eating disorder and epilepsy in mice lacking 5HT2C-serotonin receptors. Nature 1995;374:542-546.
4. Gallant DM, Bishop MP, Bishop G, O'Meallie L. U-22,394A: a controlled evaluation in chronic schizophrenic patients. Curr Ther Res Clin Exp 1967:9:579-581.
5. Smith SR, Prosser WA, Donahue DJ, Morgan ME, Anderson CM, Shanahan WR, et al. Lorcaserin (APD356), a selective 5-HT2c agonist, reduces body weight in obese men and women. Obesity 2008;17:494-503.
6. Pooley EC, Fairburg CG, Cooper Z, Sodhi MS, Cowen PJ, Harrison PJ. A 5-HT2c receptor promoter polymorphism (HTR2C-759C/T is associated with obesity in women, and with resistance to weight loss in heterozygotes. Am J Medical Genetics Part B (Neuropsychiatric Genetics) 2004;126B:124-127.
7. Yuan X, Yamada K, Ishiyama-Shigemoto S, Koyama W, Nonaka K. Identification of polymorphic loci in the promoter region of the serotonin 5HT2C receptor gene and their association with obesity and type II diabetes. Diabetologica 2000;43:373-376.
8. Risselada AJ, Mulder H, Heerdink ER, Gonera RK, Egberts ACG. Association between HTR2C polymorphisms and obesity in non-psychiatric patients. J Clin Psychopharmacol 2011 (submitted).
9. De Luca V, Mueller DJ, de Bartolomeis A, Kennedy JL. Association of the HTR2C gene and antipsychotic-induced weight gain: a meta-analysis. Int J Neuropsychopharmacol 2007;10(5):697-704.
10. Sicard MN, Zai CC, Tiwari AK, Souza RP, Meltzer HY, Lieberman JA, et al. Polymorphisms of the HTR2C gene and antipsychotic-induced weight gain: an update and meta-analysis. Pharmacogenomics 2010;11:1561-1571.
11. Risselada AJ, Mulder H, Heerdink ER, Gonera RK, Egberts ACG. Association between the HTR2C rs1414334 and ADRA2A 1291 C/G polymorphisms and lipid levels in obese patients. J Med Genetics 2011 (submitted).
12. Maldonado G, Greenland S. Simulation study of confounder-selection strategies. Am. J.Epidemiology 1993;138:923-936.

13. Perneger TV. What's wrong with Bonferroni adjustments. Br Med J 1998;316:1236-1238.
14. Rolland C, Broom I. The effects of very-low-calorie diets on HDL: a review. Cholesterol 2011, doi:10.1155/2011/306278.
15. Volek JS, Sharman MJ, Forsythe CE. Modification of lipoproteins by very low-carboydrate diets. J Nutr 2005;135:1339-1342.

Chapter 4

General Discussion

Introduction

Pharmacogenetics has been defined as the research area investigating whether and to what extent genetic variation can explain and predict the response to drugs of individual patients. Knowledge of the impact of genetic variation on drug response can help tailor pharmacotherapy by choosing the optimal drug and dose for a patient, thereby increasing treatment success and preventing side effects. Genetic variation can affect both the safety and efficacy of drugs when mutations occur in the DNA coding for drug targets (e.g. receptors and the systems involved in receptor functioning), drug transport mechanisms (e.g. P-glycoprotein pump) or drug-metabolizing enzymes (e.g. cytochrome P450). The focus in this thesis lies within genetic variation in drug targets.

There are several reasons why psychiatry was thought to be one of the first medical disciplines to implement genetic information into daily clinical practice. One of these reasons is the fact that it can take several days to several weeks before the efficacy of psychotropic drugs (e.g. antipsychotics and antidepressants) can be assessed. This means that a lot of time can be lost when the first drug of choice is ineffective, resulting in dangerous situations for both the patient (e.g. suicide risk) and its surroundings (e.g. aggressive behaviour). Another reason is the frequent occurrence of severe adverse effects associated with psychotropic drugs (especially antipsychotic drugs) including extrapyramidal symptoms, dyslipidemia, diabetes, weight gain and the metabolic syndrome. Finally, the polymorphic CYP2D6 enzyme was one of the first targets for pharmacogenetic research and has therefore been studied frequently regarding the impact of CYP2D6 genotypes on pharmacokinetics. Since the CYP2D6 enzyme is responsible for metabolizing a large proportion of the psychotropic drugs, and approximately 50% of the psychiatric patients uses at least one CYP2D6 substrate,[1] it is not surprising that the CYP2D6 genotype has been shown to be relevant for clinical psychiatric practice regarding overdosing and switching between drugs.[2-4] The Pharmacogenetics Working Group, operating on behalf of the Royal Dutch Association for the Advancement of Pharmacy (KNMP), provided several genotype based dosing recommendations for psychotropic drugs that can be used in clinical practice.

Despite these reasons and the available body of evidence, the use of genetic information in psychiatry is rare. Moreover, interindividual differences in pharmacokinetics only explain part of the variability in drug response. A larger part of the variability is likely explained by genetic variation in pharmacodynamic determinants. However, genetic tests to predict the pharmacodynamics of psychotropic drugs are not being used, despite intensive efforts to investigate associations between pharmacodynamic polymorphisms and response to psychotropic drugs as described in this thesis. If a psychiatrist could use genetic information to predict which psychotropic drug is optimal for the patient regarding efficacy and tolerance this would be of great importance. Particularly predicting the metabolic adverse effects would

be relevant given their strong association with cardiovascular disease and associated increased mortality.

In this thesis several studies have been presented that found associations between *ADRA2A* and *HTR2C* polymorphisms and metabolic complications in patient populations with or without psychotropic drugs (chapters 2 and 3 respectively). The *HTR2C* rs1414334 polymorphism was associated with prevalence of the metabolic syndrome in chapter 2.3 and triglyceride levels in chapters 2.3 and 3.2, whereas the *HTR2C* 759C/T polymorphism was associated with obesity in chapters 3.1 and 3.3. The *ADRA2A* 1291 C/G polymorphism was associated with the metabolic syndrome in chapter 2.4 and levels of LDL cholesterol in chapter 3.2. The information from these studies could potentially contribute to the development of an algorithm for chosing antipsychotic drugs based on pharmacogenetic variation in pharmacodynamic determinants. Moreover, by comparing the results between patients using antipsychotic drugs and antipsychotic naïve patients, the potential modifying influence of these psychotropic drugs on genotype associated metabolic abnormalities could be investigated.

In this final chapter the results of the individual studies will be put into a broader perspective by discussing topics that were relevant in one or more of the individual chapters. The following items will be discussed:

- Challenges in genetic association studies
  - Association versus causation
  - Study methodology
- Implementation in daily clinical practice
- Perspectives for future research

Challenges in genetic association studies

Despite the numerous studies that found associations between genetic determinants and the pharmacodynamics of psychotropic drugs regarding metabolic abnormalities, the results from those studies have not been implemented in clinical practice. The challenges that hinder the use of genetic data in daily patient care will be discussed in this part of the general discussion.

Association versus causation

Linkage disequilibrium

Linkage disequilibrium (LD) is an important and specific challenge for studies that investigate the association between genetic determinants and metabolic abnormalities. Linkage disequilibrium is the occurrence of combinations of alleles or genetic markers in a population more often or less often than would be expected based on allelic frequencies. In other words; the genotypes at two or more loci are not independent of each other.

LD introduces the uncertainty whether the SNPs studied are actually the SNPs responsible for the metabolic effects (association vs. causation). For example, it is possible that the SNPs studied in this thesis are not causative for the antipsychotic-induced metabolic effects, but are inherited together with causal SNPs. The investigated SNPs within this thesis would therefore merely be markers. The potential impact of linkage disequilibrium became most apparent in the studies investigating the two *HTR2C* polymorphisms; 759 C/T (rs3813929) and rs1414334 in this thesis. Comparing the results of the studies presented in chapters 2.3 and 3.1 and the review article in chapter 2.1 shows that these *HTR2C* polymorphisms appear to predict different phenotypes. The *HTR2C* 759 C/T polymorphism was shown to be associated with obesity in chapter 3.1 and antipsychotic-induced weight gain in chapter 2.1, whereas the *HTR2C* rs1414334 polymorphism showed an association with the metabolic syndrome in chapter 2.3 and cholesterol levels in chapters 2.3 and 3.2. Therefore the *HTR2C* 759 C/T polymorphism appears to be relevant for body weight regulation whereas the *HTR2C* rs1414334 polymorphism appears to be relevant for metabolic factors and associated cardiovascular risks. Since it is unlikely that two polymorphisms located within the *HTR2C* gene predict different phenotypes, variation in LD with a causal SNP or group of SNPs might explain these observations. The question is whether one of these polymorphisms reflects the impact of the $5HT_{2C}$-receptor, and if so; which one?

RNA editing

Answering that question is not easy because the molecular diversity in the $5HT_{2C}$-receptor is not only regulated by transcription changes due to SNPs in the DNA but also by a process called RNA editing.[5,6] RNA editing is a post-transcriptional event in which the coding potential of primary RNA transcripts is changed by mechanisms other than splicing. The human $5\text{-}HT_{2C}$ receptor mRNA undergoes Adenosine to Inosine editing at five positions (A, B, C, C′ and D), generating thirty-two protein isoforms with different distributions and signaling capabilities. This Adenosine to Inosine editing is catalyzed by enzymes including the Adenosine Deaminase Acting on RNA (ADAR) enzymes that use hydrolytic deamination for this process. The human $5\text{-}HT_{2C}$ receptor edited isoforms, which have VSV (Valine-Serine-Valine) or VGV (Valine-Glycine-Valine) at positions 156, 158 and 160, have been shown to exhibit a decrease in agonist potency and constitutive activity, as well as altered patterns of G-protein coupling.[6] This suggests that the editing process may be used as a finetuning process to regulate signaling tone in the brain, which is supported by studies that showed the editing profile of *HTR2C* mRNA to be dependent on the serotonergic tone in the central nervous system.[7] Enhanced tones are associated with edited and less responsive receptors, whereas a decreased serotonergic tone corresponds with the expression of non-edited and more responsive receptors. This illustrates that the impact of DNA mutations might not always be reflected in the phenotype when this is compensated by adaptations in RNA editing, which adds to understanding why replication of genetic association studies has proven to be difficult.

HTR2C polymorphisms

Regarding the *HTR2C* polymorphisms, it was shown in the study by Buckland et al. that the 759C allele reduced transcriptional activity and resulted in relative underexpression of *HTR2C*, which was proposed to underlie vulnerability to weight gain.[8] The studies by Hill et al. on the other hand suggested disruption of DNA-protein interactions to be the mechanism by which *HTR2C* expression is perturbed.[9,10] However, Hill et al. suggested that expression of the $5HT_{2C}$ receptor is reduced in patients carrying the *HTR2C* 759T allele, resulting in resistance to weight gain because of adaptive changes in other systems involved in feeding behaviour. The mechanisms and consequences proposed by Buckland et al. and Hill et al. are therefore conflicting. An increased expression of the *HTR2C* mRNA in patients carrying the 759T-allele would make more sense, since a stronger inhibitory role of the serotonergic system on the dopaminergic reward system involved in feeding behaviour could be expected in that situation.

The *HTR2C* rs1414334 polymorphism on the other hand is an intronic polymorphism with no known functionality in the *HTR2C* gene. However, it was pointed out recently in the study by Bai et al. who investigated the association between *HTR2C* polymorphisms and the metabolic syndrome in Asian schizophrenic patients, that the *HTR2C* rs1414334 polymorphism was not included because of its complete Linkage Disequilibrium with another well known *HTR2C* polymorphism; Cys23Ser (rs6318 C/G), according to the HapMap Project (http://hapmap.ncbi.nlm.nih.gov/).[11] In European Caucasians this LD does not appear to be complete, but it is high (R^2 0.92). This is particularly interesting because the Cys23Ser polymorphism is a functional *HTR2C* polymorphism resulting in a change in amino acids (cysteine to serine). This serine by cysteine substitution in position 23 of the $5HT_{2C}$-receptor is caused by a G to C transversion in position 68 of the *HTR2C* gene, in which C encodes serine and G encodes cysteine. The study by Okada et al. showed that high-affinity binding to a $5HT_{2C}$ agonist and serotonin was reduced in Ser23 expressing membranes.[12] Ser23 required higher serotonin concentrations to elicit the same response as Cys23, which might reflect a more extensive desensitization in the Ser23 form. However, in the study by Lappalainen et al. the Cys23Ser polymorphism had no effect on the 5-HT concentration-response curves in Xenopus oocytes expressing these receptors, which suggested that human Ser23 variants may not be functionally different under baseline physiological conditions.[13] It was proposed that the response to pharmacological agents, neuropeptides or hormones may be different, because the amino acid substitution is located in the N-terminal extracellular region of the $5HT_{2C}$-receptor with possible consequences for ligand binding. For example, Cys-Ser substitution may affect the receptor folding due to disulfide bonds (Cys-Cys), hindering the formation of a normal hydrophobic pocket and subsequently the binding of bulky ligands.

Most studies investigating the association between the *HTR2C* Cys23Ser polymorphism and antipsychotic-induced weight gain in humans did not find an association, as can be seen in Table 3 of chapter 2.1, and neither did a study investigating this polymorphism for an

association with obesity or underweight in children, adolescents and young adults.[14] This is in concordance with the *HTR2C* rs1414334 polymorphism that has only once been associated with these endpoints either,[15] compared to several negative studies (chapters 2.2 and 3.1[16]). Unfortunately, the potential association between the *HTR2C* Cys23Ser polymorphism and the metabolic syndrome has only been investigated once. The study by Bai et al. did not show an association between the *HTR2C* Cys23Ser polymorphism and prevalence of the metabolic syndrome in 456 Asian patients.[11] However, prevalence of the Cys23 variant was only 1.3% in that study and therefore the power to detect an association might have been too low. Overall, there does seem to be a large parallel in study results between the *HTR2C* Cys23Ser and *HTR2C* rs1414334 polymorphisms, which corresponds with the high LD. This means that in the studies investigating the *HTR2C* rs1414334 polymorphism we could actually have been measuring the effects of the *HTR2C* Cys23Ser polymorphism. The question remains which of the SNPs reflects functioning of the $5HT_{2C}$-receptor. Unfortunately, based on current in-vitro and in-vivo evidence no conclusions can be drawn.

Maybe a Genome Wide Association Study (GWAS) can elucidate which SNPs that are associatied with metabolic determinants are in LD with *HTR2C* 759 C/T and/or *HTR2C* rs1414334. However, for a feasible GWAS, the sample size of the study population needs to be substantially larger ($n >> 500$) than the sample sizes that have been used in this thesis ($n < 300$) and preferably in a longitudinal design.

Study methodology

As can be seen in Table 3 of chapter 2.1, the study results of genetic association studies are often inconsistent. A large part of these inconsistent results can be explained by differences in study methodology. In this section of the general discussion some of these differences in study methodology, and their effects on study results, will be discussed.

Study design

For studies investigating weight gain or the etiology of diseases like the metabolic syndrome and obesity ideally a longitudinal design is chosen, making it possible to follow the exposure to relevant confounders and metabolic changes within the patients over time. However, the inclusion for longitudinal studies has proven to be difficult, as we experienced in the study presented in chapter 2.2. Moreover, often a part of the included patients are lost to follow up, which makes it even more necessary to include large numbers of patients in these longitudinal studies. Furthermore, since obesity and the metabolic syndrome take time to develop, a long follow up period is necessary, which increases the costs of these studies. For studies investigating weight gain a longitudinal design with adequate follow up is mandatory, but for genetic association studies investigating obesity or the metabolic syndrome a cross-sectional design can also be chosen for practical reasons, as has been done in chapters 2.3, 2.4, 3.1, 3.2 and 3.3 of this thesis. Since the exposure over time to DNA mutations is constant, cross-sectional studies

can even be used to assess causality in genetic association studies. However, the exposure over time to relevant confounders like co-medication and other antipsychotic drugs is not taken into account and is therefore an important limitation of this study design. In our studies we assumed these potential confounders to be evenly divided over the genotype groups, because they are likely to be unrelated to genotype, but whether they were actually evenly divided is unknown. If the genetic determinants investigated in this thesis play a role in the occurrence of adverse effects, and possibly even efficacy, of antipsychotic drugs, it is possible that this has affected pharmacologically based switching patterns and choice of antipsychotic drugs in the past. This might explain some of the variation between study results.

Study population

The study population probably explains the largest part of the variation between study results in genetic association studies due to several reasons. First of all, regarding the studies investigating antipsychotic-induced weight gain it makes a huge difference whether first episode patients or chronic schizophrenic patients are studied, as has been described in chapter 2.1 extensively. In chronic schizophrenic patients the weight gain could have already been caused by previously used antipsychotic drugs, making it more difficult to see changes in weight gain when starting another antipsychotic drug. Moreover, on a pharmacological level it is likely that previously used antipsychotics have caused adaptive processes (e.g. receptor up regulation, down regulation, desensitization) that might obscure the impact of the genetic determinant studied. This explains why associations have most consistently been found in studies in antipsychotic naïve patients.

Secondly, gene-environment interactions can also explain part of the variation in results. These interactions occur when environmental influences on a trait differ according to a person's genetic predispositions (e.g. association between skin color and skin cancer due to ultraviolet light), or when a person's genetic predispositions are expressed differently in different environments (e.g. phenylketonuria (PKU) with or without a phenylalanine-free diet). When comparing the results from studies in psychiatric patients (chapter 2) with studies in non-psychiatric patients (chapter 3), the most obvious difference between these populations is the use of psychotropic drugs. Nevertheless, gene-environment interactions are likely to be different between these populations as well, because schizophrenic patients drink more alcohol, smoke more often and eat less healthy.[17]

However, the most important aspect of a study population in genetic association studies is the ethnicity of the patients. This is not only relevant because of environmental and behavioural differences between ethnic populations (e.g. potatoes vs. rice, Ramadan), and therefore also different gene-environment interactions, but especially because genetic differences between these populations can be large. These genetic differences between ethnic populations are clearly visible when looking at the prevalence of mutant alleles for various polymorphisms.

As an example, prevalence of the variant *HTR2C* rs1414334 C-allele in Asians was only 1.4%,[11] whereas the prevalence of this allele was 19% in chapter 2.3. Furthermore, there can also be differences in LD between ethnic populations, which can lead to inconsistent patterns of association when non causal variants are tested that are in LD with a genuine causal variant. When the noncausal variant allele is positively associated with the disease allele in a population, the noncausal allele will appear to be a risk allele. When the noncausal variant allele is negatively associated with the disease allele in another population, the noncausal allele will appear to be a protective allele. Therefore, population stratification based on ethnicity can be very relevant in genetic association studies, and differences in study results between ethnic populations can only be interpreted correctly when the LD profiles are known. The populations in the studies presented in this thesis consisted of Caucasians patients, except for a few patients in chapters 2.3 and 2.4. In- or exclusion of these patients did not alter the study results. Further population stratification within Caucasian patients is probably unnecessary.[18]

Genetic determinants

Selecting the correct genetic determinants is obviously essential in genetic association studies. It is important to select polymorphisms that modify functioning of the target gene when the impact of that gene on a selected outcome is investigated. If a non-relevant SNP is chosen, and no associations were found, the investigated genes might be falsely classified as irrelevant. To minimize that problem, most hypothesis driven genetic association studies base their SNP selection on animal studies, previous similar studies or GWAS studies that showed a potential impact of that SNP on an outcome. Apart from selecting the correct SNPs, the number of SNPs to be selected can also be relevant depending on the investigated outcome. Most hypothesis driven genetic association studies, like our own, only investigate the impact of a few genetic polymorphisms, whereas the primary endpoint is often multi-factorial. Using obesity as an example, eleven different genes have been associated with Mendelian forms of obesity in humans and more than 52 genes have been associated with obesity in knockout or transgenic mice.[19] The metabolic syndrome is likely to be that genetically complex as well. Therefore, for complex diseases like obesity and the metabolic syndrome, examining the association between a single gene and disease may produce ambiguous results because of the lack of consideration of other disease-influencing genetic loci and gene-gene interactions. This could even result in complete opposite associations, which are referred to as flip-flop associations.[20]

These flip-flop associations are often regarded as false-positive findings, especially when samples are gathered similarly from a common population. In fact, false-positive results may account for most cases of irreproducibility of genetic association studies, which is aggravated by the fact that studies finding significant results tend to attract investigators and journal editors and are hence published earlier than negative results.[21] Inconsistent findings may therefore also be attributable to publication biases. However, genuine flip-flop associations have been observed,

especially between different ethnic populations (due to differences in LD) in situations where multiple loci act in concert to cause a disease, as is the case with weight gain, obesity and the metabolic syndrome. The flip-flop associations seen between the *ADRA2A* 1291 C/G and *HTR2C* 759 C/T polymorphisms and antipsychotic-induced weight gain (Table 3, chapter 2.1) might be caused by the differences in ethnicity of study populations and psychotropic drugs used.

To tackle these problems, studies are needed that capture a larger part of the genetic variation by including large numbers of SNPs. To increase power, tagSNPs would be favorable because these SNPs predict genetic variation for a group of SNPs. Statistical power will still remain an important barrier though, since studies investigating a large number of SNPs and resultant gene-gene interactions require large sample sizes.

Data-analysis

The section of a scientific article covering the statistical analysis may often be a small part of the article, but is frequently a topic of much debate. To quote Aaron Levenstein, a professor emeritus at Baruch College: " Statistics are like a bikini. What they reveal is suggestive, but what they conceal is vital."

One of the first choices to be made within the data-analysis of genetic association studies is whether the analysis will be carried out for genotypes or for carriership of variant alleles (assuming dominance of the variant allele). In this thesis, the analyses have often been done for carriership of variant alleles instead of genotypes. In this thesis an analysis on carriership of variant alleles was used because the group of patients with the homozygous variant genotype was small in all studies. Since the largest impact of a polymorphism on a primary outcome is often expected in the group of patients homozygous for the variant allele, especially if this allele is recessive, the results within this group would be very interesting. However, when this group is small because of the number of patients included in the study, and the low frequency of the homozygous genotype, the analysis of this group of patients will not likely result in significant differences compared to the other genotype groups. Grouping the homozygous variant and heterozygous patients, resulting in an analysis on carriership of the variant alleles, can therefore be the best available option, as was the case in most studies within this thesis. However, a good example for the interpretation difficulty that can arise when reporting an association with carriership of alleles are the two meta-analyses by De Luca et al. and Sicard et al. regarding the *HTR2C* 759 C/T polymorphisms in antipsychotic-induced weight gain.[22,23] The complicating factor when comparing the results of both meta-analyses is the fact that De Luca et al. presented an odds ratio for carriership of the *HTR2C* 759T-allele, whereas Sicard et al. presented an odds ratio for carriership of the *HTR2C* 759C-allele. This makes the interpretation difficult for female patients carrying the *HTR2C* 759-CT genotype, since they would be protected against weight

gain according to the study by De Luca et al., but more vulnerable to weight gain according to the study by Sicard et al. Therefore a genotype based approach is preferable.

Multiple testing is also an important topic of debate in the data-analysis of genetic association studies and probably reflects the most frequent cause for type I errors. The study by Jassim et al. is a textbook example for this, since they investigated the impact of 76 SNPs on antipsychotic-induced weight gain on 4 separate moments in a patients disease history without correcting for multiple testing.[24] Therefore, Jassim and his colleagues basically carried out 304 separate analyses. Assuming a p-value of 0.05, the chance of not finding a statistically significant association with that number of tests is very small (<< 0.001%). Although this study serves as an extreme example to illustrate the impact of multiple testing, the same discussion can be held for the studies presented in this thesis, since nearly all studies investigated 2 SNPs or more, and used multiple endpoints. Therefore one can argue that the p-value used in these studies should have been lower than 0.05 before a result can be regarded as statistically significant. However, we did not apply any corrections for multiple testing in our studies because they were all hypothesis driven, and we didn't want to increase the type II error (false negative findings) too much. This was in accordance with the way comparable studies from other authors were presented. Furthermore, in the studies with the *HTR2C* 759C/T and rs1414334 polymorphisms one of these two polymorphisms was our main focus depending on the primary endpoint. In the studies investigating the metabolic syndrome, we expected beforehand to find an association with the *HTR2C* rs1414334 polymorphism and not so much the *HTR2C* 759 C/T polymorphism based on previous studies. In the studies investigating weight gain and obesity, we primarily expected to find an association with the *HTR2C* 759 C/T polymorphism and not so much the *HTR2C* rs1414334 polymorphism based on previous studies. Therefore the primary analysis, with the SNP of our primary focus and the primary endpoint should not need to be corrected for multiple testing. The secondary endpoints however (e.g. ATPIIIa criteria, separate antipsychotic groups) should. Correction for multiple testing would also be applicable in the *HTR2C-LEP/LEPR* interaction study in chapter 3.3, since it was a second analysis in a study population and four genotype combinations were investigated.

Several methods can be used when correction for multiple testing is required. The most frequently used correction is the Bonferroni correction, where the required p-value to dismiss the null hypothesis is calculated by dividing the regular p-value of 0.05 by the number of tests. To give an example; if 5 tests have been conducted, the required p-value to dismiss the null hypothesis would need to be smaller than 0.01. The problem with the Bonferroni correction however is that it can be too conservative especially when markers are correlated, which is often the case when multiple SNPs are investigated. This would result in an increased risk for type II errors (false negatives). Therefore a great deal of attention has been paid to develop better techniques, of which the false discovery rate method (FDR) is currently frequently used in genetic association studies.

Implementation in daily clinical practice

Several conditions have to be met before a pharmacogenetic test can be succesfully implemented in clinical psychiatric practice. The most important conditions are an evidence based motivation and a simple interpretation of test results. Ideally the genetic test is also cheap, usable for several frequently used drugs, and prevalence of variant alleles or genotypes is high. However, the latter conditions depend on the severity of the outcome. When the outcome is severe, or even life-threatening, these conditions become less important.

At present the use of genetic information to guide pharmacotherapy in psychiatry is mostly on a case by case basis and limited to pharmacokinetic strategies by looking at the drug-metabolizing enzymes (e.g. CYP2D6). Regarding pharmacokinetic strategies, lack of knowledge is still an important reason hindering the implementation, since detailed knowledge of pharmacokinetics is needed to understand how mutations in genes coding for the metabolizing enzymes can influence the metabolism of psychotropic drugs. The frequent involvement of multiple enzymes in a drugs metabolism and a 'competition' with therapeutic drug monitoring are also complicating factors.

A major problem preventing the use of pharmacodynamic strategies in psychiatry, apart from the complex pharmacology of the psychotropic drugs and the multi-factorial outcomes, is the lack of evidence due to inconsistent results of genetic association studies. Often a study finding an association is published, followed by several studies that are not able to replicate this finding or even find an association in the opposite direction, as can be seen in Table 3 of chapter 2.1. Replication problems have also been present regarding the studies within this thesis. This is illustrated by Table 1 which summarizes the main findings of the studies in and outside of this thesis for the different polymorphisms, patient populations and primary endpoints studied.

The studies investigating the association between *HTR2C* 759 C/T and *ADRA2A* 1291 C/G polymorphisms and weight gain in populations using psychotropic drugs can serve as good examples of studies with inconsistent findings. The only associations that have been replicated and are therefore more likely to reflect true association findings are the associations between the *HTR2C* rs1414334 polymorphism and the metabolic syndrome (chapter 2.3) and triglyceride values (chapter 2.3 and 3.2) and the *HTR2C* 759 C/T polymorphism and weight gain or obesity. These associations even appear to be independent of the use of psychotropic drugs (chapters 3.1 and 3.2). However, this does not automatically make genotyping these polymorphisms implementable in clinical practice. As mentioned previously, there are more conditions that have to be met before a genetic test, based on the results presented in this thesis, can be used in clinical practice. These conditions can be assessed by using the ACCE model, which consists of four components; analytical validity, clinical validity, clinical utility and ethical considerations.

Table 1. Associations found between HTR2C and ADRA2A genotypes and different outcome measures.

Outcome	*HTR2C* 759 C/T	Rs1414334 C/G	*ADRA2A* 1291 C/G
Weight gain			
Population without psychotropic drugs	Yes,C/T genotype worse [a,25] No (weight loss, chapter 3.4)	Not investigated	Not investigated
Population with psychotropic drugs	Yes, T-allele protective [26-34] Yes, T-allele worse [41,42] No (chapter 2.2, [35-40])	No (chapter 2.2)	Yes, G-allele worse [43-44] Yes, G-allele protective [45]
Obesity			
Population without psychotropic drugs	Yes, T-allele protective (chapter 3.1, [46-47]) No [48]	No (chapter 3.1)	No [b,49]
Population with psychotropic drugs	No [51-53] Yes, T-allele protective [16]	Yes, C-allele worse [15] No [16]	Not investigated
Metabolic syndrome			
Population without psychotropic drugs	In progress	In progress	In progress
Population with psychotropic drugs	No (chapter 2.3, [50-52])	Yes, C-allele worse (chapter 2.3, [50,51]) No [11]	Yes, G-allele protective (chapter 2.4)
Triglycerides			
Population without psychotropic drugs	Not investigated	Yes, C-allele worse (chapter 3.2) No (chapter 3.4)	No (chapters 3.2 + 3.4)
Population with psychotropic drugs	No (chapter 2.3, [50,51])	Yes, C-allele worse, (chapter 2.3) No [50,51]	Yes (chapter 2.4)
HDL Cholesterol			
Population without psychotropic drugs	Not investigated	No (chapters 3.2 + 3.4)	No (chapters 3.2 + 3.4)
Population with psychotropic drugs	No (chapter 2.3, [50,51])	No (chapter 2.3, [50,51])	No (chapter 2.4)
LDL cholesterol			
Population without psychotropic drugs	Not investigated	No (chapters 3.2 + 3.4)	Yes, G-allele protective (chapter 3.2) No (chapter 3.4)
Population with psychotropic drugs	Not investigated	No [c]	No [c]

a Worse stands for a negative impact on the metabolic endpoint (e.g. more weight gain, higher lipid levels etc).
b Own data; not shown. Analysis in patient sample from chapter 3.1.
c Own data; not shown. Analysis in patient sample from chapter 2.3.

Analytical validity

Analytical validity forms the basis of the ACCE model and defines a test's ability to accurately and reliably measure the genotype of interest. Elements within analytic validity include analytic sensitivity, analytic specificity, quality control and assay robustness. Meeting the criteria for analytical validity should not be a problem for most laboratories complying with Good Laboratory Practice (GLP) since they participate in collaborative trials, validate their equipment, use validated test kits, validate the genotyping procedure for each SNP, and use positive and negative control samples in each test run when these samples become available. Accurately predicting the *ADRA2A, HTR2C, LEPR* and *LEP* genotypes should therefore not be a problem.

Clinical validity

Clinical validity defines a test's ability to predict the associated phenotype. This definition points out two separate important issues in relation to association studies with pharmacodynamic parameters; the associated phenotype is often poorly understood because of its complexity and the predictive value of a single determinant (for example a genotype) is almost by definition to low for clinical validity.

The positive and negative predictive values of a genetic test determine to a large extent whether the test will be used in clinical practice. For example, if the test shows a patient to be carrying the variant *HTR2C* rs1414334 C-allele, but the metabolic syndrome does not develop the added value of doing the genetic test is limited. Unfortunately there is no predefined cutoff point for what the predictive values should be in order to make a genetic test successful. At present, it is impossible to use the results presented in this thesis to estimate the predictive values of the *HTR2C* and *ADRA2A* polymorphisms for the endpoints presented in Table 1. As mentioned before, the only associations that have been replicated are the associations between the *HTR2C* rs1414334 polymorphism and the metabolic syndrome and triglyceride values and the *HTR2C* 759 C/T polymorphism and weight gain or obesity. Furthermore, the genotypes of these polymorphisms are only a small piece of the puzzle regarding the complex phenotypes investigated. As an example, it can be seen in Table 1 of chapter 3.1 that the variant *HTR2C* 759 T-allele was present in 19% of the patients with obesity. This is less than prevalence of this allele in patients without obesity (28%), but it illustrates that the genotype of this polymorphism alone does not fully explain the phenotype. We took a small step trying to explain a larger piece of the obesity puzzle by adding leptin genotypes to the *HTR2C* genotypes, as can be seen in chapter 3.3. Unfortunately, adding the *LEP* and *LEPR* genotypes to the *HTR2C* genotypes did not result in further specifying genotype-associated risk groups, although previous studies in schizophrenic patients proved otherwise.[16,52].

If adding the leptin genotypes would have resulted in specifying the genotype associated risk groups, this still wouldn't have covered all of the genetic variation involved in the interindividual risk differences for becoming obese, since we know that more genes have already been

associated with Mendelian forms of obesity in humans as previously stated. Furthermore, a large part of the phenotype will likely also be explained by patient characteristics like ethnicity, gender, smoking, and age and environmental factors. The algorithm used for dosing warfarin can be used as an example for the integration of genetic information and patient characteristics in a predictive tool. This algorithm not only incorporates genetic information (CYP2C9 and VKORC1), but also patients characteristics like age, body surface area, ethnicity, co-medication and smoking status, explaining 53% of the variability in a warfarin dose. To develop a similar algorithm for predicting metabolic effects in patients using psychotropic drugs, large prospective studies are needed that investigate the impact of a combination of patient characteristics and several genetic determinants on developing the metabolic endpoints, resulting in an algorithm. The resultant algorithm will likely be very complicated and not fully explain the total variability in results, but it can be a start for investigating the usability of such an algorithm in intervention studies.

Clinical utility

Clinical utility defines the risks and benefits associated with a test's introduction into practice. At the moment the clinical utility for genotyping the *HTR2C* and *ADRA2A* genes in order to predict metabolic disturbances is unknown. Ideally, a genotype guided therapy could result in higher response rates and less side effects, when the most optimal drug and dose can be chosen for a patient. Genotyping *ADRA2A* does not add any usable information for choosing a treatment strategy because the results found have not been replicated yet and therefore some of the results might reflect type 1 errors. Genotyping *HTR2C* does add some information about a patients susceptibility for metabolic disturbances, but the question is whether this influences therapy. As an illustration; based on the results from chapter 3.1, carriership of the variant *HTR2C* 759T allele results in a five times reduced odds for developing obesity in a population without psychotropic drugs. However, the absolute risk increment and actual risk for obesity as well as the time interval needed to develop obesity are unknown because of the cross-sectional design of the study. Therefore we can't estimate whether or not obesity will develop in a specific patient and over what course of time.

The same applies to the association between the *HTR2C* rs1414334 polymorphism and the metabolic syndrome. Based on the results from chapter 2.3 we know that carriership of the variant *HTR2C* rs1414334 C-allele quadruples the odds for the development of the metabolic syndrome in patients using psychotropic drugs, but again the absolute risk increment, actual risk for the metabolic syndrome and time interval are unknown. Therefore, if we would genotype a patient for the *HTR2C* rs1414334 polymorphism before starting an antipsychotic drug we basically still have no idea if and when the metabolic syndrome will develop. On the other hand, one could argue that patients carrying the *HTR2C* rs1414334 C allele should be more closely monitored for metabolic abnormalities, or should not be prescriped antipsychotic

drugs with a high metabolic risk (e.g. clozapine). However, doing so would mean withholding patients the most effective antipsychotic drug, and withholding patients without the variant C-allele a closely monitoring could put them at risk since they could still develop the metabolic syndrome.

The clinical utility of genetic testing for *HTR2C* polymorphisms is therefore limited at present. For the moment, genotyping the polymorphisms investigated within this thesis should only be carried out in research projects, in combination with other potentially relevant SNPs. To assess clinical utility, genotype (algorithm) guided intervention trials could provide the necessary information and report whether interventions based on genetic information actually result in higher response rates and/or less side effects compared to standard care. Given the complexity of the endpoints chosen in this thesis it will probably take several years and possibly even decades before this is realized. Furthermore, since the algorithm is likely to incorporate several genetic factors, the costs associated with the genotyping procedure could still prove to be an important obstacle towards the implementation in clinical practice.

Ethical considerations

Ethical considerations will not be discussed in depth in this thesis, since the scope of this thesis was focused on clinical validity, but ethical considerations will sooner or later be part of the discussion regarding the implementation of genetic testing for metabolic risks in general practice. At this moment the discussion is relatively simple, since clinical validity is largely absent regarding the polymorphisms studied in this thesis, and clinical utility is unknown. Therefore it would not be ethical to screen patients for these polymorphisms and use this information to adjust pharmacotherapy accordingly, since this could lead to withholding a patient effective treatment options based on a false perception of genotype associated risks, as mentioned previously. Furthermore, it could cause unneeded commotion for the patient and his or her family since genetic predisposition involves them too. This could shift when it becomes possible to accurately predict a patients risk for metabolic side effects based on a genetic profile or algorithm. In that case, not using genetic information to tailor pharmacotherapy could put the patient at risk and might therefore be regarded as unethical.

Conclusion

Based on the criteria from the ACCE model, it would currently be premature to use the genotypes from the polymorphisms investigated in this thesis to try to predict the outcomes presented in Table 1 of this general discussion.

Perspectives for future research

Based on the results from the studies in this thesis and the discussion in the current chapter some recommendations can be made for future research.

A future research project could be to reanalyse the study in chapter 2.3 with the addition of the Cys23Ser polymorphism and investigate the LD between this polymorphism and the *HTR2C* rs1414334 polymorphism. If the LD in our population is also high between these two polymorphisms and the *HTR2C* Cys23Ser polymorphism can be associated with the metabolic syndrome it makes more sense to carry out future research projects with the *HTR2C* Cys23Ser polymorphism instead of the *HTR2C* rs1414334 polymorphism. Regarding the functionality of the *HTR2C* gene, the functional Cys23Ser polymorphism is more likely to modify the action of this gene than the intronic rs1414334 polymorphism, and there is no reason to genotype a marker (rs1414334) when the possible causal SNP (Cys23Ser) could be genotyped instead. The question is whether we are willing to invest the necessary money to carry out this analysis to prove something we are already able to predict given the high LD. At present the answer to that question is negative.

The focus for future research projects must be aimed at taking the next steps towards implementing genetic testing into daily clinical practice, since that has always been the main goal. This will not be achieved by doing genetic association studies with one or two SNPs when the endpoints have a multi factorial origin, as is the case with obesity and the metabolic syndrome. Therefore we need studies investigating several tagSNPs and carry out haplotype analyses to cover a larger piece of the genetic variation between individuals. This implies however that larger sample sizes are needed than the ones we have used so far, and preferably in a prospective longitudinal setting. Therefore, regardless of the research project, collaboration with other research centers is a necessity.

A big advantage nowadays is the fact that the metabolic disturbances caused by psychotropic drugs have received a lot of attention, which lead to the development of routine monitoring protocols. It should become common practice to enter the results from these routine monitoring protocols in a database, combined with anonymized patient characteristics, and ask the patients for informed consent to use this data and provide a blood sample for future testing. That way a goldmine for scientific research will develop, which will eventually make it possible to do the necessary analyses with multiple genetic markers and develop the algorithms needed to predict the metabolic effects. This longitudinal setup also allows following the metabolic changes and exposure to potential confounders over time, which addresses the problem of the cross-sectional studies within this thesis. Furthermore, a large part of this cohort will consist of first-episode patients which will possibly make it easier to find genetic associations with metabolic disturbances. Patients switching between antipsychotic drugs could also provide relevant data. To make an adequate data analysis possible, it will be mandatory to enter potential relevant confounders like baseline weight, ethnicity, co-medication, smoking behaviour and substance abuse into the database as well. Studies should also be stratified for ethnicity and psychotropic drugs, to minimize differences in gene-gene and gene-environment interactions.

Finally, the reporting of the study results should be compliant with the guidelines for STrengthening the REporting of Genetic Association studies (STREGA). These STREGA guidelines were build on the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement, and provide specific additions to a large number of items on the STROBE checklist. The goal of the STREGA guidelines is to enhance transparency of its reporting, regardless of choices made during design or analysis to enable assessment of the strengths and weaknesses of the evidence to help with the integration of genetic information in clinical practice.

The following research projects could be conducted within the illustrated cohort.

- GWAS for antipsychotic induced weight gain in first-episode schizophrenic patients and patients switching between antipsychotics (inception cohort).
- GWAS for the metabolic syndrome
- TagSNP/Haplotype analysis for an association between a combination of potentially relevant genes and antipsychotic induced weight gain in first-episode schizophrenic patients to address gene-gene interactions (e.g. leptin, $5HT_{2C}$, α_{2a}, GNB3 and D_2).
- TagSNP/Haplotype analysis for cholesterol elevation due to psychotropic drugs (both antidepressants and antipsychotics)
- Studies investigating the association between efficacy of psychotropic drugs and single SNPs or combinations of SNPs, depending on the drugs studied (e.g. agomelatin, a $5HT_{2C}$-blocking antidepressant and *HTR2C* genotype).

It remains important to realize that the phenotypes being investigated, as well as the interactions between genotypes, are very complex. This is illustrated by the replication problems that have arisen in laboratory controlled in vitro experiments. To reduce the chance of these replication problems in the more complex in vivo studies it might be necessary to rely on animal studies to address some of the hypotheses, because their genetic make-up and environment are known and controllable. The results from these studies can subsequently be used for hypothesis driven studies in humans. The information gathered with these studies could eventually be used to setup a genotype based intervention study for antipsychotic and antidepressant drugs. In this study, one group of patients will be treated based on common practice, and one group of patients will be treated using an algorithm or decision tree that incorporates genetic information. Comparing the treatment results with regards to efficacy and occurrence of side effects between these groups will provide information regarding the clinical utility of the intervention and possibly form a basis for a new era of common psychiatric practice that incorporates the use of genetic information.

Conclusion

In this final chapter the results from the studies within this thesis have been put in a broader perspective by discussing the challenges in genetic association studies and the steps needed for the implementation of genotyping in daily clinical psychiatric practice. Suggestions for future research have also been given.

It is clear that we are far away from using genetic information to predict metabolic adverse effects in patients using psychotropic drugs. Given the complexity of disorders like weight gain, obesity and the metabolic syndrome one could even wonder if our aim is realistic and not too high. It might be better to focus on the less complex pharmacokinetic targets in pharmacogenetics, by investigating the cost-effectiveness of genotype guided dosing for example. However, given the increasing frequency whereby studies investigating genetic determinants for antipsychotic-induced metabolic effects are published, hopefully some steps can be taken in the next few years towards genotype or algorithm guided drug therapy in psychiatry.

References

1. Mulder H, Heerdink ER, van Iersel EE, Wilmink FW, Egberts ACG. Prevalence of patients using drugs metabolised by cytochrome P450-2D6 in different patient populations: a cross-sectional study. Ann pharmacotherapy 2007;41:408-413.
2. Kirchheiner J, Nickchen K, Bauer M, Wong ML, Licinio J, Roots I, et al. Pharmacogenetics of antidepressants and antipsychotics: the contribution of allelic variations to the phenotype of drug response. Mol Psychiatry 2004;9:442-473.
3. Mulder H, Herder A, Wilmink FW, Tamminga WJ, Belitser SV, Egberts AC. The impact of cytochrome P450-2D6 genotype on the use and interpretation of therapeutic drug monitoring in long-stay patients treated with antidepressant and antipsychotic drugs in daily psychiatric practice. Pharmacoepidemiology and Drug Safety 2006;15:107-114.
4. Mulder H, Wilmink FW, Beumer TL, Tamminga WJ, Jedema JN, Egberts AC. The association between cytochrome P450-2D6 genotype and prescription patterns of antipsychotic and antidepressant drugs in hospitalised psychiatric patients: a retrospective follow-up study. J Clin Psychopharmacol 2005;25:188-191.
5. Burns CM, Chu H, Rueter SM, Hutchinson LK, Canton H, Sanders-Bush E, et al. Regulation of serotonin-2C receptor G-protein coupling by RNA editing. Nature 1997;387:303-308.
6. Niswender CM, Copeland SC, Herrick-Davis K, Emeson RB, Sanders-Bush E. RNA editing of the human serotonin 5-hydroxytryptamine 2C receptor silences constitutive activity. J Biol Chem 1999;274:9472-8.
7. Drago A, Serretti A. Focus on HTR2C: A possible suggestion for genetic studies of complex disorders. Am J Med Genet Part B 150B: 601-637.
8. Buckland PR, Hoogendoorn B, Guy CA, Smith SK, Coleman SL, O'Donovan MC. Low gene expression conferred by association of an allele of the 5-HT2c receptor gene with antipsychotic-induced weight gain. Am J Psychiatry 2005;162:613-615.

9. Hill MJ, Reynolds GP. 5-HT2c receptor gene polymorphisms associated with antipsychotic drug action alter promoter activity. Brain Research 2007;1149:14-17.
10. Hill MJ, Reynolds GP. Functional consequences of two HTR2C polymorphisms associated with antipsychotic-induced weight gain. Pharmacogenomics 2011;12(5):727-734.
11. Bai YM, Chen TT, Liou YJ, Hong CJ, Tsai SJ. Association between HTR2C polymorphisms and metabolic syndrome in patients with schizophrenia treated with atypical antipsychotics. Schizophrenia Research 2011;125:179-186.
12. Okada M, Northup JK, Ozaki N, Russell JT, Linnoila M, Goldman D. Modification of human 5-HT(2C) receptor function by Cys23Ser, an abundant, naturally occurring amino-acid substitution. Mol Psychiatry 2004;9:55-64.
13. Lappalainen J, Zhang L, Dean M, Oz M, Ozaki N, Yu DH, et al. Identification, expression, and pharmacology of a Cys23-Ser23 substitution in the human 5-HT2c receptor gene (HTR2C). Genomics 1995;27:274-279.
14. Lentes KU, Hinney A, Ziegler A, Rosenkranz K, Wurmser H, Barth N, et al. Evaluation of a Cys23Ser mutation within the human 5-HT2c receptor gene: no evidence for an association of the mutant allele with obesity or underweight in children, adolescents and young adults. Life Sci 1997;61:PL9-16.
15. Mulder H, Franke B, van der Beek-van der AA, Arends J, Wilmink FW, Egberts AC, et al. The association between HTR2c polymorphisms and obesity in psychiatric patients using antipsychotics: a cross-sectional study. Pharmacogenomics J 2007;7(5):318-324.
16. Gregoor JG, Mulder H, Cohen D, van Megen HJGM, Egberts ACG, Heerdink ER, et al. Combined HTR2C-LEP genotype as a determinant of obesity in patients using antipsychotic medication. J Clin Psychopharmacol 2010;30:702-705.
17. Leucht S, Burkard T, Henderson J, Maj M, Sartorius N. Physical illness and schizophrenia: a review of the literature. Acta Psychiatatr Scand 2007;116:317-333.
18. Wacholder S, Rothman N, Caporaso N. Population stratification in epidemiologic studies of common genetic variants and cancer: quantification of bias. J Natl Cancer Inst 2000;92:1151-1158.
19. Rankinen T, Zuberi A, Chagnon YC, Weisnagel SJ, Argyropoulos G, Walts B, et al. The human obesity gene map: the 2005 update. Obesity 2006;14:529-644.
20. Lin PI, Vance JM, Pericak-Vance MA, Martin ER. No gene is an island: the flip-flop phenomenon. Am J of Hum Gen 2007;80:531-538.
21. Dickersin K. The existence of publication bias and risk factors for its occurrence. JAMA 1990;263:1385-1389.
22. De Luca V, Mueller DJ, de Bartolomeis A, Kennedy JL. Association of the HTR2C gene and antipsychotic-induced weight gain: a meta-analysis. Int J Neuropsychopharmacol 2007;10:697-704.
23. Sicard MN, Zai CC, Tiwari AK, Souza RP, Meltzer HY, Lieberman JA, et al. Polymorphisms of the HTR2C gene and antipsychotic-induced weight gain: an update and meta-analysis. Pharmacogenomics 2010;11:1561-1571.
24. Jassim G, Ferno J, Theisen FM, Haberhausen M, Christoforou A, Havik B, et al. Association study of energy homeostasis genes and antipsychotic-induced weight gain in patients with schizophrenia. Pharmacopsychiatry 2011;44:15-20.
25. Pooley EC, Fairburg CG, Cooper Z, Sodhi MS, Cowen PJ, Harrison PJ. A 5-HT2c receptor promoter polymorphism (HTR2C-759C/T is associated with obesity in women, and with resistance to weight loss in heterozygotes. Am J Medical Genetics Part B (Neuropsychiatric Genetics) 2004;126B:124-127

26. Lane HY, Liu YC, Huang CL, et al. Risperidone-related weight gain, genetic and nongenetic predictors. J Clin Psychopharmacol 2006;26:128-134.
27. Reynolds GP, Zhang ZJ, Zhang XB. Association of antipsychotic drug-induced weight gain with a 5-HT2c receptor gene polymorphism. Lancet 2002;359:2086-87 (letter).
28. Reynolds GP, Zhang ZJ, Zhang XB. Polymorphism of the promoter region of the serotonin 5HT2c receptor gene and clozapine-induced weight gain. Am J Psychiatry 2003;160:677-679.
29. Ellingrod VL, Perry PJ, Ringold JC, Lund BC, Bever-Stille K, Fleming F, et al.Weight gain associated with the 759C/T polymorphism of the 5HT2c receptor and olanzapine. Am J Med Gen Part B (Neuropsychiatric Genetics) 2005;134B:76-78.
30. Templeman LA, Reynolds GP, Arranz B, San L. Polymorphisms of the 5HT2c receptor and leptin genes are associated with antipsychotic drug-induced weight gain in Caucasian subjects with a first-episode psychosis. Pharmacogenetics and Genomics 2005;15:195-200.
31. Miller DD, Ellingrod VL, Holman TL, Buckley PF, Arndt S. Clozapine-induced weight gain associated with the 5HT2c receptor -759C/T polymorphism. Am J Med Gen Part B (Neuropsychiatric Genetics) 2005;133B:97-100.
32. Ryu S, Cho EY, Park T, Oh S, Jang WS, Kim SK. 759 C/T polymorphism of 5-HT2C receptor gene and early phase weight gain associated with antipsychotic drug treatment. Prog Neuropsychopharmacol Biol Psychiatry 2007;31:673-677.
33. Godlewska BR, Olajossy-Hilkesberger L, Ciwoniuk M, Olajossy M, Marmurowska-Michalowska H, Limon J. Olanzapine-induced weight gain is associated with the -759 C/T and -697 G/C polymorphisms of the HTR2c gene. Pharmacogenomics J 2009;9:234-241.
34. Opgen-Rhein C, Brandl EJ, Müller DJ, Tiwari AK, Sander T, Dettling M: Association of HTR2C, but not LEP or INSIG2. genes with antipsychotic-induced weight gain in a German sample. Pharmacogenomics 2010;11:773-780.
35. Kuzman MR, Medved V, Bozina N, Hotujac L, Sain I, Bilusic H. The influence of 5-HT2c and MDR1 genetic polymorphisms on antipsychotic-induced weight gain in female schizophrenic patients. Psychiatry Research 2008;160:308-315.
36. Ujike H, Nomura A, Morita Y, Morio A, Okahisa Y, Kotaka T, et al. Multiple genetic factors in olanzapine-induced weight gain in schizophrenia patients: a cohort study. J Clin Psychiatry 2008;69:1416-1422.
37. Tsai SJ, Hong CJ, YU YWY, Lin CH. -759C/T genetic variation of 5HT2c receptor and clozapine-induced weight gain. Lancet 2002;360(30):1790 (letter).
38. Theisen FM, Hinney A, Bromel T, Heinzel-Gutenbrunner M, Martin M, Krieg JC, et al. Lack of association between the -759C/T polymorphism of the 5HT2c receptor gene and clozapine-induced weight gain among German schizophrenic individuals. Psychiatric genetics 2004;14:139-142.
39. Park YM, Cho JH, Kang SG, Choi JE, Lee SH, Kim L, et al. Lack of association between the 759C/T polymorphism of the 5-HT2c receptor gene and olanzapine-induced weight gain among Korean schizophrenic patients. J Clin Pharm Ther 2008;33:55-60.
40. Thompson A, Lavedan C, Volpi S. Absence of weight gain association with the HTR2C- 759 C/T polymorphism in patients with schizophrenia treated with iloperidone. Psychiatry Research 2010;175:271-273.
41. Basile VS, Masellis M, de Luca V, Meltzer HY, Kennedy JL. -759C/T genetic variation of 5HT2c receptor and clozapine-induced weight gain. Lancet 2002;360(30):1791 (letter).

42. De Luca V, Müller DJ, Hwang R, Lieberman JA, Volavka J, Meltzer HY, et al. HTR2C haplotypes and antipsychotics-induced weight gain: X-linked multimarker analysis. Hum. Psychopharmacol. Clin Exp 2007;22:463-467.
43. Wang YC, Bai YM, Chen JY, Lin CC, Lai IC, Liou YJ. Polymorphism of the adrenergic receptor alpha 2a-1291C>G genetic variation and clozapine-induced weight gain. J Neural Transm 2005;112:1463-1468.
44. Park YM, Chung YC, Lee SH, Lee KJ, Kim H, Byun YC, et al. Weight gain associated with the alpha2a-adrenergic receptor 1291C/G polymorphism and olanzapine treatment. Am J Med Gen Part B (Neuropsychiatric genetics) 2006;141B:394-397.
45. Sickert L, Müller DJ, Tiwari AK, Shaikh S, Zai C, De Souza R, et al. Association of the alpha2A adrenergic receptor -1291 C/G polymorphism and antipsychotic-induced weight gain in European-Americans. Pharmacogenomics 2009;10:1169-76.
46. Yuan X, Yamada K, Ishiyama-Shigemoto S, Koyama W, Nonaka K. Identification of polymorphic loci in the promoter region of the serotonin 5HT2C receptor gene and their association with obesity and type II diabetes. Diabetologia 2000;43:373-376.
47. Pooley EC, Fairburg CG, Cooper Z, Sodhi MS, Cowen PJ, Harrison PJ, et al. A 5-HT2c receptor promoter polymorphism (HTR2C-759C/T is associated with obesity in women, and with resistance to weight loss in heterozygotes. Am J Med Gen Part B (Neuropsychiatric Genetics) 2004;126B:124-127
48. Kring SII, Werge T, Holst C, Toubro S, Astrup A, Hansen T, et al. Polymorphisms of serotonin receptor 2A and 2 genes and COMT in relation to obesity and type 2 diabetes. PloS ONE 2009;4(8):e6696.
49. Rosmond R, Bouchard C, Björntorp P. A C-1291 G polymorphism in the alpha2A-adrenergic receptor gene (ADRA2A) promoter is associated with cortisol escape from dexamethasone and elevated glucose levels. J Intern Med 2002;251:252-257.
50. Mulder H, Franke B, van der Beek - van der Aart A, Arends J, Wilmink FW, Scheffer H, et al. The association between HTR2C gene polymorphisms and the metabolic syndrome in patients with schizophrenia. J Clin Psychopharmacol 2007;27:338-343.
51. Mulder H, Cohen D, Scheffer H, Gispen-de Wied C, Arends J, Wilmink FW, et al. HTR2C gene polymorphisms and the metabolic syndrome in patients with schizophrenia: a replication study. J Clin Psychopharmacol 2009;29:16-20.
52. Yevtushenko OO, Cooper SJ, O'Neill R, Doherty JK, Woodside JV, Reynolds GP. Influence of 5-HT2c receptor and leptin gene polymorphisms, smoking and drug treatment on metabolic disturbances in patients with schizophrenia. Br J Psychiatry 2008;192:424-428.
53. Iordanidou M, Tavridou A, Vasiliadis MV, Arvanitidis KI, Petridis J, Vargemezis V, et al. The 759C/T polymorphism of the 5HT2c receptor is associated with type 2 diabetes in male and female caucasians. Pharmacogenet Genomics 2008;18:153-159.

Summary

The past few decades have been characterized by a steep increase in the prevalence of obesity (defined as BMI > 30 kg/m^2), largely due to changes in eating behaviour. Obesity is a risk factor for the occurrence and prognosis of several diseases including cardiovascular disease, type 2 diabetes and hypertension. Furthermore, obesity has negative psychosocial consequences. Obesity can also be part of a larger whole of metabolic risk factors, which can ultimately lead to the metabolic syndrome. Presence of the metabolic syndrome is diagnosed when three or more of the five criteria from the National Cholesterol Education Program's Adult Treatment panel III (NCEP:ATP IIIa) criteria have been met:

- HDL-cholesterol < 1.0 mmol/L (male), or < 1.3 mmol/L (female), or use of a statin.
- Triglycerides ≥1.7 mmol/L, or use of a fibrate.
- Waist circumference ≥ 102 cm (male), or ≥ 88 cm (female).
- Blood pressure ≥ 130/85 mm Hg, or use of an antihypertensive drug.
- Glucose = fasting glucose ≥ 5.6 mmol/L, or HbA_{1c} > 6.1%, or use of an antidiabetic.

Schizophrenic patients form a subgroup within the general population with a markedly increased prevalence of obesity and the metabolic syndrome. A large part of this increased prevalence is caused by the use of antipsychotic drugs in this group of patients, since these drugs frequently cause metabolic adverse effects, including weight gain. However, not all patients experience antipsychotic-induced adverse effects to the same extent. This high interindividual variability suggests that genetic variability importantly contributes to a person's susceptibility to weight gain and the metabolic syndrome, making it a target for pharmacogenetic studies. Genetic determinants within the gene coding for the $5HT_{2C}$-receptor (*HTR2C*) have been studied most frequently for an association with antipsychotic-induced weight gain and have frequently proven to be associated with this endpoint. Therefore, the *HTR2C* gene is the most likely candidate gene to study when investigating antipsychotic-induced metabolic adverse effects. Another likely candidate gene for an association with antipsychotic-induced metabolic adverse effects is the *ADRA2A* gene coding for the adrenergic α_{2A} receptor, because studies have consistently found associations between polymorphisms within this gene and antipsychotic-induced weight gain as well.

If the associations found in psychiatric patients truly reflect a pharmacologic influence of polymorphisms in *HTR2C* and *ADRA2A* genes on a patient's metabolic state, this influence might also be visible in patients without psychotropic drugs. This could explain why some people in the general population have a higher tendency to gain weight or develop the metabolic syndrome. Moreover, comparing the effects of the genetic determinants between populations that use psychotropic drugs and populations that don't use psychotropic drugs could help clarify to what extent antipsychotic drugs modify outcome. Ultimately, knowledge of a patients metabolic risk profile increases the possibility to tailor pharmacotherapy, thereby preventing drug induced side effects and improving adherence to therapy and therapy outcome.

The objective of this thesis was to investigate whether and to what extent polymorphisms in the *HTR2C* and *ADRA2A* genes contribute to the interindividual variability in weight gain and development of the metabolic syndrome in patients that use psychotropic drugs and in patients that don't use psychotropic drugs. The first part of this thesis described the association between polymorphisms in the *HTR2C* and *ADRA2A* genes and metabolic adverse effects in psychiatric patients using psychotropic drugs.

In **chapter 2.1** we presented a systematic review summarizing the findings of studies investigating the association between genetic determinants and antipsychotic-induced weight gain up to April 2011. We also discussed the feasibility of genetic testing for antipsychotic-induced weight gain in clinical practice based on the information gathered from these studies. A total of 57 publications were included for data extraction, with the majority of these studies being published in the past 5 years. Most of these studies focussed on polymorphisms in the genes coding for adrenergic, histaminergic, dopaminergic and serotonergic receptors. Unfortunately, the results of these studies were often inconsistent, possibly owing to differences in study methodology and power issues. Study populations often varied regarding ethnicity of the patients, duration of illness, antipsychotic drugs used, follow-up duration, co-variables and data analysis. Studies in antipsychotic-naive populations probably provide most information about genetic associations with antipsychotic-induced weight gain. Polymorphisms in the genes encoding the α_{2a}-receptor, $5HT_{2c}$-receptor, G-protein ß3 subunit and leptin were most consistently associated with antipsychotic-induced weight gain. However, the utility of genotyping in clinical practice is still unknown, since studies are still focussed on clinical validity; prediction of phenotype. It is therefore too early to implement genetic testing for antipsychotic-induced weight gain in daily clinical practice. Future studies should investigate the impact of combinations of relevant polymorphisms and generate algorithms with a high predictive value to form the basis for clinical utility studies.

In **chapter 2.2** we investigated prospectively whether the rs1414334 and 759 C/T polymorphisms in the *HTR2C* gene modified mirtazapine-induced weight gain and changes in eating behaviour over a course of 12 weeks. The antidepressant mirtazapine is a strong $5HT_{2C}$-antagonist and frequently gives rise to weight gain. Therefore we expected a parallel with antipsychotic-induced weight gain regarding the impact of *HTR2C* polymorphisms. Mean weight gain in the total sample of 27 patients was 3.5 kg (95% CI 3.1 - 3.9) after 6 weeks and 4.6 kg (95% CI 3.9 - 5.3) after 12 weeks of treatment with mirtazapine, and comparable between genotype groups. However, we found a non-significant trend for patients carrying the variant rs1414334 C-allele to experience an increase in appetite more often (OR 7.7; 95% CI 0.7 - 79,8). The results of this study suggest that the *HTR2C* rs1414334 and 759 C/T polymorphisms are not significantly associated with mirtazapine-induced weight gain. However, given the small sample size, this study should be replicated in a larger setting. The trend seen in changes in eating behaviour for carriers of the variant rs1414334 C-allele also requires further study.

In **chapter 2.3** we investigated the association between the *HTR2C* rs1414334 and 759 C/T polymorphisms and prevalence of the metabolic syndrome in patients using antipsychotic drugs. In a cross-sectional study with 186, primarily schizophrenic, patients we found a significant association between carriership of the variant *HTR2C* rs1414334 C-allele and an increased prevalence of the metabolic syndrome (OR 3.73; 95%CI 1.29 - 10.79, p 0.015). No association was found between the *HTR2C* -759 C/T polymorphism and the metabolic syndrome. The association between the rs1414334 C-allele and the metabolic syndrome might be caused by an impact on lipolysis, since we also found an association between carriership of the variant rs1414334 C-allele and elevated triglyceride levels (2.4 mmol l^{-1} *vs* 1.7 mmol l^{-1}, p=0.014). Despite the limitation of its cross-sectional design, this was the third study in which we found an increased prevalence of the metabolic syndrome in patients carrying the rs1414334 C-allele. Therefore, this finding likely represents a true association. However, it is remarkable that despite both polymorphisms being located within the same *HTR2C* gene, these rs1414334 and 759 C/T polymorphisms appear to be predicting different phenotypes. The 759 C/T polymorphism has repeatedly been shown to affect antipsychotic-induced weight gain, whereas the rs1414334 polymorphism appears to affect cardiovascular risk factors including triglyceride levels, resulting in the metabolic syndrome. Differences in linkage disequilibrium with other, causal, SNP's might explain this phenomenon.

In **chapter 2.4** we investigated the association between the 1291 C/G polymorphism within the *ADRA2A* gene and prevalence of the metabolic syndrome. Since the 1291 C/G polymorphism has repeatedly been associated with antipsychotic-induced weight gain, it might also be associated with a potential consequence of this weight gain; the metabolic syndrome. The data for this cross-sectional study came from three different samples, one of which included the patients from **chapter 2.3**. The other two samples were used in two previous studies investigating the association between the *HTR2C* polymorphisms and the metabolic syndrome. The resultant sample included 497 patients of whom 27 did not use an antipsychotic drug at the moment of evaluation. We found no significant association between carriership of the variant 1291-G allele and prevalence of the metabolic syndrome (OR 0.73; 95% CI 0.49 - 1.15). We did find a trend for an association between the 1291 C/G polymorphism and 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). Furthermore, an 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 (OR 0.05; 95% CI 0.003 - 0.97, p=0.048). It is possible that antipsychotics mask the effects of the 1291 C/G polymorphism, because most antipsychotics have α_{2A}- antagonistic properties. This could explain why we did find an association with the metabolic syndrome in patients not currently using antipsychotics. However, given the small size of that group of patients, these results have to be treated with caution and need replication. Overall, this study shows that

the *ADRA2A* 1291-C/G polymorphism does not appear to be a strong predictor for long term occurrence of the metabolic syndrome in patients using antipsychotic drugs.

The second part of this thesis described studies investigating the association between polymorphisms in the *HTR2C* and *ADRA2A* genes and metabolic adverse effects in patients without psychotropic drugs. If the effects of polymorphisms in the *HTR2C* and *ADRA2A* genes are also visible in patients without antipsychotic drugs, this might help to identify patients at risk for metabolic complications. In preventive medicine this could allow for dietary adaptations as well as increased monitoring in an early stage. Furthermore, if the associations previously found in patients using psychotropic drugs are not present in patients without these drugs, this also provides information about the impact of these psychotropic drugs on the effects of the investigated polymorphisms.

In **chapter 3.1** we investigated whether prevalence of *HTR2C* rs1414334 and 759 C/T genotypes differed between obese patients (BMI > 30 kg/m^2, cases) and patients with a normal body weight (BMI < 25 kg/m^2, controls). In a cross-sectional case-control study we included 116 cases and 100 controls, making a total of 216 patients, with the majority (2/3) being female. Patients with obesity were recruited within an obesity clinic affiliated to the hospital, whereas patients with a normal body weight were recruited amongst employees of the hospital pharmacy as well as patients from the orthopaedic ward. The case-group was significantly younger than the control-group (45.7 vs. 65.1 years). We found a significantly lower prevalence of the 759T (men) or TT (women) genotype in patients with obesity (OR 0.18; 95%CI 0.04 - 0.78). Carriership of the variant 759T allele showed a protective point estimate but this was not significant (OR 0.53; 95%CI 0.24 - 1.17). No other associations were found. The results from this study match the results from studies investigating the association between the 759 C/T polymorphism and antipsychotic-induced weight gain or obesity, where the 759 T-allele was also shown to be protective. The protective effect of the *HTR2C* 759T allele in developing obesity therefore appears to be independent of antipsychotics drugs.

The objective of **chapter 3.2** was to investigate if the *HTR2C* rs1414334 and *ADRA2A* 1291 C/G polymorphisms were associated with lipid levels in obese patients without antipsychotic drugs. In the studies from **chapters 2.3** and **2.4** we found associations between these polymorphisms and triglyceride levels. If these polymorphisms truly affect lipolysis, their influence might also be visible in obese patients. In a cross-sectional study we included 130 obese patients from our obesity clinic, including the patients from the sample in **chapter 3.1**. We found that patients carrying the variant *HTR2C* rs1414334 C-allele had higher triglyceride levels than patients without this allele ($\Delta_{triglyceride}$ +0.34 (95%CI 0.02 - 0.66) mmol/L). Furthermore, patients carrying the variant *ADRA2A* 1291 G-allele had lower levels of LDL cholesterol than patients without this allele (Δ_{LDL} -0.41 (95%CI -0.77 - -0.04**)** mmol/L). In a combined genotype analysis these associations became stronger when only one of the variant alleles was present. No other

associations with lipid levels were found, and therefore we could not replicate the finding of a possible association between the *ADRA2A* 1291 C/G polymorphism and triglyceride levels. This study extends the evidence for an association between the *HTR2C* rs1414334 polymorphism and triglyceride levels, that appears to be independent of antipsychotic drugs. The other findings, including the association between *ADRA2A* and LDL cholesterol and the interaction between the *HTR2C* and *ADRA2A* polymorphisms, require further study.

In **chapter 3.3** we investigated whether prevalence of combined *HTR2C-LEP* and *HTR2C-LEPR* genotypes differs between obese and non-obese patients and if the interaction between the *HTR2C* and *LEP* genes, which was previously found in schizophrenic patients, was also present in patients without psychotropic drugs. Using a combined genotype might help in further identifying patients at risk for obesity. For this study we used a cross-sectional case-control design using the same patients population as in **chapter 3.1** (n=216). As was expected, given the results from **chapter 3.1**, there was a lower prevalence of the *HTR2C* 759T allele in obese patients compared to patients with a normal body weight. However, this was independent of *LEP* or *LEPR* genotypes. Prevalence of obesity was significantly lower in patients carrying the variant *HTR2C* 759T allele, both in absence and presence of the *LEPR* 223R allele compared to patients without the 759T allele (OR 0.22; 95%CI 0.05 - 0.97 and OR 0.31; 95%CI 0.1 - 0.998, respectively). No other significant results were found. Therefore, we were unable to replicate the interaction between the *LEP* and *HTR2C* genes that was previously found in patients using antipsychotic drugs. This interaction might therefore not be independent of antipsychotic drugs, possibly due to an interaction of antipsychotic drugs with the leptin or histaminergic system.

Chapter 3.4 describes an ongoing longitudinal study in which patients with obesity are followed during the course of their 9 month anti-obesity program. When the *HTR2C* 759 C/T polymorphism affects weight gain and obesity, and the *ADRA2A* 1291 C/G and *HTR2C* rs1414334 polymorphisms affect lipolysis and the metabolic syndrome, these polymorphisms might also affect success of the anti-obesity program regarding weight loss and improvement of lipid levels. Knowing which patients are less likely to successfully complete the program beforehand, based on their DNA profile, reduces health care costs and prevents further motivation problems in obese patients who want to loose weight. This information could help in selecting more appropriate means of losing weight in these high-risk patients, like surgery. Since the pool of included patients is still too small to analyze properly, we conducted an interim analysis using a 3-months cutoff point which enabled the inclusion of 76 patients. However, in this interim analysis we found no association between the *HTR2C* 759 C/T genotype and weight loss during the anti obesity program. Weight loss during the first period (first month) and second period (2^{nd} & 3^{rd} month) was comparable between genotype groups. Furthermore, we also did not find any associations between the *HTR2C* rs1414334 and *ADRA2A* 1291 C/G polymorphisms and triglyceride or LDL cholesterol levels, contrary to the findings presented in **chapter 3.2**. The short follow-up period is an important limitation in this interim analysis. It is

possible that differences between genotype groups become larger when the follow up period is extended, revealing the impact of the investigated polymorphisms. It might also be possible that patients with a less favourable genotype are more likely to relapse and regain weight at the end of the program or even after the program has ended. Furthermore, studies have also shown that changes in lipid levels during an anti-obesity program are often relatively stable at first, or even worsen during the first months of the program, and only improve after a longer follow-up duration. A future analysis using the 9-month cutoff points in a larger group of patients will reveal whether any associations between the *HTR2C* and *ADRA2A* polymorphisms and weight loss or lipid levels are present in obese patients during the course of an anti-obesity program.

Finally, in **chapter 4** we discussed three topics that were relevant in one or more of the individual chapters of this thesis; 'challenges in genetic association studies', 'implementation in daily clinical practice' and 'perspectives for future research'. At the moment, we are unable to use genetic information, including the genotypes of the polymorphisms investigated in this thesis, to accurately predict metabolic adverse effects in patients with or without psychotropic drugs. We have found significant associations in this thesis, but these associations only explain a small part of the variation between patients, given the (genetic) complexity of disorders like obesity and the metabolic syndrome. However, if researchers combine their efforts and patient populations and investigate combinations of polymorphisms it might be possible in the near future to uncover more pieces of the puzzle and develop an algorithm that can be used to assess a patient's metabolic risk. Such a genotype-guided algorithm could help psychiatrists make an optimal drug choice for each patient resulting in a better balance between efficacy and safety.

Samenvatting

De afgelopen decennia is de prevalentie van obesitas (gedefinieerd als BMI > 30 kg/m^2) sterk toegenomen, grotendeels veroorzaakt door veranderingen in eetgedrag. Obesitas is een risicofactor voor het ontstaan van verschillende ziekten, waaronder hart- en vaatziekten, type 2 diabetes en hypertensie. Obesitas heeft daarnaast ook nog negatieve psychosociale consequenties. Obesitas kan bovendien ook onderdeel zijn van een groter geheel aan metabole risicofactoren: het metabool syndroom. De diagnose metabool syndroom kan gesteld worden wanneer aan drie of meer van de vijf criteria van de "National Cholesterol Education Program's Adult Treatment panel III (NCEP:ATP IIIa)" voldaan wordt:

- HDL-cholesterol < 1.0 mmol/L (man), of < 1.3 mmol/L (vrouw), of gebruik van een statine;
- Triglyceriden ≥ 1.7 mmol/L, of gebruik van een fibraat;
- Buikomvang ≥ 102 cm (man), of ≥ 88 cm (vrouw);
- Bloeddruk ≥ 130/85 mm Hg, of gebruik van een antihypertensivum;
- Glucose = nuchter glucose ≥ 5.6 mmol/L, of HbA_{1c} > 6.1%, of gebruik van een antidiabeticum.

Patiënten met schizofrenie vormen een bevolkingsgroep met een sterk verhoogde prevalentie van obesitas en het metabool syndroom. Dit wordt grotendeels veroorzaakt door de antipsychotica die door deze patiënten gebruikt worden, aangezien antipsychotica frequent aanleiding geven tot metabole bijwerkingen zoals gewichtstoename. Echter, niet alle schizofrene patiënten ervaren dezelfde mate van gewichtstoename door antipsychotica of ontwikkelen het metabool syndroom. Deze grote interindividuele variabiliteit suggereert dat genetische factoren een belangrijke bijdrage leveren aan de individuele gevoeligheid voor gewichtstoename en het metabool syndroom. Farmacogenetische studies proberen deze genetische factoren te identificeren.

De meeste farmacogenetische studies die gewichtstoename door antipsychotica onderzochten hebben gekeken naar de invloed van polymorfismen binnen het gen dat codeert voor de $5HT_{2C}$-receptor (*HTR2C*). Veel van deze studies hebben hierbij associaties tussen deze polymorfismen en gewichtstoename gevonden. Bij genetisch onderzoek naar metabole bijwerkingen door antipsychotica is het *HTR2C* gen daarom de meest logische keuze. Een andere geschikt gen voor het onderzoeken van metabole bijwerkingen door antipsychotica is het gen dat codeert voor de adrenerge α_{2A} receptor (*ADRA2A*). Ook voor dit gen geldt dat verschillende studies associaties gevonden hebben tussen polymorfismen in dit gen en gewichtstoename door antipsychotica.

Als de gevonden associaties bij patiënten die antipsychotica gebruiken daadwerkelijk een farmacologische invloed van polymorfismen in de *HTR2C* en *ADRA2A* genen op de metabole status reflecteren, dan zou deze invloed mogelijk ook zichtbaar kunnen zijn bij patiënten die geen psychofarmaca gebruiken. Dit zou kunnen verklaren waarom sommige mensen in de bevolking een grotere neiging hebben tot gewichtstoename of de ontwikkeling van het metabool syndroom. Het vergelijken van de effecten van de genetische determinanten tussen populaties

die psychofarmaca gebruiken en populaties die geen psychofarmaca gebruiken verschaft bovendien inzicht in de wijze waarop antipsychotica uitkomsten kunnen beïnvloeden. Indien met behulp van genetische informatie patiënten geïdentificeerd kunnen worden die een hoog risico lopen op metabole bijwerkingen, dan zou dat kunnen helpen bij het maken van de juiste keuzes in de farmacotherapie. Dit zou kunnen helpen in het voorkomen van bijwerkingen en het verhogen van de therapietrouw, waardoor ook het effect van de therapie zou kunnen verbeteren.

In dit proefschrift is onderzocht in hoeverre polymorfismen in de *HTR2C* en *ADRA2A* genen bijdragen aan de interindividuele variabiliteit in gewichtstoename en de ontwikkeling van het metabool syndroom bij patiënten die wel of geen psychofarmaca gebruiken. Het eerste deel van dit proefschrift beschrijft de associatie tussen polymorfismen in de *HTR2C* en *ADRA2A* genen en metabole bijwerkingen in psychiatrische patiënten die psychofarmaca gebruiken.

In **hoofdstuk 2.1** presenteerden we een systematische review, waarbij we de bevindingen samengevat hebben van studies naar de associatie tussen genetische determinanten en gewichtstoename door antipsychotica. Hierbij zijn studies geselecteerd die voor april 2011 gepubliceerd zijn. Op basis van de verzamelde informatie uit de verschillende studies hebben we in dit hoofdstuk ook gediscussieerd over de huidige toepasbaarheid van genetisch onderzoek in de dagelijkse klinische praktijk om gewichtstoename door antipsychotica in te schatten. In totaal werden 57 studies geïncludeerd voor de data extractie, waarvan het merendeel in de afgelopen 5 jaar gepubliceerd is en polymorfismen in de genen coderend voor de adrenerge, histaminerge, dopaminerge en serotonerge receptoren onderzocht. Helaas zijn de resultaten van deze studies vaak tegenstrijdig, wat mogelijk veroorzaakt wordt door verschillen in studiemethodologie en problemen met de power. Studiepopulaties varieerden bijvoorbeeld vaak met betrekking tot de etniciteit van de geïncludeerde patiënten, de ziekteduur, de antipsychotica die gebruikt werden, duur van de follow-up, covariabelen en data-analyse. Studies in patiëntenpopulaties waarbij voor het eerst een antipsychoticum werd voorgeschreven zijn waarschijnlijk het meest geschikt om informatie over de invloed van genetische variatie op gewichtstoename door antipsychotica te vergaren. Polymorfismen in de genen die coderen voor de α_{2a}-receptor, $5HT_{2c}$-receptor, G-proteïne ß3 subunit en leptine zijn het vaakst geassocieerd met gewichtstoename door antipsychotica. Alle geïncludeerde studies zijn echter nog steeds gericht op het kunnen voorspellen van het fenotype; de klinische validiteit. De klinische bruikbaarheid is derhalve nog steeds onbekend. Het is daarom te vroeg om in de dagelijkse klinische praktijk te screenen op bepaalde polymorfismen om te proberen de gewichtstoename door antipsychotica te voorspellen. Toekomstige studies zouden gericht moeten zijn op het onderzoeken van de invloed van combinaties van polymorfismen op gewichtstoename door antipsychotica, om zo een hogere voorspellende waarde te krijgen en algoritmes te ontwikkelen die de basis kunnen vormen voor studies naar klinische bruikbaarheid.

In **hoofdstuk 2.2** hebben we over een periode van 12 weken prospectief onderzocht of de rs1414334 en 759 C/T polymorfismen in het *HTR2C* gen de gewichttoename en veranderingen in eetgedrag door mirtazapine beïnvloedden. Het antidepressivum mirtazapine is een sterke $5HT_{2C}$-antagonist en veroorzaakt vaak gewichtstoename. We verwachtten daarom een parallel met gewichtstoename door antipsychotica voor wat betreft de invloed van *HTR2C* polymorfismen. De gemiddelde gewichtstoename in de onderzoekspopulatie van 27 patiënten was 3.5 kg (95% BI 3.1 - 3.9) na 6 weken en 4.6 kg (95% BI 3.9 - 5.3) na 12 weken behandeling met mirtazapine, en was vergelijkbaar tussen de genotypengroepen. We vonden echter wel een niet-significante trend voor een frequentere toename van de eetlust bij patiënten die het variante rs1414334 C-allel droegen (OR 7.7; 95% BI 0.7 - 79,8). De resultaten van deze studie suggereren dat de *HTR2C* rs1414334 en 759 C/T polymorfismen niet sterk geassocieerd zijn met gewichtstoename door mirtazapine. Echter, gezien het kleine aantal patiënten zou deze studie herhaald moeten worden in een grotere setting. De trend voor een frequentere toename van de eetlust bij dragers van rs1414334 C-allel moet ook verder onderzocht worden.

In **hoofdstuk 2.3** hebben we onderzocht of er een associatie bestaat tussen de *HTR2C* rs1414334 en 759 C/T polymorfismen en de prevalentie van het metabool syndroom in patiënten die antipsychotica gebruiken. In een dwarsdoorsnede onderzoek met 186, voornamelijk schizofrene, patiënten vonden we een significante associatie tussen het *HTR2C* rs1414334 polymorfisme en prevalentie van het metabool syndroom. De prevalentie van het metabool syndroom was namelijk hoger bij patiënten die drager waren van het variante *HTR2C* rs1414334 C-allel (OR 3.73; 95%BI 1.29 - 10.79, p 0.015). We vonden geen associatie tussen het *HTR2C* 759 C/T polymorfisme en het metabool syndroom. De hogere prevalentie van het metabool syndroom bij dragers van het variante rs1414334 C-allel zou veroorzaakt kunnen worden door beïnvloeding van de lipolyse. Dragerschap van het variante rs1414334 C-allel was namelijk geassocieerd met verhoogde concentraties triglyceriden in het bloed (2.4 mmol/L *vs.* 1.7 mmol/L, p = 0.014). Ondanks de beperking van het dwarsdoorsnede ontwerp, is dit de derde studie waarin we een toegenomen prevalentie van het metabool syndroom vonden bij dragers van het variante *HTR2C* rs1414334 C-allel. Het is daarom aannemelijk dat deze bevinding een werkelijke associatie betreft. Het is echter opmerkelijk dat beide *HTR2C* polymorfismen verschillende fenotypen lijken te voorspellen, ondanks het feit dat beide polymorfismen op hetzelfde gen liggen. Van het 759 C/T polymorfisme is herhaaldelijk aangetoond dat het invloed heeft op de gewichtstoename door antipsychotica en niet op prevalentie van het metabool syndroom, terwijl het rs1414334 polymorfisme herhaaldelijk met prevalentie van het metabool syndroom en andere cardiovasculaire risicofactoren, waaronder concentraties triglyceriden in het bloed, geassocieerd is. Mogelijk kan dit fenomeen verklaard worden door verschillen in linkage disequilibrium van deze *HTR2C* polymorfisme met andere, causale, SNP's.

In **hoofdstuk 2.4** hebben we onderzocht of er een associatie bestaat tussen het *ADRA2A* 1291 C/G polymorfisme en de prevalentie van het metabool syndroom in patiënten die antipsychotica

gebruiken. Aangezien het 1291 C/G polymorfisme verschillende keren in verband is gebracht met gewichtstoename door antipsychotica, zou het mogelijk ook geassocieerd kunnen zijn met een potentieel gevolg van deze gewichtstoename; het metabool syndroom. De gegevens voor dit dwarsdoorsnede onderzoek kwamen uit drie verschillende studiepopulaties, waaronder de populatie uit hoofdstuk 2.3. De twee andere studiepopulaties waren afkomstig uit twee onderzoeken waarbij de associatie tussen de *HTR2C* polymorfismen en de prevalentie van het metabool syndroom onderzocht werd. De resulterende populatie bestond uit 497 patiënten, waarvan 27 geen antipsychoticum gebruikten op het evaluatiemoment. We vonden geen significante associatie tussen dragerschap van het variante 1291 G-allel en prevalentie van het metabool syndroom (OR 0.73; 95% CI, 0.49 - 1.15). We vonden wel een trend voor een associatie tussen het 1291 C/G polymorfisme en de concentratie triglyceriden in het bloed. Dragerschap van het variante G-allel was beschermend voor het bereiken van het afkappunt voor de concentratie triglyceriden van 1.7 mmol/L (OR 0.67; 95%CI 0.44 - 1.00; $p=0.05$). Een verkennende analyse in de groep patiënten zonder antipsychotica liet bovendien zien dat de prevalentie van het metabool syndroom bij dragers van het variante 1291 G-allel significant lager was in deze groep (OR 0.05; 95% CI 0.003 - 0.97, $p=0.048$). Mogelijk maskeren antipsychotica de effecten van het 1291 C/G polymorfisme, omdat de meeste antipsychotica α_{2A}-antagonistische eigenschappen hebben. Dit zou kunnen verklaren waarom we de associatie met het metabool syndroom wel vonden in patiënten zonder antipsychotica. Echter, vanwege het kleine aantal patiënten moet dit resultaat met voorzichtigheid benaderd worden en gerepliceerd worden. Deze studie laat zien dat het *ADRA2A* 1291 C/G polymorfisme geen sterke voorspeller lijkt te zijn voor het ontstaan van het metabool syndroom in patiënten die antipsychotica gebruiken.

Het tweede deel van dit proefschrift beschrijft studies waarin onderzocht werd of er associaties bestonden tussen polymorfismen in de *HTR2C* en *ADRA2A* genen en metabole eindpunten in patiënten die geen psychofarmaca gebruikten. Als de effecten van de polymorfismen in de *HTR2C* en *ADRA2A* genen ook zichtbaar zijn in patiënten zonder antipsychotica, dan zou dit kunnen helpen bij het in een vroeg stadium identificeren van patiënten met een verhoogd risico op metabole complicaties. Dit zou kunnen leiden tot preventieve aanpassingen van het dieet of een verhoogde monitoring op het ontstaan van de complicaties. Als de associaties die gevonden werden in patiënten met antipsychotica niet aanwezig zijn in patiënten zonder deze geneesmiddelen, dan levert dit informatie op over de invloed van deze psychofarmaca op de effecten van de onderzochte polymorfismen en de metabole status.

In **hoofdstuk 3.1** hebben we onderzocht of de prevalentie van de *HTR2C* rs1414334 en 759 C/T genotypen verschilt tussen obese patiënten ($BMI>30$ kg/m^2, cases) en patiënten met een normaal lichaamsgewicht ($BMI<25$ kg/m^2, controles). In een dwarsdoorsnede case-control onderzoek werden 116 cases en 100 controles geïncludeerd, waarvan het merendeel (2/3) vrouwen betrof. Patiënten met obesitas werden afkomstig uit de obesitaskliniek van

het ziekenhuis, terwijl patiënten met een normaal lichaamsgewicht afkomstig waren uit de ziekenhuisapotheek en orthopedie-afdeling. De casegroep was significant jonger dan de controlegroep (45.7 vs. 65.1 jaar). We vonden een significant lagere prevalentie van het 759 T (mannen) of TT (vrouwen) genotype in patiënten met obesitas (OR 0.18; 95%CI 0.04 - 0.78). Dragerschap van het variante 759 T-allel liet een beschermende puntschatting zien, maar deze was niet significant (OR 0.53; 95%CI 0.24 - 1.17). Er werden geen andere associaties gevonden. De resultaten van deze studie komen overeen met de resultaten van studies naar gewichtstoename of obesitas door antipsychotica, waarbij het 759 T-allel ook beschermend was. Het beschermende effect van het *HTR2C* 759 T-allel lijkt daarom onafhankelijk te zijn van de aanwezigheid van antipsychotica.

Het doel van **hoofdstuk 3.2** was het onderzoeken van een mogelijke associatie tussen de *HTR2C* rs1414334 en *ADRA* 1291 C/G polymorfismen en lipidenconcentraties in obese patiënten zonder antipsychotica. In de studies van hoofdstukken 2.3 en 2.4 vonden we associaties tussen deze polymorfismen en triglyceriden concentraties in het bloed. Als deze polymorfismen werkelijk de lipolyse beïnvloeden zou hun invloed ook zichtbaar kunnen zijn in obese patiënten. In een dwarsdoorsnede onderzoek werden 130 obese patiënten vanuit de obesitaskliniek geïncludeerd, waaronder de patiënten van de populatie in hoofdstuk 3.1. Patiënten die het variante *HTR2C* rs1414334 C-allel droegen hadden significant hogere concentraties triglyceride in het bloed dan patiënten zonder dit allel ($\Delta_{triglyceride}$ +0.34 (95%BI 0.02 - 0.66) mmol/L). Patiënten die het variante *ADRA2A* 1291 G-allele droegen hadden lagere concentraties LDL-cholesterol in het bloed dan patiënten zonder dit allel (Δ_{LDL} -0.41 (95%BI -0.77 - -0.04 mmol/L). In een gecombineerde genotypenanalyse werden deze associaties sterker wanneer slechts één van de variante allelen aanwezig was. Er werden geen andere associaties met lipidenspiegels gevonden. De in hoofdstuk 2.4 gevonden associatie tussen het *ADRA2A* 1291 C/G polymorfisme en triglyceriden concentraties werd derhalve niet gerepliceerd. Deze studie versterkt het bewijs voor een associatie tussen het *HTR2C* rs1414334 polymorfisme en triglyceriden spiegels, dat onafhankelijk lijkt te zijn van antipsychotica. De andere bevindingen, waaronder de associaties tussen *ADRA2A* en LDL-cholesterol en de interactie tussen de *HTR2C* en *ADRA2A* polymorfismen, vergen verder onderzoek.

In **hoofdstuk 3.3** hebben we onderzocht of de prevalentie van gecombineerde *HTR2C-LEP* en *HTR2C-LEPR* genotypen verschilt tussen obese en niet-obese patiënten en of de interactie tussen deze polymorfismen, zoals die gevonden was in schizofrene patiënten, ook zichtbaar was in patiënten zonder psychofarmaca. Het gebruiken van een gecombineerd genotype zou kunnen helpen in het verder kunnen identificeren van patiënten met een hoog risico op obesitas. Voor dit onderzoek gebruikten we een dwarsdoorsnede case-control ontwerp met dezelfde patiëntenpopulatie als in hoofdstuk 3.1 (n=216). Zoals verwacht, gezien de resultaten van hoofdstuk 3.1, was er een lagere prevalentie van het *HTR2C* 759 T-allel in obese patiënten dan in patiënten met een normaal lichaamsgewicht. Dit was echter onafhankelijk van de *LEP*

of *LEPR* genotypen. Prevalentie van obesitas was significant lager in patiënten met het variante *HTR2C* 759 T-allel, zowel in aan- als afwezigheid van het *LEPR* 223R allel, vergeleken met patiënten zonder het 759 T-allel (OR 0.22; 95%BI 0.05 - 0.97 en OR 0.31; 95%CI 0.1 - 0.998, respectievelijk). Er werden geen andere significante resultaten gevonden. We waren daarom niet in staat om de interactie tussen *LEP* en *HTR2C* te repliceren zoals deze gevonden was in patiënten met antipsychotica. Deze interactie zou daarom afhankelijk kunnen zijn van antipsychotica. Wellicht speelt een invloed van antipsychotica op het leptine of histaminerge systeem hier een rol bij.

Hoofdstuk 3.4 beschrijft een lopend longitudinaal onderzoek waarin patiënten met obesitas gevolgd worden tijdens hun 9 maanden durende afslankprogramma binnen de obesitaskliniek. Wanneer het *HTR2C* 759 C/T polymorfisme invloed heeft op gewichtstoename en de prevalentie van obesitas, en de *ADRA2A* 1291 C/G en *HTR2C* rs1414334 polymorfismen lipolyse en het metabool syndroom beïnvloeden, dan zouden deze polymorfismen ook het succes van het afslankprogramma kunnen beïnvloeden voor wat betreft gewichtsverlies en verbetering van lipidenconcentraties. Als we op basis van het DNA profiel zouden kunnen weten welke patiënten minder kans hebben op het succesvol doorlopen van het afslankprogramma, dan vermindert dit de kosten voor de gezondheidszorg en voorkomt het verdere motivatieproblemen bij obese patiënten die graag gewicht willen verliezen. Dergelijke hoog risico patiënten zouden wellicht eerder in aanmerking kunnen komen voor alternatieve behandelingen, zoals chirurgie. Aangezien het aantal geïncludeerde patiënten nog te klein is om een adequate data-analyse uit te voeren, hebben we een interim-analyse uitgevoerd bij een afkappunt van 3 maanden follow-up. Dit maakte het mogelijk 76 patiënten te includeren. We vonden in deze interim-analyse echter geen associatie tussen het *HTR2C* 759 C/T polymorfisme en gewichtsverlies tijdens het afslankprogramma. Gewichtsverlies tijdens de eerste periode (1^{e} maand) en tweede periode (2^{e} en 3^{e} maand) was vergelijkbaar tussen de genotypengroepen. We vonden ook geen associaties tussen de *HTR2C* rs1414334 en *ADRA2A* 1291 C/G polymorfismen en concentraties triglyceriden of LDL-cholesterol, in tegenstelling tot de resultaten van hoofdstuk 3.2. De korte follow-up periode is een belangrijke beperking in deze interim-analyse. Het is mogelijk dat verschillen tussen genotypengroepen groter worden, en bloot komen te liggen, naarmate de follow-up periode verlengd wordt. Het is ook mogelijk dat patiënten met een minder gunstig genotype een hoger risico lopen op terugval na afloop van het programma of weer aankomen in gewicht aan het einde van het afslankprogramma. Studies hebben bovendien laten zien dat veranderingen in lipidenconcentraties tijdens een afslankprogramma in het begin klein zijn, of zelfs initieel een verslechtering laten zien, om pas te verbeteren na een langere follow-up periode. Een toekomstige analyse in een grotere groep patiënten met een langere follow-up periode zou uitsluitsel kunnen geven over het al dan niet bestaan van associaties tussen de *HTR2C* en *ADRA2A* polymorfismen en gewichtsverlies of lipidenconcentraties in obese patiënten die een afslankprogramma doorlopen.

Tot slot hebben we in hoofdstuk 4 drie onderwerpen besproken die relevant waren in een of meer van de andere hoofdstukken van dit proefschrift; 'uitdagingen in genetische associatie studies', 'implementatie in de dagelijkse praktijk' en 'vooruitzichten over toekomstig onderzoek'. Op dit moment is het niet mogelijk om op basis van genetische informatie, waaronder de *HTR2C* en *ADRA2A* genotypen, klinisch relevante voorspellingen te doen over het ontstaan van metabole bijwerkingen in aan- of afwezigheid van antipsychotica. Er zijn significante associaties gevonden in dit proefschrift, maar deze associaties verklaren maar een klein gedeelte van de variatie tussen patiënten gezien de (genetische) complexiteit van aandoeningen zoals obesitas en het metabool syndroom. Echter, als onderzoekers hun inspanningen en patiëntenpopulaties combineren, en combinaties van polymorfismen onderzoeken, zou het in de nabije toekomst mogelijk kunnen zijn om meer stukjes van de genetische puzzel te leggen en een algoritme te ontwikkelen dat gebruikt kan worden voor het inschatten van het metabole risico bij een patiënt. Zo'n algoritme zou psychiaters kunnen helpen bij het maken van een optimale keuze tussen de psychofarmaca, waardoor een betere balans ontstaat tussen effectiviteit en veiligheid.

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Publications related to this thesis

Risselada AJ, Mulder H. Genetische beïnvloeding van antipsychotica-geïnduceerde gewichtstoename. Psyfar 2008 (4):15-21.

Risselada AJ, Mulder H, Heerdink ER, Grube AM, Wilmink FW, Egberts TCG. The association between serotonin 2C receptor polymorphisms and weight gain and eating behaviour in patients using mirtazapine. A prospective follow-up study. Journal of Clinical Psychopharmacology 2010; 30: 207-209.

Risselada AJ, Vehof J, Bruggeman R, Wilffert B, Cohen D, Al Hadithy AF, Arends J, Mulder H. Association between HTR2C gene polymorphisms and the metabolic syndrome in patients using antipsychotics: a replication study. The Pharmacogenomics Journal 2010; doi:10.1038/tpj.2010.66 (advance online publication).

Risselada AJ, Vehof J, Bruggeman R, Wilffert B, Cohen D, Al Hadithy AF, Arends J, Mulder H. Association between the 1291-C/G polymorphism in the adrenergic α-2a receptor and the metabolic syndrome. Journal of Clinical Psychopharmacology 2010;30: 667-671.

Risselada AJ, Mulder H, Heerdink ER, TCG Egberts. Pharmacogenetic testing to predict antipsychotic-induced weight gain: a systematic review. Pharmacogenomics 2011;12(8):1213-1227.

Risselada AJ, Mulder H, Heerdink ER, Gonera RK, Egberts TCG. Association between HTR2C polymorphisms and obesity in patients without antipsychotic drugs. Journal of Clinical Psychopharmacology 2011. *Article under revision*.

About the author

Arne Jouke Risselada was born on the 24th of December 1976 in Kollum, the Netherlands. In 1995 he completed secondary school (Atheneum) at the Lauwerscollege in Buitenpost and began his study in Pharmacy at the University of Groningen. He obtained his Pharmacist degree in 2002 and started working at the department of Clinical Pharmacy at the 'Ziekenhuisgroep Twente' in Almelo and Hengelo, where he was trained as a hospital pharmacist.

After completing his training as a hospital pharmacist in 2007 he began working as a hospital pharmacist at the Wilhelmina Hospital Assen, where he is still currently employed.

In 2008 he started his PhD research at the Division of Pharmacoepidemiology and Clinical Pharmacology of the Utrecht Institute for Pharmaceutical Sciences of Utrecht University. During this period he also obtained a Master of Science degree in Epidemiology at the EMGO Institute of the VU University Medical Center in Amsterdam and was registered as an epidemiologist.

Arne is married to Barbara Lutz and they are the proud parents of Amélie Clarijn (2008) and Lennart Herre (2010).

About the research group

The CNS clinical pharmacoepidemiology research group

Background

Central Nervous System Clinical Pharmacoepidemiology is one of the research themes of the division of Pharmacoepidemiology & Clinical Pharmacology of the Utrecht Institute for Pharmaceutical Sciences (UIPS). The division of Pharmacoepidemiology & Clinical Pharmacology consists of a multidisciplinary team of young and internationally oriented researchers. The research program is directed at the epidemiological, therapeutic and policy aspects of drug use and their effects. The mission of the research program is to contribute to the knowledge of and decision-making in the effectiveness, safety and economics of drug usage. In bridging the gap between the science of pharmacoepidemiology and the 'real world' of patients' drug usage and public health, the program covers a variety of methods and approaches from (molecular) epidemiology, pharmacovigilance, practice research and policy analysis. The myriad of research strategies provides an excellent environment for thoughtful learning and innovation in system therapeutics.

The Central Nervous System Clinical Pharmacoepidemiology research group focuses on the use and effects of psychotropic drugs in psychiatry and neurology, both in ambulatory care and in clinical settings. Principle investigators of this research group are Dr Eibert R Heerdink and Prof dr Toine CG Egberts. There is close collaboration with psychiatric hospitals including Altrecht and GGZ Centraal and with the University Medical Centre Utrecht.
Contact: www.uu.nl/science/pharmacoepidemiology

Theses from the CNS clinical pharmacoepidemiology research group:

Dr Bart Kleijer (2011)
Balancing the benefits and risks of antipsychotics. (Co)promotores: Prof dr ACG Egberts, Prof dr MW Ribbe, Dr ER Heerdink, Dr R van Marum.

Dr Wilma Knol (2011)
Antipsychotic induced parkinsonism in the elderly: assessment, causes and consequences. (Co)promotores: Prof dr AFAM Schobben, Prof dr ACG Egberts, Dr PAF Jansen, Dr R van Marum.

Dr Inge van Geijlswijk (2011)
Melatonin in sleepless children. Everything has a rhythm? (Co)promotores: Prof dr ACG Egberts, Prof dr H Vaarkamp, Dr M Smits.

Dr Maurits Arbouw (2010)
Assessment of pharmacotherapy in Parkinson's disease. (Co)promotores: Prof dr ACG Egberts, Prof dr HJ Guchelaar, Prof dr C Neef, Dr KLL Movig.

Dr Laurette Goedhard (2010)
Pharmacotherapy and aggressive behaviour in psychiatric patients. (Co)promotores: Prof dr ACG Egberts, Prof dr H Nijman, Dr ER Heerdink, Dr JJ Stolker.

Dr Jeroen Derijks (2009)
Effects of antidepressants on glucose homeostasis. Effects and mechanisms. (Co)promotores: Prof dr ACG Egberts, Dr ER Heerdink, Dr GHP de Koning, Dr R Janknegt.

Dr Helga Gardarsdottir (2009)
Drug treatment episodes in pharmacoepidemiology: antidepressant use as a model. (Co)promotores: Prof dr ACG Egberts, Dr ER Heerdink.

Dr Kim Gombert - Handoko (2009)
Treatment failure in epilepsy: exploring causes of ineffectiveness and adverse effects. (Co)promotores: Prof dr ACG Egberts, Prof dr YA Hekster, Dr J Zwart-van Rijkom, Dr W Hermens.

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Dr Mirjam Knol (2008, summa cum laude)
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