Association of genetic variants of the histamine H1 and muscarinic M3 receptors with BMI and HbA1c values in patients on antipsychotic medication

Jelle Vehof · Arne J. Risselada · Asmar F. Y. Al Hadithy · Huibert Burger ·
Harold Snieder · Bob Wilffert · Johan Arends · Lex Wunderink ·
Henrikus Knegtering · Durk Wiersma · Dan Cohen · Hans Mulder ·
Richard Bruggeman

Abstract

Rationale Antipsychotic affinity for the histamine H1 receptor and the muscarinic M3 receptor have been associated with the side effects weight gain, and development of diabetes, respectively.

Objectives We investigated polymorphisms of the histamine H1 (*HRH1*) and muscarinic acetylcholine receptor M3 (*CHRM3*) receptor genes for an association with body mass index (BMI) and glycated hemoglobin (HbA1c).

Methods We included 430 Caucasian patients with a non-affective psychotic disorder using antipsychotics for at least 3 months. Primary endpoints of the study were cross-sectionally measured BMI and HbA1c; secondary endpoints were obesity and hyperglycaemia. Two single-nucleotide polymorphisms (SNPs) in the *HRH1* gene, rs346074 and rs346070, and one SNP in the *CHRM3* gene, rs3738435, were genotyped. Our primary hypothesis in this study was an interaction between genotype on BMI and antipsychotic affinity for the H1 and M3 receptor.

Results A significant association of interaction between haplotype rs346074–rs346070 and BMI (p value 0.025) and obesity (p value 0.005) in patients using high-H1 affinity antipsychotics versus patients using low-H1 affinity antipsychotics was found. There was no association of

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J. Vehof (✉) · H. Burger · D. Wiersma · R. Bruggeman
Department of Psychiatry, University Medical Center Groningen,
University of Groningen,
Groningen, The Netherlands
e-mail: j.vehof@epi.umcg.nl

J. Vehof · H. Burger · H. Snieder
Department of Epidemiology,
University Medical Center Groningen, University of Groningen,
Groningen, The Netherlands

A. J. Risselada · H. Mulder
Department of Clinical Pharmacy, Wilhelmina Hospital Assen,
Assen, The Netherlands

A. J. Risselada · H. Mulder
Department of Pharmacoepidemiology and Pharmacotherapy,
Utrecht Institute for Pharmaceutical Sciences (UIPS),
Utrecht University,
Utrecht, The Netherlands

A. F. Y. Al Hadithy · B. Wilffert · R. Bruggeman
Department of Pharmacotherapy and Pharmaceutical Care,
University of Groningen,
Groningen, The Netherlands

A. F. Y. Al Hadithy
Hospital Pharmacy, Erasmus University Medical Center,
Rotterdam, The Netherlands

J. Arends
Mental Health Services Drenthe,
Assen, The Netherlands

L. Wunderink
Mental Health Services Friesland,
Leeuwarden, The Netherlands

H. Knegtering
Mental Health Services Lentis,
Groningen, The Netherlands

D. Cohen
Mental Health Services GGZ-NHN,
Heerhugowaard, The Netherlands

CHRM3 gene variant rs3738435 with BMI, and we observed no association with HbA1c or hyperglycaemia in any of the variants.

Conclusions This study, for the first time, demonstrates a significant association between *HRH1* variants and BMI in patients with a psychotic disorder using antipsychotics. In future, genotyping of *HRH1* variants may help predicting weight gain in patients using antipsychotics.

Keywords Antipsychotics · BMI · HbA1c · Schizophrenia · Polymorphism · Histamine · Muscarine · Pharmacogenetics · Weight gain · Hyperglycaemia

Introduction

The majority of patients with schizophrenia or other psychotic disorder use antipsychotic medication. Antipsychotic treatment, especially the use of clozapine and olanzapine, increases the risk of developing obesity (Allison et al. 1999; Lieberman et al. 2005; Parsons et al. 2009) and type 2 diabetes mellitus (T2DM) (Leslie and Rosenheck. 2004; Holt et al. 2005; Lieberman et al. 2005; Miller et al. 2005b; Newcomer. 2005; Gianfrancesco et al. 2006; Nasrallah. 2006). The underlying mechanisms of antipsychotic-induced weight gain and diabetes mellitus are unknown, and may involve different pathways. As in the general population, obesity may have an unfavorable impact on glucose homeostasis in patients using antipsychotics. However, several studies have shown elevated serum insulin levels following atypical antipsychotic medication independent of body mass index (BMI) (Melkersson et al. 2000; Arranz et al. 2004; Henderson et al. 2005). This finding suggests that antipsychotics may directly affect glucose homeostasis by mechanisms other than by weight gain alone. There is also a considerable variability among users of the same antipsychotic in weight gain and T2DM (e.g., not all patients on clozapine ultimately develop T2DM). It is plausible that this variability in patient propensity to these side effects is determined by a combination of genetic and environmental factors.

Atypical antipsychotics may differ highly in their affinities for the dopaminergic, serotonergic, histaminergic, adrenergic, and muscarinic acetylcholine receptors (Roth et al. 2004). Combining receptor affinities and clinical data, several authors have concluded that histamine H1 antagonism showed the best correlation with drug-induced weight gain and diabetes mellitus (Wirshing et al. 1999; Kroeze et al. 2003; Matsui-Sakata et al. 2005). Likewise, antagonism of the muscarine acetylcholine receptor was suggested to play an important role, especially in the development of diabetes mellitus (Matsui-Sakata et al. 2005; Silvestre and Prous. 2005). Interactions with serotonergic (5-HT2C and

5-HT6) and adrenergic (alpha1A) receptors were also significantly correlated with metabolic parameters (Kroeze et al. 2003; Matsui-Sakata et al. 2005). To date, pharmacogenetic studies have shown the most consistent evidence for polymorphisms in the 5-HT2C receptor and leptin genes to be associated with antipsychotic-induced weight gain (Reynolds et al. 2003; Ellingrod et al. 2005; Miller et al. 2005a; Templeman et al. 2005; Zhang et al. 2007; Kang et al. 2008; Gregoor et al. 2009) and the metabolic syndrome (Mulder et al. 2007a; Yevtushenko et al. 2008; Mulder et al. 2009; Risselada et al. 2010). So far, only two studies (Basile et al. 2001; Hong et al. 2002) have reported on histamine H1 polymorphisms and antipsychotic-induced weight gain, both finding no association. Thus, the contribution of genetic variations of the histamine and muscarine acetylcholine receptors on the emergence of weight gain and diabetes in antipsychotic-treated patients remains to be elucidated.

The ventromedial hypothalamus and the paraventricular nucleus of the brain, where H1 receptors are localized in high density (Sakata et al. 1995), play a central role in the development of obesity by regulating energy expenditure and food intake (Masaki et al. 2004). Clozapine, olanzapine, and quetiapine exhibit the highest affinities for the H1 receptor, whereas risperidone and aripiprazole exhibit lower, and ziprasidone and haloperidol exhibit hardly any affinity towards the H1 receptor (Roth et al. 2004; Nasrallah. 2008). Clozapine and olanzapine are also known to induce most weight gain, followed by quetiapine and risperidone. Aripiprazole, ziprasidone, and haloperidol are known to cause little or no weight gain at all (Wirshing et al. 1999; Nasrallah. 2008). Tricyclic antidepressants with a high antihistaminergic effect (e.g. amitriptyline) are found to induce weight gain as well (Zimmermann et al. 2003). The histamine H1 receptor may therefore play a role in the etiology of medication-induced weight gain.

The M3 receptor is expressed on pancreatic β cells. These receptors seem to play a critical role in regulating insulin release and glucose homeostasis (Gautam et al. 2006). Impaired glucose tolerance and reduced levels of insulin were found in mice with targeted deletions in the *CHRM3* gene (Gautam et al. 2006). This might indicate that antagonism of the β-cell M3 receptor leads to a higher risk of hyperglycemia and developing diabetes in humans. Olanzapine and clozapine, which have the highest binding affinities with the M3 receptor, have been associated with highest risk of developing T2DM (Citrome et al. 2004; Leslie and Rosenheck. 2004; Holt et al. 2005; Newcomer. 2005) and higher levels of glycated hemoglobin (HBA1c) and blood glucose (Lieberman et al. 2005; Gianfrancesco et al. 2006; Nasrallah. 2008). Risperidone, quetiapine, ziprasidone, haloperidol, and aripiprazole have weak to absent M3 receptor antagonistic activity (Roth et al. 2004;

Nasrallah. 2008) and are associated with lower levels of HbA1c and blood glucose in patients (Lieberman et al. 2005; Nasrallah. 2008).

Out of the known H1 receptor gene (*HRH1*) splice variants, we studied two polymorphisms in the B/K variant, which is by far the most prevalent (95%) in the brain (Swan et al. 2006). Rs346070 is a single-nucleotide polymorphism (SNP) and may be functional as it is located in the exonic splicing enhancer region. SNP rs346074 is located in the transcription factor binding sites of the *HRH1* gene and may thus affect transcription rates. The muscarinic acetyl-choline receptor M3 (*CHRM3*) variant rs3738435 is located in the 5′ untranslated region of the first exon. Its C allele was found to be associated with increased risk of early-onset type 2 diabetes and a reduced acute insulin response in a family-based sample of Pima Indians (Guo et al. 2006).

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

Materials and methods

Setting

For this study, three similar psychiatric patient populations from the Netherlands were pooled. The majority of patients were from the ongoing 'Pharmacotherapy Monitoring and Outcome Survey' (PHAMOUS). PHAMOUS is an initiative from the Rob Giel Research centre, including three Mental Health Care Institutions and the University Centre of Psychiatry of Groningen. It combines a yearly somatic screening with routine outcome assessment in patients using antipsychotics included. Subjects included in this study originated from the northern part of the Netherlands. The two other study populations have been described in detail elsewhere (Cohen et al. 2006; Mulder et al. 2007a; Mulder et al. 2009). In brief, these populations consisted of patients from a Department of Psychiatric Disorders of a general hospital in the North of the Netherlands (Mulder et al. 2007a; Mulder et al. 2009), and patients from a Mental Health Care Organisation in the West of the Netherlands (Cohen et al. 2006).

Design and patients

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

Outcome measures

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

Determinants

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

Genotyping

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

Statistical analysis

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

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

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

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

We first compared demographic characteristics between the genotypes of the three variants. To test our primary hypothesis, we applied linear regression to test whether genotype in users of high-affinity antipsychotics has a significantly different outcome on BMI and HbA1c than in users of low-affinity antipsychotics. We used the interaction term affinity x genotype in our model to test this association, where affinity was coded as 1 or 0 when the patient used a high- or a low-affinity antipsychotic, respectively. A pKi > 7 defined a high-affinity antipsychotic for a certain receptor (Nasrallah. 2008), the other antipsychotics were considered having a low affinity. We adjusted for age, gender, and patient population in our analyses. Similarly, logistic regression was used to analyze the associations with obesity (BMI > 30 kg/m^2) and hyperglycemia (HbA1c $\geq$ 6.1% or the use of antidiabetics). Additionally, for the two *HRH1* variants haplotype analysis using the haplotype trend regression approach (Zaykin et al. 2002) was performed, with haplotypes inferred by the software package PHASE (Stephens et al. 2001; Stephens and Donnelly. 2003). Pairwise linkage disequiliburum (LD)

was tested by calculating D′, as well as r^2. All of the analyses were performed using standard software (SPSS 16.0 for Windows). The level of significance was set at 0.05, two-sided.

Results

Subjects

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

Medication

Patients used monotherapy clozapine (21.9%), olanzapine (22.6%) or risperidon (22.1%), aripiprazole (2.3%), quetiapine (4.2%), typical antipsychotics (14.4%), or had a combination of more than one antipsychotic (12.6%). No substantial differences in BMI (range 27.4–29.3 kg/m^2) were found between users of the various antipsychotics (*p* value ANOVA 0.58) or between different diagnoses. HbA1c values (range 5.5–6.8%) were significantly differ-

ent between the various antipsychotics (p value ANOVA 0.033). Between users of typical and atypical antipsychotics, no differences in BMI and HbA1c were found (p value Student's t test 0.93 and 0.82, respectively). Of all antipsychotics used in our population, clozapine, olanzapine, and quetiapine were defined as high H1 receptor affinity antipsychotics, and clozapine and olanzapine as high M3 receptor affinity antipsychotics.

Association analyses

Genotype distributions were consistent with the Hardy–Weinberg equilibrium (p values 0.59, 0.88, and 1.00 for rs346074, rs346070, and rs3738435, respectively). Age (increase of 0.055 kg/m^2 per year, p value 0.021) and gender (increase of 2.97 kg/m^2 if female, p value <0.001) were significantly associated with BMI. Patient population

was not associated with BMI. HbA1c was not associated with patient population, age, or gender. Demographic characteristics, DSM-IV-diagnosis, and antipsychotic distributions did not differ between genotype groups in all three variants.

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

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

Variables	No. of patients	Mean (SD)/proportion			p value β genotype	p value β interaction genotype x affinity
HRH1 rs346074	GG/GA/AA	GG	GA	AA		
BMI	182/189/55	28.0 (5.2)	27.8 (5.3)	28.5 (5.0)	0.93	**0.046**
High aff.	83/97/28	27.5 (4.2)	27.7 (5.3)	30.1 (5.3)	0.27	
Low aff.	99/92/27	28.4 (5.9)	27.9 (5.2)	26.8 (4.0)	0.10	
Obesity	182/189/55	34%	30%	31%	0.58	**0.005**
High aff.	83/97/28	25%	30%	46%	0.14	
Low aff.	99/92/27	40%	30%	15%	**0.015**	
HRH1 rs346070	CC/CT/TT	CC	CT	TT		
BMI	286/128/15	28.0 (5.1)	28.2 (5.6)	27.4 (4.8)	0.74	**0.044**
High aff.	139/58/12	27.6 (4.7)	29.0 (5.9)	28.5 (4.2)	0.10	
Low aff.	147/70/3	28.4 (5.5)	27.5 (5.3)	22.9 (4.9)	0.22	
Obesity	286/128/15	34%	29%	20%	0.22	**0.009**
High aff.	139/58/12	28%	38%	25%	0.36	
Low aff.	147/70/3	39%	21%	0%	**0.006**	
CHRM3 rs3738435	TT/TC/CC	TT	TC	CC		
BMI	276/137/17	28.0 (5.2)	27.6 (5.2)	30.4 (5.5)	0.60	0.88
High aff.	127/57/7	27.8 (4.9)	27.8 (4.9)	30.7 (6.1)	0.33	
Low aff.	149/80/10	28.3 (5.5)	27.5 (5.4)	30.2 (5.3)	0.90	
Obesity	276/137/17	31%	32%	53%	0.15	0.56
High aff.	127/57/7	28%	32%	57%	0.16	
Low aff.	149/80/10	34%	33%	50%	0.56	

BMI (kg/m^2, mean and standard deviation) and obesity (%) are given per genotype group, separated in users of antipsychotics with low and high affinity for the histamine H1 receptor (in rs346074 and rs346070 high affinity clozapine, olanzapine, and quetiapine) and the muscarine M3 receptor (in rs3738435 high affinity clozapine and olanzapine).

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

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

Genotype was tested additive in rs346074, and dominant for the minor allele in rs346070 and rs3738435
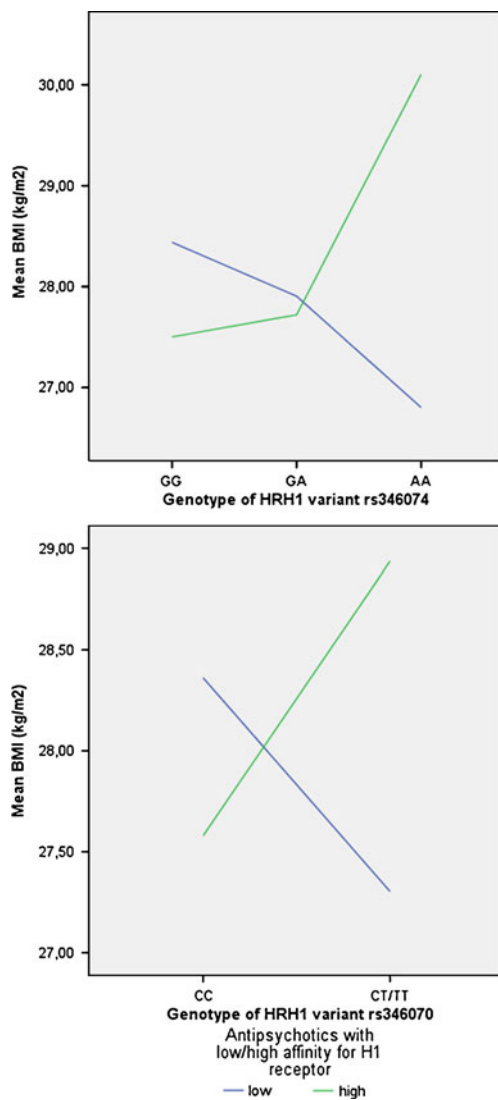
Significant p values are shown in bold

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

0.046 and 0.033, respectively), and when using a dominant model for the T allele of rs346070 (*p* value 0.044).

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

The two *HRH1* SNPs were found to be in substantial LD (D'=1.00, r^2=0.42). Haplotype analyses of the two polymorphisms showed similar opposite effects of haplotype on

BMI and obesity in low and high H1 affinity antipsychotic users (see Table 3). For each AT-haplotype, having a high H1 affinity antipsychotic means a more than three times higher risk of obesity (*p* value 0.005) compared to the reference haplotype G-C, than when having a low H1 affinity antipsychotic.

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

Discussion

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

Although it has been proposed that histamine H1 receptor antagonism causes weight gain (Kroeze et al. 2003; Matsui-Sakata et al. 2005), earlier studies on other histamine H1 receptor variants showed no relationship with clozapine-induced weight gain (Basile et al. 2001; Hong et al. 2002). Of note, post-hoc analysis in our study showed similar direction and effect size of the risk alleles on BMI in all three high H1 affinity antipsychotics studied (clozapine, olanzapine, and quetiapine), emphasizing the role of the histamine receptor.

Regarding the metabolic consequences of antipsychotic treatment, several receptors other than the H1 receptor are of importance (Reynolds and Kirk. 2010), especially the 5-HT2C receptor. Previously, we have shown a significant association between 5-HT2C polymorphism rs1414334 and obesity (Mulder et al. 2007b) and the metabolic syndrome (Mulder et al. 2007a; Mulder et al. 2009; Risselada et al. 2010). The association with obesity of this polymorphism also tested significant in the present population (data not shown). We additionally included this polymorphism as a covariate in our regression analysis on obesity. This did not alter the results of the H1 polymorphisms on obesity, implying a 5-HT2C rs1414334 independent, additive effect of our H1 polymorphisms.

Within the hypothalamus, histamine and the H1 receptor are part of the leptin-signaling pathway (Sakata et al. 1988;

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

BMI	Haplotype (rs346074-rs346070)	β in high H1 affinity AP users	p value	β in low H1 affinity AP users	p value	β of interaction term haplotype * affinity	p value
	G-C	–		–		–	
	A-C	+0.569	0.38	−0.129	0.85	0.795	0.39
	A-T	+0.941	0.10	−1.093	0.13	2.043	**0.025**
Obesity	Haplotype (rs346074-rs346070)	e^{β} in high H1 affinity AP users	p value	e^{β} in low H1 affinity AP users	p value	e^{β} of interaction term haplotype * affinity	p value
	G-C	–		–		–	
	A-C	1.672	0.10	0.795	0.43	2.110	0.07
	A-T	1.256	0.42	0.375	**0.004**	3.331	**0.005**

The unstandardized coefficients (β) of haplotype in linear regression with BMI and the odds ratios (e^{β}) of haplotype in logistic regression with obesity are given, in high and low H1 affinity antipsychotic (*AP*) users, respectively

Haplotypes A-C and A-T are compared with the most frequent haplotype G-C as a reference. Haplotype G-T was not prevalent

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

Significant *p* values are shown in bold

Masaki et al. 2001). Leptin is an adipocyte-specific hormone that regulates the mass of adipose tissue through hypothalamic effects on satiety and energy expenditure (Forbes et al. 2001). Polymorphisms in the leptin and leptin receptor gene have been associated with antipsychotic-induced weight gain (Templeman et al. 2005; Zhang et al. 2007; Kang et al. 2008; Gregoor et al. 2009). Templeman et al. (2005) demonstrated that a genetic variation in the 5-HT2C receptor resulted in different pre-treatment leptin levels. Of note, an interaction between two polymorphisms in the 5-HT2C receptor and leptin gene was showed to influence the risk of metabolic disturbances during antipsychotic treatment (Yevtushenko et al. 2008). Future studies investigating gene–gene interactions between histamine H1, 5-HT2C and leptin genes may help unravel the exact role of the histamine system in antipsychotic-induced weight gain.

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

Next to antipsychotics, several other risk factors for hyperglycaemia are overrepresented in psychotic patients, such as a positive family history, high BMI, and reduced physical activity. It has been hypothesized that patients with schizophrenia may already have β-cell defects prior to antipsychotic treatment (Bergman and Ader. 2005). Since several factors, involving multiple metabolic pathways, may contribute to hyperglycaemia in psychosis patients, examining genetic associations with antipsychotic-induced alterations in glucose homeostasis may be difficult to perform.

The present study has some limitations. First, we did not have complete quantitative information on the cumulative exposure to currently and previously used antipsychotics. Therefore, the relationship between BMI and users of antipsychotics with H1 affinity may be partly biased by earlier use of a previous other antipsychotic. However, since all patients used the antipsychotic for at least 3 months, we do not expect this limitation to be a serious deficit. Second, since this study is cross-sectional, we did not have information on BMI or HbA1c before antipsychotic treatment was started, suggesting that results might

reflect non-antipsychotic-mediated pathways. However, this is very unlikely, since we decided to test the interaction between genotype and antipsychotic affinity for the certain receptor. We found significantly different genotype effects on BMI values between users of antipsychotics with high and low affinity for the H1 receptor. Since one would expect genotype effect on baseline BMI values to be similar between future users of low and high H1 affinity antipsychotics, non-antipsychotic-mediated effects of genotype would not lead to differences in genotype effect on BMI between users with high and low H1 affinity antipsychotics. Also, genotype distributions did not differ between users of low and high H1 affinity antipsychotics, ruling out the possibility of confounding by indication because of genotype. Despite its limitations this study has also several merits. First, compared to previous studies, we have a big sample size (more than 400 patients). Second, we have a very homogeneous group of Caucasian patients of Northern European ancestry, all diagnosed with a non-affective psychosis.

In conclusion, the *HRH1* gene haplotype consisting of rs346074 and rs346070 might be associated with BMI and obesity in patients using antipsychotics with high affinity for the histamine H1 receptor. These findings need to be replicated in independent samples. In none of the variants an association with HbA1c or hyperglycaemia was found. Genotyping for *HRH1* variants may help predicting weight gain in patients using atypical antipsychotics. Further longitudinal studies are warranted to investigate the potential role on BMI of the *HRH1* gene.

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