Association of orexin receptor polymorphisms with antipsychotic-induced weight gain

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ABSTRACT

Objectives: Antipsychotic-induced weight gain (AIWG) is a common side effect of treatment with antipsychotics such as clozapine and olanzapine. The orexin gene and its receptors are expressed in the hypothalamus and have been associated with maintenance of energy homeostasis. In this study, we have analysed tagging single nucleotide polymorphisms (SNPs) in orexin receptors 1 and 2 (HCRTR1 and HCRTR2) for association with AIWG.

Methods: Schizophrenia or schizoaffective disorder subjects (n = 218), treated mostly with clozapine and olanzapine for up to 14 weeks, were included. Replication was conducted in a subset of CATIE samples (n = 122) treated with either olanzapine or risperidone for up to 190 days. Association between SNPs and AIWG was assessed using analysis of covariance (ANCOVA) with baseline weight and duration of treatment as covariates.

Results: Several SNPs in HCRTR2 were nominally associated with AIWG in patients of European ancestry treated with either clozapine or olanzapine (P < 0.05). In the replication analysis two SNPs rs3134701 (P = 0.043) and rs12662510 (P = 0.012) were nominally associated with AIWG. None of the SNPs in HCRTR1 were associated with AIWG.

Conclusion: This study provides preliminary evidence supporting the role of HCRTR2 in AIWG. However, these results need to be confirmed in large study samples.

INTRODUCTION

Development of severe weight gain and metabolic syndrome continues to be a major hindrance in the use of second-generation antipsychotics (SGA) such as clozapine and olanzapine. Weight gain and obesity have a detrimental effect on the physical and psychological health of the patients and contribute to non-adherence to antipsychotic medication (Crisp et al. 2000; Lieberman et al. 2005a). Concordance of weight gain in monozygotic twins and sibling pairs exposed to antipsychotics suggests a role of genetic factors in AIWG (Theisen et al. 2005; Wehmeier et al. 2005; Gebhardt et al. 2010). Genetic association studies from our laboratory and others have shown an important influence of genetic variation in genes involved in the maintenance of energy homeostasis. Two of the most important and best replicated findings to date are association of genetic variation in the melanocortin 4 receptor (rs489693) and in the serotonin 5HT2c receptor genes (rs3813929) with AIWG (Muller and Kennedy 2006; Lett et al. 2012). In this study, we investigate the impact of genetic variation in the important but less studied orexin/hypocretin system genes on AIWG.

The orexin system includes the orexin gene coding for pre-pro-orexin which is cleaved into two polypeptides, orexin A (OXA, hypocretin 1, 33 amino acids) and orexin B (OXB, hypocretin 2, 28 amino acids). The biological action of the orexin peptides is mediated through two G-protein coupled receptors: orexin receptor 1 (OX1R or HCRTR1) and orexin receptor 2 (OX2R or HCRTR2; Sakurai and Mieda 2011; Kukkonen 2013; Perez-Leighton et al. 2013). Orexin receptors are expressed in several regions...
in the brain. OX1R, compared to OX2R, are predominant in the locus coeruleus, paraventricular thalamic nucleus and bed nucleus of the stria terminalis. OX2R are mainly expressed in the arcuate nucleus (ARC), paraventricular nucleus and lateral hypothalamic area (Marcus et al. 2001; Funato et al. 2009). The OX2R has been shown to play a major role in preventing high-fat diet-induced obesity and insulin insensitivity in mice (Funato et al. 2009). Continuous infusion of an OX2R selective agonist to the lateral ventricles of wild-type mice on a high-fat diet suppresses food intake, leads to significantly less fat mass and greater energy expenditure. In the same study mice with OX1R deletion showed improved glucose tolerance and insulin sensitivity on a high-fat diet suggesting that OX1R may also have a role in mediating the effect of high-fat diet on glucose metabolism (Funato et al. 2009). Overall, OX2R appears to play a major role in adverse dietary conditions with OX1R making minor contribution. The orexin gene and its receptors have also been associated to narcolepsy in mice, dogs and humans (Kukkonen 2013). Interestingly, individuals with narcolepsy have decreased caloric intake but have a higher body mass index and increased incidence of metabolic syndrome (Schuld et al. 2000; Nishino 2007). However, the orexin receptors have not been investigated for association with obesity in the general population using focussed comprehensive candidate gene studies.

The orexin system is modulated by leptin (Funato et al. 2009), and sends excitatory signals to neuropeptide Y (NPY) expressing neurons in the ARC increasing food intake (Muroya et al. 2004). In addition, it has been shown that the orexin system also interacts with endocannabinoids as injection of the cannabinoid receptor type 1 (CB1 or CNR1) antagonist, rimonabant, abolishes feeding induced by intracerebroventricular OXA injection (Crespo et al. 2008). Recently, Cristino et al. (2013), reported that in murine models of obesity (leptin deficient), increased endocannabinoid synthesis causes activation of CB1 receptors (Cristino et al. 2013). This reduces inhibition of orexinergic neurons and enhances OXA release leading to hyperphagia and increased body weight gain. Thus, the orexin system interacts with both NPY and the CB1 expressing neurons. We have previously shown that SNPs in CNR1 (rs806378) and NPY (rs16147) were associated with AIWG and significantly interact with each other to increase the risk for AIWG (Tiwari et al. 2010, 2013).

More importantly, the potential role of orexin system genes in AIWG is underlined by the observations that antipsychotics associated with weight gain increase neuronal activity in orexin neurons compared to antipsychotics with no weight gain liability (Fadel et al. 2002). In addition, antipsychotics associated with higher risk of weight gain (e.g., clozapine and olanzapine) activate orexin neurons significantly more than antipsychotics with relatively less AIWG risk (e.g., risperidone, Fadel et al. 2002). Similarly, in female Sprague-Dawley rats injected with olanzapine, 50% of the neurons activated in the perifornical region of lateral hypothalamus were OXA positive (Stefanidis et al. 2009). This suggests that antipsychotics with weight gain risk modulate orexin neurons. However, the impact of genetic variation in the orexin system on AIWG has not been investigated to date.

Based on the important role of the orexin system in energy homeostasis and on the influence of antipsychotics with high risk for AIWG on orexin neurons, we analysed tagSNPs in HCRTR1 and HCRTR2 genes for association with AIWG. We hypothesised that the SNPs in these two genes are likely to be associated with AIWG caused by high weight gain risk medications such as clozapine and olanzapine.

Methodology

Discovery subjects

Patients were diagnosed with schizophrenia or schizoaffective disorder according to DSM-III-R or DSM-IV criteria (n = 218). Written informed consent was obtained from all participants. Detailed demographic and clinical characteristics are provided in Table I and have been published previously (Tiwari et al. 2010, 2013). Patients included in this study were 18–60 years old and were recruited from Charité University Medicine, Berlin, Germany (Sample A, n = 88); Case Western Reserve University, Cleveland, OH, USA (Sample B, n = 74); Hillside Hospital in Glen Oaks, NY, USA (Sample C, n = 56). Patients from sample A were given mixed antipsychotic medication and assessed for up to 6 weeks. In sample B patients were either treatment refractory or intolerant to treatment with typical antipsychotics with relatively less AIWG risk (e.g., risperidone) following a 7–14-day washout period (Masellis et al. 1998). In sample C, patients who had suboptimal response to previous treatment with typical antipsychotic drugs, defined by persistent positive symptoms and poor level of functioning over the past 2 years, were included. Patients were randomly assigned to receive either clozapine (500 mg/day), olanzapine (20 mg/day), risperidone (8 mg/day) or haloperidol in a 14-week double-blind study (for details see Volavka et al. 2002). Exclusion criteria for these studies included pregnancy, organic brain disorder, severe head
injuries, previous medical conditions which required treatment and were not stable (hepatitis C, HIV, thyroid disorder or diabetes mellitus), substance dependence, clinically relevant intellectual disability and severe personality disorder.

**Genotyping**

Genomic DNA was extracted from blood samples using the high-salt method (Lahiri and Nurnberger 1991). TagSNPs were selected from the CEU population in HapMap (Haploview 4.2, Barrett et al. 2005) using a region ~10 kb upstream and 2 kb downstream of the HCRTR1 and HCRTR2 genes (minor allele frequency >0.05, \( r^2 \geq 0.8 \)). A total of 5 tagSNPs covering a 20-kb region including HCRTR1 (~9.6 kb) and 28 tagSNPs covering 120 kb including HCRTR2 (~108 kb) were investigated in this study. All genotyping was carried out using customised Golden Gate Genotyping Assays (Illumina, Inc. San Diego, CA, USA). As a quality control, 5% of the total sample was re-genotyped and 100% concordance rate was observed in this study.

**Replication subjects**

The replication subjects were a subset of patients that participated in the Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE, Lieberman et al. 2005b). Briefly, CATIE was a multicenter, double-blind, multiphase study conducted at 57 sites in the USA between January 2001 and December 2004. A genome-wide association study for pharmacogenomic factors has been conducted (Adkins et al. 2011) and the genome wide genotyping data are available for 741 choric schizophrenia patients. These patients were randomly assigned to olanzapine, risperidone, quetiapine, perphenazine or ziprasidone. These drugs have different propensities to cause weight gain and the patients may have been exposed to weight gain associated antipsychotics (e.g., olanzapine) prior to randomisation. In addition, if patients are markedly obese at baseline, the probability to gain significant amounts of weight during the study is low. Therefore, using the CATIE Phase I information, we selected a subset of patients suitable for antipsychotic induced weight gain studies. This refined sample \( (n = 122) \) consisted of patients of European ancestry who were not being treated with high weight gain risk medication (e.g., olanzapine) for more than 14 days before baseline assessment, body mass index (BMI) <40, randomised to either risperidone or olanzapine and had more than one weight measure available after baseline (Table II).

**Orexin receptor SNPs found to be nominally associated with AIWG were not genotyped in the GWAS. Therefore, we performed imputation. The quality control measures included removing individuals with less than 95% of the markers genotyped and excluded SNPs that were less than 95% genotyped or had a minor allele frequency >0.05.**

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### Table I. Demographic and clinical characteristics of the Discovery samples.

| Characteristics | Sample A \( n = 88 \) n (%) | Sample B \( n = 74 \) n (%) | Sample C \( n = 56 \) n (%) | \( P \) value | Total \( n = 218 \) n (%) |
|-----------------|-----------------------------|-----------------------------|-----------------------------|-------------|-----------------------------|
| Gender          |                             |                             |                             |             |                             |
| Female          | 38 (43.2)                   | 27 (36.5)                   | 9 (16.1)                    | 0.003       | 74 (33.9)                   |
| Male            | 50 (56.8)                   | 47 (63.5)                   | 47 (83.9)                   |             | 144 (66.1)                  |
| Age             | 35.78 ± 12.2                | 33.41 ± 8.5                 | 41.3 ± 7.4                  | <0.001*     | 36.37 ± 10.4                |
| Initial bodyweight (kg) | 79.47 ± 15.9             | 75.05 ± 13.8                | 84.95 ± 17.4                | 0.002       | 79.32 ± 16.0                |
| Weight change (kg) | 3.27 ± 3.9                | 3.76 ± 4.4                  | 4.43 ± 6.4                  | 0.631*      | 3.73 ± 4.8                  |
| Weight change (%) | 4.01 ± 4.7                 | 5.35 ± 6.3                  | 5.73 ± 8.4                  | 0.511*      | 4.90 ± 6.4                  |
| Weight gain <7%  | 64 (72.7)                   | 47 (63.5)                   | 37 (66.1)                   | 0.327       | 148 (67.9)                  |
| ≥7%             | 24 (27.3)                   | 27 (36.5)                   | 19 (33.9)                   |             | 70 (32.1)                   |
| Baseline BPRS^   | 51.0 ± 14.4                 | 51.23 ± 14.2                | 54.17 ± 7.7                 | 0.025*      | 52.19 ± 12.4                |
| Study duration (weeks) | 5.09 ± 1.6                 | 6.00                        | 11.76 ± 3.7                 | <0.001*     | 7.09 ± 3.5                  |
| Ethnicity       |                             |                             |                             |             |                             |
| European-American | 87 (98.9)                  | 52 (70.3)                   | 12 (21.4)                   | <0.001      | 151 (69.3)                  |
| African-American | 1 (1.1)                    | 22 (29.7)                   | 33 (58.9)                   |             | 56 (25.7)                   |
| Others          | –                           | –                           | 11 (19.7)                   | <0.001      | 11 (5.0)                    |
| Drugs prescribed |                             |                             |                             |             |                             |
| Clozapine       | 11 (12.6)                   | 74 (100)                    | 12 (21.4)                   | 97 (44.7)   |                             |
| Haloperidol     | 6 (6.9)                     | –                           | 11 (19.6)                   | 17 (7.8)    |                             |
| Olanzapine      | 15 (17.2)                   | –                           | 21 (37.5)                   | 36 (16.6)   |                             |
| Risperidone     | 27 (31.0)                   | –                           | 12 (21.4)                   | 39 (18.0)   |                             |
| Others^          | 28 (32.2)                 | –                           | –                           | 28 (12.9)   |                             |

*Kruskal–Wallis test. ^BPRS, Brief Psychiatric Rating Scale. Baseline BPRS scores was available for a subset of the patients \( n = 155 \). To achieve comparability with sample B, BPRS total scores were extracted from PANSS ratings for samples A and C. ^Includes antipsychotic drugs: fluphenazine; aripiprazole, quetiapine, ziprasidone and amisulpride. Drug information for one individual was not available.
frequency of less than 5%. We checked for cryptic relatedness and, calculated mean heterozygosity and outliers (4 SD from the mean) were removed. In a subsequent step, some SNPs were removed if the $\chi^2$-test for Hardy–Weinberg equilibrium was $< 1 \times 10^{-3}$. Multi-dimensional scaling (MDS) analysis was used to cheque for population stratification, and outliers were removed and only subjects of European ancestry were selected. Finally, we updated the map position for build 37 and conducted imputation in 1 Mb segments upstream and downstream HCRTR2 using IMPUTE v2.3.2 (Howie et al. 2012) using the 1000 Genome Project (Genomes Project et al. 2010) build 37 (Haplotype release date: June 2014 (ALL.integrated_phase1_SHAPEIT_16-06-14.nosing.tgz) data as reference panel. The SNPs of interest were successfully imputed in all the patients and had good imputation accuracy (IMPUTE INFO >0.95).

Statistical analysis

Pearson’s $\chi^2$-test for categorical variables and Student’s t-test or analyses of variance for continuous variables were used for statistical comparisons (IBM Statistical Package for the Social Sciences (IBM SPSS Statistics, version 20). Analysis of covariance (ANCOVA) was applied to test association between genotype and weight change (%) from baseline as the dependent variable ([Weight at the end of study - weight at Baseline] / weight at Baseline) $\times 100$). Genotypes were entered as fixed factor and baseline weight and duration of treatment as covariates. Linkage disequilibrium (LD) was calculated using Haploview 4.2 (Barrett et al. 2005) and haplotypes were analysed with UNPHASED version 3.1.5 (Dudbridge 2003, 2008). Corrections for multiple tests were done using Single Nucleotide Polymorphism Spectral Decomposition (SNPSpD, Nyholt 2004). Power calculations were performed in Quanto 1.2.4 (Gauderman and Morrison 2006). Assuming a minor allele frequency of 0.25 a sample size of $n = 218$, we had more than 80% power to detect a mean difference of over 2% between carriers and non-carriers of the risk genotype in an additive model. In the subsample of European ancestry patients treated with either clozapine or olanzapine ($n = 86$), we had more than 80% power to detect over 3.25% difference in an additive model. In the discovery sample, the Programme mbmdr was applied to detect gene–gene interaction between HCRTR2, NPY and CNR1 and significance was estimated using permutation test (Calle et al. 2010).

Results

All the SNPs analysed in this study were in Hardy–Weinberg equilibrium ($P>0.05$; Supplementary Table 1 available online). The SNPs rs77324737 (monomorphic), rs12057176, rs3134712 and rs12111299 occurred at a minor allele frequency of <5% and were excluded from the study. LD plots among the SNPs in HCRTR1 and HCRTR2 are provided in Supplementary Figures 1 and 2, respectively (available online). Among the clinical sites, no significant difference in the amount of weight gained was observed (Tables I and II). The baseline weight and the duration of treatment were significantly different between the three sites and were entered as covariates in all the association analysis.

Association study in the total and European ancestry sample

We performed an exploratory analysis in the total sample and did not observe association of any of the SNPs with AIWG ($P>0.05$). Since the sample consisted of patients of different ancestry, we focussed our further analyses on the subset of patients of European ancestry only ($n = 151$). In this subset, we observed nominal genotypic association of rs4467775 ($P = 0.007$), rs3134701 ($P = 0.026$) and rs4142972 ($P = 0.007$) in HCRTR2 with weight change.

Association study in patients of European ancestry treated with either clozapine or olanzapine

Considering that clozapine and olanzapine carry the highest risk of weight gain and have similar pharmacology, we stratified our sample and conducted an analysis on patients of European ancestry treated with clozapine or olanzapine ($n = 86$). This subset consisted primarily of individuals who have had no or minimal prior exposure to atypical antipsychotic drugs. We observed nominal genotypic association of the above 3 SNPs as well as rs6922310, rs12662510 and rs2653350 (Table III). Patients with risk genotypes, for example the AA genotype of...

### Table II. Demographic and clinical characteristics of the CATIE replication sample.

| Characteristic                  | Total n=122 | $N (%)$/mean ± SD |
|---------------------------------|-------------|-------------------|
| Gender                          |             |                   |
| Male                            | 98 (80.3)   |                   |
| Female                          | 24 (19.7)   |                   |
| Medication                      |             |                   |
| Olanzapine                      | 63 (51.6)   |                   |
| Risperidone                     | 59 (48.4)   |                   |
| Age                             | 41.47 ± 11.2|                   |
| PANSS at baseline               | 78.17 ± 18.4|                   |
| BMI at baseline                 | 28.53 ± 5.2 |                   |
| BMI change from baseline (%)    | 0.75 ± 1.95 |                   |

BMI, Body mass index.
rs6922310 or A-allele carriers for rs4142972 gained \( \sim 2.6 \) and \( \sim 2.8 \) kg more weight than the non-risk genotype. In this subsample, nominal allelic associations of several SNPs with weight change (%) were also observed (Table III). None of the SNPs in HCRTR1 were associated with AIWG (Supplementary Table 1 available online).

Haplotype analysis within this subsample of European ancestry patients treated with clozapine or olanzapine (\( n = 86 \)) revealed that haplotypes of the HCRTR2 SNPs rs12111375, rs4142972 and rs6922310 were significantly associated with AIWG after correction for multiple testing (Supplementary Figure 3 available online). Carriers of the T-G-G haplotype (versus all the others pooled together) gained less weight (\( n_{\text{chr}}=29 \), frequency \( =0.173; P = 9.1 \times 10^{-4} \)) whereas the T-A-A haplotype was associated with higher weight gain (\( n_{\text{chr}}=36 \), frequency \( =0.214; P = 0.0064 \)). Considering that a majority of the significant SNPs are present in \( \sim 12 \) kb LD block (Supplementary Figure 2, Block 3, available online), we also explored association of the five SNPs haplotypes with AIWG. The five SNP haplotype (rs3134701, rs12111375, rs4142972, rs6922310 and rs12662510) was significantly associated with AWIG (\( P = 0.012 \)). Carriers of the G-T-G-G-G haplotype gained less weight (\( n_{\text{chr}}=23 \), frequency \( =0.160; P = 0.0074 \)) whereas the A-T-A-A-A haplotype carriers gained significantly more weight (\( n_{\text{chr}}=33 \), frequency \( =0.229; P = 0.025 \)).

The correction for multiple comparisons was carried out using SNPSpD. This method takes the linkage disequilibrium between SNPs into account and determines the effective number of independent SNPs. The number of independent tests (MeffLi) for HCRTR1 and HCRTR2 were 2 and 14.84, respectively (Nyholt 2004; Li and Ji 2005). The association of weight change and SNPs rs4142972 and rs6922310 remained nominally significant in a dominant model at a gene wide level (\( P_{\text{corrected}}=0.03 \) and 0.045, respectively; Table III). However, if we consider all the tests done in this study none of the observations are statistically significant.

We also carried out an exploratory gene–gene interaction analysis between rs4142972 in HCRTR2 with rs16147 in NPY and rs806378 in CNR1 in the subsample of European ancestry patients treated with either clozapine or olanzapine. The SNPs rs806378 (CNR1) and rs16147 (NPY) were observed to be significantly associated with AIWG in our earlier studies (Tiwari et al. 2010, 2013). We observed a trend for interaction between the three SNPs (Permutation \( P \) value \( =0.05 \)). Carriers of the TT genotype at rs16147, the CC genotype of rs806378 and the GG genotype at rs4142972 gained the least weight (\( \beta =-7.11, P = 0.00012 \)). An ANCOVA of patients carrying the low risk (TT, CC and GG) genotypes vs. other genotypic combinations pooled together further supported the finding (TT, CC, GG vs. others; \( -1.48 \pm 4.3\% \) vs. \( 5.7 \pm 5.3\% \), \( n = 10 \) vs. 69, \( P = 6.13 \times 10^{-5} \)). Interaction between rs6922310 in HCRTR2 with rs16147 in NPY and rs806378 in CNR1 were not significant (Permutation \( P \)-value \( =0.1 \)).

**Association study in the replication sample**

We carried out the replication analysis of the HCRTR2 SNPs nominally associated with AIWG in the refined CATIE sample (Table IV). In the overall sample of patients treated with either olanzapine or risperidone, rs3134701 (\( P = 0.043 \)) and rs12662510 (\( P = 0.012 \)) were nominally associated with BMI change (%). Although the remaining SNPs were not statistically significant, the risk genotypes were similar to the discovery sample. In addition, among the subset of patients treated only with olanzapine the risk genotypes and associations continued to be similar to the discovery sample (Table IV).

**Discussion**

Our study is the first to investigate genetic variation in the HCRTR1 and HCRTR2 genes and their role in antipsychotic-induced weight gain. In the discovery sample, we observed nominal association of several SNPs, in particular rs4142972 and rs6922310, in the orexin 2 receptor gene (HCRTR2) with AIWG. The SNP rs4142972 remained significant after accounting for all the independent tests in the discovery sample. The SNPs rs4142972 and rs6922310 are intronic, present within 250 bp of each other but are not correlated (\( r^2=0.06 \)), and have no known functional effect. The SNP rs6922310 is correlated with SNPs rs12662510 (\( r^2=0.84 \)) and rs3134701 (\( r^2=0.75 \)) which are also intronic and nominally associated with AIWG (Table III). In addition, rs6922310 is primarily correlated with SNPs in the introns 1 region only (Supplementary Figure 4 available online). The SNP rs4142972 is moderately correlated with rs4467775 (\( r^2=0.49 \)) and other SNPs in the putative promoter region of HCRTR2 gene (Supplementary Figures 2 and 5 available online). These distinct correlation patterns and independent associations suggest that HCRTR2 may be contributing to the development of AIWG via two independent mechanisms.

In the replication analysis rs3134701 and rs12662510 was nominally associated with AIWG. In addition, the remaining SNPs nominally associated with AIWG in the discovery sample had the same genotypes associated with higher weight gain in the replication sample (Table IV). The trends in the replication sample are notable as these observations are made in a sample of chronic schizophrenia patients that may have been exposed to...
Table III. SNPs in orexin receptor 2 (HCRTR2) associated with antipsychotic induced weight gain at genotypic and/or allelic level.

| SNP      | Location | Genotype | Weight change (%)* | Weight change (%)* | Weight change (%) | P value | P value (dominant model)* | P value | Variance explained* | Allele | Frequency | P-value | HWE* | P-value |
|----------|----------|----------|--------------------|--------------------|-------------------|---------|--------------------------|---------|---------------------|--------|-----------|--------|-------|---------|
| rs4467775| 55036599 | GG       | 4.70 ± 6.6 (154)   | 0.366              | 3.14 ± 5.4 (105)  | 0.015   | 3.59 ± 4.9 (55)          | 0.025   | 0.031              | 0.052  | G         | 0.8    | 0.135 | 0.956  |
|          |          | GC       | 5.72 ± 6.1 (56)    |                    | 6.12 ± 5.8 (41)  |         | 7.05 ± 6.6 (27)          |         |         |        | C      | 0.2    |       |         |
|          |          | CC       | 1.69 ± 5.0 (6)     |                    | 3.30 ± 3.5 (5)   |         | 1.88 ± 1.7 (4)           |         |         |        |        |        |       |         |
| rs3134701| 55067318 | AA       | 5.22 ± 5.8 (108)   | 0.786              | 4.82 ± 5.5 (82)  | 0.026   | 6.08 ± 5.6 (51)          | 0.021   | 0.006              | 0.085  | A       | 0.77   | 0.0065 | 1      |
|          |          | AG       | 4.43 ± 6.2 (91)    |                    | 3.40 ± 4.9 (61)  |         | 2.30 ± 4.8 (31)          |         |         |        | G      | 0.23   |       |         |
|          |          | GG       | 5.14 ± 10.6 (17)   |                    | (-10.67 ± 9.3) (8) |         | 3.45 ± 6.4 (4)           |         |         |        |        |        |       |         |
| rs4142972| 55076937 | GG       | 4.70 ± 6.7 (161)   | 0.559              | 3.07 ± 5.5 (109) | 0.007   | 3.27 ± 5.3 (55)          | 0.005   | 0.002              | 0.109  | G       | 0.79   | 0.0102 | 0.301  |
|          |          | GA       | 5.23 ± 5.6 (47)    |                    | 6.27 ± 5.58 (35) |         | 7.29 ± 5.8 (25)          |         |         |        | A      | 0.21   |       |         |
|          |          | AA       | 6.50 ± 4.0 (8)     |                    | 6.02 ± 4.0 (7)   |         | 5.53 ± 4.2 (6)           |         |         |        |        |        |       |         |
| rs6922310| 55077184 | AA       | 4.95 ± 6.1 (127)   | 0.279              | 4.42 ± 6.0 (92)  | 0.28    | 5.92 ± 5.4 (57)          | 0.013   | 0.003              | 0.098  | A       | 0.82   | 0.0016 | 0.914  |
|          |          | AG       | 4.32 ± 6.4 (79)    |                    | 3.45 ± 4.9 (55)  |         | 2.10 ± 5.3 (27)          |         |         |        | G      | 0.18   |       |         |
|          |          | GG       | 8.41 ± 9.9 (10)    |                    | 0.11 ± 1.7 (4)   |         | 0.42 ± 2.7 (2)           |         |         |        |        |        |       |         |
| rs12662510| 55079891 | AA       | 5.42 ± 6.6 (158)   | 0.319              | 4.43 ± 6.1 (94)  | 0.35    | 5.71 ± 5.7 (97)          | 0.02    | –                  | 0.058  | A       | 0.84   | 0.0186 | 0.165  |
|          |          | AG       | 3.88 ± 6.1 (60)    |                    | 3.36 ± 4.8 (51)  |         | 2.41 ± 4.9 (27)          |         |         |        | G      | 0.16   |       |         |
| rs2653350| 55141402 | AA       | 6.07 ± 6.7 (64)    | 0.257              | 5.09 ± 6.1 (45)  | 0.231   | 6.48 ± 5.8 (31)          | 0.017   | 0.016              | 0.065  | A       | 0.59   | 0.0063 | 0.864  |
|          |          | AG       | 4.38 ± 6.4 (104)   |                    | 3.33 ± 5.4 (75)  |         | 4.08 ± 5.0 (40)          |         |         |        | G      | 0.41   |       |         |
|          |          | GG       | 4.47 ± 6.0 (47)    |                    | 3.80 ± 5.3 (31)  |         | 2.07 ± 5.9 (15)          |         |         |        |        |        |       |         |

*Based on Genome Reference Consortium Human Genome Build 37 Patch Release 13 (GCRh37.p13, June 28, 2013).
*Mean ±SD (number of individuals); HWE, Hardy–Weinberg equilibrium.
*Values calculated for the patients with European ancestry on clozapine and olanzapine. P values significant after accounting for the 14.84 independent tests are in bold font. Cloz or Olz, clozapine- or olanzapine-treated patients. The variance explained is based on the dominant model and includes baseline weight and duration of treatment as covariates.
atypical antipsychotics in the past. Our discovery sample consisted primarily of patients undergoing first exposure to atypical antipsychotic drugs. Generally larger effect sizes are observed in patients undergoing first exposure to antipsychotics compared to chronically treated patients and genome-wide significant genetic polymorphisms have been detected in small sample sizes (e.g., rs489693, n = 139) (Malhotra et al. 2012). These observations suggest that HCRTR2 gene is likely to play a role in AIWG development.

We also observed a nominal gene–gene interaction between functional SNPs in NPY and CNR1 with rs4142972 in HCRTR2. This interaction may be biologically relevant since NPY neurons in the arcuate nucleus received projections from orexin neurons in the lateral hypothalamus (LH) and i.c.v. injection of NPY in the lateral hypothalamus activates orexin neurons (Kageyama et al. 2012). Administration of an HCRTR2 agonist reduces NPY and AGRP expression in mice on a high fat diet compared to those on a low fat diet (Funato et al. 2009). Furthermore, endocannabinoids cause increased OXA (Cristino et al. 2013) and NPY release (Gamber et al. 2005) suggesting that these neurotransmitters interact with each other and can together influence feeding behaviour and energy homeostasis. The rs806378 and rs16147 polymorphisms were not genotyped in the CATIE samples so replication analysis for this interaction was not performed.

Limitations of our study include a relatively small sample size after refining the samples by ethnicity and antipsychotic medication, use of self-reported ancestry in the discovery sample, and limited power to detect small gene effects. In addition, if we consider all the tests performed in this and previous studies, none of the observation will meet the statistical threshold of P < 0.05. The other limitation of this study is that genetic polymorphisms in the orexin gene were not tested for association with AIWG. The orexin gene is small (~1.4 kb), and highly conserved with no known common variations (>5%). Therefore, we did not investigate this gene in our study. However, this gene is a good candidate for sequencing studies in subjects exhibiting extremely high weight gain.

In summary, we provide preliminary evidence that genetic variation in the orexin receptor 2 gene (HCRTR2) is associated with AIWG. Analysis of this gene in larger sample sets will provide a clearer picture on its contribution to AIWG.

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### Statement of interest

NF reports no competing interests. AKT/EJB/CCZ/VFG/NIC/ DJM/JLK are authors on a patent application for a multigene model (including SNPs presented here) predicting antipsychotic induced weight gain. HYM has received grants or is or was a consultant to: Abbott Labs, ACADIA, Alkermes, Bristol Myers Squibb, DaiNippon Sumitomo, Eli Lilly, EnVivo, Janssen, Otsuka, Pfizer, Roche, Sunovion, and BiolineRx. HYM is a shareholder of ACADIA and Glaxo Smith Kline. In the past 3 years JAL reports having received research funding or is a member of the advisory board of Allon, Alkermes Bioline, GlaxoSmithKline Intracellular Therapies, Lilly, Merck, Novartis, Pfizer, Pierre Fabre, Psychogenics, F. Hoffmann-La Roche LTD, Sepracor (Sunovion) and Targacept. JAL receive no direct financial compensation or salary support for participation in these

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**Table IV. Replication analysis of the SNPs in orexin receptor 2 (HCRTR2) in the selected CATIE sample (n=122).**

| SNP       | Location | Genotype | BMI change (%)* European Americans on Olz or Risp (n=122) | P value | BMI change (%) European Americans (Olz) (n=63) | P value | Imputation info score |
|-----------|----------|----------|-----------------------------------------------------------|---------|-----------------------------------------------|---------|-----------------------|
| rs4467775 | 55036599 | GG       | 2.79±6.8 (85)                                            | 0.200   | 4.48±6.8 (43)                                 | 0.341   | 0.99                  |
| rs3134701 | 55067318 | AA       | 4.09±7.3 (75)                                            | 0.043   | 6.37±7.9 (44)                                 | 0.025   | 0.99                  |
| rs4142972 | 55076937 | AG + GG  | 3.10±7.1 (46)                                            | 0.894   | 4.20±6.7 (49)                                 | 0.131   | 1                      |
| rs6922310 | 55077184 | AA       | 3.91±7.1 (81)                                            | 0.046   | 6.01±7.7 (47)                                 | 0.056   | 0.98                  |
| rs12662510| 55079891 | AA       | 3.80±7.2 (90)                                            | 0.012   | 5.85±7.6 (49)                                 | 0.019   | 0.96                  |
| rs2653350 | 55144102 | AA       | 3.54±7.0 (36)                                            | 0.476   | 5.46±6.9 (21)                                 | 0.652   | 0.98                  |

BMI, Body mass index; Olz, olanzapine; Risp, risperidone. 
*Based on Genome Reference Consortium Human Genome Build 37 Patch Release 13 (GCRh37.p13, June 28, 2013).
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