Association of ADRA2A and MTHFR gene polymorphisms with weight loss following antipsychotic switching to aripiprazole or ziprasidone

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Objectives Various genetic polymorphisms have been reported to be associated with antipsychotic-induced weight gain. In this study, we aimed to determine whether risk polymorphisms in 12 candidate genes are associated with reduction in body mass index (BMI) of patients following switching of antipsychotics to aripiprazole or ziprasidone.

Methods We recruited 115 schizophrenia patients with metabolic abnormalities and who have been on at least 1 year treatment with other antipsychotics; they were then switched to either aripiprazole or ziprasidone. They were genotyped, and their BMI monitored for 6 months.

Results Significant associations with reduction in BMI at 6 months following switching were found in two of these genes: with rs1800544 of the ADRA2A gene (CC + CG vs GG, p = 0.013) and with rs1801131 of the MTHFR gene (AA vs AC + CC, p = 0.015).

Conclusion The study data indicated that carriage of the ADRA2A rs1800544 GG genotype and the MTHFR rs1801131 C allele are associated with BMI reduction in this population following switching of antipsychotics to aripiprazole and ziprasidone. Copyright © 2013 John Wiley & Sons, Ltd.

KEY WORDS—schizophrenia; antipsychotic-induced weight gain; -1291C/G adrenergic receptor; 1298A/C methylenetetrahydrofolate reductase; aripiprazole; ziprasidone

INTRODUCTION

Weight gain as a side effect of antipsychotic treatment varies substantially between different antipsychotics (Allison et al., 1999). It was reported that 80% of patients taking antipsychotics over a period of at least 6 to 12 months gained more than 20% more than their ideal body weight (Umbricht et al., 1994, Masand, 2000). Weight gain has been strongly associated with health complications such as obesity, insulin resistance and diabetes that could lead to cardiovascular events and may reduce life expectancy by up to 20–30 years (Stahl et al., 2009, Lett et al., 2012). Apart from the physical health issues, increases in body weight can also have psychological influences resulting in discontinuation of antipsychotic medication, hence increasing the chances of relapse (Panarelli et al., 2011). Among the atypical antipsychotics, clozapine and olanzapine have long been known to cause the greatest weight increase, with quetiapine and risperidone having intermediate effects in causing weight gain and metabolic disturbances. Aripiprazole and ziprasidone, on the other hand, are reported to cause little or no gain in weight (Allison et al., 1999, Coccurello and Moles, 2010, Reynolds and Kirk, 2010, Correll et al., 2011). The different extent to which weight gain occurs following treatment by the different antipsychotics is likely due to differences in their affinities for neurotransmitter receptors. Generally, agonist action at serotonin (5HT) 2C and 1B receptors (De Vry and Schreiber, 2000, Clifton et al., 2000) and the histamine H1 receptor have been associated with decreased food intake (Lecklin et al., 1998), whereas the 5HT1A receptor promotes food intake (Dourish et al., 1985). The receptor mechanisms underlying antipsychotic...
drug-induced weight gain remain unclearly defined, although action at 5-HT2C and H1 receptors, along with dopamine D2 antagonism, are the strongest candidates (Reynolds and Kirk, 2010).

Genetic factors have been suggested to be involved in weight gain induced by antipsychotics, and the genes studied include those involved in maintenance of food intake and energy expenditure in the hypothalamus (Lett et al., 2012). Polymorphisms in the dopamine D2 receptor, adrenergic alpha-2A receptor (ADRA2A), pro-melanin concentrating hormone, leptin, 5HT 2A receptor, 5HT2C receptor, melanocortin 4 receptor, brain-derived neurotrophic factor and the fat mass and obesity associated genes have been associated with increased weight in antipsychotic-treated schizophrenia patients (Reynolds, 2012). The melanocortin 4 receptor, pro-melanin concentrating hormone and leptin receptor gene polymorphisms have also been implicated in antipsychotic-induced obesity (Chagnon et al., 2007, Gregoor et al., 2011, Kuo et al., 2011), whereas polymorphisms in the adiponectin and methylenetetrahydrofolate reductase genes have shown limited evidence indicating their involvement in antipsychotic-induced metabolic problems (Jassim et al., 2011, Ellingrod et al., 2008).

There are indications that patients with metabolic disturbances which might be a consequence of antipsychotic drug treatment can benefit from being switched to aripiprazole or ziprasidone (Alptekin et al., 2009, Kim et al., 2010, Takeuchi et al., 2010, Chen et al., 2012), although not all patients will benefit equally from such a strategy. It seems likely that, in the weight loss which accompanies such a switch, genetic factors are also involved and the genes implicated may include those associated with drug-induced weight gain and metabolic problems. The current study aims to determine whether one or more polymorphisms in a series of such candidate genes might be associated with weight loss following switching to ziprasidone or aripiprazole in antipsychotic-treated patients with metabolic abnormalities.

MATERIALS AND METHODS

Subject

A total of 115 patients who fulfilled criteria for the diagnosis of schizophrenia according to the Diagnostic and Statistical Manual of Mental Disorders Fourth Edition, Text Revision (American Psychiatric Association, 2000) were recruited from the University of Malaya Medical Centre and from five other participating Ministry of Health hospitals in the country; Terendak Army Hospital (Melaka), Lumut Navy Hospital (Perak), Hospital Bahagia Ulu Kinta (Perak), Hospital Permai (Johor) and Hospital Sentosa Kuching (Sarawak). The study involved only outpatients who had received at least 1 year of treatment with antipsychotic drugs and were not on any mood stabilizers. The study protocol was approved by the ethics committees of the University of Malaya Medical Centre and Ministry of Health. All subjects gave written informed consent after receiving a copy of the study information sheet approved by the ethical committees. A thorough assessment was conducted by trained research assistants on potential candidates which included questions regarding their family history of medical and physical illness and information on their previous and concomitant antipsychotics used, together with other medications for chronic illness such as diabetes and hypertension. Those with any metabolic abnormality (according to the National Cholesterol Education Program Adult Treatment Panel III [Grundy et al., 2005] criteria-modified for diagnosis of metabolic syndrome in Asian subjects) to which prior antipsychotic treatment may have contributed was included as study subjects and was then switched to either aripiprazole or ziprasidone. Other inclusion criteria included, between 18 and 65 years old and, where treated with antihypertensives, anti-diabetics or anti-hyperlipidemics prior to the study, the treatment was initiated 3 months prior to screening with no dosage changes 30 days before study recruitment. Patients were randomised to either aripiprazole or ziprasidone using a Web-based randomisation protocol (www.randomisation.com). Once included, the switching of medication was undertaken gradually by slowly reducing the dose of their previous approval process medication while introducing the study medication in their treatment regime. Subjects were followed up at monthly intervals until 6 months after switching.

Clinical and laboratory assessments

Measurements of height, weight, blood pressure, waist and hip circumference were recorded according to standard protocol at the initial visit and each subsequent visit. The body mass index (BMI), derived according to the formula, BMI = weight/height squared (kg/m²), was used as the measure of body weight and weight change.

Genotyping

Peripheral blood was obtained, and DNA was extracted according to the manufacturer’s protocol (QIAamp DNA Blood Mini Kit, QIAGEN®, Hilden,
Germany). Sixteen selected polymorphisms from 12 candidate genes (Table 2) were then genotyped using the Sequenom MassARRAY technology platform (Sequenom, San Diego, California) carried out at the Genome Research Centre (University of Hong Kong).

**Statistical analyses**

The primary outcome measure was weight change following 6 months of treatment with ziprasidone or aripiprazole; the main hypothesis being that this would be significantly associated with genotype of candidate gene polymorphisms. With a priori evidence supporting the involvement of each gene in drug-induced weight changes, association with weight change was considered a separate hypothesis for each gene. SPSS 16.0 (SPSS Inc., Chicago, IL, USA) and Microsoft Excel software were used for statistical analysis. A $p$-value of $<0.05$ was considered significant. The sample size of 115 was adequate to obtain 89% power with an effect size of 0.6 between two approximately equal genotype groups, calculated using the G Power software. Data are expressed as percentage (%) or mean ± standard deviation where appropriate. The chi-square test was used for categorical data. The Kolmogorov–Smirnov test was used for normality testing. For variables with normal distribution (BMI changes at 6 months), the analysis of variance test was used to compare the mean BMI changes between three groups. Stepwise linear regression analysis was used to investigate the influence of the dose and duration of the study drugs (aripiprazole and ziprasidone) and the influence of other covariables towards BMI changes. The univariate analysis of variance test was used to test the interaction between the associated polymorphisms with BMI changes. The $X^2$ goodness of fit test was used for the Hardy Weinberg Equilibrium calculation with one degree of freedom.

**RESULTS**

Out of the 115 patients recruited, there were 65 men (40.12 ± 12.22 years) and 50 women (40.32 ± 12.11 years). After randomization, 62 patients (53.9%) were switched to aripiprazole (mean dose: 21.14 ± 7.01 mg/day), whereas 53 (46.1%) patients were switched to ziprasidone (mean dose: 125.59 ± 36.09 mg/day). There was no association between the mean dose of either study drugs with BMI change ($p > 0.05$). The age, age of onset, duration of illness, smoking, exercise, sex, ethnicity, prior treatment and baseline BMI of these patients in each treatment group are shown in Table 1. No significant difference between the groups in any of these prior measures was observed. The mean doses of prior antipsychotic used by these patients are as follow: olanzapine = 14.51 ± 6.46 mg/day, risperidone = 28.91 ± 112.53 mg/day, sulpiride = 372.86 ±...
309.02 mg/day, paliperidone = 6.64 ± 2.10 mg/day, quetiapine = 350.00 ± 252.13 mg/day, chlorpromazine = 385.00 ± 476.84 mg/day and trifluoperazine = 10.00 ± 0.00 mg/day. Of the 115 patients recruited, all were followed up at 1-month intervals for 6 months; attrition resulted in 75 subjects completing the assessment at 6 months with the total group having a mean period of monitoring of 4.95 months. The reasons for attrition include withdrawal of consent (n = 8), lost to follow-up (n = 10), adverse event (n = 7), hospitalisation (n = 1), lack of efficacy (n = 6) and non-compliance (n = 3), whereas no information was available for five patients. To preserve the power of the study, a last-observation-carried-forward approach was used in the weight change analyses.

At baseline, there was no significant correlation of BMI and other demographic variables with any of the polymorphisms (p > 0.05).

Significant weight loss was observed at end point with a mean BMI change of −0.68 ± 1.56 kg/m². No difference was observed in the extent of weight loss induced by aripiprazole and ziprasidone (Table 1); hence, in subsequent analyses the two treatment groups are considered together. Stepwise linear regression analysis showed that age, gender and baseline BMI did not make any significant contribution to BMI change at 6 months.

The 16 polymorphisms with minor allele frequencies >5% are listed, and the association of each polymorphism with change in BMI is shown in Table 2. Two polymorphisms rs1800544 of ADRA2A and rs1801131 of MTHFR showed significant association of genotype with BMI change. No trends to an effect within the other polymorphisms were apparent (all p > 0.10). Two-genotype genetic modelling showed that, for ADRA2A, those with the GG genotype showed significantly greater reductions in mean BMI than the C allele carriers. For the MTHFR polymorphism, the C allele carriers had a greater reduction in BMI than the AA genotype (Figure 1). Analysing the

<table>
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<th>Gene</th>
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<th>MAF</th>
<th>HWE (P)</th>
<th>Genotype (N)</th>
<th>Genotype (N)</th>
<th>Genotype (N)</th>
<th>P</th>
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BMI, body mass index; HWE, Hardy-Weinberg equilibrium; Gene abbreviations: ADIPOQ, Adiponectin; ADRA2A, Alpha-2A adrenergic receptor; BDNF, Brain-derived neurotrophic factor; DRD2, Dopamine D2 receptor; FTO, Fat mass and obesity associated; HTR2A, Serotonin 2A receptor; HTR2C, Serotonin 2C receptor; LEP, Leptin; LEPR, Leptin receptor; MC4R, Melanocortin 4 receptor; MTHFR, Methylene-tetra-hydro-folate reductase; PMCH, Pro-melanin concentrating hormone.

*HTR2C is located on the X chromosome, thus the HWE values are not included.

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association of weight loss with both polymorphisms together, there is a strongly significant overall effect ($p=0.001$) but no significant interaction term ($p=0.183$) indicating an additive effect of the two polymorphisms ($R^2=0.13$). The subgroup with carriers of the ADRA2A GG genotype and MTHFR C allele showed the greatest weight loss ($-1.30 \pm 1.93 \text{ kg/m}^2$, $n=23$) and the group with neither ‘risk’ genotype showed the least ($0.27 \pm 1.45 \text{ kg/m}^2$, $n=28$). Including drug treatment (i.e. aripiprazole or ziprasidone) as a further factor in the association analysis with each genotype showed no significant drug effect or drug-genotype interaction on BMI change (data not shown). Similarly, sex included in the association analysis showed no significant effect or interaction with genotype on BMI change (data not shown).

DISCUSSION

In the present study, the GG genotype of the ADRA2A and the C allele of the MTHFR gene polymorphisms conferred a significant reduction in the mean BMI of the patients switched to either aripiprazole or ziprasidone for 6 months. Our finding is consistent with the randomised controlled trials reported in Italian (Rossi et al., 2008) and Spanish populations (Montes et al., 2007) in which improvements reported in weight and metabolic parameters of patients following switching to ziprasidone.

The G allele (ADRA2A) and the C allele (MTHFR) frequencies in our Malaysian population are 0.72 and 0.27, respectively, which are consistent with other Asian ethnicities as has been reported in the HapMap project; Han Chinese in Beijing (0.64), Han Chinese South (0.68) and Japanese (0.71) for the ADRA2A gene polymorphism and Han Chinese in Beijing (0.19), Chinese in Metropolitan Denver Colorado (0.21) and Japanese (0.19) for the MTHFR gene polymorphism.

The association of ADRA2A-1291C/G polymorphism and antipsychotic-induced weight gain has been studied previously in three populations. In European-Americans, the GG genotype protects against weight gain in schizophrenia patients (Sickert et al., 2009). On the other hand, carriage of the G allele was associated with >7% weight gain after at least 3 months treatment in clozapine-treated Chinese and >10% weight gain in olanzapine-treated Koreans (Wang et al., 2005, Park et al., 2006). The apparent inconsistency here may relate to differences in ethnicity, although the length of prior treatment, current treatment drug or other factors may also influence the findings. That the G allele was associated with weight gain in these Asian groups and with weight loss in our study might indicate that carriage of the G allele or GG genotype confers a more labile response to drug effects on body weight and food intake.

Although other studies did not reveal any association between the ADRA2A-1291C/G and prevalence of metabolic syndrome (MS) in antipsychotic-treated patients (Risselada et al., 2010, Cheng et al., 2012), this polymorphism has been associated with hypertension (Li et al., 2006), fasting glucose levels and changes in the accumulation of visceral fat (Rosmond et al., 2002, Garenc et al., 2002). The underlying mechanism of the association that is observed in this study may relate to the role of the adrenergic system in maintaining energy balance via the control of thermogenesis and lipolysis (Park et al., 2006, Arner, 1992). Effects at the ADRA2As inhibit heat production and lipolysis (Hellstrom et al., 1996, Arner, 1992). As it is situated in the regulatory promoter sequence of the gene, this -1291C/G polymorphism may influence transcription factor control of gene expression.

We also found association of the MTHFR 1298C allele with greater improvements in weight. A previous report found that the 1298C allele was associated with increased risk of MS (van Winkel et al., 2010b) and predicted increase in weight after 3 months atypical antipsychotic treatment (van Winkel et al., 2010a). The 1298A/C was not associated with MS in two separate studies of adults and children patients receiving antipsychotic treatment but association was seen in the 677C/T polymorphism (Ellingrod et al., 2008, Devlin et al., 2012). However, significant association of 677C/T with weight changes is not seen in this study, nor by van Winkel and colleague (2010) (van Winkel et al., 2010a).

Figure 1. Mean BMI changes following six months treatment with aripiprazole or ziprasidone associated with ADRA2A rs1800544 and MTHFR rs1801131 polymorphisms.
The MTHFR catalyses the conversion of 5,10-methylenetetrahydrofolate to 5-methylenetetrahydrofolate used in the synthesis of methionine from homocysteine. The methionine is further converted to S-adenosylmethionine, which is a methyl donor in a wide variety of enzymatic processes including neurotransmitter and hormone synthesis, signal transduction and DNA methylation (Fontecave et al., 2004). The ubiquity of this methylation has resulted in MTHFR activity being implicated in a wide variety of biological processes. The MTHFR polymorphisms studied here are coding region missense mutations that affect enzyme activity (Weisberg et al., 1998). These polymorphisms have long been associated with many disorders including cancer, neurodevelopmental abnormalities and cardiovascular and metabolic disorders (Bailey and Gregory, 1999); in these genetic associations an interaction with folate concentrations is often apparent.

Ziprasidone and aripiprazole, in addition to their relative freedom from drug-induced weight gain, may also have a protective effect against the weight-inducing effects of some other antipsychotics. The underlying mechanisms are not understood, but have been suggested to include actions at 5-HT1A and/or 5-HT1B receptors (Reynolds and Kirk, 2010). It has been postulated that antagonists of ADRA2A, like clozapine and risperidone (Reynolds and Kirk, 2010), may conceal the functional effect of the -1291C/G polymorphism (Risselada et al., 2010); as ziprasidone and aripiprazole have a low affinity for the ADRA2A (Miyamoto et al., 2005) the genotype effect on change in weight may emerge as patients are switched to these drugs.

There are several limitations to the present study. One is that of sample size, although this is inevitably restricted by the specific nature of the clinical sample, namely antipsychotic-treated patients with risk factors for possible drug-induced metabolic disease. Equivalent sample sizes have identified reproducible pharmacogenetic associations with weight changes following treatment initiation (Reynolds et al., 2002, Templeman et al., 2005, Zhang et al., 2007). The study had approximately 90% power to detect the estimated effect size of 0.6, somewhat less than the effect sizes of 0.7–0.8 observed in previous studies; in fact, the effect sizes obtained here were somewhat less, resulting in a statistical power of approximately 70% for each genotype. Nevertheless, the highly significant combined effect of the two genotypes makes the possibility of a type II error highly unlikely. In order to avoid type I errors, we did not apply a correction for multiple testing; however, each gene and polymorphism chosen for investigation had evidence implicating its role in antipsychotic drug-induced effects on body weight, thus a separate hypothesis for each gene provided a justification for this approach. Nevertheless, replication in further patient cohorts is necessary before we can generalise these genetic associations with weight loss following switching to aripiprazole or ziprasidone.

The patient sample was heterogeneous in several ways, but the study was not powered to investigate possible differences between ethnicity, sex, prior treatments and current drug treatment. Nevertheless, we found no significant differences between the treatment groups in terms of weight loss (Table 1), nor were there significant differences apparent in weight loss between the ethnic and the sex groups (data not shown).

One advantage of this work is that it is a longitudinal study in a single sample, rather than a single cross-sectional study or a case-control study. Although subjects underwent a fairly homogeneous procedure in which they were switched randomly to either aripiprazole or ziprasidone, the study is otherwise a naturalistic one that may well extrapolate to routine clinical treatment. Further verification in a larger sample and over a longer evaluation period is needed to confirm these findings.

CONCLUSION
The finding of the association of rs1800544 ADRA2A and rs1801131 MTHFR with mean BMI changes following antipsychotic-induced weight gain.

CONFLICT OF INTEREST
The authors have declared no conflict of interest.

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AUTHOR CONTRIBUTIONS
Design of the experiment: MAS, AH, ZM, NZZ, SNR
Performed the experiment: SNR, ASAD
Analysed the data: SNR, GPR
Wrote the paper: SNR, GPR
Revision of the paper: ZM, GPR, NZZ, MAS, SNR
REFERENCES


