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The influence of 5-HT_{2C} and MDR1 genetic polymorphisms on antipsychotic-induced weight gain in female schizophrenic patients

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Abstract

We investigated the relationships between functional genetic variants of 5-HT_{2C} receptor and multi drug resistant protein (MDR1), coding for P-glycoprotein, and second generation antipsychotic (SDA)-induced weight gain among 108 female schizophrenic patients treated with olanzapine or risperidone for up to 4 months. No significant differences in -759C/T allelic and genotype variants of $5HT_{2C}$ were found between patients who gained more than 7% of their initial weight compared to those who gained less. Haplotype based analysis of two MDR1 loci - exon 21 G2677T and exon 26 C3435T revealed borderline less representation of the G2677/C3435 haplotype in ≥7% group. In the subgroup of patients treated with risperidone, we found borderline overrepresentations of 2677T and significant overrepresentations of 3435T variant and borderline overrepresentation of 2677T/3435T haplotype the ≥7% group, whereas G2677/C3435 haplotype was found to be less represented in the ≥7% group. Our data indicate a non significant role of 759C/T $5HT_{2C}$ in SDAs-induced weight gain, and stronger influence of MDR1 G2677T and C3435T polymorphisms on risperidone-induced weight gain in female schizophrenic patients. 3435T and 2677T MDR1 variants, both associated with lower P-gp function might predispose to higher risperidone accessibility to the brain allowing its stronger effects, including weight gain.

Key words: antipsychotics, genetic polymorphism, $5HT_{2C,}$ MDR1, schizophrenia, weight gain

1. Introduction

1.1. Weight gain and serotonin receptor 5-HT_{2C}

Weight gain is one of the major side effects of treatment with second generation antipsychotics (SDAs) (Sussman, 2001) increasing morbidity and often leading to the lack of compliance with the patient thus influencing the outcome of the treatment itself (Lee and Paffenbarger, 1992). Gene polymorphisms of target receptors that could serve as potential gene markers in the prediction of side-effects, including weight gain, have been extensively studied. Serotonin receptor 5-$HT_{2C,}$ on which, both olanzapine and risperidone have strong antagonistic effects (Stahl, 2000; Di Matteo et al., 2002), is one of the major gene candidates for SDAs-induced weight gain (Tecott et al.,1995). According to several studies the role of -759C/T promoter polymorphism in SDAs-induced weight gain is particularly interesting, however, the results are far from being consistent (Basile et al., 2002; Reynolds et al., 2002; Tsai et al., 2002; Theisen et al., 2004; Ellingrod et al., 2005; Miller et al., 2005; Godlewska et al., 2006).

1.2. Weight gain and P-glycoprotein

SDAs-induced weight gain is thought to be dose independent (Kinon et al., 2001). However, apart from dose, plasma olanzapine and risperidone levels and their access to the brain might depend upon multidrug resistant protein gene (MDR1 or ABCB1), which codes for P-glycoprotein (P-gp), the transmembrane efflux transporter. P-gp plays a major role in absorption, distribution and elimination of drugs (Lin and Yamazaki, 2003). Furthermore, it is widely localized in the luminal membranes of endothelial cells in cerebral capillaries forming the blood-brain barrier (Cordon-Cardo et al., 1989). Studies using mice models showed P-glycoprotein-deficient mice to have 10 times higher drug concentrations (Uhr et al., 2006). P-gp may in various degrees influence the access of antipsychotics to the brain, and olanzapine was ranked as an intermediate (Boulton et al., 2002) and risperidone as a strong P-gp substrate (Mahar Doan et al., 2002).

Two functional single nucleotide polymorphisms (SNP), silent mutation C3435T in exon 26 and exon 21 SNP G2677T were extensively studied through their influence on expression of MDR1 (Hoffmeyer et al., 2000; Tanabe et al., 2001), their association with pharmacokinetics, bioavailability and clinical effects of drugs (Uhr et al., 2006; Roberts et al., 2002). As to our knowledge, only one recent association study of weight gain and MDR1 polymorphisms was performed reporting negative findings (Lin et al., 2006). Considering the role of P-gp in forming blood-brain barrier which influences olanzapine and risperidone access, we hypothesized that functional polymorphisms of MDR1 gene might influence weight gain.

Therefore, the aim of the study was to investigate the potential influence of 5-HT_{2C} (promoter region -759C/T) and MDR1 polymorphisms (exon 26 C3435T and exon 21 G2677T) on olanzapine and risperidone-induced weight gain in female schizophrenic patients.

2. Methods

2.1. Subjects

This study included 108 adult schizophrenic female patients, of Croatian descent, referred to the Department of Psychiatry, University Hospital Centre Zagreb and psychiatric hospital "Sveti Ivan". 52 out of 108 female patients were included in a previous study by our group, described in Bozina et al., (2006). Inclusive criteria, diagnosis and illness description were the same as described in the mentioned publication. All patients were experiencing their acute phase of the illness. 96 patients had received no antipsychotic medication prior to olanzapine or risperidone admission, and 12 patients had received typical antipsychotics (haloperidol or fluphenazine) during the course of their illness, but have stopped taking their medication prior to this study (mean+/-SD was 35+/-10 days). The mean +/- SD age, years of education, and duration of illness (defined by anamnestic data on the first occurrence of the symptoms) were 33.6 +/- 11.5 years, 12.0+/- 2.8 years and 6.7+/-7.8 years, respectively, with no differences between patients who received olanzapine and risperidone.
All participants gave informed written consent for participation in the study, prior to blood sampling and after the remission of the illness were achieved. The study protocol was approved by the Hospital Ethical Committee of the Hospital Centre Zagreb and Hospital Ethical Committee of the psychiatric hospital "Sveti Ivan".

2.2. Procedure

All subjects were weighted, their height was measured and body mass index (BMI) was calculated for each patient prior to olanzapine/risperidone administration. Body weight increase was assessed four months afterwards on outpatient visits. Olanzapine and risperidone were administered in open treatment, in fixed doses (olanzapine 10 mg/day (n=56), or 20 mg/day (n=5) and risperidone 4mg/day (n=47). To assess and evaluate improvement of clinical psychotic symptoms and therapeutic response to an antipsychotic, all patients were rated using the Positive and Negative Syndrome Scale (PANSS) at two time points (initial and four months afterwards), by two experienced psychiatrists (VM and IS). Interrater reliability score was high, above 0.87. Clinical improvement was measured with total PANSS and positive, negative and general PANSS subscales percentages of change. Weight gain related to olanzapine/risperidone was defined as increase in bodyweight more than 7% of baseline body weight, according to The Federal Drug and Food Administration definition.

2.3. Genotyping

Genomic DNA was freshly isolated from leukocytes (EDTA-anticoagulated blood) by the salting out method (Miller el al., 1988). For analysis of -759C/T allelic variants of 5-HT2C gene PCR-RFLP method was applied according to previously described procedure (Yuan et al., 2000; Pooley et al., 2004) with some modifications. By PCR method a fragment from –885 to -634 of the gene sequence was amplified using the following specific primers: forward 5'-ATC TCC ACC ATG GGT CTC GC-3' and

reverse 5'-CAA TCT AGC CGC TCC AAA GG-3'. PCR was performed in 20µl reaction volume containing 1x PCR reaction buffer, 200ng of genomic DNA, 200µmol/L of each dNTP, 0.3 µmol/L of each primer, 1.5 mmol/L $MgCl_2$, and 2U of Taq DNA polymerase. The conditions for amplification were as follows: initial denaturing step of 94°C for 5 min, followed by 35 cycles of 95°C for 30 sec, 57°C for 45 sec, 72°C for 1 min, and a final elongation step of 72°C for 10 min. 252 bp PCR product was then digested with Aci I restriction endonuclease into fragments depending on genotype (C→T substitution at –759 abolishes Aci I restriction site). With regard to the –759 polymorphisms, the C allele is denoted by 126 bp fragment and the T allele by a 160 bp fragment.
Analysis of 2677 G/T/A polymorphisms in exon 21 of MDR1 and 3435C/T polymorphism in exon 26 were previously described in Bozina et al., (2006).

2.4. Data analysis

Binary logistic regression was used to predict weight gain from sociodemographic data (age, years of education), duration of illness, baseline body weight, baseline BMI (BBMI), baseline PANSS and all PANSS subscales, and dose of antipsychotics needed to achieve the remission of the symptoms. Differences among subjects with various genotype groups were determined by using analysis of variance. Fischer exact test was used for pair-wise comparisons of the allele (2X2) and genotype (2X3) frequencies between ≥7% and <7% groups. P value < 0.05 was considered statistically significant. Odds ratios (ORs) and confidence intervals (CIs) were calculated when appropriate. All statistical analyses were carried out using SPSS 11.5 (SSPS inc., Chicago, IL, USA) statistical software package. The sequential Bonferroni adjustments (Rice, 1989), using two different methods appropriate for dependent (genotype distribution depending on allelic frequency, and frequency of particular haplotype depending on the overall haplotype distribution) (Holm, 1979) and independent variables (loci and haplotypes) (Simes, 1986; Hochberg, 1988) were applied to correct for the effect of multiple tests.
Afterwards, a test for Hardy-Weinberg equilibrium using Markov chain method (Guo and Thompson, 1992), as well as linkage-disequilibrium (LD) likelihood-ratio test between loci (Slatkin and Excoffier, 1996) were performed, as implemented in Arlequin ver. 3.01 (Excoffier et al., 2006). MDR1 haplotype frequencies were estimated using Expectation-Maximization algorithm implemented in the same program, leading to maximum likelihood estimates of haplotype frequency. Log likelihood ratio tests were performed to compare distributions of the estimated haplotypes between ≥7% and <7% groups.

3. Results

3.1. Allele and genotype frequencies of $5HT_{2C}$ and MDR1 genes

Frequencies of CC, CT and TT genotypes of -759C/T were 60, 42 and 6 respectively, of GG, GT, TT genotypes of exon 21 G2677T/A were 42, 53 and 13, respectively, whereas of CC, CT and TT genotypes of exon 26 C3435T MDR1 polymorphic locus were 24, 55 and 29, respectively. No significant deviations from the expected Hardy–Weinberg proportions were observed in the sample (exon 21 G2677T, p=0.68; exon

26 C3435T, p=1) and also in ≥7% (exon 21 G2677T, p=0.38; exon 26 C3435T, p=1), or in <7% groups (exon 21 G2677T, p=1; exon 26 C3435T, p=0.79). Test results for linkage disequilibrium between loci exon 21 G2677T and exon 26 C3435T were found to be significant in the total sample (r^2=0.3261, p<0.0001, χ^2=32.56, d.f.=1), but also ≥7% (r^2=0.2595, p=0.011, χ^2=6.49, d.f.=1) and <7% groups (r^2=0.3764, p<0.0001, χ^2=25.80, d.f.=1) in the total sample. Only in ≥7% group among patients treated with olanzapine, when analyzed separately, LD between loci was not significant (p=0.134, χ^2=2.23, d.f.=1).

3.2. Allele, genotype and haplotype associations with weight gain

After 4-months of treatment with SDAs, patients gained 3.5+/-4.2 kg or 5.5+/-6% (mean +/-SD) of their baseline body weight with no differences between patients treated with risperidone and olanzapine (data not shown).
No differences in allele and genotype distributions of all studied genes were found when weight gain was divided as dichotomous trait in group of patients who gained more than 7% of their initial body weight and group of patients who gained less than 7% of their initial body weight (Table 1). No statistical differences were found in distributions of the estimated MDR1 haplotypes between ≥7% and <7% groups, either. However, comparison of carriers of G2677/C3435 haplotype vs. others showed this haplotype to be less represented in the ≥7% group (χ^2= 5.009, d.f.=1, p=0.025; OR=0.54, 95% CI 0.31-0.93). After correction for multiple testing this association would be close to statistical significance, but no reaching it (α=0.0125) (Table 2).

3.3. Allele, genotype and haplotype associations with weight gain in risperidone treated patients

Pair-wise comparisons of the allele and genotype frequency between ≥7% and <7% groups in the group of patients treated with risperidone revealed statistical difference in the distribution of 26 C3435T variants (allele comparison with Fischer exact test 2X2, p=0.015; OR=0.35, 95% CI 0.15-0.80 and genotype comparison with Fischer exact test 2x3 p=0.022) and statistical difference in the distribution of exon 21 G2677T (Fischer exact test 2X2, p=0.031; OR=0.36, 95% CI 0.15-0.87). After correction for multiple testing, all mentioned association remained significant, with the exception of difference in the distribution of exon 21 G2677T (Table 1).
Statistical differences were found in distributions of the estimated MDR1 haplotypes between ≥7% and <7% groups among risperidone treated patients (Log likelihood ratio test, χ^2 = 7.928, d.f. =3, p=0.048). Subsequent analyses showed G2677/C3435 haplotype to be less represented (Log likelihood ratio test, H1 vs. others χ^2 = 7.509, d.f. =1, p= 0.006; OR=0.31, 95% CI 0.13-0.73) and 2677T/3435T haplotype to be overrepresented in the ≥7% group (Log likelihood ratio test, H4 vs. others χ^2 = 4.539, d.f. =1, p= 0.033; OR=2.59, 95% CI 1.06-6.30). After correction for multiple testing in haplotype based analysis among the risperidone treated patients, all mentioned association remained significant, with the exception of difference in the distribution of 2677T/3435T haplotype between ≥7% and <7% (Table 2).

3.4. Allele and genotype associations with treatment response

Although we observed similar trends in treatment response measured with changed PANSS score comparing G2766T and C3435T genotypes (GG<GT<TT and CC<CT<TT), differences reached statistical significance only when comparing changed positive PANSS sub scores between G2766T (mean +/- SD of GG:GT:TT were 53+/-16:53+/-15:64+/-9, p=0.047) and C3435T genotype (mean +/- SD of CC:CT:TT were 48+/-17:57+/-16:59+/-16, p=0.016). Binary logistic regression analysis established no confounding effects of age, duration of illness, dose of antipsychotics, baseline weight, BBMI, baseline PANSS and baseline values of all PANSS subscales on weight gain. There were no differences between groups of patients treated with olanzapine and risperidone in aforementioned variables. There were no statistically significant differences between genotype groups for each SNP in terms of age, duration of illness, dose of antipsychotics, baseline weight, BBMI and baseline PANSS.

4. Discussion

Based on the main findings of this study, two conclusions might be drawn: (1) 759C/T polymorphism of the $5HT_{2C}$ receptor might not play a significant role in susceptibility to weight gain in female schizophrenic patients; (2) MDR1 exon 21 G2677T and exon 26 C3435T polymorphisms might have significant influence on SDAs-induced weight gain in female schizophrenic patients.

We found no associations between SDAs-induced weight increase and -759C/T genotype in female schizophrenic patients. So far, results of association studies of 759C/T variants and SDAs-induced weight gain produced conflicting results. Recent studies found less weight gain in subjects bearing T allele (Ellingrod et all., 2005; Miller et al., 2005; Templeman et al., 2005, Godlewska et al., 2006), while other reported opposite results (Basile et al., 2002, in male subjects), or found no associations (Basile et al., 2002, Tsai et al., 2002; Theisen et al., 2004; Godlewska et al., 2006 in females). A variety of possible factors could provide explanations for these differences.

Compared to other studies, the majority of our patients had received no prior antipsychotic treatment, which limits the influence of one of the most confounding factors on the results. We included in the study only female subjects. Previous studies reporting associations between T allelic variant and lower weight gain included male (Reynolds et al., 2002) or predominantly male subjects (Ellingrod et al., 2005; Miller et al., 2005; Templeman et al., 2005). In the recent study by Godlewska et al., (2006), authors found significant associations between T allelic variant and lower weight gain only in male subjects, whereas no association was found in female subjects. Since $5HT_{2C}$ is situated on X chromosome, sex of subjects might influence study results, i.e. the associations with $5HT_{2C}$ might be more evident among male subjects. It is possible that SDAs induced weight gain in female schizophrenic patients may result from contributions from both allele variants. Study duration might greatly contribute to divergence among mentioned results. Templeman et al., (2005) found T variant to be overrepresented in <7% group at week 6 and after 9 months, but not at after 3-month SDAs treatment suggesting that additional mechanisms of weight increase, including gene-environment interactions (Caspi et al., 2002) might be implicated during long term SDAs-treatment in schizophrenia. Those effects might be especially important for female population (Caspi et al., 2002). Our results are consistent with previous reports which are compatible in terms of sample size and sex (Basile et al., 2002; Tsai et al., 2002; Theisen et al., 2004; Godlewska et al., 2006). Allele and genotype frequencies of 759C/T polymorphism in the studied population did not differ from reported results in other studies among Caucasian populations (i.e. Basile et al., 2002; Godlewska et al., 2006).

Studies reporting the influence of polymorphic loci in exon 26 C3435T on MDR1 expression so far produced conflicting results, as 3435T variant was found to be associated with lower MDR1 expression in the duodenum, and increased plasma levels of digoxin (Hoffmeyer et al., 2000), but also with lower plasma concentration of the digoxin in 3435T carriers according to other authors (Sakaeda et al., 2001). The same concerns studies which reported the influence of the exon 21 G2677T polymorphism on MDR1 expression and its effects on drug blood levels (Kim et al., 2001; Tanabe et al., 2001).

According to our findings, both studied MDR1 polymorphic loci, G2677T and

C3435T might contribute to weight gain, possibly producing a combined effect. Although we found less representation of G2677/C3435 haplotype in the ≥7% group, it did not reach statistical significance after correction for multiple testing. It is possible that observed associations account for the strong statistical significance which was revealed in the group of patients treated with risperidone. We found borderline overrepresentation of 2677T allele and overrepresentation 3435T allele in ≥7% group among patients treated with risperidone. The strongest statically significant differences were observed in haplotype-based analyses, which showed G2677/C3435 haplotype to be significantly less represented and 2677T/3435T haplotype to be non significantly overrepresented in the ≥7% group. Our results might indicate lower P-gp expression of 3435T and 2677T allele compared to C3435T and G2677 allele, compatible with the studies by Tanabe et al., (2001) and Hoffmeyer et al., (2000). According to our results, we could assume that both, 3435T and 2677T (in lesser extent) variants were associated with lower P-gp function and higher risperidone accessibility to the brain. Higher risperidone concentrations in the brain of 3435T and 2677T carriers compared to C3435 and G2677 carriers would allow stronger effects of risperidone, including its effects on risperidone-induced weight gain. Carriers of 2677T/3435T haplotypes would be prone to effects of risperidone treatment, including effects on weight gain, having higher risperidone brain concentrations, whereas carriers of G2677/C3435 haplotype would be more "resistant" to risperidone effects, including induced weight gain. This hypothesis is also supported by the finding of significantly better treatment response among carriers of 2677TT and 3435TT genotype, which was observed in this study, and compatible with previous studies (Bozina et al., 2006, Lin et al., 2006). That would suggest a pharmacokinetic effect of MDR1 on weight gain. Although no effects of antipsychotics' dose on weigh gain were found in this study, it might be because the majority of the patients were receiving the same dose in either risperidone and olanzapine treated groups. In contrast, $5HT_{2C}$ genotype had no influence on treatment response. It might be possible several mechanisms (one of which is pharmacokinetic) are involved in SDAs induced weight increase.

Compared to risperidone, which is considered a strong MDR1 substrate (Mahar Doan et al., 2002); olanzapine is an intermediate MDR1 substrate (Boulton et al., 2002). This fact could provide an explanation for the lack of associations between studied MDR1 variants and olanzapine-induced weight gain in our study and also in the study conducted by Lin et al., (2006), where negative association of olanzapine-induced weight gain and exon 26 C3435T and exon 21 G2677T MDR1 polymorphisms were reported.

Although G2677T and C3435T genotype seem to influence MDR1 expression, other explanations are possible. Considering that exon 26 C3435T is a silent mutation (Hoffmeyer et. al., 2000), it might be possible that observed associations are due to its linkage disequilibrium with exon 21 G2677T, or it might be possible that both polymorphic loci are in linkage disequilibrium with another MDR1 mutation, for example exon 12 (Hoffmeyer et. al., 2000). Therefore, although we determined suggestive factors for susceptibility to risperidone induced weight gain in schizophrenia in females, these results require replication, also including analysis of other MDR1 polymorphic loci. Allele and genotype frequencies in the studied population did not differ from results reported in other European regions (Hoffmeyer et. al., 2000; Fromm, 2002).

There were several limitations in this study that should be acknowledged. First, as olanzapine and risperidone blood concentrations were not measured in this study, we are able to present only indirect associations between the MDR1 polymorphisms and risperidone-induced weight gain. Furthermore, we carefully observed the factors that could have influenced antipsychotic dose as smoking, metabolic disturbances and renal functions..). It could also be argued that plasma level of prescribed drug is useful for indirect assessment of compliance, as treatment compliance might be critical for psychiatric patients (Licinio and Wong, 2005). However, since none of the patients showed the worsening of symptoms after the initial improvement and after they were discharged and they came to outpatients visits regularly as proscribed by the psychiatrist, we assumed they were taking drugs as proscribed. Second, we point out the relatively small number of subjects, especially after their division in different SDAs groups. This effect could be especially important in the analysis of -759C/T polymorphic loci, especially considering that its effects might be less evident in female compared to male patients. Third, caloric input was not measured and patients were not controlled for this, which might have confounded the results.
Finally, we included only female subjects in the study, who, although setting a limitation to generalizing the findings to male subjects with schizophrenia, contributed at the same time to the homogeneity of the study sample since gender differences in the course of illness and also in the SDAs-induced weight gain have been described (Babigian, 1985; DeLisi, 1992; Basson et al., 2001). As a strong point of this study we emphasize that the majority of our patients were drug naive prior to the beginning of the study, and the rest stopped taking their medication prior to their enrolments in the study, limiting the influence of one of the most confounding factors in previous studies.

Conclusion

In sum, we found no associations between olanzapine and risperidone-induced weight increase and -759C/T genotype. However, we found less representation of the G2677/C3435 among schizophrenic female patients who gained significant weight probably accounting for the statistically significant less representation of that haplotype in risperidone treated patients. Furthermore, we found borderline overrepresentation of 2677T, significant overrepresentation of 3435T variant and borderline overrepresentation of 2677T/3435T haplotype among patients who gained significant weight following risperidone treatment. Therefore, carriers of MDR1 2677T and 3435T variants, and especially 2677T/3435T haplotype might be prone to risperidone induced weight gain. However, because of the limitations of our sample, these results should be taken as suggestive and require replication studies with careful characterization of confounding factors like food intake and energy expenditure, especially with samples of different ethnic origin.

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Table 1. Distribution of allele and genotype frequencies of $5HT_{2C}$ receptor -759C/T and MDR1 exon 21 G2677T and exon 26 C3435T polymorphisms in weight gain ≥ 7% and weight gain < 7% groups in female schizophrenic patients in total sample and risperidone-treated patients

			Total sample (n=108)			*Risperidone-treated patients (n=47)*	
Locus			*≥ 7% n=49*	*< 7% n=59*		*≥ 7% n=23*	*< 7% n=24*
$5HT_{2C}$ -759C/T	Allele	[a]C	67	95	[c]C	30	39
		T	31	23	T	16	9
	Genotype	[b]CC	22	38	[d]CC	9	15
		CT	23	19	CT	12	9
		TT	4	2	TT	2	0
MDR 1 exon 21 G2677T	Allele	[a]G	56	81	[c]G*	24	36
		T	42	37	T	22	12
	Genotype	[b]GG	14	28	[d] GG	6	13
		GT	28	25	GT	12	10
		TT	7	6	TT	5	1
MDR 1 exon 26 C3435T	Allele	[a]C	40	63	[c]C*	16	29
		T	58	55	T	30	19
	Genotype	[b]CC	8	16	[c]CC*	2	7
		CT	24	31	CT	12	15
		TT	17	12	TT	9	2

[a]Fischer exact 2X2: $5HT_{2C}$ -759C/T p=0.057; exon 21 G2677T, p=0.089; exon 26 C3435, p=0.076
[b]Fischer exact 2X3: $5HT_{2C}$ -759C/T p=0.103; exon 21 G2677T, p=0.133; exon 26 C3435, p=0.186
[c]Fischer exact 2X2: $5HT_{2C}$ -759C/T p=0.103; exon 21 G2677T, p=0.031*; exon 26 C3435, p=0.015*
[d]Fischer exact 2X3: $5HT_{2C}$ -759C/T p=0.129; exon 21 G2677T, p=0.072; exon 26 C3435, p=0.022*

Table 2. Haplotype frequencies (proportions) of the two SNP loci: exon 21 G2677T and exon 26 C3435T in weight gain ≥ 7% and weight gain < 7% groups in female schizophrenic patients in total sample and risperidone-treated patients. Haplotype frequency was determined using the statistical program based on the EM algorithm.

			[a] *Total sample n=108*		[b] *Risperidone-treated patients n=47*	
Haplotype	G2677T MDR1	C3435T MDR1	weight gain ≥ 7% *n=49*	weight gain < 7% *n=59*	weight gain ≥ 7% *n=23*	weight gain < 7% *n=24*
H1	G	C	35 (33)	60 (50)	14 (28)	28 (58)
H2	G	T	21 (24)	21 (19)	10 (24)	8 (17)
H3	T	C	5 (8)	3 (3)	2 (6)	1 (3)
H4	T	T	37 (35)	34 (28)	20 (42)	11 (22)

Log likelihood ratio test, [a]χ^2 = 5.435, d.f. =3, p=0.143, H1 vs. others χ^2= 5.009, d.f.=1, p= 0.025
Log likelihood ratio test, [b]χ^2 = 7.928, d.f. =3, p=0.048; H1 vs. others χ^2 = 7.509, d.f.=1, p= 0.006; H4 vs. others χ^2 = 4.539, d.f.=1, p= 0.033