Polymorphisms of the *HTR2C* gene and antipsychotic-induced weight gain: an update and meta-analysis

Aims: This study aims to test for possible associations between the gene coding for the 5-HT2C receptor and antipsychotic-induced weight gain. **Materials & methods:** Four *HTR2C* polymorphisms (rs498207, C-759T, G-697C and Ser23Cys) were investigated in our sample of 205 chronic schizophrenia patients. **Results:** Significant over-representation of the C–G–Cys23 haplotype in patients with weight gain (OR: 1.93; 95% CI: 1.04–3.56; p = 0.0015) was found. Similarly, haplotype analyses of percentage weight change were also significant (p = 0.029) for the C–G–Cys23 haplotype associated with the highest average percent weight gain. Observations in the polymorphisms are consistent with previous studies. An updated meta-analysis of nine previous studies plus our current sample suggest that the -759C allele is associated with antipsychotic-induced weight gain. **Conclusion:** Additional studies, including the resequencing of the region surrounding the *HTR2C* promoter, and functional studies of the promoter polymorphisms, may elucidate the mechanism underlying this genetic association.

KEYWORDS: 5-HT2C receptor ■ antipsychotics ■ genetics ■ *HTR2C* ■ meta-analysis ■ weight gain

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Schizophrenia and schizoaffective disorder are serious conditions that affect 1% of the general population. Treatment of schizophrenia symptoms has been marred by adverse effects [1,2].

Yevtushenko and coworkers found that 99 out of 133 schizophrenia or schizoaffective patients that were taking antipsychotics had central obesity [3]. Moreover, De Hert *et al.* observed that treated schizophrenia patients had a two- to four-fold higher frequency of insulin resistance, elevated triglycerides, decreased high-density lipoprotein and hypertension compared with the nonschizophrenics [4]. Consequently, the potentially fatal consequence of antipsychotic-induced weight gain requires investigation. Antipsychotic-induced weight gain could lead to complications that are associated with obesity, including an increased risk for diabetes Type 2 and metabolic syndromes [5]. Fontaine and coworkers estimated that the use of clozapine, a second-generation antipsychotic drug for schizophrenia patients, could potentially prevent 492 suicide deaths per 100,000 patients over a 10-year period [6,7]. However, close to 85% of these patients would instead succumb to complications arising from clozapine-induced weight gain [6,7]. In addition, weight gain may add to the stigmatization of schizophrenia and reduce the medication compliance. For example, owing to side effects, olanzapine was found to have the highest rate of discontinuation compared with other antipsychotics (e.g., risperidone, perphenazine and quetiapine) in the Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) trial [8]. For these reasons, the importance of determining susceptibility to antipsychotic-induced weight gain is a critical aspect in the decision-making process informing prescription choice.

Animal studies have demonstrated that the 5-hydroxytryptamine 2C (5-HT2C) receptor is involved in the regulation of food intake [9]. For example, 5-HT2C receptor agonists decrease food intake [10,11], while 5-HT2C receptor antagonists increase food intake despite satiety, causing weight gain [12,13]. Mice with a 5-HT2C receptor deficiency display adult-onset hyperphagia, depressed metabolic rate and develop midlife obesity [14]. It has been demonstrated that antipsychotic-induced weight gain has a genetic component [15–17]. Thus, these observations have led to the hypothesis that antagonism of the 5-HT2C receptor by antipsychotics leads to increased food intake, which can ultimately lead to weight gain, and that variants in the *HTR2C* gene, which have been mapped to human chromosome Xq24, may explain part of the variation observed in antipsychotic-induced weight gain [18,19].

Previous studies indicate that specific antipsychotics induce weight gain more so than others [20,21]. Several such studies have demonstrated that there is a high correlation between

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clozapine and olanzapine treatment and weight gain in schizophrenia patients [21]. Moreover, these antipsychotics have a high antagonistic affinity for the 5-HT2C receptor [22,23]. Polymorphisms that may predispose towards weight gain are thought to be located in the 5′ region of the *HTR2C* gene [24].

Multiple past studies have shown that the C-759T polymorphism in the *HTR2C* promoter region has been significantly associated with antipsychotic-induced weight gain in schizophrenia or schizoaffective disorder patients. Specifically, the -759T allele was protective against weight gain in regards to kg of weight gained or BMI gain [25–32]. Conflicting results were obtained in two previous studies, which suggested that the -759C allele is protective against weight gain, yet these results were not significant [33,34]. Our research group reviewed previous findings on C-759T and found an overall weak protective effect of the T allele in a cohort of schizophrenia patients collected in the USA [35]. The mixed results could have been the result of the use of different ethnicities and/or different antipsychotics. In addition, antipsychotic-induced weight gain could be influenced by additional *HTR2C* variants, yet few studies have investigated polymorphisms other than C-759T of *HTR2C* for association with antipsychotic-induced weight gain. The C allele of the G-697C polymorphism was found to be protective against a 10% BMI gain or higher [28]. In addition, the nonsynonymous Ser23Cys polymorphism has also been investigated in antipsychotic-induced weight gain with mixed findings [29,35–40].

A recent study found that patients who carried the *HTR2C* haplotype with the -759C, -697C and 23Ser alleles had a higher BMI [29]. Functional studies of the *HTR2C* promoter region revealed that haplotypes containing the -759T and -697C polymorphisms were associated with reduced *in vitro* promoter activity [41]. Our current study aims to investigate four *HTR2C* polymorphisms (including rs498207, C-759T, G-697C and Ser23Cys) by extending our previous analyses to a larger cohort including four different samples.

Materials & methods

Subjects

A total of 205 schizophrenia or schizoaffective disorder patients were recruited in Berlin, Germany (Sample A; n = 68) and North America (Cleveland, OH, USA, New York, NY, USA and North Carolina, NC, USA, Samples B and C; n = 137). Sample A (n = 68) was assessed for up to 6 weeks using a variety of antipsychotics. Sample B (n = 78) was treatment resistant or intolerant to typical antipsychotic medications. This group of patients started treatment with clozapine after a washout period of 2–4 weeks with no medication unless clinically required. Weight gain was assessed after 6 weeks of clozapine treatment. Sample C (n = 59) consisted of schizophrenia or schizoaffective disorder patients who did not respond adequately to typical antipsychotics in terms of positive symptoms and functional impairment. They were treated with four antipsychotics (clozapine, olanzapine, haloperidol and risperidone), and weight gain was assessed on average for 11 weeks. We have previously published findings on Samples B and C [34,35]. We are including results on two additional *HTR2C* markers (rs498207 and G-697C) for these samples. We have also added results from analysis of all four markers on Sample A to our analysis.

The diagnosis of schizophrenia or schizoaffective disorder was made according to the Diagnostic and Statistical Manual of Mental Disorders III or IV criteria. Informed consent was acquired for all patients and clearance from the institutional ethics committee was obtained.

We included only patients who were treated for 6 weeks, of which 141 were male and 64 were female, and 130 were treated with clozapine or olanzapine. Furthermore, we analyzed a subgroup containing only Europeans taking clozapine or olanzapine. This resulted in a total of 76 patients, in which 50 were male and 26 were female. The samples' characteristics are displayed in more detail in Table 1.

Patients were assessed for weight in kg throughout the study and percentage weight gain was calculated from baseline. In addition we grouped the patients into a category of having gained weight or not gained weight according to at least a 7% weight gain for those who gained weight, which is the weight gain threshold as set by the US FDA.

Genetic analysis

The genomic DNA was extracted and purified from 10 ml of blood using the high-salt method [42]. Genotypes were determined using commercially available PCR-based genotyping assays (Applied Biosystems, Inc., CA, USA) followed by allelic discrimination using the accompanying software on the ABI7500® Sequence Detection System (Applied Biosystems). A total

of 10% of the genotypes were regenotyped and 100% cross validation was indicated. The C-759T (rs3813929), G-697C (rs518147), Ser23Cys (rs6318) SNPs were selected through a literature review, and the rs498207 SNP was chosen owing to its relatively high minor allele frequency and its location in the upstream region of the *HTR2C* gene.

Statistical analysis

The data analysis consisted of the χ^2 analysis and Fisher's Exact Test on SPSS v15 (SPSS, Inc., IL, USA) and UNPHASED v3 for the categorical and percentage weight change data versus genotype, allele and haplotype frequencies [43]. In addition, one-way analysis of variance/Kruskal–Wallis test on SPSS v15 was conducted to determine percentage weight change data. The linkage disequilibrium for the markers was calculated using Haploview v4 [44]. The odds ratios and their 95% CI for the genotypes were calculated from 2 × 2 tables in case–control format using the Program RelRisk v2.33 written by Jurg Ott. This analysis was performed on the entire sample with ethnicity (European or African–American or others) and medication (clozapine/olanzapine or others) as covariates, as well as a subgroup containing only Europeans taking clozapine or olanzapine. Since the *HTR2C* gene is X-linked, we performed the analyses in both females and males together while taking into consideration the X chromosome (Haploview, UNPHASED v3), as well as analyzing the sexes separately. We have also performed analysis of covariance using sex, ethnicity, medication, treatment duration and baseline weight as covariates to confirm the genotypic findings from UNPHASED (SPSS).

Meta-analysis

We added samples to our previously published meta-analysis [45]. We searched for the terms 'weight gain', '5-HT2C', 'HTR2C' in the literature, which had been published upto February 2010 on the National Library of Medicine's PubMed online search engine. The following information was extracted from these papers: names of first authors, year of publication, sex ratio, mean age, study duration, ethnicity (European, African–American, east Asian), percentage of patients on clozapine or olanzapine, end point measure (BMI or weight gain), and genotype frequency of C-759T (CC/C versus CT or TT/T) in weight gain and nonweight gain groups. Data was analyzed under the more conservative random-effects model [46] on STATA version 8.0 statistical software package (StataCorp, TX, USA) as previously described [45]. Meta-regression analysis was conducted to assess any moderating effects of ancestry (mainly European versus east Asian), mean age, gender ratio and percentage of patients treated with clozapine or olanzapine. Publication bias was assessed using the method as described by Egger *et al.* [47].

Results

The percentage weight change in patients compared between the North American and German samples is not significantly different. Similarly, the number of individuals with significant (>7%) weight gain is not significantly different between North American and German samples. Only the number of males and females compared between the German and the North American samples is significant ($p = 0.029$) (Table 1).

Our results are in accordance with previous studies; demonstrating that patients who are taking clozapine or olanzapine gain more weight (weight gained: $p = 0.028$; percentage weight change: $p = 0.043$) than patients taking other antipsychotic medications [48]. African–Americans were found to gain significantly more weight than European Americans ($p = 0.002$), a finding that correlates with previous studies [49]. We also found that those who gained weight (classified as at least 7% weight gain) underwent longer study duration than

Table 1. Demographic information of our current sample.

Demographic information	Sample A (Germany)	Samples B + C (North America)	p-value
Sex (male/female)	41/27	100/37	0.065
Age (years ± standard deviation)	34.70 ± 12.16	36.55 ± 8.93	0.27
Weight gain (% ± standard deviation)	5.28 ± 4.35	5.34 ± 7.18	0.94
Weight gain of at least 7% (yes/no)	24/44	47/90	0.89
Clozapine or olanzapine (yes/no)	18/23	112/25	<0.001
Ethnicity (European/African–American)†	62/2	64/56	<0.001

†*Self-reported.*

Table 2. Results from single-marker analysis on our entire sample.

SNP	Genotypes	Genotypic weight gain (yes/no)		Weight change ± SD (%)		Alleles	Allelic weight gain M + F (yes/no)
		M	F	M	F		
C-759T (rs3813929)	TT	11/15	0/1	5.57 ± 7.29	-2.18	T	13/26
	TC		2/9		4.58 ± 6.34		
	CC	38/74	19/32	5.22 ± 6.48	5.73 ± 5.98	C	78/147
	p-value	0.421	0.537†	0.811	0.387		
	p-value (M + F)	0.401		0.874			0.871
G-697C (rs518147)	GG	31/48	14/15	5.86 ± 6.78	6.60 ± 5.45	G	66/98
	GC		7/20		4.95 ± 6.88		
	CC	18/39	0/6	4.54 ± 6.47	2.53 ± 3.79	C	25/71
	p-value	0.358	0.044†	0.255	0.273		
	p-value (M + F)	0.113		0.206			**0.019**
Ser23Cys (rs6318)	GG (CysCys)	37/69	16/24	5.39 ± 6.57	5.78 ± 5.90	Cys	73/103
	GC (CysSer)		4/13		5.11 ± 7.14		
	CC (SerSer)	11/19	0/5	4.74 ± 6.66	3.03 ± 3.41	Ser	15/42
	p-value	0.859	0.141†	0.635	0.630		
	p-value (M + F)	0.564		0.693			0.167

The reported p-values were obtained from SPSS for males and females separately (χ^2 for weight gain yes/no and analysis of variance for percentage weight change), as well as for the entire sample from UNPHASED version 3.1.3 with a number of parameters (X-linked option, with ethnicity and clozapine/olanzapine yes/no as factor covariates). Bolded numbers represent significant findings.
†Fisher's exact test.
F: Female; M: Male; SD: Standard deviation.

those who did not gain weight (p = 0.016); these results are consistent with previous studies (e.g., [50]).

The genotypes for the rs498207 marker were highly correlated to those for the G-697C polymorphism, so we analyzed three polymorphisms (G-697C, C-759T and Ser23Cys) in this study. Ethnicity, gender (as the *HTR2C* gene is X-linked) and the type of antipsychotic medication administered were all taken into account.

We found the G allele of the G-697C marker to be associated with the dichotomous weight gain variable (at least 7% weight gain change; OR: 1.91; 95% CI: 1.10–3.32). Carriers of at least one copy of the G allele at G-697C were also associated with significant weight gain and a higher percentage weight gain; however, the genotypic association results were not statistically significant, either with UNPHASED or analysis of covariance (UNPHASED results shown in Tables 2 & 3). Results for the other two polymorphisms were not significant for 7% weight gain (yes/no) or percentage weight change. As the three polymorphisms are in high linkage disequilibrium with each other (D′ >0.6), we analyzed haplotypes containing all three analyzed polymorphisms. The results were significant with the dichotomous variable (p = 0.005), with the C–G–Cys haplotype being over-represented in patients with weight gain (haplotype-specific p = 0.0015; OR: 1.93; 95% CI: 1.04–3.56). The C–G–Cys haplotype was also associated with the highest average percentage weight gain; however, the results were not statistically significant (p = 0.123; Tables 2 & 3). When we added baseline weight, treatment duration and sex as covariates, the haplotype results remained similarly significant (p = 0.005).

Table 3. Results from haplotype analysis on our entire sample.

C-759T–G-697C–Ser23Cys haplotypes	Weight gain (yes/no)	% Weight change (variance)
1–2–1 (T–C–Cys)	10/19	4.86 (43.59)
2–1–1 (C–G–Cys)	51/61	6.57 (43.59)
2–1–2 (C–G–Ser)		5.04 (43.59)
2–2–2 (C–C–Ser)	8/27	4.43 (43.59)
p-value	**0.005**	0.123

The reported p-values were obtained for the entire sample from UNPHASED version 3.1.3 with a number of parameters (X-linked option, with ethnicity and clozapine/olanzapine yes/no as factor covariates). Bolded numbers represent significant findings.

Table 4. Summary of results from single-marker analysis on a subset of our sample who are of European origin and are taking clozapine or olanzapine.

SNP	Genotypes	Genotypic weight gain (yes/no)		Weight change ± SD (%)		Alleles	Allelic weight gain M + F (yes/no)
		M	F	M	F		
C-759T (rs3813929)	TT	3/7	0/1	5.29 ± 3.51	-2.18	T	5/16
	TC		2/7		5.96 ± 6.21		
	CC	12/25	6/10	4.77 ± 6.30	4.29 ± 6.17	C	26/52
	p-value	0.884	0.765†	0.805	0.445		
	p-value (M + F)	0.732		0.819			0.404
G-697C (rs518147)	GG	10/17	5/5	5.12 ± 6.31	6.17 ± 6.35	G	23/35
	GC		3/8		4.31 ± 6.91		
	CC	5/14	0/4	4.56 ± 5.29	2.74 ± 3.70	C	8/30
	p-value	0.445	0.226†	0.750	0.625		
	p-value (M + F)	0.287		0.713			0.057
Ser23Cys (rs6318)	GG (CysCys)	14/26	7/12	5.07 ± 5.51	5.46 ± 6.49	G (Cys)	29/56
	GC (CysSer)		1/6		2.34 ± 4.75		
	CC (SerSer)	1/6		2.57 ± 5.69	4.62 ± 6.14	C (Ser)	2/12
	p-value	0.405†	0.375†	0.277	0.259		
	p-value (M + F)	1.000		0.242			0.214†

The reported p-values were obtained from SPSS for males and females separately (χ^2 for weight gain yes/no and analysis of variance for percentage weight change), as well as for both sexes together from UNPHASED version 3 (X-linked option).
†Fisher's exact test.
F: Female; M: Male.

Results in males and females indicated the same risk alleles, as did results in the North American and German samples. Refining the sample for Europeans taking clozapine or olanzapine resulted in the same risk alleles, but the results were not significant with regards to allelic, genotypic and haplotypic analysis as depicted in Table 4 & 5. Furthermore, refining the sample for African–Americans did not yield significant findings in an exploratory analysis. The minor frequency of C-759T in this group is low, but the risk alleles for the other two markers were the same as those observed in the overall sample.

Our PubMed search found 13 previous papers investigating the *HTR2C* gene in antipsychotic-induced weight gain (Table 6), of which nine had genotype data for C-759T. Our analysis on the nine samples, plus our own three samples, revealed the absence of the T allele association with risk for antipsychotic-induced weight gain (p = 0.02; Figure 1). Meta-regression analysis did not reveal significant effect of sex ratio, mean age, study duration, ethnicity, percentage of patients on clozapine or olanzapine or end point measure on the results. However, significant heterogeneity was detected (p = 0.003). A relevant publication bias was also detected (Egger's Test p = 0.009), suggesting preferential publication of significant findings over nonsignificant observations. When we analyzed samples consisting of mostly or all Europeans, the results became more significant with the presence of the C allele as the risk allele (p = 0.006; Figure 1). Both heterogeneity (p = 0.074) and publication bias (Egger's Test p = 0.370) dissipated with the removal of the Asian samples. In an exploratory meta-analysis on the four Asian samples, the results were not significant (p = 0.39; OR_{-759C}: 2.00; 95% CI: 0.41).

Table 5. Summary of results from haplotype analysis on a subset of our sample who are of European origin and are taking clozapine or olanzapine.

C-759T–G-697C–Ser23Cys haplotypes	Weight gain (yes/no)	Weight change (%)
1–2–1 (T–C–Cys)	5/15	4.87 (33)
2–1–1 (C–G–Cys)	23/35	5.33 (33)
2–2–2 (C–C–Ser)	2/12	2.46 (33)
p-value	0.117	0.230

The reported p-values were obtained for both sexes together from UNPHASED version 3 (X-linked option).

Table 6. Previous genetic studies of the *HTR2C* gene in antipsychotic-induced weight gain.

M/F	Antipsychotics	Ethnicity	Study duration	SNP	Risk allele	Summary of results	Ref.
180/36	Iloperidone	106 African–American, 85 European, 17 Asian	4 weeks	C-759T	C	Weight change from baseline = NS	[51]
79	Olanzapine	Korean	3 months	C-759T	N/A	Change in BMI (kg/m^2) = NS; weight gain (>7%) = NS	[52]
139	Clozapine (n = 92), haloperidol (n = 13), olanzapine (n = 21) or risperidone (n = 13)	Majority European (not specified)	6–14 weeks	C-759T, Ser23Cys, (GT) 12–18/ (CT) 4–5	N/A	-759T allele showed no significant relationship with lower weight gain (p = 0.614), long-C-Ser haplotype protective (p = 0.042)	[35]
39/45	Risperidone (n = 53), olanzapine (n = 12), amisulpride (n = 5), quetiapine (n = 4) or typical antipsychotics (n = 10)	Korean	4 weeks	C-759T	C	BMI gain (>5%) -759T allele with less weight gain group (p = 0.030) BMI gain (>7%) -759T allele with less weight gain group (p = 0.048) BMI risperidone (>5%) -759T allele with less weight gain group (p = 0.059) BMI risperidone (>7%) -759T allele with less weight gain group (p = 0.200)	[27]
54/53	Olanzapine	N/A	6 weeks	G-697C, C-759T	-697G, -759C	BMI gain <10% have higher presence of -697C and -759T (p = 0.0006, p = 0.002)	[28]
13/14	Olanzapine	N/A	6 months	C-759T, G-697C, Ser23Cys	-759C, -697C, 23Ser	Patients who carried a *HTR2C* haplotype, including -759C, -697C and 23Ser alleles had higher BMI (p = 0.046)	[29]
34/8	Olanzapine	European	6 weeks	C-759T	C	Weight gain (>10%) -759T allele with no weight gain group (p = 0.0035)	[26]
55/18	Risperidone (n = 26), olanzapine (n = 19), amisulpride (n = 1), quetiapine (n = 11), haloperidol (n = 10) or ziprasidone (n = 6)	Spanish	6 weeks, 3 months, 9 months	C-759T	C	Less BMI gain (6 weeks, 3 months, 9 months) with -759T (p = 0.003, p = 0.018, p = 0.031) without outliers (p = 0.05, p = 0.03, p = 0.15) <7% BMI gain (6 weeks, 3 months, 9 months) with -759T (p = 0.04, p = 0.163, p = 0.01)	[30]
26/15	Clozapine	35 European, 5 African–American, 1 Hispanic	6 months	C-759T	C	BMI gain >7% have lower presence of -759T than BMI gain <7% (p = 0.0026) The presence or absence of -759T had effects on BMI at 6 months (p = 0.029)	[31]
57/40	Clozapine	German	12 weeks	C-759T	C	-759C allele with change in BMI (p = 0.77), CC associated with weight gain (>7%) group (p = 0.29)	[53]
21/11	Clozapine	Han Chinese	6 weeks	C-759T	C	ANOVA -759T allele less BMI gain (p ≤ 0.02), p(m) = 0.008 for BMI gain	[32]
52/28	Clozapine	Han Chinese	4 months	C-759T	T	For change in BMI (kg/m^2) = NS; weight gain (>7%) = NS	[33]

ANCOVA: Analysis of covariance; ANOVA: Analysis of variance; F: Female; M: Male; N/A: Not available; NS: Not significant; p(f): p-value female; p(m): p-value male.

Table 6. Previous genetic studies of the *HTR2C* gene in antipsychotic-induced weight gain.

M/F	Antipsychotics	Ethnicity	Study duration	SNP	Risk allele	Summary of results	Ref.
61/62	Chlorpromazine (n = 69), risperidone (46), clozapine (4), fluphenazine (3) or sulpiride (1)	Han Chinese	6 weeks, 10 weeks	C-759T	C	-759T allele less BMI gain 6 weeks ($p < 0.0001$, p[m] = 0.004, p[f] = 0.002) -759T allele less BMI gain 10 weeks ($p = 0.0003$, p[m] = 0.005, p[f] = 0.018) -759T allele less weight gain 6 weeks and 10 weeks compared with -759C allele ($p = 0.002$ and $p = 0.001$, respectively)	[22]
45/28	Clozapine	58 white, 22 black	6 weeks	C-759T	T	ANCOVA (sex, ethnicity, response/nonresponse, age and baseline weight) for percentage weight gain = NS, weight gain (>7%) = NS, p(m) = 0.047 for weight gain (kg), black>white	[34]
60/33	Clozapine	Taiwanese	4 months	Ser23Cys	G	Weight change in kg related to genotypes = NS	[36]
100/64	Olanzapine	Japanese	8–24 weeks	C-759T Ser23Cys	C 23Ser	Percentage BMI change (kg/m^2) = NS Possession of the 23Ser allele predicted olanzapine-induced weight gain	[37]

ANCOVA: Analysis of covariance; ANOVA: Analysis of variance; F: Female; M: Male; N/A: Not available; NS: Not significant; p(f): p-value female; p(m): p-value male.

Discussion

In the current study, we analyzed three selected polymorphisms in the *HTR2C* gene for possible association with antipsychotic-induced weight gain, including sex (X-linked), ethnicity and medication as covariates. We found a significant association between the C–G–Cys haplotype with the occurrence of antipsychotic-induced weight gain. Upon adding treatment duration and baseline weight as covariates, the haplotype results remained significant. These findings suggest a predisposition to antipsychotic-induced weight gain in schizophrenic individuals with this haplotype. The risk alleles are the same as those reported in most previous studies and our current meta-analysis of C-759T with antipsychotic-induced weight gain [45]. Our positive allelic findings with G-697C are also in agreement with the only previous report on the G-697C marker and BMI gain in patients treated with olanzapine [28].

Here, we also provide an update of the meta-analysis our research group performed in 2007 [45]. We confirmed the significant association between the -759C allele and antipsychotic-induced weight gain. C-759T may not be the only marker that confers risk for antipsychotic-induced weight gain as we did not find this marker to be associated by itself in our sample. Additional markers across *HTR2C* must be explored so that future meta-analysis of multiple markers and haplotypes can be conducted to better explain the role of *HTR2C* in this serious side effect.

Functional studies regarding *HTR2C* and associated polymorphisms have been conducted with mixed results. In one study it was reported that haplotypes, including the assumed protective alleles for weight gain, -759T or -697C, resulted in reduced promoter activity, and consequently reduced receptor expression [41]. However, in an earlier study, an increase in promoter activity was observed for haplotypes that included Z-6, -995A, -759T and -697C compared with a haplotype including Z, -995G, -759C and -697G [24]. Similarly, another study found that haplotypes including the -759C allele display reduced transcriptional activity compared with the -759T allele [51]. The observed mixed findings may be owing to the fact that the 5-HT2C receptor mRNA undergoes editing and splicing [23]. Furthermore, it was observed that the Ser23 allele might influence interindividual variation in behavior, response and predisposition to mental disorder as it was found that the Ser23 allele was constitutively more active than its Cys23 allele counterpart [52].

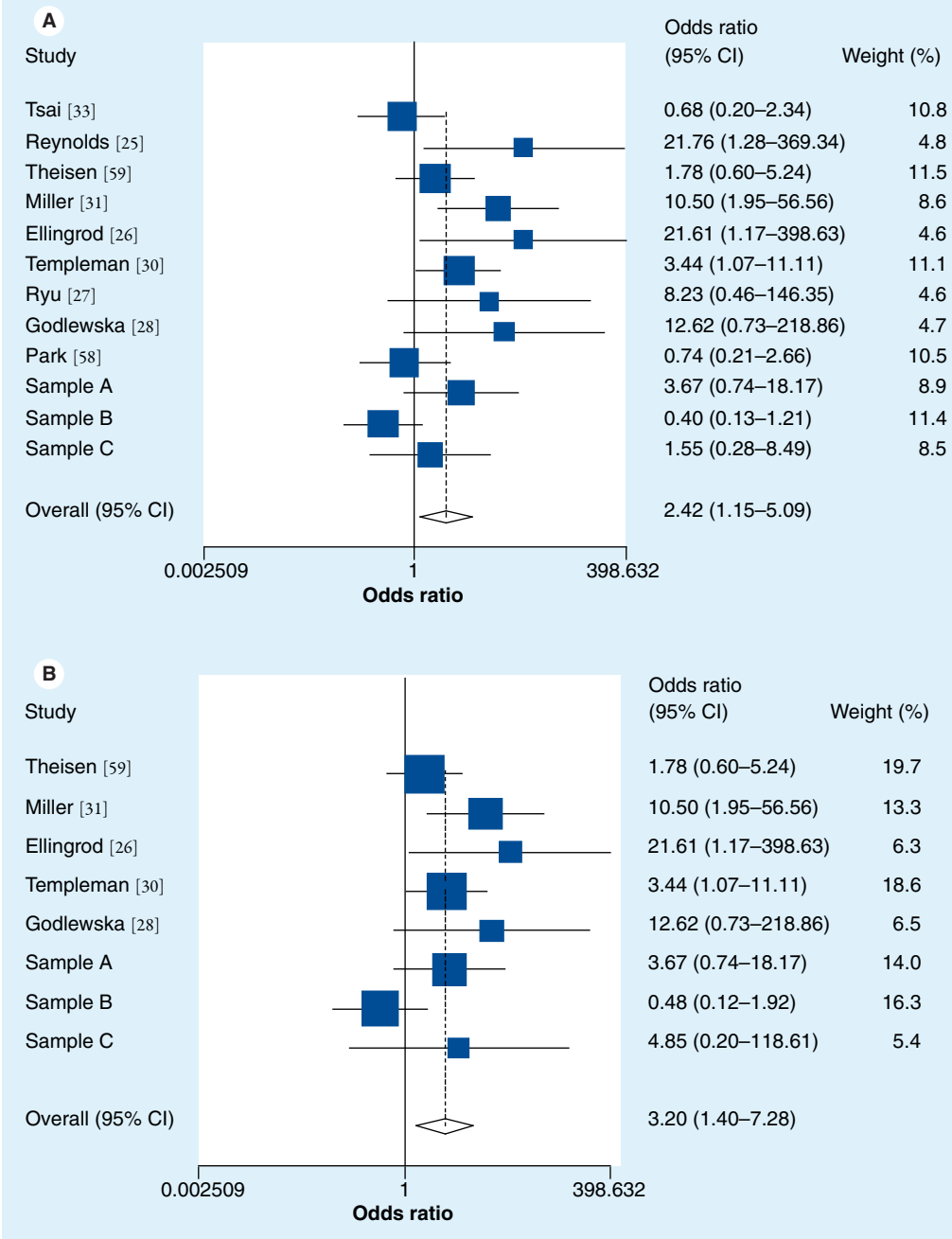

Figure 1. Forest plots of the meta-analysis of the *HTR2C* C-759T polymorphism C allele in antipsychotic-induced weight gain across nine samples plus our three samples (samples A, B and C). (A) Results for all available samples. **(B)** Results for studies on mostly or all European samples.

Follow-up weight-gain studies, along with our current findings, indicate that study duration may be associated with experiencing weight gain (at least 7% weight gain) or not experiencing weight gain and BMI gain ($p = 0.03$ and 0.016, respectively). Thus, it is important to realize that long-term atypical antipsychotic treatment will potentially have very harmful effects. We believe that if *HTR2C* studies were to be repeated for longer treatment duration, for at least 10 weeks, the significance found for the polymorphisms in regards to percentage weight gain, and at least 7% weight gain change, would become more apparent. This is because antipsychotic-induced weight gain tends to further increase during long-term treatment [53].

Conclusion

In conclusion, antipsychotic-induced weight gain appears to be a complex side effect that involves several intricate neuronal pathway circuits [54]. Thus, various genes, including *HTR2C*,

dopamine receptors, leptin [55] and cannabinoid receptor 1 [56], as well as environmental and social factors must be further studied in order to gain a comprehensive understanding of the mechanisms that are responsible [57–59]. It is important that scientists find ways to predict the outcome of a patient taking certain medications in order to prevent harmful side effects. This can be achieved through pharmacogenetic research. This research is important because obesity in the population taking antipsychotic medication has become an increasing major health problem.

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Executive summary

- The C-759T, G-697C and Ser23Cys polymorphisms in the *HTR2C* gene were tested for possible associations with antipsychotic-induced weight gain.
- Haplotypes containing -759C, -697G and Cys23 were found to be associated with antipsychotic-induced weight gain in our sample.
- Increased weight gain in patients treated with clozapine/olanzapine was found compared to with those treated with other antipsychotic medications, in African–American patients compared with patients of European ancestry, and in patients having undergone longer duration of treatment. These findings are in accordance with previous reports. When these results are included as covariates, the haplotype results remained significant.
- -759C was found to be associated with antipsychotic-induced weight gain in an updated meta-analysis on European Caucasian samples.
- It can be concluded *HTR2C* is an important candidate gene in antipsychotic-induced weight gain.

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