Available online at www.sciencedirect.com
ScienceDirect
Metabolism Clinical and Experimental 59 (2010) 1156–1163
Metabolism
Clinical and Experimental
www.metabolismjournal.com

Further exploration of the possible influence of polymorphisms in *HTR2C* and *5HTT* on body weight

Jessica Bah[a,*], Lars Westberg[a], Fariba Baghaei[b], Susanne Henningsson[a], Roland Rosmond[b], Jonas Melke[a], Göran Holm[b], Elias Eriksson[a]

[a]*Department of Pharmacology, Institute of Neuroscience and Physiology, The Sahlgrenska Academy at Göteborg University, Box 431, SE- 405 30 Göteborg, Sweden*
[b]*The Cardiovascular Institute, The Sahlgrenska Academy at Göteborg University, Göteborg, Sweden*

Received 27 March 2009; accepted 5 November 2009

Abstract

Receptors of the 5-HT2C subtype are of importance for the influence of serotonin on food intake, and 2 single nucleotide polymorphisms in this gene (*HTR2C*)—Cys23Ser (*rs6318*) and −759C>T (*rs3813929*)—have been reported to be associated with weight and/or antipsychotic-induced weight gain. The present study aimed to replicate these associations; in addition, the 5-HTTLPR polymorphism in the promoter region of the serotonin transporter gene (*SLC6A4*) was assessed. The polymorphisms were genotyped in subjects recruited from the normal population (n = 510), and possible associations between genotype and body mass index (BMI) were assessed. The Ser23 allele was more common in underweight subjects (BMI <20) than in normal- and overweight (BMI ≥20) subjects (P = .006). The T allele of the −759C/T polymorphism was less common in the overweight group (BMI ≥25) (P = .007). Homozygosity for the short allele of 5-HTTLPR was more frequent in underweight subjects (P = .015). Our results are in agreement with previous studies, suggesting polymorphisms in *HTR2C* to be associated with body weight, particularly in women; and they also suggest that 5-HTTLPR may influence this phenotype. Further studies on the importance of the investigated genes for eating disorders and drug-induced weight gain are warranted.

1. Introduction

The neurotransmitter serotonin (5-HT) is known to exert substantial inhibitory influence on the regulation of appetite and food intake, as supported by the efficacy of drugs increasing synaptic serotonin availability, such as fenfluramine and subutramine, for the treatment of obesity [1]. Genes encoding proteins involved in the serotonergic transmission are hence reasonable candidates in studies aiming to elucidate the genetic influence on obesity, eating disorders, and weight gain induced by antidepressant or antipsychotic drugs.

The 5-HT2C receptor is a G protein–coupled receptor that is expressed in many brain regions, such as prefrontal cortex, hippocampus, amygdala, and hypothalamus; in contrast, it is not found outside the central nervous system. It is assumed to play an important role in the regulation of neuronal network excitability and has been implicated in the pathophysiology of several psychiatric disorders, including depression and schizophrenia [2,3].

Pharmacologic studies in rats [4], as well as experiments using knockout mice [5], suggest that the inhibitory influence of serotonin on food intake is partly mediated by 5-HT2C receptors. This notion also gains support from the observation that weight gain is a common side effect of antidepressant and antipsychotic drugs acting as antagonists at 5-HT2C receptors [6] and by the finding that dieting may increase 5-HT2C receptor responsiveness in humans [7]. Importantly, preclinical studies suggest 5-HT2C receptor agonists to reduce food intake and body weight in rodents and, at doses lower than those influencing body weight, to improve glucose tolerance and insulin sensitivity in obese animals [1].

The gene encoding 5-HT2C (*HTR2C*) has been mapped to human chromosome X, band q24, and is the only G protein–coupled receptor gene known to be the subject of

* Corresponding author. Tel.: +46 31 786 34 48; fax: +46 31 82 10 85.
E-mail address: jessica.bah@pharm.gu.se (J. Bah).

0026-0495/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2009.11.007

adenosine-to-inosine RNA editing [2,3]. Two polymorphisms in this gene have been extensively studied: a cysteine to serine substitution at amino acid 23, Cys23Ser (rs6318), and a C>T single nucleotide polymorphism (SNP) in the promoter region, 759C/T (rs3813929) [8,9].

Whereas in vitro studies addressing the possible functional consequences of the Cys23Ser polymorphism have been conflicting [10,11], one clinical study comparing individuals with different Cys23Ser genotype with respect to cerebral blood flow suggests this polymorphism to be of functional importance [12]. We have previously reported an association between the Cys23Ser polymorphism and low weight in teenage girls who did or did not fulfill the diagnostic criteria for anorexia nervosa [13], the less common Ser23 allele occurring in a higher frequency in underweight girls regardless of diagnosis. This finding was later confirmed by a study by Hu and coworkers [14]. Moreover, a similar relationship was found in a population of female patients with seasonal affective disorder [15], but not in another cohort comprising mainly children [16].

The −759C>T polymorphism is situated in the promoter region of the gene and has been suggested to influence the transcription rate of the 5-HT2C receptor; this is however not an undisputed finding [17-19]. It has been associated with decreased weight gain in patients treated with antipsychotics [6,19,20], and with resistance to obesity and type 2 diabetes mellitus [9,18,21].

The serotonin transporter (5-HTT) is the protein responsible for the reuptake of serotonin from the synaptic cleft into the presynaptic neuron and the target for the most widely used antidepressants, that is, the selective serotonin reuptake inhibitors. An extensively studied functional deletion in the promoter region of the gene coding for 5-HTT (*SLC6A4*), 5-HTTLPR, has also been associated with weight regulation in several studies, but with conflicting results [22-26].

The present study was primarily aimed at replicating the previously reported associations between the Cys23Ser SNP and underweight and between the −759C/T SNP and overweight, our a priori hypothesis hence being that the 23Ser of the Cys23Ser SNP allele should be associated with underweight and that the T allele of the −759C/T SNP should protect against overweight. In addition, the possible influence of 5-HTTLPR on body mass index (BMI)—per se and when analyzed in conjunction with the *HTR2C* polymorphisms—was to be assessed, our prior hypothesis being that this polymorphism may be associated with underweight, overweight, or both. To this end, BMI and genotypes were measured in 270 women, all aged 42 years, and in 240 men, all aged 51 years, who were recruited from the population register.

2. Material and methods

2.1. Subjects

Both the male and the female cohorts were originally recruited for a study on body anthropometry, the results of which have been reported elsewhere [27,28]. The primary cohort from which the female group was recruited comprised all women born on uneven days in the year of 1956 and living in Göteborg, Sweden (n = 1137). Of these subjects, 80% reported self-measured circumferences over the waist and hips. From this group, 450 women in total with low, medium, and high self-assessed waist-hip ratio, respectively, were selected; of these, 270 (60%) volunteered to provide blood samples for genotyping. In the population that was genotyped, waist-hip ratio, as assessed at the clinic, was almost normally distributed [27]. Body weight was measured to the nearest 0.1 kg, with the women in underwear; height was measured to the nearest 0.01 m; and BMI was calculated. At the time of investigation, all women were 42 years old. The male subjects (n = 240) were also selected from a larger cohort, comprising all men born during the first 6 months of 1944 and living in Göteborg, Sweden (n = 1302), using a similar strategy as for the women; for details on this population, see a previous article by Rosmond and coworkers [28]. At the time of investigation, the men were 51 years old. The vast majority (>95%) of all subjects were white. All participants provided written informed consent. The study protocol was approved by the ethical committee of Göteborg University.

2.2. Molecular genetics

Venous blood was collected from each subject, and genomic DNA was isolated using the QIAamp DNA blood Mini Kit (Qiagen, Solna, Sweden). The 3 different regions were amplified in a Perkin-Elmer 9700 thermal cycler (Perkin-Elmer, Upplands Väsby, Sweden). The polymerase chain reaction (PCR) products were generated using HotstarTaq polymerase (Qiagen) in a total volume of 20 μL containing 1.5 mmol/L magnesium chloride, approximately 50 ng genomic DNA, and 200 mmol/L each dATP, dCTP, dGTP, and dTTP. The reactions contained 150 mmol/L of each primer.

The Cys23Ser fragment was genotyped using the protocol described by Lappalainen et al [8]. The −759C>T polymorphism was amplified by using the primers 5′-AATGCT-GAGTGCTGATTGGC-3′ and 5′-biotine-CTAGCAATCT-AGCCGCTCCA-3′, yielding a 174–base pair (bp) product surrounding the −759C/T polymorphism. The PCR conditions comprised an initial denaturing step at 95°C for 15 minutes; 40 cycles each at 95°C for 15 seconds, 63°C for 15 seconds, and 72°C for 15 seconds; and a final extension at 72°C for 7 minutes. The polymorphism was detected using Pyrosequencing (PSQ 96 System; Pyrosequencing, Uppsala, Sweden). A total of 20 μL of PCR product was used for Pyrosequencing in accordance with the manufacturer's instructions; 15 pmol of the sequencing primer 5′-GCTCCTCCCCTCATC-3′ was used to detect the corresponding polymorphism. The 5-HTTLPR polymorphism was amplified using the PCR primers 5′-ATGCCA-GCACCTAACCCCTAATGT-3′ and 5′-GGACCG-CAAGGTGGGCGGGA-3, yielding a product of 419 bp

for the 16-repeat allele (L) and 375 bp for the 14-repeat allele (S). After an initial denaturation step of 15 minutes at 95°C, 35 cycles were performed, including 30 seconds at 95°C, 30 seconds at 66°C ,and an elongation step at 72°C for 1 minute. Genotyping was performed on 2% agarose gels. DNA was visualized by ethidium bromide. Genotyping failed for 4 subjects with respect to −759C/T and for 9 subjects with respect to 5-HTTLPR.

2.3. Statistical analysis

On the basis of previous studies, all primary analyses were undertaken with the assumption that the Ser23, −759T, and the 5-HTTLPR S alleles were dominant. Thus, Cys23/Cys23 was compared with the combined group of Cys23/Ser23 and Ser23/Ser23, −759C/C with the combined group of −759C>T and −759T/T, and the 5-HTTLPR L/L with the combined group of S/L and S/S genotypes [6,10,17,23]. It should be noted that the assumptions of dominance only apply to women because men are hemizygous for *HTR2C*. In addition, based on previous results [13], it was decided a priori that, for analyses of possible associations between BMI and the Cys23Ser SNP, the individuals should be grouped as underweight vs not underweight. When analyzing the −759C>T SNP, on the other hand, the plan was to compare those that were overweight with those that were not; also, this a priori decision was based on earlier observations [21]. *Underweight* was defined as BMI less than 20; *normal weight*, as BMI at least 20 but less than 25; and overweight, as BMI at least 25. The analyses of possible differences between genotypes in the distribution of underweight vs not underweight, or overweight vs not overweight, were undertaken using χ^2-test or Fisher exact test, depending on the expected number of subjects in each group. A logistic regression was also performed including all 3 SNPs in the same model and controlling for sex.

Body mass index was also assessed as a continuous variable. Possible deviation of BMI values from Gaussian distribution in the studied population was assessed with the Kolmogorov-Smirnov test. Because BMI was not normally distributed (Kolmogorov-Smirnov $Z = 1.744$, $P = .005$), the potential associations between the *HTR2C* and 5-HTTLPR polymorphisms on the one hand and BMI on the other were analyzed using the nonparametric Mann-Whitney test, with genotype coded as 0 and 1, indicating homozygosity for the more common allele and presence of the less common allele, respectively, as well as with linear regression analysis, with genotypes coded as 0, 1 (=heterozygous), and 2, respectively.

Because *HTR2C* is situated on the X chromosome, linkage disequilibrium (LD) between the 2 *HTR2C* SNPs was estimated separately in men and women. In the female cohort, estimations were done by using the expectation maximization algorithm and a likelihood ratio test procedure in R, version 1.9.0 (www.r-project.org). Linkage disequilibrium in the male cohort was assessed on the basis on the actual haplotypes.

Possible influence of the combined effect of the 2 *HTR2C* SNPs on BMI, both as continuous variable and with subjects divided into underweight, normal weight, or overweight, coded as 0, 1, and 2 respectively, was assessed using the Mann-Whitney test, the Kruskal-Wallis test, or the χ^2 test. For this analysis, genotypes were grouped as Cys23/Cys23 + −759C/C, Cys23/Cys23 + −759T present, Ser23 present + −759C/C, and Ser23 present + −759T present.

Possible interacting effects between the *HTR2C* polymorphisms and the 5-HTTLPR on BMI and weight were analyzed using the Mann-Whitney test, the Kruskal-Wallis test, or the χ^2 test. For these analysis, genotypes were grouped as Cys23/Cys23 + 5-HTTLPR-L present, Cys23/Cys23 + 5-HTTLPR-SS, Ser23 present + 5-HTTLPR-L present and Ser23 present + 5-HTTLPR-SS and −759C/C + 5-HTTLPR-LL, −759C/C + 5-HTTLPR-S present, −759T present + 5-HTTLPR-LL, and −759T present + 5-HTTLPR -S present.

The significance level was set to .01. The data analyses were carried out using SPSS (Version 13.0; SPSS, Chicago, IL).

3. Results

3.1. Cys23Ser and BMI

Genotype and allele distributions for Cys23Ser, −759C>T, and 5-HTTLPR are presented in Tables 1-3. In

Table 1
BMI weight groups, genotype, and allele distribution of Cys23Ser (number, percentage)

	Total						Women						Men					
	UW		N		OW		UW		N		OW		UW		N		OW	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Cys23Cys	18	4.3	190	46.5	198	49.2	14	7.0	109	54.2	78	38.8	4	2.0	81	39.5	120	58.5
Ser23 present	12	10.9*	48	48.5	44	40.6	11	15.9	33	47.8	25	36.2	1	2.9	15	42.9	19	54.3
Cys23	42	6.3	332	49.4	298	44.3	38	8.1	251	53.7	178	38.1	4	2.0	81	39.5	120	58.5
Ser23	13	12.0†	48	44.4	47	43.5	12	16.4†	33	45.2	28	38.4	1	2.9	15	42.9	19	54.3

UW indicates underweight (BMI <20); N, normal weight (20 ≤ BMI<25); OW, overweight (BMI ≥25).
Underweight compared with non-UW (ie, N and OW) subjects:
* $P \leq .01$.
† $P \leq .05$.

Table 2
BMI weight groups, genotype, and allele distribution of −759C/T (number, percentage)

	Total						Women						Men					
	UW		N		OW		UW		N		OW		UW		N		OW	
CC	19	4.9	171	44.4	195	50.6[‡]	18	9.4	94	48.7	81	41.9[§]	2	1.0[†]	78	40.0	115	59.0
T present	9	7.6	66	55.9	43	36.4	7	9.1	48	62.3	22	28.6	3	7.3	17	41.5	21	51.2
C	44	6.8	305	47.1	298	46.1[§]	42	9.3	227	50.2	183	40.5[§]	2	1.0	78	40.0	115	59.0
T	11	8.5	74	57.4	44	34.1	8	9.1	57	64.8	23	26.1	3	7.3	17	41.5	21	51.2

Underweight compared with non-UW (ie, N and OW) subjects: [†]$P \leq .05$.
Overweight compared with non-OW (ie, N and UW) subjects: [§]$P \leq .01$, [‡]$P \leq .05$.

the total cohort, the Ser23 allele occurred more frequently in underweight individuals ($\chi^2 = 7.549$, $P = .006$, odds ratio [OR] = 0.356, 95% confidence interval [CI] = 0.166-0.764). When splitting for sex, this association was found in women ($\chi^2 = 4.927$, $P = .026$, OR = 0.395, 95% CI = 0.170-0.917) but not in men (Fisher exact test, $P = .549$, OR = 0.677, 95% CI = 0.073-6.238). A logistic regression including the SNPs and sex as independent variables displayed significant effects for Cys23Ser ($P = .015$) and for sex ($P = .008$); the Ser allele was thus more common in the underweight group (OR = 2.75), and women were more often underweight (OR = 3.90). On the other hand, Cys23Ser was not associated with BMI as a continuous variable. Moreover, a post hoc analysis assuming the Cys23 allele to be dominant showed no significant associations with BMI (data not shown).

3.2. −759T and BMI

The −759T allele was more prevalent in nonoverweight subjects than in the overweight group ($\chi^2 = 7.192$, $P = .007$, OR = 0.562, 95% CI = 0.367-0.859). As for Cys23Ser, after splitting for sex, this relationship was significant for the female group only (women: $\chi^2 = 4.187$, $P = .041$, OR = 0.553, 95% CI = 0.312-0.979; men: $\chi^2 = 0.834$, $P = .361$, OR = 0.730, 95% CI = 0.372-1.435). A logistic regression examining the difference between overweight and nonoverweight subjects and including the 3 SNPs and sex displayed significant effects for the –759C/T ($P = .015$) and sex ($P = .0003$), the −759T allele being less frequent in the overweight group (OR = 0.59) and men more often being overweight (OR = 0.51). −759C/T was associated also with BMI as a continuous variable, carriers of the −759T allele displaying lower BMI ($Z = -2.585$, $P = .010$). This association was not significant when men and women were analyzed separately (data not shown). A post hoc analysis, assuming −759C allele to be dominant, showed no significant associations with BMI (data not shown).

3.3. 5-HTTLPR and BMI

No significant association between weight group and 5-HTTLPR was found when the S allele was considered dominant. However, the SS genotype tended to be more frequent in underweight subjects ($\chi^2 = 5.934$, $P = .015$, OR = 0.391, 95% CI = 0.180-0.852). When analyzing men and women separately, this association was close to significance only in men (Fisher exact test, $P = .040$, OR = 0.135, 95% CI = 0.022-0.834). There was no significant association between 5-HTTLPR and BMI when BMI was regarded as a continuous variable.

3.4. Two-locus genotypes: Cys23Ser and −759C/T

In both men and women, LD between Cys23Ser and −759C/T as measured by $|D'|$ was equal to one ($P = .0002$), indicating a high LD. However, LD as measured by R^2 was equal to 0.03, indicating presence of 3 haplotypes; that is, all carriers of the −759T allele also carried the Cys23 allele, but not vice versa.

When analyzing the possible effect of 2-locus genotypes on BMI and weight group, the −759T- and Ser23-containing genotypes were found to be more frequent in nonoverweight individuals ($\chi^2 = 18.776$, $P = .005$). The relationship was additive, those carrying both the Ser23 allele and the −759T allele having the lowest BMI (linear regression, $P = .013$) (Fig. 1A). These associations however were significant only in the combined population and hence not when men and women were analyzed separately.

Table 3
BMI weight groups, genotype, and allele distribution of 5-HTTLPR (number, percentage)

	Total						Women						Men					
	UW		N		OW		UW		N		OW		UW		N		OW	
SS	11	11.2[†]	41	41.8	46	46.9	8	14.0	25	43.9	24	42.1	3	7.3[†]	16	39.0	22	53.7
L present	19	4.7	196	48.6	188	46.7	17	8.0	117	54.9	79	37.1	2	1.1	79	41.6	109	57.4
L	28	4.9	279	49.1	261	46.0	24	7.7	176	56.6	111	35.7	4	1.6	103	40.1	150	58.4
S	32	7.4	195	44.9	207	47.7	26	11.4	108	47.2	95	41.5	6	2.9	87	42.4	112	54.6

Underweight compared with non-UW (ie, N and OW) subjects: [†]$P \leq .05$.
Overweight compared with non-OW (ie, N and UW) subjects: [§]$P \leq .01$, [‡]$P \leq .05$.

3.5. Two-locus genotypes: −759C/T and 5-HTTLPR

A trend for a significant interaction between −759C>T and 5-HTTLPR was found in the sense that only those homozygous for the L allele had lower BMI when carrying the T allele (Kruskal-Wallis, $\chi^2 = 8.474$, $P = .037$) (Fig. 1B). However, this association was not significant when analyzing men and women separately.

3.6. Two-locus genotypes: Cys23Ser and 5-HTTLPR

In addition, an additive effect between 5-HTTLPR and Cys23Ser was found ($\chi^2 = 20.391$, $P = .0001$), the combination of 5-HTTLPR-SS + Ser23 present being significantly overrepresented in the underweight group (16.7% vs 2.5%, Fisher exact test, $P = .002$) (Fig. 2). When analyzing men and women separately, this association remained significant for women and was close to significance for men (women: $\chi^2 = 11.635$, $P = .009$, men; $\chi^2 = 9.315$, $P = .025$).

The power of finding a 2-times–higher frequency of any genotype group in either the overweight or the underweight group was approximately 90% and 30%, respectively, for all 3 SNPs. Because of the low number of underweight men, the power of finding an effect for this contrast in men was less than 10% for all SNPs.

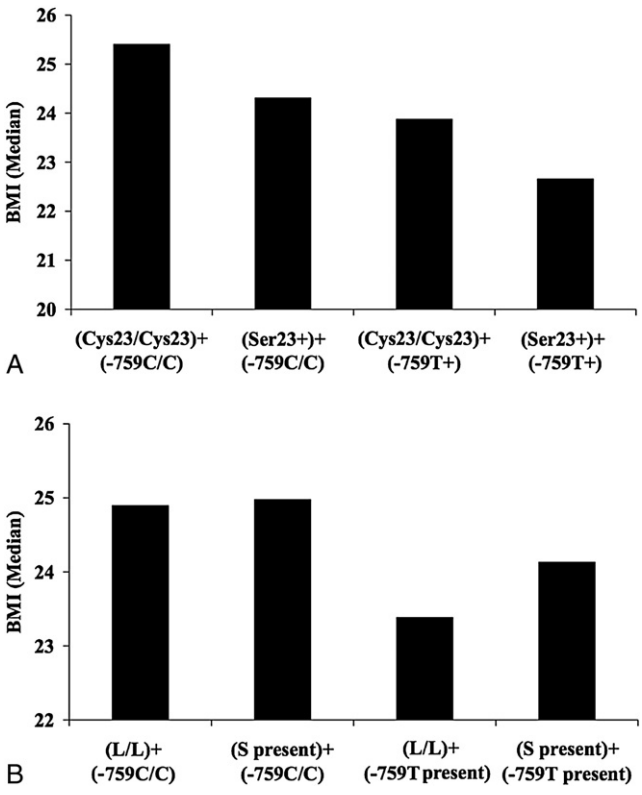

Fig. 1. Additive effects of *HTR2C*-Cys23Ser, *HTR2C*−759C/T, 5-HTTLPR on BMI. Two-locus genotype analysis of *HTR2C*-Cys23Ser, *HTR2C* −759C/T and BMI (A); and 5-HTTLPR, *HTR2C*−759C/T and BMI (B). Ser23+ = Ser23/Ser23 + Cys23/Ser23; −759T+ = T/T+ C/T; S+ = S/S+L/S.

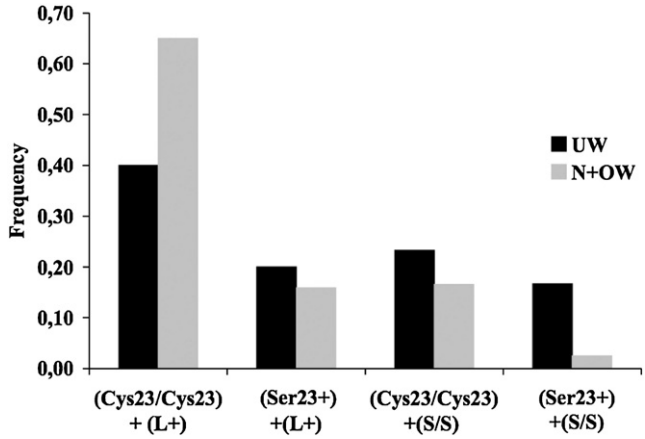

Fig. 2. Effects of *HTR2C*-Cys23Ser and 5-HTTLPR on BMI weight group. Two-locus genotype analysis of *HTR2C*-Cys23Ser and 5-HTTLPR and *HTR2C*−759C/T and BMI (B). L+ = L/L + L/S; Ser23+ = Ser23/Ser23 + Cys23/Ser23.

4. Discussion

The main findings of this study are that the Ser23 allele of the Cys23Ser polymorphism in the gene encoding the 5-HT2C receptor is overrepresented in women with low BMI and that the T allele of the −759C>T in the same gene is overrepresented in nonobese women; moreover, these 2 polymorphisms displayed an additive, negative effect on BMI. We only found weak associations between the 5-HTLLPR and BMI per se, but a significant additive effect of the Ser23 allele in *HTR2C* and the shot variant of the 5-HTLLPR in the transporter gene with respect to BMI, subjects carrying both alleles being overrepresented in the underweight group.

The finding that the Ser23 allele is associated with low BMI in women is in consonance with 3 previous studies all suggesting the Ser23 allele to be overrepresented in underweight female subjects with or without anorexia nervosa [13-15]. The notion that the −759T allele exerts a protective effect regarding overweight, being more frequent in the normal- and underweight groups than in subjects with obesity, is also in line with previous studies, most (but not all) of which have studied antipsychotic-induced weight gain [6,9,19,20]. The association between underweight and the S allele of the 5-HTTLPR genotype is to some extent in line with a study showing the S allele to be associated with anorexia nervosa [23], a study showing the L allele to be associated with binge eating [29], and a recent study in female nonhuman primates showing S carriers to display enhanced weight reduction when exposed to stress [23]. However, there are also previous studies on this topic that have yielded entirely different results [22,26,30,31].

The fact that some of the significant associations regarding the *HTR2C* polymorphisms were found only in women, but not in men, suggests that men and women differ regarding the expression or function of appetite-regulating 5-HT2C receptors. However, with respect to the −759C>T polymorphism, previous studies have been able to find associations with resistance to overweight in both men and women [6]. To some extent, the lack of significant associations in the male group in this study could be the result of the anthropometric characteristics of this cohort, very few subjects being underweight and a substantial number being overweight. When considering the possible role of sex for the outcome of the analyses, it should however be noted that the male cohort was 12 years older than the female cohort, which obviously also may be a matter of importance for the difference in results.

Notably, significant associations were found only when the Cys23Ser SNP was tested in relationship to underweight and when the −759C>T was tested with respect to overweight, and not vice versa (data not shown). This observation should be taken into consideration when interpreting studies failing to detect a direct association between the studied SNPs and BMI.

Whereas data on the possible functional relevance of the Cys23Ser polymorphism are conflicting [10,11], one study suggests that the Ser23 allele leads to a higher constitutive activity; if it is assumed that the 5HT2C receptor exerts a tonic inhibitory influence on food intake, this would be in accordance with our results. With respect to the −759C>T promoter polymorphism, one recent study suggests −759T-containing haplotypes to be related to enhanced transcriptional activity [19]; this result also is in agreement with the present findings. In line with a previous study, we did not show complete LD between Cys23Ser and −759C>T [18].

If the 5-HTTLPR, as is generally assumed, influences synaptic levels of serotonin and if serotonergic synapses exert an important 5-HT2C-receptor–mediated inhibitory influence on food intake that is modulated by polymorphisms in this gene, it is not surprising, but rather to be expected, that the latter polymorphisms display an additive effect or a synergistic interaction with the former with respect to influence on body weight. To our knowledge, this is however the first study assessing the possible issue of gene × gene interaction with respect to the influence of these 2 genes on weight. The observation that the SS genotype of 5-HTTLPR and the Ser allele of the Cys23Ser polymorphism displayed an additive influence on body weight, so that this combination was clearly overrepresented in underweight subjects, is in the expected direction, the S allele tentatively being associated with low transporter expression and hence with high extracellular levels of serotonin, leading to enhanced 5-HT2C receptor activity. One should however be cautious when discussing the 5-HTTLPR alleles in terms of enhanced or reduced serotonergic transmission, one reason being that this polymorphism may influence not only the inactivation of serotonin in the adult organism, but also the early development of serotonergic neurons.

Although the S allele of 5-HTTLPR and the Ser allele of the Cys23Ser polymorphism exerting an additive effect with respect to low body weight is well in line with the observed association between low weight and 5-HTTLPR per se, the observation that homozygosity with respect to the L allele of the 5-HTTLPR was a prerequisite for the T allele of the −759C>T to prevent weight gain was less predictable. It is however noteworthy that both the L allele of the 5-HTTLPR [19] and the T allele of the −759C>T [3,16,17] have previously been reported to prevent antipsychotic-induced weight gain.

Although the preclinical studies supporting an influence of 5-HT2C receptors on food intake in rodents make it tempting to suggest that the observed associations reflect an influence on serotonin on appetite and satiety, the possible importance of other mechanisms should not be ignored. An association between certain polymorphisms and low BMI may hence also reflect an influence of these polymorphisms on various personality traits influencing dieting behavior, as well as on susceptibility for eating disorders. Whereas the literature on the possible influence of *HTR2C* on personality traits is far from unanimous [32-35], there are 2 independent studies suggesting the Ser allele of the Cys23Ser polymorphism to be more common in women with anorexia nervosa than in controls [13,14].

For 2 of the 3 polymorphisms assessed, the aim of this study was to explore if previous associations would be possible to confirm. Apart from setting the cutoff for significance to .01 rather than .05, we have hence not found it appropriate to correct *P* values for multiple comparisons, the reason being that we had a strong a priori hypothesis regarding the outcome. The fact that multiple secondary analyses were undertaken should however be taken into consideration when interpreting the findings.

One limitation of the present study is the relatively small number of studies subjects; especially all data regarding underweight men should be interpreted with caution because of the very small number of subjects in this group. The fact that we managed to replicate previous findings regarding the influence of the studied polymorphisms on weight however does indicate that the statistical power was indeed sufficient for our purpose. A second limitation is that all studied women were 42 years of age and all studied men were 51 years of age; to what extent the results are valid also for other age groups hence is unclear. However, because a significant increase in body weight has taken place in the population during the past decade, studying subjects of the same age may also be an advantage that increases the likelihood of detecting significant associations. A third limitation is that only 2 polymorphisms were assessed in the *HTR2C* gene. The reason for this was that our aim was to replicate previous findings regarding these 2 polymorphisms; assessment of additional SNPs would have required correction for

multiple comparisons and hence reduced the power of the study. In future studies comprising larger cohorts, haplotype-based analyses of both the *HTR2C* and the *SLC6A4* in relation to weight would however be useful, as well as relating these genetic data with environmental factors such as diet, smoking habits, alcohol consumption, medication, and exercise.

To conclude, we report 2 polymorphisms in *HTR2C* and the 5-HTTLPR in *SLC6A4* to be weakly associated with BMI. Because of the fact that the observed associations were replications of previously reported findings, in conjunction with the large body of evidence suggesting that serotonin acts via the 5-HT2C receptor to influence food intake and body weight, there are reasons to believe that the reported influence of these polymorphisms on body weight is factual.

Acknowledgment

Inger Oscarsson and Gunilla Bourghardt are gratefully acknowledged for their excellent technical assistance. This study was sponsored by the Swedish Medical Research Council (grant 8668), Torsten & Ragnar Söderberg's Foundation, Hållsten's Foundation, The Swedish Brain Foundation, and The Swedish Brain Power Initiative.

References

[1] Marston OJ, Heisler LK. Targeting the serotonin 2C receptor for the treatment of obesity and type 2 diabetes. Neuropsychopharmacol 2009;34:252-3.

[2] Berg KA, Clarke WP, Cunningham KA, Spampinato U. Fine-tuning serotonin 2C receptor function in the brain: molecular and functional implications. Neuropharmacol 2008;55:969-76.

[3] Werry TD, Loiacono R, Sexton PM, Christopoulos A. RNA editing of the serotonin 5HT23C receptor and its effects on cell signalling, pharmacology and brain function. Pharmacol. Ther 2008;119:7-23.

[4] Kennett GA, Curzon G. Potencies of antagonists indicate that 5-HT1C receptors mediate 1-3(chlorophenyl)piperazine-induced hypophagia. Br J Pharmacol 1991;103:2016-20.

[5] Tecott LH, Sun LM, Akana SF, Strack AM, Lowenstein DH, Dallman MF, et al. Eating disorder and epilepsy in mice lacking 5-HT2c serotonin receptors. Nature 1995;374:542-6.

[6] Reynolds GP, Hill MJ, Kirk SL. The 5-HT2C receptor and antipsychotic-induced weight gain—mechanisms and genetics. J Psychopharmacol 2006;20:15-8.

[7] Cowen PJ, Clifford EM, Walsh AE, Williams C, Fairburn CG. Moderate dieting causes 5-HT2C receptor supersensitivity. Psychol Med 1996;26:1155-9.

[8] Lappalainen J, Zhang L, Dean M, Oz M, Ozaki N, Yu DH, et al. Identification, expression, and pharmacology of a Cys23-Ser23 substitution in the human 5-HT2c receptor gene (*HTR2C*). Genomics 1995;27:274-9.

[9] Yuan X, Yamada K, Ishiyama-Shigemoto S, Koyama W, Nonaka K. Identification of polymorphic loci in the promoter region of the serotonin 5-HT2C receptor gene and their association with obesity and type II diabetes. Diabetologia 2000;43:373-6.

[10] Fentress HM, Grinde E, Mazurkiewicz JE, Backstrom JR, Herrick-Davis K, Sanders-Bush E. Pharmacological properties of the Cys23Ser single nucleotide polymorphism in human 5-HT2C receptor isoforms. Pharmacogenomics J 2005;5:244-54.

[11] Okada M, Northup JK, Ozaki N, Russell JT, Linnoila M, Goldman D. Modification of human 5-HT(2C) receptor function by Cys23Ser, an abundant, naturally occurring amino-acid substitution. Mol Psychiatry 2004;9:55-64.

[12] Kuhn KU, Joe AY, Meyer K, Reichmann K, Maier W, Rao ML, et al. Neuroimaging and 5-HT2C receptor polymorphism: a HMPAO-SPECT study in healthy male probands using mCPP-challenge of the 5-HT2C receptor. Pharmacopsychiatry 2004;37:286-91.

[13] Westberg L, Bah J, Rastam M, Gillberg C, Wentz E, Melke J, et al. Association between a polymorphism of the 5-HT2C receptor and weight loss in teenage girls. Neuropsychopharmacology 2002;26: 789-93.

[14] Hu X, Giotakis O, Li T, Karwautz A, Treasure J, Collier DA. Association of the 5-HT2c gene with susceptibility and minimum body mass index in anorexia nervosa. Neuroreport 2003;14:781-3.

[15] Praschak-Rieder N, Willeit M, Zill P, Winkler D, Thierry N, Konstantinidis A, et al. A Cys 23-Ser 23 substitution in the 5-HT (2C) receptor gene influences body weight regulation in females with seasonal affective disorder: an Austrian-Canadian collaborative study. J Psychiatr Res 2005;39:561-7.

[16] Lentes KU, Hinney A, Ziegler A, Rosenkranz K, Wurmser H, Barth N, et al. Evaluation of a Cys23Ser mutation within the human 5-HT2C receptor gene: no evidence for an association of the mutant allele with obesity or underweight in children, adolescents and young adults. Life Sci 1997;61:PL9-PL16.

[17] Hill MJ, Reynolds GP. 5-HT2C receptor gene polymorphisms associated with antipsychotic drug action alter promoter activity. Brain Res 2007;1149:14-7.

[18] McCarthy S, Mottagui-Tabar S, Mizuno Y, Sennblad B, Hoffstedt J, Arner P, et al. Complex *HTR2C* linkage disequilibrium and promoter associations with body mass index and serum leptin. Hum Genet 2005;117:545-57.

[19] Buckland PR, Hoogendoorn B, Guy CA, Smith SK, Coleman SL, O'Donovan MC. Low gene expression conferred by association of an allele of the 5-HT2C receptor gene with antipsychotic-induced weight gain. Am J Psychiatry 2005;162:613-5.

[20] Reynolds GP, Zhang ZJ, Zhang XB. Association of antipsychotic drug-induced weight gain with a 5-HT2C receptor gene polymorphism. Lancet 2002;359:2086-7.

[21] Pooley EC, Fairburn CG, Cooper Z, Sodhi MS, Cowen PJ, Harrison PJ. A 5-HT2C receptor promoter polymorphism (*HTR2C* −759C/T) is associated with obesity in women, and with resistance to weight loss in heterozygotes. Am J Med Genet B Neuropsychiatr Genet 2004;126:124-7.

[22] Bozina N, Medved V, Kuzman MR, Sain I, Sertic J. Association study of olanzapine-induced weight gain and therapeutic response with SERT gene polymorphisms in female schizophrenic patients. J Psychopharmacol 2007;21:728-34.

[23] Hoffman JB, Kaplan JR, Kinkead B, Berga SL, Wilson ME. Metabolic and reproductive consequences of the serotonin transporter promoter polymorphism (5-HTTLPR) in adult female rhesus monkeys (*Macaca mulatta*). Endocrine 2007;31:202-11.

[24] Matsushita S, Suzuki K, Murayama M, Nishiguchi N, Hishimoto A, Takeda A, et al. Serotonin transporter regulatory region polymorphism is associated with anorexia nervosa. Am J Med Genet B Neuropsychiatr Genet 2004;128:114-7.

[25] Mergen H, Karaaslan C, Mergen M, Deniz Ozsoy E, Ozata M. ALEPR, ADBR3, IRS-1 and 5-HTT genes polymorphisms do not associate with obesity. Endocr J 2007;54:89-94.

[26] Sookoian S, Gemma C, Garcia SI, Fernandez Gianotti T, Dieuzeide G, Roussos A, et al. Short allele of serotonin transporter gene promoter is a risk factor for obesity in adolescents. Obesity (Silver Spring) 2007;15: 271-6.

[27] Rosmond R, Bjorntorp P. Psychiatric ill-health of women and its relationship to obesity and body fat distribution. Obes Res 1998;6:338-45.

[28] Rosmond R, Dallman MF, Bjorntorp P. Stress-related cortisol secretion in men: relationships with abdominal obesity and endocrine,

metabolic and hemodynamic abnormalities. J Clin Endocrinol Metab 1998;83:1853-9.

[29] Monteleone P, Tortorella A, Castaldo E, Maj M. Association of a functional serotonin transporter gene polymorphism with binge eating disorder. Am J Med Genet B Neuropsychiatr Genet 2006;141:7-9.

[30] Di Bella DD, Catalano M, Cavallini MC, Riboldi C, Bellodi L. Serotonin transporter linked polymorphic region in anorexia nervosa and bulimia nervosa. Mol Psychiatry 2000 May;5:233-4.

[31] Hinney A, Barth N, Ziegler A, von Prittwitz S, Hamann A, Hennighausen K, et al. Serotonin transporter gene-linked polymorphic region: allele distributions in relationship to body weight and in anorexia nervosa. Life Sci 1997;61:PL295-303.

[32] Ebstein RP, Segman R, Benjamin J, Osher Y, Nemanov L, Belmaker RH. 5-HT2C (*HTR2C*) serotonin receptor gene polymorphism associated with the human personality trait of reward dependence: interaction with dopamine D4 receptor (D4DR) and dopamine D3 receptor (D3DR) polymorphisms. Am J Med Genet 1997;74:65-72.

[33] Evans J, Reeves B, Platt H, Leibenau A, Goldman D, Jefferson K, Nutt D. Impulsiveness, serotonin genes and repetition of deliberate self-harm (DSH). Psychol Med 2000;30:1327-34.

[34] Tochigi M, Hibino H, Otowa T, Ohtani T, Ebisawa T, Kato N, Sasaki T. No association of 5-HT2C, 5-HT6, and tryptophan hydroxylase-1 gene polymorphisms with personality traits in the Japanese population. Neurosci Lett 2006;403:100-2.

[35] Serretti A, Mandelli L, Giegling I, Schneider B, Hartmann AM, Schnabel A, Maurer K, Möller HJ, Rujescu D. *HTR2C* and HTR1A gene variants in German and Italian suicide attempters and completers. Am J Med Genet B Neuropsychiatr Genet.;144B:291-9.