Serotonin receptor 2A gene polymorphisms and schizophrenia: association with family history, diagnostic subtype and height in patients

Kristina Melkersson [1*], Anna-Lena Hulting [2]

1. Department of Molecular Medicine and Surgery, Karolinska Institute, Stockholm, Sweden
2. Department of Endocrinology, Metabolism and Diabetes, Karolinska University Hospital, Stockholm, Sweden

Correspondence to: Kristina Melkersson, MD, PhD
Department of Molecular Medicine and Surgery, Karolinska Institute,
c/o Sollentuna Psychiatric Polyclinic,
Nytorpsvägen 10, SE-191 35 Sollentuna, Sweden.
PHONE: +46 8 58730405; FAX: +46 8 351318; E-MAIL: kristina.melkersson@ki.se

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Abstract

OBJECTIVES: The 102T/C single nucleotide polymorphism (SNP) in the 5-hydroxytryptamine receptor 2A (*HTR2A*) gene has been reported to be associated with schizophrenia. However, SNPs of the *HTR2A* gene other than the 102T/C have attracted only limited studies in relation to schizophrenia, and also on the whole SNPs of the *HTR2A* gene have been little studied in relation to clinical parameters in patients. Therefore, the aim of this study was to evaluate the impact of main functionally characterized SNPs of the *HTR2A* gene on both the schizophrenia and clinical parameters.

METHODS: Ninety-four patients with schizophrenia and 57 control subjects were genotyped for the -1438A/G, -783A/G, 102T/C and His452Tyr SNPs of the *HTR2A* gene. The four SNPs were then investigated in relation to the schizophrenia and clinical parameters.

RESULTS: No differences were found in genotype-, allele- or haplotype frequencies between schizophrenia patients and control subjects. However, the 452Tyr variant of the His452Tyr polymorphism occurred more often in patients with a family history of schizophrenia compared with patients without heredity (p=0.028). The 452Tyr variant was also more common in female patients with paranoid schizophrenia than in those with non-paranoid schizophrenia (p=0.018). Moreover, the male patients carrying the A/A or T/T genotypes of the -1438A/G and 102T/C polymorphisms were shorter than those carrying the G/A or C/T genotypes (p=0.007; p=0.006).

CONCLUSION: The present findings bring further support to the view that the -1438A/G, 102T/C and His452Tyr polymorphisms of the *HTR2A* gene are connected with a constitutive cellular change that causes susceptibility to schizophrenia.

ORIGINAL ARTICLE

INTRODUCTION

The human 5-hydroxytryptamine (serotonin) receptor 2A (HTR2A) is a cell membrane-bound receptor that is widely distributed in both central and peripheral tissues (Hoyer *et al.* 2002). This receptor is essential for mediating a variety of physiological processes including platelet aggregation, capillary permeability, smooth muscle contraction, neuroendocrine regulation and modulation of cognition, perception and mood (Roth *et al.* 1998; Van de Kar *et al.* 2001; Hoyer *et al.* 2002).

The gene coding for the HTR2A is located on chromosome 13q14-q21 and consists of three exons and two introns (Hsieh *et al.* 1990). To date, as many as 299 single nucleotide polymorphisms (SNPs) in the gene have been described (http://www.ensembl.org). However, as yet only some of these SNPs have been functionally characterized and related to impaired expression and function of the receptor. The -1438A/G polymorphism in the promoter-region, which is in close linkage disequilibrium with the synonymous coding 102T/C polymorphism in exon 1 (Spurlock *et al.* 1998), along with the modifier -783A/G polymorphism also located in the promoter-region, has been suggested in recent studies to alter promoter activity and expression of HTR2As (Parsons *et al.* 2004; Myers *et al.* 2007). Another polymorphism of the *HTR2A* gene, the His452Tyr in exon 3, causing an amino acid substitution within the cytoplasmic C-terminal tail of the receptor, has been shown to be associated with decreased serotonin-induced calcium mobilization and reduced activation of phospholipases C and D in cells (Ozaki *et al.* 1997; Hazelwood & Sanders-Bush, 2004).

Given that HTR2As are present on cells in the brain as well as in the body (Hoyer *et al.* 2002), it is not surprising that SNPs in the *HTR2A* gene have been implicated in both psychiatric and somatic disorders, such as anorexia nervosa, major depressive disorder, panic disorder, schizophrenia, the metabolic syndrome, myocardial infarction and stroke (Inayama *et al.* 1996; Collier *et al.* 1997; Yamada *et al.* 2000; Inada *et al.* 2003; Choi *et al.* 2004; Olesen *et al.* 2006; Halder *et al.* 2007). Regarding schizophrenia and the *HTR2A* gene, the 102T/C polymorphism is the most studied, whereas other polymorphisms such as -1438A/G, His452Tyr, 516C/T and Thr25Asn have been investigated only in a small number of studies (for review, see ***Table 1***). In a meta-analysis of 31 case-control studies, a significant association between the C allele of the 102T/C polymorphism and schizophrenia was found (Abdolmaleky *et al.* 2004). This finding is of potential interest, although the C allele of the 102T/C polymorphism (or the linked G allele of the -1438A/G polymorphism) seems to confer only a small effect on the liability to develop schizophrenia. Probably, this also explains why more recent studies not included in the meta-analysis by Abdolmaleky *et al.* (2004) have failed to separately replicate this positive finding (Table 1). Nevertheless, the 102T/C polymorphism (or the linked -1438A/G polymorphism) also has been associated with poor response to clozapine treatment (Arranz *et al.* 1998), akathisia and parkinsonism during perphenazine therapy (Gunes *et al.* 2007), and tardive dyskinesia during unspecified antipsychotic treatment (Lerer *et al.* 2005). Thus, the 102T/C or linked -1438A/G SNPs of the *HTR2A* gene seem to be related to a constitutive cellular change which might cause susceptibility to schizophrenia, poor response to clozapine treatment and increased risk for both acute and delayed antipsychotic-induced extrapyramidal symptoms (EPS). However, SNPs of the *HTR2A* gene other than the 102T/C SNP have not been widely studied in relation to schizophrenia (Table 1). Also on the whole SNPs of the *HTR2A* gene have been little studied in relation to clinical parameters in patients with schizophrenia. Therefore, the aim of this study was to evaluate the impact of main functionally characterized SNPs of the *HTR2A* gene both on the schizophrenia and on clinical parameters in patients.

PATIENTS & METHODS

Patients and control subjects

Consecutive out-patients from psychiatric polyclinics in the region of Stockholm, Sweden and with the diagnosis of schizophrenia according to DSM-IV criteria (American Psychiatric Association, 1994) were asked to participate in the study. In total 94 patients, 47 males and 47 females, gave their written informed consent to participate. The patients were structurally interviewed about mental and physical health in themselves and their relatives, and the patient group is described elsewhere in detail (Melkersson, 2009). In brief, all patients were unrelated Caucasian individuals. They were in full or partial remission regarding psychotic symptoms, and were all receiving long-term therapy with antipsychotics. Their mean (S.D.) age was 44 (9) years, and their duration of schizophrenia illness ranged from 0.5 to 42 years [mean (S.D.) = 18 (9) years, median = 17 years]. Control subjects were 57 unrelated Caucasian individuals (15 males and 42 females) who lived in the Stockholm County or in the nearby located Uppsala County and gave written informed consent to participate in the study. The control subjects were also interviewed about their own mental and physical health and also about that of their relatives. They were all healthy individuals with no family history of psychotic disorder and diabetes mellitus (DM) (type 1, type 2 or other types). Their mean (S.D.) age was 45 (11) years. The study was approved by the Ethics Committee of the Karolinska Institute, Stockholm, Sweden.

Collection of blood samples

Venous blood was taken in EDTA-containing tubes from all patients and control subjects and stored at –20°C until preparation of DNA.

Table 1. Published studies on *HTR2A* gene polymorphisms and schizophrenia[a]

Number of studies	*HTR2A* gene polymorphism examined	Results		Publications
		Genotype distribution	Allele frequency	
31 (meta-analysis)	102T/C [exon 1]	102C/C ↑ in P vs C	102C ↑ in P vs C	Abdolmaleky et al. 2004
10		No difference P vs C	No difference P vs C	Herken et al. 2003
		102T/T ↑ in P vs C	102T ↑ in P vs C	Baritaki et al. 2004
		No difference P vs C	No difference P vs C	Mata et al. 2004
		No difference P vs C	No difference P vs C	Zhang et al. 2004
		No difference P vs C	Not described	Pae et al. 2005
		102C/C ↑ in P vs C	102C ↑ in P vs C	Vaquero Lorenzo et al. 2006
		No difference P vs C	Not described	Correa et al. 2007
		102T/T ↑ in P vs C	102T ↑ in P vs C	Peñas-Lledo et al. 2007
		102T/T ↑ in P vs C	102T ↑ in P vs C	Sáiz et al. 2007
		No difference P vs C	No difference P vs C	Ji et al. 2008
4	-1438G/A [promoter-region]	Not described	-1438A ↑ in P vs C	Semwal et al. 2002
		No difference P vs C	No difference P vs C	Mata et al. 2004
		-1438A/A ↑ in P vs C	-1438A ↑ in P vs C	Peñas-Lledo et al. 2007
		-1438A/A ↑ in P vs C	-1438A ↑ in P vs C	Sáiz et al. 2007
2	His452Tyr [exon 3]	Not described	No difference P vs C	Erdmann et al. 1996
		No difference P vs C	No difference P vs C	Mata et al. 2004
2	516C/T [exon 2]	Not described	No difference P vs C	Erdmann et al. 1996
		No difference P vs C	No difference P vs C	Bertola et al. 2007
1	Thr25Asn [exon 1]	Not described	No difference P vs C	Erdmann et al. 1996

Abbreviations: P = patients, C = control subjects
[a]Publications were sought from January 1966 to December 2008 using the Medline database

Table 2. Data regarding the four single nucleotide polymorphisms (SNPs) studied in the *HTR2A* gene

SNP	SNP numbering[a]	SNP position[a]	Polymorphism[b]	Coding or promoter-region located
-1438A/G	rs6311	46369479 [5′ untranslated region]	G/A	Promoter-region located
-783A/G	rs6312	46368825 [5′ untranslated region]	G/A	Promoter-region located
102T/C	rs6313	46367941 [exon 1]	C/T	Synonymous coding (serine)
His452Tyr	rs6314	46307035 [exon 3]	C/T	Non-synonymous coding (histidine → tyrosine)

[a] SNP rs numbers and positions from the NCBI and Ensembl SNP databases
[b] Allele 1/ allele 2

SNP-typing

Genomic DNA was extracted from peripheral blood leukocytes using a Genomic DNA Purification Kit (Gentra Systems Inc., Minneapolis, MN, USA). The four SNPs in the *HTR2A* gene: rs6311 (-1438A/G) (Spurlock *et al.* 1998), rs6312 (-783A/G) (Zhu *et al.* 1995; Myers *et al.* 2007), rs6313 (102T/C) (Warren *et al.* 1993) and rs6314 (His452Tyr) (Ozaki *et al.* 1997), also described in ***Table 2***, were typed by TaqMan® Genotyping Assays according to the instructions of the manufacturer (Applied Biosystems, Foster City, CA, USA).

Determination of BMI

Body mass index (BMI) was calculated as kg body weight/ m^2, where m denotes the height in meters (Labhart, 1986).

Statistical methods

Categorical data were summarized using frequency counts and percentages. Continuous data were presented as mean and standard deviation or as median and inter-quartile range (P_{25}-P_{75}). Haplotypes based on the -1438A/G, -783A/G, 102T/C and His452Tyr polymorphisms of the *HTR2A* gene were calculated using PHASE version 2.1. (Stephens *et al.* 2001; Stephens & Scheet, 2005). Associations between genotype-, allele-

Table 3A. Genotype and allele frequencies regarding the four single nucleotide polymorphisms (SNPs) in the *HTR2A* gene in all schizophrenia patients (P) and control subjects (C)

SNP	Polymorphism[a]	Numbers of P and C	Genotype frequencies (%) P			C				Allele frequencies (%)[c] P	C	
			1-1	1-2	2-2	1-1	1-2	2-2	P-value[b]			*p*-value
-1438A/G	G/A	94/ 57	39.36	47.87	12.77	47.37	42.11	10.53	0.62 [0.66]	63.30	68.42	0.39
-783A/G	G/A	94/ 55	2.13	11.70	86.17	0.00	9.09	90.91	0.64 [0.71]	92.02	95.45	0.34
102T/C	C/T	94/ 57	40.43	46.81	12.77	47.37	42.11	10.53	0.70 [0.71]	63.83	68.42	0.45
His452Tyr	C/T	94/ 57	90.43	9.57	0.00	89.47	10.53	0.00	0.85 [0.93]	95.21	94.74	1.00

[a] Allele 1/ allele 2
[b] *p*-value in square brackets refers to analysis controlled for gender
[c] Only highest allele frequency is shown

Table 3B. Genotype and allele frequencies regarding the four single nucleotide polymorphisms (SNPs) in the *HTR2A* gene in schizophrenia patients without both diabetes mellitus (DM) and a family history of DM (P_{NoDM}), and control subjects (C)

SNP	Polymorphism[a]	Numbers of P_{NoDM} and C	Genotype frequencies (%) P_{NoDM}			C				Allele frequencies (%)[c] P_{NoDM}	C	
			1-1	1-2	2-2	1-1	1-2	2-2	P-value[b]			*p*-value
-1438A/G	G/A	44/ 57	29.55	61.36	9.09	47.37	42.11	10.53	0.14 [0.22]	60.23	68.42	0.24
-783A/G	G/A	44/ 55	0.00	13.64	86.36	0.00	9.09	90.91	0.47 [0.38]	93.18	95.45	0.54
102T/C	C/T	44/ 57	31.82	59.09	9.09	47.37	42.11	10.53	0.23 [0.29]	61.36	68.42	0.30
His452Tyr	C/T	44/ 57	84.09	15.91	0.00	89.47	10.53	0.00	0.42 [0.18]	92.05	94.74	0.57

[a] Allele 1/ allele 2
[b] *p*-value in square brackets refers to analysis controlled for gender
[c] Only highest allele frequency is shown

or haplotype frequencies and schizophrenia were analysed with Chi-square test and Fisher's exact test, and with logistic regression analysis when controlling for gender. The same statistical methods were used to investigate the association between the different variables such as family history of schizophrenia, subtype of schizophrenia and type of antipsychotics on one hand and the groups of genotype or haplotype on the other. In comparison between different groups of genotypes or haplotypes regarding age at onset, height and BMI, one-way analysis of variance (ANOVA) was used and when controlling for gender, two-way ANOVA was employed. In case of a significant interaction between group and gender, simple effects were tested within gender. The variation in drug dose was skewed distributed and therefore Mann-Whitney *U* test and Kruskal-Wallis ANOVA by ranks were used in comparison between patients with different genotypes or haplotypes. A *p* value of less than 0.05 was considered statistically significant. All calculations were made with the statistical programs Statistica for Windows 8.0 (Statsoft, Inc., Tulsa, OK, USA) and SAS System 9.1 (SAS Institute Inc., Cary, NC, USA).

HTR2A SNP				
-1438A/G	-1438A/G			
-783A/G	0.006	-783A/G		
102T/C	0.985	0.008	102T/C	
His452Tyr	0.031	0.000	0.032	His452Tyr

Figure 1. Pairwise linkage disequilibrium analyses (r^2) between the ***HTR2A*** gene single nucleotide polymorphisms (SNPs) -1438A/G (rs6311), -783A/G (rs6312), 102T/C (rs6313) and His452Tyr (rs6314).

RESULTS

Association of the HTR2A gene polymorphisms with schizophrenia

The genotype distributions and allele frequencies of the -1438A/G, -783A/G, 102T/C and His452Tyr polymorphisms of the *HTR2A* gene in schizophrenia patients and healthy control subjects are shown in ***Tables 3A and 3B***. A close linkage disequilibrium was found between the -1438A/G and 102T/C polymorphisms, but not between the other pairwise-combinations of polymorphisms (***Figure 1***). There were no significant differences found in genotype distributions or allele frequencies, either when all schizophrenia patients were compared with control subjects or when a subgroup of schizophrenia patients without DM and a family history of DM (n=44) were compared with control sub-

Table 4. The genotype distributions of the four single nucleotide polymorphisms (SNPs) in the *HTR2A* gene in association with clinical parameters in 94 schizophrenia patients

	-1438A/G			-783A/G			102T/C			His452Tyr		
SNP Polymorphism[a]	**G-G**	**G-A**	**A-A**	**G-G**	**G-A**	**A-A**	**C-C**	**C-T**	**T-T**	**C-C**	**C-T**	**T-T**
Family history of schizophrenia												
Percentage of patients [b] (%)												
Presence (n=37)	43.24	40.54	16.22	0.00	8.11	91.89	43.24	40.54	16.22	81.08	18.92	0.00*
Absence (n=55)	38.18	50.91	10.91	3.64	14.55	81.82	40.00	49.09	10.91	96.36	3.64	0.00
Subtype of schizophrenia												
Percentage of patients (%)												
Paranoid (n=36)	41.67	41.67	16.67	2.78	13.89	83.33	41.67	41.67	16.67	M: 100.00	0.00	0.00
										F: 70.59	29.41	0.00 **
Non-paranoid (n=58)	37.93	51.72	10.34	1.72	10.34	87.93	39.66	50.00	10.34	M: 89.29	10.71	0.00
										F: 96.67	3.33	0.00
Age at onset of schizophrenia												
Number of patients (n)	37	45	12	2	11	81	38	44	12	85	9	0
Mean ± SD (years)	26 ± 8	25 ±7	29 ±9	29 ±1	26 ±8	26 ±8	26 ±8	25 ±7	29 ±9	26 ±8	26 ±7	-
Type of antipsychotics												
Percentage of patients (%)												
1st generation agents (n=31)	38.71	41.94	19.35	3.23	6.45	90.32	38.71	41.94	19.35	87.10	12.90	0.00
2nd generation agents [c] (n=44)	45.45	45.45	9.09	2.27	15.91	81.82	47.73	43.18	9.09	90.91	9.09	0.00
1st+ 2nd generation agents [c] (n=19)	26.32	63.16	10.53	0.00	10.53	89.47	26.32	63.16	10.53	94.74	5.26	0.00
Dose of antipsychotics[d]												
Number of patients (n)	37	44	12	2	11	80	38	43	12	84	9	0
Median	200	218	229	293	250	200	200	225	229	203	300	-
P_{25}- P_{75}	133-300	197-278	138-315	286-300	125-286	150-300	125-300	200-286	138-315	150-286	200-381	-
Height												
Number of patients[e] (n) [M/F]	[18/18]	[23/22]	[5/7]	[1/1]	[7/4]	[38/42]	[19/18]	[22/22]	[5/7]	[43/41]	[3/6]	0
Mean ± SD (m) A:	1.73	1.75	1.70	1.75	1.74	1.74	1.73	1.75	1.70	1.74	1.74	-
	±0.10	±0.11	±0.06	±0.10	±0.14	±0.10	±0.10	±0.11	± 0.06	±0.10	±0.12	-
M:	1.80	1.83	1.74***	1.82	1.82	1.81	1.80	1.83	1.74****			
	±0.08	±0.09	±0.05	±0.00	±0.10	±0.08	±0.08	±0.09	±0.05			
F:	1.66	1.67	1.67	1.68	1.61	1.67	1.66	1.67	1.67			
	±0.05	±0.06	±0.04	±0.00	±0.07	±0.05	±0.05	±0.06	±0.04			
Body mass index												
Number of patients[e] (n)[M/F]	[18/18]	[23/22]	[5/7]	[1/1]	[7/4]	[38/42]	[19/18]	[22/22]	[5/7]	[43/41]	[3/6]	0
Mean ± SD (kg/m^2) A:	29 ±7	29 ±7	29 ±4	27 ±7	29 ±5	29 ±7	29 ±7	29 ±7	29 ±4	29 ±6	29 ±7	-
M:	31 ±7	28 ±5	27 ±4	32 ±0	29 ±5	29 ±6	31 ±7	28 ±5	27 ±4			
F:	26 ±6	30 ±8	31 ±4	22 ±0	27 ±5	29 ±7	26 ±6	30 ±8	31 ±4			

Abbreviations: A = all patients, M = male patients, F = female patients

[a] Allele 1/ allele 2

[b] Two patients were adoptees and lacked knowledge about their relatives

[c] Clozapine, olanzapine, risperidone or ziprasidone

[d] Expressed as mg chlorpromazine equivalent doses, calculated as previously described (Melkersson et al., 2001; Woods, 2003)

[e] Data on height and weight was missing in one patient

* Significantly different compared to the patients without a family history of schizophrenia, p=0.028 (not controlled as well as controlled for both gender and occurrence of diabetes and a family history of diabetes in patients)

** Significantly different compared to the female patients with non-paranoid schizophrenia, p=0.018

*** Significantly different compared to the male patients carrying the G/A genotype, p=0.007, and a tendency towards a significant difference compared to the male patient carrying the G/G genotype, p=0.072

**** Significantly different compared to the male patients carrying the C/T genotype, p=0.006, and a tendency towards a significant difference compared to the male patient carrying the C/C genotype, p=0.070

Table 5. Estimated haplotypes based on the -1438A/G, -783A/G, 102T/C and His452Tyr single nucleotide polymorphisms (SNPs) of the *HTR2A* gene, together with their frequencies in the overall study population[a]

Haplotypes[b]	Frequency (%)
1. G A C C	58.4
2. A A T C	29.7
3. G G C C	5.7

[a] All patients (n=94) and control subjects (n=54) who had data on all four SNPs in the haplotype set were included in the haplotype calculations.
[b] Haplotypes with estimated frequencies >5 % in the overall study population are described.

jects. Similarly, no significant differences were noted when the genotype analyses were controlled for gender (Tables 3A and 3B). The interactions Gender x respective Genotype were also not significant.

Association of the HTR2A gene polymorphisms with clinical parameters

The associations between clinical parameters of schizophrenia patients and their genotypes of the four SNPs are shown in ***Table 4***. A significant difference in genotype distribution was found for the His452Tyr polymorphism between patients with and without a family history of schizophrenia (p=0.028), but not for the -1438A/G, -783A/G or 102T/C polymorphisms. It was found that the 452Tyr variant was more common in patients with a family history (Table 4). Significant differences in genotype distributions for all SNPs except the -783A/G were also noted when schizophrenia patients with DM and a family history of DM (n=46) were compared with schizophrenia patients without DM and a family history of DM (n=44) (data not shown). Therefore, the genotype comparisons between patients with and without a family history of schizophrenia were in addition controlled for both gender and occurrence of DM and a family history of DM in patients; however the result remained unchanged (p=0.028) (Table 4). For subtype of schizophrenia (paranoid vs non-paranoid), the interaction Gender x Genotype group was significant for the His452Tyr polymorphism (p=0.024), leading to separate analyses of male and female patients. It was found that the 452Tyr variant also was more common in female patients with paranoid schizophrenia than in female patients with non-paranoid schizophrenia (p=0.018) (Table 4). For male patients, none of the 19 male patients (0 %) with paranoid schizophrenia versus three of the 28 male patients (10.7%) with non-paranoid schizophrenia were carrying the 452Tyr variant, but this difference was not significant (p=0.262) (Table 4). Regarding age at onset of schizophrenia, the interactions Gender x respective Genotype group were not significant, and no significant associations were found between the genotype groups of the SNPs and age at onset of disease (controlled for gender). Neither were any significant associations found between genotype groups of the SNPs and the type of antipsychotics used (1st generation-, 2nd generation- or both 1st and 2nd generation antipsychotics) or dose of antipsychotics (Table 4).

Regarding height, the interactions Gender x respective Genotype group were not significant, and no significant associations were found between genotype groups of the -783A/G or His452Tyr polymorphisms and the height of patients (correlated for gender). However, for the -1438A/G and 102T/C polymorphisms there were tendencies towards significant associations between genotype groups and patients' height (correlated for gender) (p=0.067 and p=0.062, respectively), and when analysing male and female patients separately, significant associations were found for male patients (p=0.020 and p=0.018, respectively), but not for female patients. Further pair-wise analyses revealed that male patients carrying the A/A or T/T genotypes were significantly shorter than the male patients carrying the G/A or C/T genotypes (p=0.007 and p=0.006, respectively), and that they also tended to be shorter than males carrying the G/G or C/C genotypes (p=0.072 and p=0.070, respectively) (Table 4). Regarding BMI, the interactions Gender x respective Genotype group were significant for the -1438A/G and 102T/C polymorphisms (p=0.038 and p=0.046, respectively), but not for the -783A/G or His454Tyr polymorphisms. However, no significant associations were found between the genotype groups of the four SNPs and patients' BMI in analyses controlled for gender or when male and female patients were analysed separately (Table 4).

Association of the HTR2A gene -1438A/G, -783A/G, 102T/C and His452Tyr polymorphism-based haplotypes with the schizophrenia and clinical parameters

The estimated haplotypes based on the -1438A/G, -783A/G, 102T/C and His452Tyr polymorphisms of the *HTR2A* gene and their frequencies in the overall study population are described in ***Table 5***. No significant differences were found in haplotype frequencies either when all schizophrenia patients (n=94) were compared with control subjects (n=54) or when a subgroup of schizophrenia patients without DM and a family history of DM (n=44) were compared with control subjects (n=54). Similarly, no significant differences were noted when the haplotype analyses were controlled for gender (data not shown). Neither were any significant associations found between haplotype groups and patients´ family history of schizophrenia, subtype of schizophrenia, age at onset of disease, antipsychotic dose, type of antipsychotics, height or BMI (data not shown).

DISCUSSION

There are three main findings arising from this study. Firstly, we found that the 452Tyr variant of the His452Tyr polymorphism was more frequently occurring in patients with a family history of schizophrenia than in patients without heredity, whereas no differences in genotype distributions of the -1438A/G, -783A/G or 102T/C polymorphisms between patients with or without a family history were found. This finding is of interest, since it has been reported that patients with familial schizophrenia are a more homogeneous patient group from an aetiological view than are patients with sporadic, non-familial schizophrenia (Melkersson, 2009). Consequently, the familial form of schizophrenia may also be expected to be associated with a higher genetic loading than the non-familial form (Faraone *et al.* 2000). Therefore, our finding of a higher frequency of the 452Tyr variant in patients with familial schizophrenia compared with those with the non-familial form may point to that this *HTR2A* gene variant and the change it causes in the HTR2A, may be connected with a constitutive cellular change that causes susceptibility to schizophrenia. Recently, it was demonstrated that the Ca^{2+} homeostasis is altered in peripheral lymphocytes from schizophrenia patients (Genius *et al.* 2008). Possibly, the 452Tyr variant of the His452Tyr polymorphism, causing an amino acid change in the HTR2A that is associated with decreased serotonin-induced calcium mobilization in cells (Ozaki *et al.* 1997; Hazelwood & Sanders-Bush, 2004), will further affect an already altered intracellular Ca^{2+} homeostasis in individuals predisposed to schizophrenia, thereby further increasing the risk for schizophrenia development. To our best knowledge, neither the His452Tyr polymorphism, nor the -1438A/G and -783A/G polymorphisms, have been studied before in relation to a family history of schizophrenia. On the other hand, the 102T/C polymorphism has been examined in this respect in four studies, but as in this study, has not been found to be associated with a family history of schizophrenia (Chen *et al.* 2001; Herken *et al.* 2003; Baritaki *et al.* 2004; Pae *et al.* 2005).

Secondly, although the frequency of the 452Tyr variant of the His452Tyr polymorphism did not differ between male and female patients (3/47 vs 6/47, p=0.49), this gene variant was more common in female patients with paranoid schizophrenia than in female patients with non-paranoid subtypes of schizophrenia. In contrast, no significant association of the 452Tyr variant with schizophrenia subtypes was found in the male patients. Thus, the 452Tyr variant of the *HTR2A* gene seems to modify the clinical phenotype at a diagnostic level especially in the female patients. As yet, there are no other studies published on the association of the His452Tyr polymorphism with schizophrenia subtypes or psychotic symptomatology, but regarding the 102T/C polymorphism, a significant association between the 102T/T genotype and negative, but not positive, psychotic symptoms has been reported in first-episode patients with schizophrenia (Wang *et al.* 2008). However, in at least six other studies, as in this study, association of the 102T/C polymorphism (or the linked -1438A/G polymorphism) with schizophrenia subtypes or psychotic symptomatology has not been found (Chen *et al.* 2001; Herken *et al.* 2003; Baritaki *et al.* 2004; Pae *et al.* 2005; Correa *et al.* 2007; Peñas-Lledo *et al.* 2007).

Thirdly, we found that male, but not female, patients with schizophrenia carrying the A/A genotype of the -1438A/G polymorphism (or the T/T genotype of the linked 102T/C polymorphism) had shorter stature compared with male schizophrenia patients carrying the G/A or G/G (C/T or C/C) genotypes. Worthy of note is that no significant associations of any of the four *HTR2A* gene polymorphisms with height were found in our healthy male or female control subjects (data not shown). Expression of HTR2As on cells is subject to considerable developmental regulation (Roth *et al.* 1998). Hence, it might be possible that the A/A genotype of the -1438A/G polymorphism (or the T/T genotype of the linked 102T/C polymorphism) of the *HTR2A* gene, implying increased expression of HTR2As on cells (Parsons *et al.* 2004), may confer effect on height exclusively in boys who have a genetic predisposition to schizophrenia. Several lines of evidence suggest that a slower rate of growth during childhood is associated with adult psychosis and that this effect would be modified by gender (Gunnell *et al.* 2003, 2005; Perrin *et al.* 2007). In males, short stature at age 18 was associated with increased risk for schizophrenia, whereas in females, slower growth velocity during early life (birth to age 2.5 years), but not height in adulthood, was associated with schizophrenia development (Gunnell *et al.* 2003, 2005; Perrin *et al.* 2007). Interestingly, individuals with the rare Coffin-Lowry syndrome, who have loss-of-function mutations in the X-chromosome-located p90 ribosomal S6 kinase (*RSK2*) gene, have mental retardation, pathognomonic craniofacial deformalities, movement disorders, psychotic illness almost exclusively in females and short stature that is most pronounced in the males (Sivagamasundari *et al.* 1994; Hanauer & Young, 2002). The *RSK2* gene is coding for the RSK2 protein that interacts with and reduces intracellular signalling of HTR2As (Sheffler *et al.* 2006). Thus, it is a striking parallel between on one hand, the loss of a reduction (i.e. potentiation) of intracellular signalling via HTR2As as in the Coffin-Lowry syndrome, leading to short stature especially in males, and on the other hand, increased expression of HTR2As (implying enhanced intracellular signalling via the receptor) in schizophrenia patients carrying the A/A genotype of the -1438A/G (or the T/T genotype of the linked 102T/C) polymorphisms, resulting in short stature in male schizophrenia patients.

Although haplotype analyses considering all four polymorphisms (i.e. -1438A/G, -783A/G, 102T/C and

His452Tyr) did not show any associations with the schizophrenia or clinical parameters, the -1438A/G (or linked 102T/C) and His452Tyr polymorphisms may have an independent effect on HTR2A function (Arranz *et al.* 1998), thereby differently affecting clinical parameters of schizophrenia patients as found in this study.

CONCLUSIONS

We conclude that in addition to previous studies of the *HTR2A* gene and schizophrenia, showing associations between schizophrenia, poor response to clozapine therapy or EPS and the C allele of the 102T/C polymorphism, and between poor response to clozapine therapy and the 452Tyr variant of the His452Tyr polymorphism, this present study demonstrates associations both between a family history of schizophrenia as well as a diagnostic subtype of schizophrenia in female patients and the 452Tyr variant of the His452Tyr polymorphism, and also between height in male patients and the A/A genotype (or linked T/T genotype) of the -1438A/G (or linked 102T/C) polymorphisms. Taken together, these present findings bring further support to the view that the polymorphisms -1438A/G, 102T/C and His452Tyr of the *HTR2A* gene are connected with a constitutive cellular change that causes susceptibility to schizophrenia.

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