European Neuropsychopharmacology (2010) **20**, 655–662

www.elsevier.com/locate/euroneuro

Seasonality and winter-type seasonal depression are associated with the rs731779 polymorphism of the serotonin-2A receptor gene

Eszter Molnar [a], Judit Lazary [b,c], Anita Benko [a], Xenia Gonda [b,c], Dorottya Pap [a], Krisztina Mekli [d,e], Gabriella Juhasz [e], Gabor Kovacs [f], Tamas Kurimay [g], Zoltan Rihmer [c], Gyorgy Bagdy [a,h,i,*]

[a] *Department of Pharmacodynamics, Semmelweis University, Budapest, Hungary*
[b] *Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, Hungary*
[c] *Department of Clinical and Theoretical Mental Health, Kutvolgyi Clinical Center, Semmelweis University, Budapest, Hungary*
[d] *Centre for Integrated Genomic Medical Research, School of Translational Medicine, Faculty of Medical and Human Sciences, The University of Manchester, UK*
[e] *Neuroscience and Psychiatry Unit, School of Community Based Medicine, Faculty of Medical and Human Sciences, The University of Manchester, UK*
[f] *Honved Hospital, Department of Psychiatry, Hungary*
[g] *Saint John's Hospital, Department of Psychiatry, Budapest, Hungary*
[h] *Group of Neurochemistry, Semmelweis University and Hungarian Academy of Science, Hungary*
[i] *Group of Neuropsychopharmacology, Hungarian Academy of Sciences and Semmelweis University, Hungary*

Received 16 February 2010; received in revised form 12 April 2010; accepted 23 April 2010

KEYWORDS
Seasonal affective disorder;
5-HT2A receptor gene;
rs731779;
rs985934;
rs6311;
depression

Abstract

Seasonal Affective Disorder (SAD), seasonality and increased sensitivity to the fluctuation of seasons in biological and psychological parameters can manifest to varying degrees across a normal population. The serotonin-2A (5-HT2A) receptor gene has long been suggested as a candidate for the genetic basis of this phenomenon. We hypothesized that functional sequence variation in this gene could contribute to seasonality and the development of winter- and/or summer-type seasonal depression. Seasonality was measured by the self-rating Global Seasonality Score (GSS) of the Seasonal Pattern Assessment Questionnaire, and SAD by the Seasonal Health Questionnaire (SHQ). We analysed associations between GSS or SAD scores and 5-HTR2A receptor gene polymorphisms rs731779, rs985934 and rs6311, in 609 individuals. People carrying the GG genotype of rs731779 were six times more likely to manifest winter or summer SAD compared to GT or TT genotypes (OR = 6.47), and the chance of having winter-type SAD was almost nine-fold (OR = 8.7) with the GG genotype. GG subjects of rs731779 also scored

* Corresponding author. Department of Pharmacodynamics, Semmelweis University, Nagyvárad tér 4, 1089, Hungary. Tel.: +36 14591495.
E-mail address: bag13638@iif.hu (G. Bagdy).

doi:10.1016/j.euroneuro.2010.04.009

significantly higher on the GSS scale compared to carriers of the T allele. In the haplotype analysis subjects carrying the G allele of rs731779 scored higher on the GSS scale, while the presence of the T allele leads to lower scores. These results suggest that variations in the 5-HTR2A gene play a significant role in the development of seasonality and especially in winter-type SAD. The fact that the above polymorphism showed association not only with clinical SAD but also seasonality symptoms in a general population provides evidence for the spectrum nature of this connection.

1. Introduction

Seasonal fluctuation, which affects mood, behavior, different psychological parameters and neurovegetative symptoms (such as sleep, appetite, taste-preference and energy) is present in the general population (Madden et al., 1996). The term seasonality denotes an increased sensitivity to normal seasonal fluctuation, and the extreme end of this continuum is known as annual depression or seasonal affective disorder (SAD) (Lee et al., 2006). There are two primary, opposite seasonal patterns of annual depressions with opposite vegetative symptoms: winter depression (hypersomnia, hyperphagia, carbohydrate craving and weight gain) and summer depression (insomnia, loss of appetite and weight loss) (Goodwin and Jamison, 2007; Wehr and Rosenthal, 1989), of which the lifetime prevalence of winter depression (winter SAD) is much higher than summer depression in Europe and in North America (Goodwin and Jamison, 2007; Mersch et al., 1999b) Mersch et al. (1999b).

Although the genetic base for SAD has not been established so far, several studies strongly suggest that seasonality and SAD have an inherited component (Madden et al., 1996; Sher et al., 1999). Previous molecular genetic studies have focused on the serotonergic system because it is associated with many of the major symptoms of winter depression, such as overeating, carbohydrate craving, weight gain, and oversleeping (Sher et al., 1999). Furthermore, the hypothalamic level of serotonin in the human brain shows seasonal variations and a general decrease during winter (Carlsson et al., 1980). The therapeutic efficacy of SSRIs in winter depression (winter SAD) also supports the assumption that there is a connection to serotonin (Lee et al., 1997); sertraline (Moscovitch et al., 2004) and fluoxetine (Lam et al., 2006) both decreased depressive symptoms, while escitalopram reduced the atypical symptoms of SAD (Pae et al., 2008). Moreover, rapid tryptophan depletion reverses the good clinical effects of bright light therapy (Lam et al., 2006) and therapy with serotonergic (but not with noradrenergic) antidepressants (Spillmann et al., 2001) in patients with winter-type SAD. The significantly higher activity of serotonin transporter binding potential during fall and winter in healthy volunteers, which reflects a significantly lower level of synaptic serotonin level, also supports the strong relationship between central serotonergic dysregulation and winter depression (Praschak-Rieder et al., 2008).

The 5-HTR2A receptor gene is the major candidate gene in association studies of seasonality and SAD (Arias et al., 2001; Enoch et al., 1999). Although, to date, the exact mechanism of action is unclear, one possibility is that downregulation of 5-HT2A receptors may underlie the therapeutic effects of SSRIs (Maj et al., 1996).

It has also been suggested that the effectiveness of light therapy, another useful treatment for SAD, may be due to an alteration in the sensitivity of 5-HT2A receptors (Yatham et al., 1997) and, as a consequence, the serotonin-2A receptor gene (Lee et al., 2006), has received much attention as a candidate gene for seasonality and SAD.

The 5-HTR2A gene is located on chromosome 13q14-q21, consists of 3 exons separated by 2 introns, and spans over 20 kb (Chen et al., 1992). It has two alternative promoters, one of which is TATA-less with four putative transcriptional start sites and a silencer element just downstream of the second promoter element (Zhu et al., 1995).

To date, most of the studies concerning the 5-HTR2A gene have investigated three single nucleotide polymorphisms (SNPs), namely −1438 A/G (rs6311), 102 T/C (rs6313) and His452Tyr (C/T 1354 or rs6314). The His452Tyr polymorphism failed to show any association either with seasonality or with SAD (Johansson et al., 2001; Serretti et al., 2007). The SNP rs6311 (−1438 A/G) is a variant close to the promoter, in the regulatory region of HTR2A, and 102 T/C is reported to be in linkage disequilibrium with the rs6311 (Spurlock et al., 1998), but the role of these SNPs in seasonality and SAD is still not clear. In a case–control study, Enoch et al. (1999) found that the −1438 A allele is associated with SAD but not with seasonality (as measured by the Seasonal Pattern Assessment Questionnaire, SPAQ). Arias et al. (2001) reported that major depression with a seasonal pattern was 7-fold more frequent in 102C-allele carriers than in 102T homozygous individuals. Levitan et al. (2002) studied adult women with childhood attention deficit hyperactivity disorder (ADHD) and adulthood SAD, and found that both childhood ADHD and the later development of SAD were associated with the 102C-allele variant. The only study which investigated a general population sample in this field is that of Lee et al. (2006) who examined a healthy Korean population and found that the Global Seasonality Scores of the SPAQ among three genotypes of the −1438 A/G polymorphism were not different. On the other hand, winter-type seasonals showed a significantly higher frequency of the −1438A allele.

Early studies used a case–control design to investigate potential associations with the 5-HTR2A gene. Enoch et al. (1999) however warned about possible false-positive findings caused by stratification of the case–control association method and suggested population genetic studies instead.

We have chosen the rs6311, the most investigated SNP in the regulatory region of 5-HTR2A gene, and the two closest tagSNPs (rs731779 and rs985934) which are located in the

second intron close to the promoter region in a population genetic study.

Therefore, the first aim of our study was to investigate the possible association between 5-HT2A receptor gene polymorphisms (rs6311, rs985934 and rs731779) and the phenotype of seasonality, which we measured by the Seasonality Pattern Assessment Questionnaire (Rosenthal et al., 1984), in a general population. The second aim was to explore the effect of these polymorphisms on SAD, which we measured by Seasonal Health Questionnaire (Thompson and Cowan, 2001). Finally, the present study aimed to apply a candidate gene approach with haplotype analysis to determine the potential involvement of 5-HTR2A haplotypes on seasonality and SAD—a method which has not been used previously.

2. Experimental procedures

2.1. Subjects

Our study involved a total of 643 unrelated volunteers, (515 women and 122 men), aged 18–60 years, with a mean age of 30.0±10.34. Socio-demographic data of the population are shown in Table 1. All subjects were Hungarian and of Caucasian origin. Participants were recruited from patient lists of general practitioners, students of higher education, and from a community-based population. The inclusion of subjects was independent of any positive psychiatric anamnesis. Each subject was given an oral and written summary of the aims and procedures of the project and gave formal written consent before entering the study. The study protocol was approved by the Central Ethics Committee in charge of genetic experimentation with human subjects. Measurements of the phenotypes were performed between September 2005 and May 2007, in Budapest, which lies at the latitude of 47.5°N.

Table 1 Depression, family history and socio-demographic data of the population.

Socio-demographic variables	*n*	%
Children under 16		
None	512	74.6
1 or 2	146	21.3
3 or more	17	2.5
Marital status		
Single	344	50.1
Married	217	31.6
Couple	70	10.2
Divorced	33	4.8
Separated	13	1.9
Widow/widower	4	0.6
Education		
No qualification	4	0.6
Technical school	50	7.3
High school	520	75.8
Degree	183	26.7
Prevalence of depression		
Anamnesis depression	142	20.7
Anamnesis bipolar	9	1.3
One year prevalence for depression	58	8.5
Psychiatric family history		
Depression in family history	101	14.7
Bipolarity in family history	15	2.2

2.2. Independent questionnaires

All volunteers completed a background questionnaire, the Seasonal Pattern Assessment Questionnaire (SPAQ) (Rosenthal et al., 1984) and the Seasonal Health Questionnaire (SHQ) (Thompson and Cowan, 2001). We used a background questionnaire to obtain detailed data from our subjects. The background questionnaire was adapted from a version developed by the Epidemiology Unit of the University of Manchester for studies with a large number of participants. This questionnaire consists of 22 items and collects detailed information about medical history including psychiatric history (depression, suicide attempt, manic/bipolar episode, anxiety, panic, phobia, obsessive–compulsive disorder, psychotic episode, schizophrenia, eating and drug or alcohol problems). Detailed information is registered concerning possible depressive episodes in the history (lifetime, 1 year prevalence, whether professional help was needed and/or medication was prescribed). Furthermore, family psychiatric history and socio-economic background, such as marital status, number of children, employment status, education and financial problems were also recorded.

The Seasonal Pattern Assessment Questionnaire (SPAQ) is a widely used instrument for investigating the history of seasonal changes. Six behaviors with strong seasonal influence (sleeping, appetite, mood, energy level, weight and social behavior) are evaluated by a 0 to 4-point scale. The six scores are summed to create the Global Seasonality Score (GSS), which ranges from 0 to 24 (Rosenthal et al., 1984). We used this GSS scale to investigate the sensitivity for seasonality in our population.

The Seasonal Health Questionnaire, designed more recently (Thompson and Cowan, 2001), has a stricter criteria system than the SPAQ. It has 6 sections with probe questions and cut-off points between each, in order to allow individuals to complete the questionnaire more quickly and easily. The sections examine the major depressive episodes (parts A and B), the number of these episodes (part C), they select the seasonal types of depression (part D), detail the seasonal pattern (part E) and finally screen for rapid cycling depressive episodes (part F). The questionnaire uses four different sets of criteria (DSM-IIIR, DSM-IV, ICD-10 and Rosenthal's criteria), and the overall scores are both sensitive and specific for diagnosing SAD. Therefore we used the SHQ to separate the SAD vs. non-SAD groups and also those with winter SAD vs. summer SAD, whereas we used the GSS (which cannot differentiate between SAD and non-SAD) to measure general sensitivity to seasonality (Thompson and Cowan, 2001).

2.3. Genotyping

Three polymorphisms of the 5-HTR2A gene were selected for genotyping using data from the International HapMap Project; we use HaploView software to select tagging SNPs in the 5′ region. The SNP rs6311 is in the promoter region of the gene, and it is wildly investigated, while rs731779 and rs985934 are haplotype tags in intron 2, close to the promoter region. Buccal mucosa samples were collected from each subject and genomic DNA was extracted according to a protocol previously described (Freeman et al., 2003). DNA quality and quantity was determined with a NanoDrop B-100 spectrophotometer, and all samples were diluted to a DNA concentration of 20 ng/μl.

The rs731779, rs985934 and rs6311 polymorphisms were genotyped at the Centre for Integrated Genomic Medical Research at The University of Manchester using the Sequenom® MassARRAY technology (Sequenom Inc., San Diego, CA, USA). The iPLEX™ assay, based on post-PCR single base primer extension, was performed according to the manufacturer's instructions. Forward, reverse and extension primers (Table 2) were designed using the Assay Design 3.0 software of Sequenom®. The iPLEX™ reaction products were dispensed onto a 384-well SpectroChip (Sequenom Inc.), processed and analyzed in a Compact Mass Spectrometer by MassARRAY Workstation 3.3 software (Sequenom Inc., San Diego, CA, USA).

Table 2 Summary of the HTR2A gene polymorphisms.

SNP	*n*	%	Position	Region	Alleles	Primer sets
rs731779	638	99.2%	13:46350039	Intron 2	G/T	f: 5-GTCATGTCATTTCACTCCCAC-3 r: 5-AAAGTAGAAGGCAGCTAGGC-3 e: 5-ATGTCATTTCACTCCCACACTTTCA-3
rs985934	631	98.1%	13:46353726	Intron 2	C/T	f: 5-GACTTCCACTTCTGGGAGATC-3 r: 5-AAACCAAAGGAGACTTGGAC-3 e: 5-CCGGTAGCATCTACCAGAATACAAA-3
rs6311	598	93.0%	13:46369479	Promoter	C/T	f: 5-TGGGCTAGAAAACAGTATGTCC-3 r: 5-TGGACACAAACACTGTTGGC-3 e: 5-GAGTGCTGTGAGTGTC-3

The number of successfully genotyped samples (*n*, %), is presented. Primer sets denote forward (f), reverse (r) and extension (e) primers used for genotyping.

2.4. Statistical analysis

HaploView 4.1 software was used for computing Hardy–Weinberg equilibrium, minor allele frequency and pair-wise linkage disequilibrium (LD) between the genotyped polymorphisms (Barrett et al., 2005; Wigginton et al., 2005). For computation of descriptive statistics, ANOVAs were calculated using SPSS 15.0 (SPSS Inc., Chicago, USA). Single marker association studies on SPAQ-GSS were performed under three common genetic models (codominant, dominant and recessive) using generalized linear models (GLM) in the SNPassoc software package of R (available at CRAN from http://cran.r-project.org). All tests were adjusted for age and gender. An α-level was considered significant if the *p*-value was less than 0.05. Results are presented as Mean ± S.D.

The effect of SNPs on SHQ scale was investigated with logistic regression with an enter method. Two types of calculations have been done: firstly the 'winter&summer-type SAD' group vs. 'non-SAD' group, and secondly the 'winter-type SAD' group vs. 'non-SAD' group. Odds ratio (OR) and 95% confidence intervals (CI) were also calculated. Both phenotype–genotype associations were performed according to three models (codominant, dominant, and recessive).

Haplotype analysis of the 5-HTR2A gene was performed in 609 individuals with at least two successfully genotyped polymorphisms to avoid the false results in haplotype estimation. We built the haplotypes by using HaploStats 1.4.0. software package of R. The individual effect of each haplotype was analyzed by performing a score test for the difference in effect between a haplotype and all the others pooled together. To assess the reliability of the results, permutation procedures with 1000 random permutations were performed to generate empirical *p*-values. Permuted *p*-values less than 0.05 were considered significant in haplotype analyses. All analyses were adjusted for age and gender. The power of the study was computed using Quanto 1.2.3. (http://hydra.usc.edu/gxe/).

3. Results

4. Descriptive statistics

In our study the mean score of GSS was 7.87 ± 4.12, with females having significantly higher point scores on GSS scale compared to males ($Mean_{male}$ = 6.46 ± 3.96; $Mean_{female}$ = 8.20 ± 4.08; F = 0.047; $p < 0.001$). Using the SHQ, the frequency of winter-type SAD was 3.27% in males and 3.49% in females, while the summer-type frequency was 0.81% and 0.97% respectively. Overall, 4.6% of the total population was affected by some form of SAD.

Each genetic marker was genotyped with a success rate over 90% (Table 2). There was no significant deviation from the Hardy–Weinberg equilibrium, and the minor allele frequencies of polymorphisms were more than 5% (Table 3). Table 3 shows the genotype and allele frequencies of each SNP. Pairwise LD D′ values are shown in Fig. 1.

5. Single marker association between Global Seasonality Score and Seasonal Health Questionnaire

First we analyzed the individual effects of each polymorphism on seasonal phenotype. Significant associations between rs731779 and GSS scores were found using both the recessive genetic model (Table 4; $Mean_{GG}$ = 9.68 ± 0.90 vs. $Mean_{GT+TT}$ = 7.87 ± 0.17; p = 0.018) and the codominant genetic model (Table 4; $Mean_{GG}$ = 9.68 ± 0.90 vs. $Mean_{GT}$ = 8.09 ± 0.31 vs. $Mean_{TT}$ = 7.77 ± 0.20; p = 0.035), with GG homozygous individuals scoring significantly higher compared to T allele carriers. On the basis of the

Table 3 Frequency of genotypes and alleles at the HTR2A polymorphisms.

SNP	Genotypes	Frequency	Percentage	Alleles	Frequency	Percentage	HWE	MAF
rs731779	T/T	432	67.70%	T	1050	82.28%	1	0.177
	G/T	186	29.15%	G	226	17.71%		
	G/G	20	3.13%					
rs985934	T/T	266	42.15%	T	815	64.58%	0.663	0.354
	C/T	283	44.84%	C	447	35.41%		
	C/C	82	12.99%					
rs6311	C/C	214	35.78%	C	706	59.03%	0.352	0.410
	C/T	278	46.48%	T	490	40.96%		
	T/T	106	17.72%					

p-value of chi-square tests for Hardy–Weinberg equilibrium (HWE) minimal allele frequency (MAF) are presented.

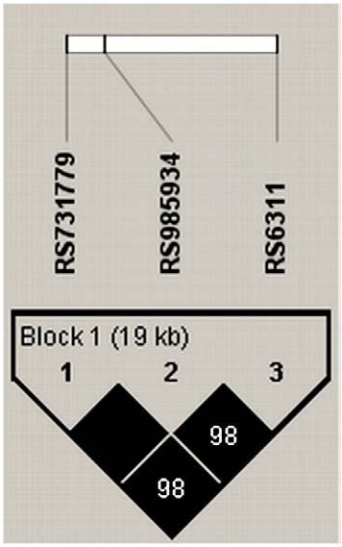

Figure 1 Linkage disequilibrium (LD) map of genotyped polymorphisms in 5-HTR2A gene.

Table 4 Single marker associations between HTR2A polymorphisms and Global Seasonality Score (GSS).

GSS				
SNP	Model and genotype	*n*	Mean ± SEM	*p*-value
rs731779	Codominant			
	T/T	406	7.77 ± 0.20	0.035*
	G/T	179	8.09 ± 0.31	
	G/G	19	9.68 ± 0.90	
	Dominant			
	T/T	406	7.77 ± 0.20	0.104
	G/T-G/G	198	8.24 ± 0.29	
	Recessive			
	T/T-G/T	585	7.87 ± 0.17	0.018*
	G/G	19	9.68 ± 0.90	
rs985934	Codominant			
	T/T	247	8.02 ± 0.27	0.871
	C/T	269	7.79 ± 0.24	
	C/C	81	7.93 ± 0.47	
	Dominant			
	T/T	247	8.02 ± 0.27	0.704
	C/T-C/C	350	7.82 ± 0.22	
	Recessive			
	T/T-C/T	516	7.90 ± 0.18	0.828
	C/C	81	7.93 ± 0.47	
rs6311	Codominant			
	C/C	205	7.91 ± 0.30	0.340
	C/T	265	7.79 ± 0.24	
	T/T	97	8.58 ± 0.41	
	Dominant			
	C/C	205	7.91 ± 0.30	0.990
	C/T-T/T	362	8.00 ± 0.21	
	Recessive			
	C/C-C/T	470	7.85 ± 0.19	0.168
	T/T	97	8.59 ± 0.42	

*$p<0.05$.
Data are adjusted by age and gender.

sample size, the power of the study was 71.6% to detect a significant effect of rs731997 interaction on GSS score under a recessive genetic model.

Significant associations were also found between that same polymorphism (rs731779) and SHQ scores, again using both the recessive and the codominant genetic models (Table 5); the chance of having winter- or summer-type depression was more than five-fold greater for GG carriers compared to GT or TT genotypes (Table 5; OR: 6.47; 95% CI: 1.94–21.57). Moreover, if subjects with summer-type SAD ($n=6$) were eliminated, the chance of having winter-type SAD increased to almost 8-fold for GG compared to GT or TT genotype (Table 5; OR = 8.67; 95% CI: 2.53–29.74). Gender and age had no significant effect on the models.

No significant associations were found between GSS scores and either of the other two SNPs (rs985934 and rs6311) (data shown in Table 4), nor between SHQ scores and either of these two SNPs (data shown in Table 5).

6. Haplotype analysis

The haplotype analysis were performed on 609 subjects, the haplotypes were constructed using data from rs731779, rs985934 and rs6311. Four haplotypes were built, each had a frequency greater than 10% (Table 6). The global *p*-value for haplotypes was 0.0104 and the permutated *p*-value of global association was 0.038. Two of the haplotypes had significant individual effects on GSS; the 'TCC' haplotype had a protective effect (hap-score = −2.389, permuted *p*-value = 0.013), whereas the 'GCC' haplotype had risk effect (hap-score = 2.049, permuted *p*-value = 0.040).

7. Discussion

Our study provides evidence for a relationship between 5-HTR2A receptor gene, SAD and seasonality in a Hungarian general population sample. This association is driven by winter-type SAD.

To our knowledge, this is the first study which investigates the epidemiology of seasonality and SAD among Hungarians. The prevalence of SAD in our study was similar to the prevalence observed in a Middle-European population (Mersch et al., 1999a; Muscettola et al., 1995; Wirz-Justice et al., 2003) and on the same latitude (Agumadu et al., 2004). A previous study found that women had about 1.5 times higher prevalence than men for SAD (Chotai et al., 2004), but we found no difference between the genders although this might be explained by the small number of men in our study. Using the GSS scale, we found that women scored significantly higher than men, a result which has been found in previous studies also (Chotai et al., 2004; Muscettola et al., 1995).

Enoch et al. (1999) found a significant association between the A allele of rs6311 and SAD but not with seasonality, however Lee et al. (2006) found association with only winter-type seasonality. In our study rs6311 was associated neither with SPAQ, nor with SHQ scales. The possible functional role of rs6311 is not clear, but the discordance could be explained by Myers et al. (2007) who found no significant difference in the promoter activity between the A and G alleles investigated alone, or in conjunction with the major allele

Table 5 Odds ratios for seasonal affective disorder (SAD; winter- and summer-type seasonal depression, as assessed by the Seasonal Health Questionnaire) according to genotypes of the three 5-HTR2A gene polymorphisms. Data are adjusted for age and gender.

SNP	Model and genotype	Winter- and summer-type SAD								Winter-type SAD							
		No SAD		SAD		OR	CI 95%		*p*-value	No SAD		SAD		OR	CI 95%		*p*-value
		n	%	*n*	%		Lower	Upper		*n*	%	*n*	%		Lower	Upper	
rs731779	Codominant																
	T/T	385	67.5%	18	64.3%	1.00				385	67.5%	12	54.5%	1.00			
	G/T	171	30.0%	6	21.4%	0.79	0.31	2.05	**0.028** *	171	30.0%	6	27.3%	1.2	0.44	3.30	**0.013** *
	G/G	14	2.5%	4	14.3%	6.06	1.78	20.72		14	2.5%	4	18.2%	9.2	2.56	33.13	
	Dominant																
	T/T	385	67.5%	18	64.3%	1.00			0.639	385	67.5%	12	54.4%	1.00			0.172
	G/T-G/G	185	32.5%	10	35.7%	1.21	0.54	2.70		185	32.5%	10	45.5%	1.9	0.78	4.40	
	Recessive																
	T/T-G/T	556	97.5%	24	85.7%	1.00			**0.008** **	556	97.5%	18	81.8%	1.00			**0.003** **
	G/G	14	2.5%	4	14.3%	6.47	1.94	21.57		14	2.5%	4	18.2%	8.7	2.53	29.74	
rs985934	Codominant																
	T/T	235	41.7%	11	40.7%	1.00			0.410	235	41.7%	7	33.3%	1.00			
	C/T	255	45.2%	10	37.0%	0.86	0.36	2.08		255	45.2%	8	38.1%	1.1	0.39	3.11	0.175
	C/C	74	13.1%	6	22.2%	1.8	0.64	5.07		74	13.1%	6	28.6%	2.88	0.93	8.94	
	Dominant																
	T/T	235	41.7%	11	40.7%	1.00			0.863	235	41.7%	7	33.3%	1.00			0.387
	C/T-C/C	329	58.3%	16	59.3%	1.07	0.49	2.37		329	58.3%	14	66.7%	1.50	0.59	3.80	
	Recessive																
	T/T-C/T	490	86.9%	21	77.8%	1.00			0.195	490	86.9%	15	71.4%	1.00			0.063
	C/C	74	13.1%	6	22.2%	1.93	0.75	4.98		74	13.1%	6	28.6%	2.7	1.02	7.34	
rs6311	Codominant																
	C/C	193	35.9%	11	45.8%	1.00			0.628	193	35.9%	9	45.0%	1.00			
	C/T	251	46.7%	9	37.5%	0.65	0.26	1.60		251	46.7%	8	40.0%	0.7	0.27	1.88	0.736
	T/T	93	17.3%	4	16.7%	0.72	0.22	2.36		93	17.3%	3	15.0%	0.7	0.17	2.56	
	Dominant																
	C/C	193	35.9%	11	45.8%	1.00			0.343	193	35.9%	9	45.0%	1.00			0.437
	C/T-T/T	344	64.1%	13	54.2%	0.67	0.29	1.53		344	64.1%	11	55.0%	0.70	0.28	1.72	
	Recessive																
	C/C-C/T	444	83.7%	20	83.3%	1.00			0.851	444	82.7%	17	85.0%	1.00			0.72
	T/T	93	17.3%	4	16.7%	0.90	0.30	2.73		93	17.3%	3	15.0%	0.80	0.23	2.82	

* $p<0.05$.
** $p<0.01$.

Table 6 Analyses of HTR2A haplotypes in condition of Global Seasonality Score (GSS).

n	Global statistic	*df*	Global permuted *p*-value
609	10.019	4	0.038 *
Haplotypes	**Frequency (%)**	**Hap-score**	**Permuted *p*-value**
TCC	18.2%	−2.389	0.013 *
TTC	21.1%	−0.622	0.544
TTT	40.6%	0.716	0.481
GCC	17.9%	2.049	0.040 *

Haplotypes show alleles of rs731779 T/G, rs985934 C/T and rs3611 C/T. Frequencies of haplotypes, coefficients of GSS (hap-score), permutated *p*-value of the global and the individual effect of haplotypes are represented. Data are adjusted by age and gender.
* $p<0.05$.

(A) of the −783A/G polymorphism, but when the minor allele G at −783 was expressed with the major allele G at rs6311, the promoter activity was significantly decreased.

We demonstrated that subjects with the GG genotype of rs731779 are five-fold more likely to be affected by SAD, the extreme end of seasonality, compared to subjects with the GT or TT genotype. Moreover our findings suggest that this association is with winter-type rather than summer-type SAD, although the number of summer-type SAD is very low ($n=6$) in our study, and any statistical analysis for this group must be considered with caution. However, our low number is in good agreement with another community-based epidemiological study, showing that the lifetime prevalence of summer SAD is much lower than that of winter SAD (Mersch et al., 1999a), although studies at this latitude are difficult because the prevalence is less than 1% (Mersch et al., 1999b). Summer-type SAD has opposite symptomatology to winter-type (Wehr and Rosenthal, 1989), and it is likely that in the development of summer-type SAD other serotonin and/or monoamine receptors play a significant role. However, we found that both seasonality and SAD are associated with the same receptor

polymorphism and this adds further support to the hypothesis that sensitivity to seasonality and SAD have a shared biological basis but differ in the severity of the symptoms.

Our findings show that people who carry the GG genotype of rs731779 have an increased vulnerability to seasonality compared to GT or TT genotypes. However, we could not replicate the previously described association between seasonality and rs6311 (−1438 A/G) (Lee et al., 2006), nor could we find an association between the rs985934 polymorphism and seasonality. The haplotype analysis showed a significant global effect of haplotypes on GSS scale; two haplotypes—GCC and TCC—had significant individual effects, the difference being driven by the rs731779 T/G polymorphism as discussed above.

SSRIs have been the main pharmacotherapy for depression, including the winter-type SAD (Lam et al., 2006; Moscovitch et al., 2004; Pae et al., 2008), for many years. Earlier studies indicate that 5-HT2A receptors may play a role in mediating SSRI effects (Maj et al., 1996; Yamauchi et al., 2006). Our results suggest that the 5-HTR2A gene could play a significant role in SAD or seasonality which raises the possibility that 5-HT2A antagonists may have a role in the therapy of SAD, too. Further research is required to better understand the pathomechanism of SAD and the genetic component of non-responders using genetic studies in relation to treatment outcome. However, the 5-HTR2A gene alone cannot be responsible for the development of seasonality and SAD. A recent study of 88 drug-naïve healthy individuals showed that the activity of brain serotonin transporter exhibits a significant seasonal variation (higher in autumn and winter) as the reflection of lower synaptic level of serotonin during these two seasons (Praschak-Rieder et al., 2008). This recently described physiologic mechanism is in good agreement with our present knowledge on SAD in general (Goodwin and Jamison, 2007; Wehr and Rosenthal, 1989) and on low brain serotonin function in winter SAD in particular (Carlsson et al., 1980; Goodwin and Jamison, 2007; Wehr and Rosenthal, 1989), suggesting that the development of SAD in vulnerable individuals is the consequence of the interaction of at least two genetically mediated and one or more exogenous (photoperiod) factors.

The strength of our study is that we investigated this phenomenon with a continuum approach, not in a case–control study design, to avoid false-positive findings by stratification. In addition, this is the first report of genetic association with seasonality in a Hungarian population. A potential limitation of our study was that we used only self-rating scales to measure SAD, without any clinical inventory. Another important limitation of our study is that the questionnaires we used have not been validated in Hungarian.

In conclusion, our data confirm that the 5-HTR2A receptor gene plays a significant role in the phenomenon of seasonality in general, and in the development of winter-type SAD in particular. Our findings support the relevance for further molecular biological, genetic and pharmacological investigations in this field.

Role of the funding source

This study was supported by the Sixth Framework Program of the EU, LSHM-CT-2004-503474, TAMOP-4.2.1.B-09/1/KMR-2010-0001, ETT 318/041/2009 and ETT 318-0412009 and the PhD Fellowship Program of Semmelweis University, Ministry of Culture and Education, Hungary.

Contributors

Professor Bagdy, Kovacs, Gonda, Molnar designed the study. Professor Kurimay and Kovacs selected the subjects. Authors Molnar, Lazary, Benko and Pap recruited the subjects and analyzed data. Authors Mekli and Juhasz managed the DNA analyses. Molnar undertook the statistical analysis. Professor Rihmer and Bagdy managed the literature searches and corrected the manuscript. All authors contributed to and have approved the final manuscript.

Conflict of interest

All authors reported no biomedical financial interests or potential conflicts of interest.

Acknowledgements

Special thanks to Hazel Platt for contributing in genotyping procedures, and to Dr. Diana Chase for language correction of the manuscript.

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