Prevalence, characteristics and associations of serotonin-2A and cannabinoid-1 receptor genes with seasonality and seasonal affective disorder

Ph.D. theses

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1. Introduction

Seasonal affective disorder (SAD) is a clinical subtype of recurrent major depression, with episodes of depression tending to recur at specific times of the year. Two opposite patterns have been described, the fall-winter and the spring-summer type depression. The degree of seasonal changes in mood, behavior and pychovegetative symptoms (changes in sleep, weight, eating, energy level, social activity) is termed seasonality while SAD is usually considered to be at the extreme end of the spectrum of seasonality. Validated screening tools and epidemiological studies are not available in a Hungarian general population.

In the genetic background of the development of seasonality and seasonal affective disorder the serotonin transporter gene (SLC6A4) polymorphism 5HTTLPR S (short) allele, located in the promoter region of the gene, is the most investigated variant, however the role of the S allele in the development of seasonality and SAD has not been demonstrated clearly. Further studies also investigated rs6311 (-1439 A/G), a single nucleotide polymorphism (SNP) in the promoter region of the serotonin-2A receptor gene (5HTR2A). The A allele of rs6311 proved to be the more active variant, was more frequent among seasonality and/or SAD groups, these results however, were not supported by other studies. The role of the cannabinoid-1 receptor gene (CNR1) was not investigated in the development of SAD and seasonalitys far but its contribution is hypothesized in vulnerability for non-seasonal depression and anxiety.

2. Objectives

The main objectives of our research were to measure the prevalence of seasonality and seasonal affective disorder in the Hungarian general population (1st study). Then we examined the role of specific gene polymorphisms (2nd study), including the serotonin transporter gene, serotonin-2A receptor gene and cannabinoid-1 receptor gene.

Our objectives in the above studies were:

1st study

- What is the validity and reliability of the Seasonal Pattern Assessment Questionnaire (SPAQ) and the Seasonal Health Questionnaire (SHQ) in Hungarian general population?
- What are the prevalence rates of SAD and seasonality in the Hungarian general population?
- What is the relationship between gender, age, seasonality and SAD?
- How can the pattern and the average length of a SAD depressive episode be characterized?
- Which psychiatric disorders are comorbid with seasonality and SAD?
- Which familial psychiatric disorders are comorbid with seasonality and SAD?

- What is the predictive power of the independent variables of personality, such as affective temperaments and personality traits in the development of seasonality and SAD?

2nd study:

- Is there an association between serotonin transporter gene variants (5HTTLPR), seasonality and SAD (single marker association studies)?
- Is there an association between the polymorphisms of the serotonin-2A receptor gene, seasonality and SAD (single marker association and haplotype analyzes)?
- Is there an association between the polymorphisms of the cannabinoid-1 receptor gene, seasonality and SAD (single marker association and haplotype analyzes)?
- Do the polymorphisms of 5HTTLPR, HTR2A and CNR1 receptor genes show any interaction with seasonality and SAD (GxG model)?

3. Materials and Methods

Subjects

Our study involved volunteers aged between 18-60 years, Hungarian and of Caucasian origin. Measurements of phenotypes were performed between September 2005 and May 2007.

923 subject participated (641 women, 282 men, mean age 31.27±10.65) in the first study. Not all volunteers gave genetic sample thus the examined group size depended on the number of available genetic samples and the success rates of genotyping. The sample sizes in our analyzes were 640-700 subjects.

Instruments

The first part of the Seasonal Pattern Assessment Questionnaire (SPAQ) consists of six items measuring the seasonal changes of symptoms in a four-point scale. The six scores are summed to create the Global Seasonality Score (GSS). The second part of the scale investigates the severity of symptoms.

The Seasonal Health Questionnaire (SHQ) is a screening test with 6 parts and cut-off points and designed to filter out subjects with seasonal affective disorder. The A and B sections examine the major depressive episodes, 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 affective episodes (part F).

All volunteers filled out the Background questionnaire, the Zung Self-rating Depression Scale (ZSDS), the Spielberger State Trait Anxiety Inventory (STAI-S & STAI-T), the Brief Symptom Inventory (BFI), the TEMPS-A (Temperament Evaluation of the Memphis, Pisa, Paris and San Diego-Autoquestionnaire) and the Big Five Inventory-44 (BFI-44).

Genotyping

Buccal mucosa samples were collected and the extraction and genotyping process was carried out in our partner laboratory at the University of Manchester. Genomic DNA was extracted using conventional phenol-chloroform extraction protocol. For genotyping the 5HTTLPR, the genomic region containing the polymorphism was amplified using PCR and Taqman method. The single nucleotide polymorphisms (SNPs) (in case of HTR2A gene: rs731779, rs985934, rs6311; in case of CNR1 gene: rs2180619, rs806379, rs1535255, rs2023239, rs806369, rs1049353, rs4707436, rs12720071, rs806368, rs806366, rs7766029) were genotyped with Sequenom® MassARRAY technology. After the iPLEX^{TM} reaction, based on post-PCR single base primer extension, 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, USA).

Statistical Analyzes

After the linguistic adaptation of SHQ and SPAQ, tests of homogeneity (Cronbach-α) were performed and item validity, item-total correlations were analyzed. GSS distribution, Kurtosis and skewness of the GSS scale were investigated. Convergence validity of SPAQ and SHQ was measured by Pearson's correlations using ZSDS, BFI and STAI. We used chi-square tests, Fisher exact tests, linear regression models, two sample t-tests and

ANOVA to analyze the effect of age, gender, comorbid psychiatric illnesses and family history of psychiatric illnesses on seasonality and SAD. In case of the SPAQ, linear regression models with stepwise method were used to calculate the predictive power of independent personality variables. In case of SHQ, binary logistic regression with stepwise method was applied. Statistica 8.0 (StatSoft Inc., Tulsa, USA) and SPSS 20 (IBM SPSS Inc., Chicago, USA) programs were used for statistical analyses.

Hardy-Weinberg equilibrium and linkage disequilibria were computed using Haploview 4.1 software. Allele and genotype frequencies and single marker associations with the phenotypes were performed by SNPassoc software package for R (available at CRAN from http://cran.r-project.org). The SPAQ-GSS was used as a continuous variable and the SHQ was used as a dichotomous variable (existence of SAD – SHQ1, or not – SHQ0) in our models and they were adjusted for age and gender in all analyzes. Allele and genotype frequencies and single marker association studies on SPAQ were performed using generalized linear models (GLM), the effect of SNPs on SHQ scale was investigated with logistic regression with an enter method in the SNPassoc software package of R. Score tests of HaploStat package of R were used for haplotype analysis of 5HTR2A and CNR1 genes. Gene-gene interactions were tested with loglikelihood ratio tests (LTR) using the SNPassoc R package software, and GLM analyzes were performed to validate our results by SPSS software.

4. Results

1st study

Internal consistency of the GSS scale, the subscale of SPAQ, is appropriate (Cronbach-α=0.830, item-total correlations r>0.5). The distribution of GSS is approximately normal (skewness=0.216±0.92 SD; Kurtosis= 0.527±0.185 SD). Prevalence of seasonality measured by SPAQ indicates that 71.83% of the population has no seasonality, 22.75% has a moderate form and 5.42% has a severe form of seasonality. Mean score of the population on GSS was 7.87±4.12, and significant gender differences were detected ($Mean_{men}$=6.46±3.96; $Mean_{women}$=8.20±4.08; F=0.047; p<0.001). A mild negative correlation was found between GSS and age (r= -0.140; p<0.01), while in case of the whole SPAQ a slightly positive correlation was measured (r= 0.103; p<0.01).

Cronbach-α value of A and B part of the SHQ was 0.911 and item-total correlations were r>0.45. Prevalence of the fall-winter type depression was 3.36% and prevalence of spring-summer type depression was 1.73% as measured by SHQ. Average length of a seasonal depressive episode was 4.28±2.48 months. In case of fall-winter type depression episodes have biphasic pattern, characteristically with a mild remission in December then worsening in January. Women are affected by SAD three times as frequently as men (74.47% vs. 25.53%), and symptoms worsen with age. Convergence validity showed poor to medium correlation with ZSDS, STAI and BSI scales (SPAQ: r=0.265-0.405, SHQ: r=0.400-0.550).

Populations screened with either SPAQ and SHQ have shown comorbidity with depression, suicide, anxiety and eating disorders, while bipolar disorder was only comorbid with moderate and severe seasonality. Positive family history of depression and eating disorders in the SHQ1 group and moderate and severe SPAQ groups were significantly higher. Additionally, suicide attempts and anxiety disorders in the SPAQ groups were also significantly higher.

Cyclothymic and anxious affective temperaments, STAI-T and neuroticism had a significant predictive effect on GSS scores ($AdjR^2$=0.163; $F_{4;918}$ = 45.974, p<0.001). States of depression (Wald=15.57, OR=5.520, p<0.001), Cyclothymic temperament (Wald=4.02, OR=1.08; p=0.045), age (Wald=14.12, OR=1.053, p<0.001) and GSS scale (Wald=12.07, OR=1.146, p=0.001) had a significant predictive effect on SHQ1 (Nagelkerke R^2=0.272).

2nd study

The 5HTTLPR polymorphism of the serotonin transporter gene failed to show any significant associations either with GSS or with SHQ in association and gene-gene interaction analyzes.

The rs731779 GG homozygous genotype of serotonin-2A receptor gene was associated with high GSS scores under a recessive genetic model ($Mean_{TT-GT}$= 7.87±0.17 vs. $Mean_{GG}$=9.68; p=0.0184). In case of SHQ the GG homozygous genotype carriers' chance of having SAD was six-fold greater compared to GT or TT carriers (OR=6.47; 95% CI=1.94-21-57).

Moreover, if subjects with summer-type SAD were eliminated, the chance of having winter-type SAD increased to 8-fold for GG compared to GT or TT genotype (OR= 8.67; 95% CI= 2.53-29.73). Neither rs985934, nor rs6311 showed any individual association with SPAQ or SHQ.

Haplotype analyzes of the 5HTR2A gene (rs7317779, rs985934 and rs6311) resulted in two significant haplotypes, the susceptible 'GCC' (hap-score= 2.049, p=0.04) and the protective 'TCC' (hap-score= -2.389, p=0.013). Besides the 'CC' alleles, carrying the G allele of rs731779 resulted in higher, while presence of T allele resulted in lower GSS points.

The polymorphisms of CNR1 gene failed to show any significant association or haplotypes either with GSS, or with SHQ.

Gene-gene interaction analyzes on seasonality phenotype were tested between the SNPs of 5HTR2A and CNR1 genes. Among the TT genotype carriers of the rs6311 SNP of 5HTR2A, significantly higher GSS scores were found in the presence of rs7766029 TT genotype ($F_{2;126}$=7.898; p<0.01); rs4707436 AA genotype ($F_{2;126}$=7.002; p=0.001); rs1049353 AA genotype ($F_{2;126}$=7.039; p=0.001) and rs806369 CC genotype ($F_{2;126}$=7.322; p<0.001) of the CNR1 gene, respectively.

Haplotype analyzes of the CNR1 gene polymorphisms in the group of rs6311 TT carriers (N=129) resulted in significant haplotypes on the GSS scale. We analyzed the first haploblock of the CNR1 gene constructed by 7 SNPs (in order: rs806369, rs1049353, rs4707436, rs12720071, rs806368, rs806366 and rs7766029). Two significant haplotypes were identified, the 'TGGATCT' which was associated with low GSS scores

(hap-score= -2.578, p=0.01) and the 'CAAATTC' which implied high seasonality (hap-score=3.579, p<0.001). The second haploblock consisted of the SNPs from conventional and alternative promoter regions (in order: rs2180619, rs806379, rs1535255 and rs2023239). We found that the 'GTGC' haplotype was associated with high seasonality (hap-score=3.089, p=0.003).

5. Conclusions

The most important results were:

1. Psychometric properties of both SPAQ and SHQ were proven to be appropriate in Hungarian samples. Both of them are valid and reliable tools for measuring seasonality and seasonal affective disorder.
2. Lifetime prevalence of seasonal affective disorder in the Hungarian general population corresponds with measurements from the same latitude (approximately 4-6% of the population are affected); except for the spring-summer type depression rates (1.73%), as it reflects data of the Mediterranean region (~2%).
3. Although the two instruments yield similar screening results, they differentiate based on different theoretical concepts. The SHQ is based on the mood component of depression, while psycho-vegetative symptoms of depressive states are the base of the SPAQ.

4. Women are more affected and show a more severe fluctuation of symptoms than men. Severity of symptoms decreases by age, however, their subjective relevance increases.
5. Among Hungarians October-November and January are the months most affected by depression, while in December a tendency of remission was found, which resulted in a biphasic pattern of the fall-winter type depression.
6. Both seasonality and SAD were proven to be comorbid with depression, bipolar disorders, suicide, anxiety disorders and eating disorders in our population. These results are in a good agreement with data form literature.
7. In our study, frequency of depression, suicide, anxiety disorders and eating disorders were significantly higher in the family history of subjects suffering from the severity form of seasonality. In the family history of SAD group, higher frequency of depression and eating disorders was observed.
8. Both in case of seasonality and seasonal affective disorder a robust tendency for affective lability was found. In case of seasonality it was manifested by presence of cyclothymic temperament, anxiety, neuroticism and physical-vegetative symptoms of depression. Our measurements validate seasonality as an independent construct.
9. The serotonin transporter gene polymorphism 5HTTLPR is not directly involved in the development of seasonality and SAD phenotypes.

10. Presence of serotonin-2A receptor gene SNP rs731779 GG homozygous genotype conveys susceptibility to seasonality and SAD. The association was more robust if only winter-type depression was investigated. These findings further strengthen the fact that summer and winter type depression has different biological backgrounds.
11. Investigating the 5HTR2A gene polymorphisms susceptible and protective haplotypes for seasonality were found. The fact that these polymorphisms showed an association not only with clinical SAD but also seasonality symptoms in a general population provided evidence for the spectrum nature of this relationship.
12. The cannabinoid-1 receptor gene polymorphisms failed to show any direct significant relationship either with seasonality or with SAD in association or haplotype studies.
13. Gene-gene interaction analyzes between 5HTTLPR and CNR1 gene allele variants on seasonality scores yielded negative results.
14. The 5HTR2A and CNR1 genes interactions affected the phenotype of seasonality. We suggest that these genetic variants might be manifest in the number and/or in the structure of receptor proteins of the 5-HT2A and CB1 receptors that may influence synaptic serotonin transmission. The investigated genotypes and haplotypes may play a significant role in the background of development of vulnerability for seasonality. These findings provide evidence for

the biological/genetic determination of seasonality and seasonal affective disorders.

6. Publications

Publications relevant to the dissertation

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3. Gonda X, Fountoulakis K N, Csukly G, Bagdy G, Pap D, Molnár E, Laszik A, Lazary J, Sarosi A, Faludi G, Sasvari-Szekely M, Szekely A, Rihmer Z
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JOURNAL OF AFFECTIVE DISORDERS 132:(3) pp. 432-437. (2011)
IF: 3.517

4. Benko A, Lazary J, Molnar E, Gonda X, Tothfalusi L, Pap D, Mirnics Z, Kurimay T, Chase D, Juhasz G, Anderson IM, Deakin JFW, Bagdy G **Significant Association Between the C(-1019)G Functional Polymorphism of the HTR1A Gene and Impulsivity** AMERICAN JOURNAL OF MEDICAL GENETICS PART B-NEUROPSYCHIATRIC GENETICS 153B:(2) pp. 592-599. (2010) IF: 4.156