

# Association between the C(-1019)G functional polymorphism of the HTR1A gene and impulsivity and subthreshold depression

Ph.D. Theses

ANITA BENKŐ

Semmelweis University, Ph.D. School of Mental Health Sciences



**Supervisor:**

György Bagdy, Ph.D., D.sc.  
László Tóthfalusi, Ph.D.

**Director of Comprehensive exam:** Mária Kopp, Ph.D., D.sc.

**Members of Comprehensive exam:** Zsuzsanna Mirnics, Ph.D.  
György Purebl, Ph.D.

**Official reviewers:**

Róbert Bódizs, Ph.D.  
Viktor Vörös, Ph.D.

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## 1. INTRODUCTION

Serotonin-1A (5-HT<sub>1A</sub>) receptors are known to play a role in impulsivity-related behaviour and depression as well. The C(-1019)G functional polymorphism (rs6295) has been suggested to regulate the 5-HT<sub>1A</sub> receptor gene (HTR1A) expression in presynaptic raphe neurons. In a series of experiments, it was shown that the part of the HTR1A gene promoter that contains SNP rs6295 binds to the transcription factor DEAF-1/suppressin (NUDR), that this factor represses transcription of HTR1A and that this inhibitory action was impaired for the G-allele. The implication is that failed inhibition by NUDR at the G-allele leads to higher expression of HTR1A. This could enhance the negative feedback inhibition of serotonergic raphe neurons exerted by HTR1A autoreceptors and lead to a lower serotonergic neurotransmission.

Previous studies indicate that this polymorphism is associated with aggression, suicide, and several psychiatric disorders, yet its association with impulsivity has rarely been investigated. Furthermore, studies reported that dysfunction of the serotonin 1A receptor may play a role in the background of depression but its association with subclinical forms of depression has not been investigated yet. Some authors suggested a direct relationship between depression and impulsivity. It was noted that there are phenotypic associations occurring in childhood and adolescence between constructs relating to impulsivity and depression and impulsivity shares similar biological background, both have been shown to be related to decreased serotonin levels. Despite these associations, it remains unclear whether impulsivity is a predictor of the onset of depression in adulthood. Prior research on this issue has typically focused on the relationship between impulsivity and suicide in clinical populations or in children or adolescents with attention deficit hyperactivity disorder (ADHD) or conduct disorders. There have been only a few studies addressing the question of associations between impulsivity and depressive symptoms in subclinical depressive or non-clinical populations.

Subthreshold depression (SSD) patients have a high risk of developing major depression, more than 25% of the subthreshold patients develop major depression over a period of 2 years. The risk of suicide is significantly higher in patients with SSD compared to normal subjects, but lower than in major depression or minor depression. Subthreshold depression patients have a relatively high risk of developing major depression, thus recognizing

subthreshold depressive symptoms and to investigate its psychological and biochemical background is also important in the prevention of major depression.

## **2. OBJECTIVES**

The aim of the present study was to test the hypothesis of the involvement of the C(-1019)G polymorphism in impulsivity related behaviour and subthreshold depression. Furthermore, we aimed to investigate the relationship between impulsivity and subthreshold depression and the effect of genotype on the relationship between impulsivity and subthreshold depression. The aims of our study were:

- Previous studies indicate that the C(-1019)G functional polymorphism of the HTR1A gene is associated with aggression, suicide, depression and several psychiatric disorders. Is there any relationship between the C(-1019)G functional polymorphism and impulsivity?
- Since it is well known that the C(-1019)G polymorphism is related to major depression, is subthreshold depression also associated with the C(-1019)G functional polymorphism?
- Impulsivity is known to be associated with depression but the nature of this relationship is not elucidated. Is there a relationship also between impulsivity and subthreshold depression and which facets of impulsivity play a role this relationship?
- Does genotype (subjects with the GG genotype compared to subjects with the GC or CC genotype) affect the relationship between impulsivity and subthreshold depression?

### **3. MATERIALS AND METHODS**

#### **3.1. Subjects**

Eight hundred fifty-one unrelated Hungarian volunteers were recruited for the study. Subjects whose DNA sample was not successfully genotyped and subjects with missing questionnaire data were excluded from all statistical tests. Finally 725 subjects remained, 596 women and 129 men. The participants were aged 18–60 years, the mean age was  $30.26 \pm 10.601$  years. Participants were recruited from general practices, universities, and 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. All subjects were Hungarian and of Caucasian origin. The study protocol was approved by the Central Ethics Committee in charge of genetic studies with human subjects.

#### **3.2. Procedures and Measures**

##### ***3.2.1. Background information***

Background information was obtained from all participants. The background questionnaire was adapted from the version developed by the Epidemiology Unit at the University of Manchester. The self-rating questionnaire consisted of 22 items and collected detailed information about socioeconomic background, and medical history including personal and family psychiatric history.

##### ***3.2.2. IVE-I and BIS-11***

The Eysenck IVE scale consists of 54 true/false items and contains 3 unidimensional subscales: Impulsiveness, Venturesomeness, and an Empathy scale. In our study we only used the Impulsiveness subscale (IVE-I), which contains 19 items (e.g., “Do you often do things on the spur of the moment?”). The total score is the sum of the points; a high score indicates a high level of impulsivity.

The most recent version of the Barratt Impulsiveness Scale (BIS-11) consists of 30 items asking about impulsivity-related behaviours and cognitions. According to Barratt's most recent proposal that there are three subtraits (measured by three subscales) which are Motor Impulsiveness (e.g., "I do things without thinking," 11 items), Cognitive Impulsiveness (e.g., "I don't pay attention," 8 items), and Nonplanning Impulsiveness (e.g., "I plan tasks carefully," inverted item, 11 items). The BIS total score is the sum of the three scales. Each item is measured on a 4-point scale, 4 indicates the most impulsive response. The total score is the sum of the points.

### ***3.2.3. Zung Self-Rating Depression Scale***

Subjects completed the standardised Hungarian version of the Zung Self-Rating Depression Scale. The Zung Self-Rating Depression Scale is a 20-item short self-administered test and its items cover affective, psychological and somatic features of depression. Each question is scored on a scale of 1 through 4 ("a little of the time," "some of the time," "good part of the time," "most of the time"). Non-depressed individuals typically score less than 40, while 40 to 80 cover various grades of depressive symptomatology. Between 40-47 points individuals show mild depressive symptoms and above 48 points moderate and severe depressive symptoms can be observed. The main reason we have chosen the Zung Self-Rating Depression Scale (ZSDS) was that it contains more items relating to the vegetative and physical aspects of depression than other self-rating depression scales.

### **3.3. Genotyping**

Buccal mucosa samples were collected from each subject, and genomic DNA was extracted according to a protocol previously described. DNA quality and quantity were determined with NanoDrop B-100 spectrophotometer, and all samples were diluted to a DNA concentration of 20 ng/ml. The SNP rs6295 was genotyped at the Centre for Integrated Genomic Medical Research at the University of Manchester using Sequenom MassARRAY technology (Sequenom, San Diego, CA). The iPLEX assay, based on post-PCR single base

primer extension, was performed according to manufacturer's instructions. Forward, reverse and extension primers were designed using the Assay Design 3.0 software of Sequenom. The iPLEX reaction products were dispensed onto a 384-well SpectroChip (Sequenom), processed and analyzed in a Compact Mass Spectrometer by MassARRAY Workstation 3.3 software (Sequenom).

### **3.4. Statistical analysis**

#### **3.4.1 Genetic association analysis**

Association tests were performed by means of analysis of variance (ANOVA) with the rs6295 SNP (GG vs. GC vs. CC) as an independent variable and IVE-I, BIS-11 and the ZSDS scales entered as dependent variables. Tukey HSD post-hoc test was used for multiple comparisons. Age and gender were included in the ANOVA model as covariates. Statistical analyses were carried out using SPSS 15.0 (SPSS Inc., Chicago, IL).  $P < 0.05$  was accepted as level of significance. For power calculations we used G\*Power 3. Statistical Analysis Haploview 4.0 software was used for computing Hardy–Weinberg equilibrium and minimal allele frequency.

#### **3.4.2 Structural Equation Modeling**

The latent structural relationship between impulsivity and subthreshold depression was analysed by structural equation modelling (SEM). In the first step we analysed the relationship between the two latent variables, impulsivity and depression. In the next step we divided the sample according to genotype (GG, GC and CC) and analysed the model separately. We used AMOS (ver 18, SPSS Inc.) for SEM analysis.

## 4. RESULTS

There was no significant deviation from the Hardy–Weinberg equilibrium ( $P=0.655$ ), and minimal allele frequency of rs6295 polymorphism was more than 5%. Frequencies of the GG, GC, and the CC genotypes were 27% ( $n=193$ ), 51% ( $n=368$ ), and 22% ( $n=163$ ), respectively. Frequencies of the G and C alleles were 52% ( $n=754$ ) and 48% ( $n=694$ ), respectively.

### 4.1. The association of IVE-I and BIS-11 Scales with genotypes

Using the IVE-I scale, there were significant differences ( $F=4.302$ ,  $df=2$ ,  $p=0.014$  observed power=0.97) in the test scores between the three genotype groups. Test results indicate that subjects with the GG genotype show significantly higher impulsivity ( $6,47\pm 0,291$ ), and with no over-lap at 95% confidence, compared to CC subjects ( $5,228\pm 0,318$ ); the scores for the GC heterozygotes ( $5,75\pm 0,210$ ) lying approximately midway.

A very similar pattern with significant difference ( $F=4.862$ ,  $df=2$ ,  $p=0.008$ , observed power=0.98) was observed using the BIS total scores; subjects with the GG genotype show significantly higher impulsivity ( $59,178\pm 0,698$ ), and with no overlap at 95% confidence, compared to CC subjects ( $56,112\pm 0,759$ ); the scores for the GC heterozygotes lying approximately midway ( $57,093\pm 0,505$ ). On the Motor Impulsiveness subscale of BIS-11, the GC subjects ( $19,40\pm 0,171$ ) behaved like CC individuals ( $19,34\pm 0,258$ ), with a significant difference ( $F=3.886$ ,  $df=2$ ,  $p=0.021$ , observed power=0.88) between the GC and the GG ( $20,15\pm 0,236$ ) genotypes; whereas on the Cognitive Impulsiveness subscale, GC subjects ( $15,97\pm 0,148$ ) behaved more like GG individuals ( $16,31\pm 0,204$ ), with significant differences ( $F=6.337$ ,  $df=2$ ,  $p=0.002$ , observed power=0.99) between each of these two groups and the CC homozygotes ( $15,25\pm 0,223$ ); however, there was no significant difference ( $F=0.654$ ,  $df=2$ ,  $p=0.520$ , observed power=0.29) between the different genotype groups in the Nonplanning Impulsiveness subscale (GG:  $22,70\pm 0,313$ ; GC:  $22,34\pm 0,228$ ; CC:  $22,21\pm 0,343$ ).

Although age and gender were included in the ANOVA model as covariates, separate analyses of males and females were also performed. These analyses revealed that on the IVE-I

scale, genotype had significant effect in both groups (males:  $P=0.017$ , females:  $P=0.005$ ). On the Motor and the Cognitive Impulsiveness subscales of the BIS-11, the effect of genotype remained significant only among females ( $P=0.016$  and  $P=0.006$ ) but not among males ( $P=0.09$  and  $P=0.23$ ). Because the ratio of females to males was higher than 4 in our study group, we attribute the lack of significance in males due to the smaller sample size in the latter case. Indeed, the general patterns were the same for both groups and subjects carrying GG genotypes had the highest impulsivity scores in all comparisons.

#### **4.2. The association of Zung Self-rating Depression Scale with genotype**

686 subjects (123 male and 570 female) who had Zung Self-Rating Depression Scale scores below the level indicating depression (48 points) remained in the statistical analysis. 258 (37%) subjects scores between 40-48 points indicating subthreshold depressive symptoms. We found no significant difference ( $F=2.370$ ,  $df=2$ ,  $p=0.094$ ), between the subjects carrying the GG ( $38,83\pm 0,342$ ), GC ( $37,95\pm 0,249$ ) and CC ( $38,54\pm 0,379$ ) genotype and ZSDS total scores.

#### **4.3 The association between impulsivity and subthreshold depression and the effect of genotype on the relationship between impulsivity and subthreshold depression**

Depression and impulsivity were treated as latent variables and the relationship between these latent variables was analyzed by setting up a structural equation model. The observed variables were subscales of the Zung scores below 48 points and the subscales of BIS 11.

##### **4.3.1 Model for the whole study cohort: association between the BIS-11 and the ZSDS**

In overall the model is plausible (Chi-square = 17.576,  $DF=13$ ,  $p=0.174$ ) because the p value is much larger than 0.05. We computed the 90% confidence intervals of the corresponding parameters as well.



Based on the confidence intervals the relationship between impulsivity and depression can be considered significant because the corresponding 90% confidence interval does not cross zero. This confidence interval-based, reasoning corresponds to a one-sided test at 0.05 level. In classical two-sided hypothesis setting the alpha error is 0.054 which is at the border of being significant. The estimated standardized coefficient between impulsivity and depression is 0.064 meaning that about 6.4% percent of the total variability can be attributed to differences in impulsivity. All other connection between the observed and latent variables are highly significant ( $p < 0.05$ ) which support the content validity of the applied scales. Still it is noteworthy to observe that there are significant differences among the strengths of association of sub-scales to latent variables they measure. For example, the association between depression (as latent variable) and self-esteem (as an observed variable) is much stronger than association between depression and mood because the corresponding correlations are 0.762 and 0.223 respectively.

#### **4.3.2 The effect of genotype (CC, GG and GC) on the relationship between BIS-11 and the ZSDS**

There is a clear trend can be observed between genotype and impulsivity-->depression beta weights. The association is the weakest in the GG group (0.037), strongest is the CC group (0.08) and as expected the GC group occupies the middle position (0.068). The regression coefficients were significant in case of all three groups ( $p < 0.05$ ), indicating that the studied genotype does not influence the association between the ZSDS scores and impulsivity.

## 5. CONCLUSIONS

In the present study we investigated the involvement of the C(-1019)G polymorphism in impulsivity related behaviour and subthreshold depression. Furthermore, we investigated the relationship between impulsivity and subthreshold depression and the effect of genotype on the relationship between impulsivity and subthreshold depression.

- We found a significant association between the C(-1019)G polymorphism, and the Impulsiveness subscale of the Eysenck IVE Scale, and also the Motor and Cognitive Impulsiveness subscales but not the Nonplanning Impulsiveness subscale of the BIS-11. Subjects with the GG genotype are significantly more impulsive compared to subjects with the GC or CC genotypes, our results suggest the involvement of the HTR1A gene in the continuum phenotype of impulsivity.
- The majority of work that has been done in this area has focused on impulsive aggression. This focus is partly the result of the fact that aggressive acts are more easily measured than other aspects of impulsivity. Repeatable measures of impulsivity that capture the core aspects of this behaviour are needed.
- Impulsivity is a key factor in so many disorders and an important factor in treatment, but the biological and psychological research is limited by current diagnostic categories and that a dimensional approach may be more appropriate than the categorical approach used in psychiatric diagnosis and treatment.
- In the present study we found no significant association between the C(-1019)G functional polymorphism of the HTR<sub>1A</sub> gene and the ZSDS scores below the level indicating depression.
- The ZSDS scores below the level indicating major depression is significantly associated with impulsivity in a non-clinical population suggesting that impulsivity could be a risk factor for depression in healthy adults.

- The mood items of the ZSDS have a smaller influence on subthreshold depression, which is in line with previous studies reporting that in case of subthreshold depression mood symptoms are often absent.
- Impulsivity measured by the BIS-11 is determined by the Motor Impulsiveness subscale in 62%.
- There is a statistically significant connection between impulsivity and depression as latent variables. This connection seems to be influenced by the polymorphism of HTR<sub>1A</sub> gen however we could not demonstrate that this polymorphism effect is statistically significant. Standardised regression coefficients concerning the relationship between impulsivity and depression for GG, GC and CC genotype were 0.037, 0.068 and 0.08 respectively. These regression coefficients were significant in case of all three groups, suggesting some influence on the association between impulsivity and depression.
- From a clinical point of view, recognizing subthreshold depression is important as subthreshold depression causes considerable psychological suffering, thus treatment is necessary. The goal of this treatment is to reduce depressive symptomatology and to improve quality of life.
- Another reason why subthreshold depression is important from a clinical viewpoint, is the increased risk of developing major depression and an increased risk of suicide.

## 6. PUBLICATIONS

### 6.1 Publications relevant to the dissertation

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### 6.3 Book chapters

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