THE SEROTONIN TRANSPORTER GENE AND PERSONALITY:

ASSOCIATION OF THE 5HTTLPR S ALLELE, ANXIETY, DEPRESSION
AND AFFECTIVE TEMPERAMENTS

PhD theses

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1. Introduction

The serotonin transporter (5HTT) has been proven to play a crucial role in the background of mood and anxiety disorders. Besides pharmacological evidence, genetic association studies also point towards this conclusion. Fewer studies, however, have been carried out to investigate the relationship between the 5HTT gene and psychological traits and phenomena within a psychiatrically healthy population. In several studies it has been described that the s allele of the 5HTTLPR polymorphism of the serotonin transporter gene has been associated with the neuroticism trait in healthy subjects. Both the neuroticism trait and psychiatric disorders are very complex both in their manifestation and in their neurobiological background, with several, mutually interrelated genetic, neurochemical, psychological and social factors playing a role. Therefore, to have a better understanding of what role the 5HTTLPR polymorphism plays in the background of mood disorders and anxiety disorders on one hand, and in the background of the neuroticism trait on the other, it seems more reasonable to find better described, more distinct and atomic entities composing these phenomena, so that the contribution of the serotonin transporter gene in the manifestation of psychological characteristics and psychiatric disorders, and the role of these psychological characteristics in the background of psychiatric disorders can be outlined and described more precisely.

2. Objectives

The aim of our studies was to outline the role of the s allele of the 5HTTLPR polymorphism of the serotonin transporter gene in the background of subclinical depression, affective temperaments and anxiety traits in a psychiatrically healthy population. The tendency for emotionality, increased anxiety and lower mood has been conceptualised in the neuroticism personality trait and in earlier studies the association of the s allele with neuroticism has been confirmed. Since neuroticism this way is a complex phenomenon, it was
not possible to determine whether its different aspects, emotional reactivity, anxiety and depressiveness and their subtypes and symptoms are equally responsible for this genetic association, or only one or a few of them is markedly related to this polymorphism. The aim of our studies thus was to investigate the relationship of these aspects of neuroticism and the s allele independently. The main objectives in our studies were:

1. Is subthreshold depression, as indicated by elevated Zung Self-rating Depression Scale scores related to the s allele in a psychiatrically healthy sample?
2. Is the relationship between the s allele and subclinical depression valid if we consider only physical and vegetative symptoms of depression?
3. Are the psychological and physical-vegetative symptoms equally or differentially associated with the s allele?
4. Is there any relationship between the s allele and increased anxiety in a psychiatrically healthy population?
5. Is the s allele also related to affective temperaments in the general population? It is accepted that extreme affective temperaments can be considered the subsyndromal manifestations of affective disorders, and affective disorders are likely to be associated with the s allele.
6. Is there any difference in the 5HTTLPR / anxiety relationship between migraineurs and non-migraineurs in a psychiatrically healthy sample?

3. METHODS

3.1 Subjects and measures

3.1.1 Investigation of the association between the 5HTTLPR s allele and subthreshold depression

128 healthy Caucasian female subjects participated in the study. All subjects were genotyped and completed the standardised Hungarian version of the Zung Self-rating Depression Scale (Zung, 1965; Simon, 1998). By the consensus of our group of researchers,
we have identified a Physical-vegetative Symptoms Subscale within the ZSDS consisting of 8 items. The groups carrying different genotypes were compared with respect to their total score on the ZSDS as well as their score on the Physical-vegetative Symptoms Subscale and a subscale containing the remaining 12 items of the original scale.

3.1.2 *Investigation of the association between the 5HTTLPR s allele and anxiety*

45 migraine without aura patients, referred from the Headache Clinics of the National Institute for Psychiatry and Neurology and Sport Hospital, Budapest, Hungary, and 52 non-migraineurs (with rare, less than 1/month and mild if any headaches) were included in the study. All subjects completed the standardised Hungarian version of Spielberger’s State Trait Anxiety Inventory (STAI) (Spielberger, 1970; Sipos et al., 1998).

3.1.3 *Investigation of the association between the 5HTTLPR s allele and affective temperaments*

139 unrelated females of Caucasian origin participated in the study. All subjects completed the standardized Hungarian version of the original, 110-item TEMPS-A (Temperament Evaluation of Memphis, Pisa, Paris and San Diego) questionnaire (Akiskal and Akiskal, 2005a; Rozsa et al., 2006) and were genotyped for the 5HTTLPR polymorphism.

3.2 Genotyping

- DNA was obtained from all subjects by buccal swabs (Walsh et al., 1991). Polymerase chain reaction (PCR) amplification of 5HTTLPR was performed on genomic DNA extracted from buccal mucosal cells. The 5HTTLPR genotypes were identified as previously reported (Heils et al., 1996).
- PCR was conducted in a Perkin-Elmer GeneAmp 2400 thermal cycler. The amplification products were resolved on an 8% non-denaturating polyacrylamide gel by electrophoresis and visualized by silver staining (Figure 2). Fragment sizes were
determined by comparison with molecular length standards (100 bp ladder, Invitrogen).

### 3.3 Statistical analyses

All statistical analyses were carried out using Statistica 7.0 for Windows. In all cases our subjects were divided into genotype groups (subject with the 3 different genotypes: ss, sl, ll) and phenotype groups (subjects carrying the s allele and subjects not carrying the s allele). We compared test scores in the sample according to both types of distribution of the subjects. Analysis of variance test was used to compare the different genotype and phenotype groups and Tukey Honest Significant Test were used for post hoc comparisons. In case of the study investigating migraineurs and non-migraineurs, Yates’ corrected chi-square test was used to test for the distribution of alleles and genotypes in case of migraineurs and non-migraineurs. MANOVA and ANOVA tests were used to test for the difference of psychometric scores between migraineurs and non-migraineurs and between subjects carrying and not carrying the s allele.

Deviations from the Hardy-Weinberg equilibrium were calculated for each sample.

### 4. RESULTS

#### 4.1 Association of the 5HTTLPR s allele with trait and state anxiety

In our sample subjects carrying the s allele (sl and ss genotype) scored significantly higher on the state anxiety scale than subjects not carrying the s allele (ll genotype) (F=7.6288, df=1, p=0.0069). A strong tendency emerged for subjects carrying the s allele to have a higher trait anxiety (F=3.2032, df=1, p=0.0767).

The MANOVA test indicated a significant association between the genotype and the psychometric measures in our sample (F=4.0605, df=2, p=0.0204). There was, however, no significant difference in this association between the diagnostic groups (migraineurs vs. non-migraineurs, F=0.2802, df=2, p=0.7563) in either of the STAI subscales. Differences on the anxiety scales emerged within the two diagnostic groups with respect to the presence of the s
allele. Migraineurs carrying the s allele scored significantly higher on the State Anxiety Scale compared to migraineurs not carrying the s allele (p=0.0338), and non-migraineurs carrying the s allele showed a strong tendency to score higher on the state anxiety scale than non-migraineurs with no s allele (p=0.0678). In case of the Trait Anxiety Scale, there was no significant difference between any of the subgroups studied.

4.2 Association of the 5HTTLPR s allele with subthreshold depression

Comparing the two phenotype groups, subjects carrying the s allele (sl and ss genotypes) had a significantly higher score on the Zung Self-rating Depression Scale (F=5.0162, df=1, p=0.0268) and also had a significantly higher score on the Physical-vegetative Symptoms Subscale (F=7.3804, df=1, p=0.0075) but not on the remaining items of the scale (F=2.0830, df=1, p=0.1514).

There was a significant difference on the Zung Self-rating Depression Scale (F=3.4490, df=2, p=0.0348) and on the Physical-vegetative Symptoms Subscale (F=3.9384, df=2, p=0.0219) when the three different genotype groups were compared. No significant difference occurred in case of the remaining items of the scale (F=2.0191, df=2, p=0.1371). Post-hoc Tukey Honest Significant Distance Test indicated that there was a significant difference between the ss and the ll genotype in the case of the Total score on the Zung Self-rating Depression Scale, with the ss group scoring higher (p=0.0292). There was also a significant difference between ll and sl (p=0.0487), and ll and ss genotypes (p=0.0407) on the Physical-vegetative Symptoms Subscale, in both cases the ll group had the lower scores.

4.3 Association of affective temperaments with the 5HTTLPR s allele

We found a significant association between the phenotype groups and the TEMPS scores in case of four of the five affective temperaments studied. Subjects carrying the s allele scored significantly higher in all four of these temperaments: depressive (F=3.9812, df=1, p=0.0480), cyclothymic (F=4.8107, df=1, p=0.0299), irritable (F=4.0041, df=1, p=0.0474), anxious (F=3.9607, df=1, p=0.0486). No significant difference between the two phenotype groups was found in case of the hyperthymic temperament (F=0.3302, df=1, p=0.5665).
When the 3 genotype groups were compared, a significant difference between the 3 groups emerged on the scale measuring the cyclothymic temperament (F=3.8899, df=2, p=0.0228). Post hoc Tukey HSD test revealed that there is a significant difference between the ll and sl groups (p=0.0080). A strong trend (F=2.6815, df=2, p=0.0721) was observed in case of the irritable temperament, with post hoc Tukey HSD test indicating that subjects with sl genotype had a significantly higher score than subjects with ll genotype (p=0.0227). No significant association emerged in case of the other three temperaments: depressive (F=1.2320, df=2, p=0.2950), hyperthymic (F=0.2737, df=2, p=0.7610), anxious (F=1.6910, df=2, p=0.1882).

5. CONCLUSIONS

The results of our research, which has focused on the relationship between the s allele of the 5HTTLPR polymorphism and depression, anxiety and affective temperaments, lead us to the following conclusions:

- The s allele of the serotonin transporter gene is significantly associated with subthreshold forms of depression in a psychiatrically healthy sample. The s allele of the 5HTTLPR shows even stronger association with subthreshold depression if only the physical and vegetative symptoms associated with depression are taken into account. This result suggests that physical and vegetative symptoms might carry the association between the 5HTTLPR s allele and subthreshold depression.

- The s allele of the 5HTTLPR is significantly associated with state anxiety, and there is a strong tendency in case of trait anxiety. This result points to the association of the s allele and anxiety in general, but it also emphasises that this association in case of the s allele is not primarily valid for a constant increased level of anxiety, but rather for anxiety proneness and stress sensitivity.

- The s allele of the 5HTTLPR is significantly associated with those affective temperaments which carry a depressive component (depressive, cyclothymic, anxious, irritable), but not with the hyperthymic temperament. This result strengthens the association of the s allele with affective lability.
Our results indicate that the s allele is significantly associated with several independent facets, such as anxiety, anxiety proneness, depressiveness, tendency for depression to manifest in the form of physical and vegetative symptoms and affective lability, which comprise the neuroticism / emotional lability personality dimension. Our research thus supports the relationship between neuroticism and s allele and indicates that neuroticism as a whole and solid construct and not only one of its factors is responsible for this association. Our results thus indicate that neuroticism is also genetically (and not only phenomenologically) a solid construct.

Our results have important implications for everyday clinical practice and the way psychiatric disorders are viewed. It seems reasonable from our research that the category of neurosis and neurotic disorders, which is not used in modern classification systems, might be considered solid from a genetic point of view.

Our results can also be viewed from the aspect of gene-environment interactions: the strong presence of affective temperaments themselves and neuroticism may confer an increased vulnerability to chronic stress and other life events that play an important causal role in the emergence of depression, and thus preventive psychological and biological therapies may be helpful in people carrying the s allele and subjected to chronic stress.

Furthermore, our findings open the potential to identify within the community those people with temperamental inclination to mood instability with the joint use of measures of putative behavioural endophenotypes and molecular genetic markers. Given that 20-30% of the population is at risk for affective spectrum disorders based on temperamental vulnerability (Akiskal et al., 1998; Akiskal and Akiskal, 2005), and given that 60–70% of the population appears to carry the s allele, which under permissive “stressful” circumstances might express clinical affective phenotypes (Caspi et al., 2003), the present findings bring psychiatric genetics closer to the aim of delineating risk profiles for such expression in more precise quantitative measures. This further emphasises the need for future research to delineate the genetic background of traits and characteristics observable in the healthy population as endophenotypes related to neuropsychiatric disorders.
6. PUBLICATIONS

6.1 Publications relevant to the dissertation

6.1.1 Journal articles


6.1.2 Posters and presentations


23. Gonda X. (2006) A szerotonin transzporter gén szerepe és a neurózis: az 5HTTLPR s allél, az affektív temperamentumok, a szorongás és a depresszióra való hajlam


6.2 Other publications

6.2.1 Journal articles


6.2.2. Posters and presentations


6.3 Book chapters


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