

Association Between 5-HTTLPR Gen Polymorphisms of Serotonin Transporter Gene and Threatening Life Events in Undergraduate Students

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Abstract— A functional polymorphism in the promoter region of the serotonin transporter gene (5-HTTLPR) has been a highly suspect genetic marker for personality. In this study, a functional polymorphism in the promoter region of the serotonin transporter gene was used to characterize genetic vulnerability to negative life events in a representative nonclinical undergraduate students. We observed 9-repeat allele, 10 repeat allele and 12 repeat allele for VNTR polymorphism of this gene. A number of genetic variants moderates the effects of environmental risk. Frequencies of short (s/s) and long alleles (l/l and l/s) of 5-HTTLPR were found as mean: 17% and mean: 83%. Short allele (“s”) in the 5-HTTLPR gene was significantly associated with environmental experiences of these students. The study suggests a gene–environment interaction whereby biological determinants of attachment disorganization are moderated by social experiences. We determined that the interaction reached a higher level of significance when the threatening life events effect on depression in (s/s) genotype carriers was compared with the other two genotypes combined (l/l or l/s). The 5-HTTLPR gene can interact with environmental conditions. There is the relation between learning perceptual style and 5-HTTLPR gene polymorphism.

Index Terms— Gene-environment interaction, 5-HTTLPR gene polymorphism, Threatening life events, VNTR polymorphism.

I. INTRODUCTION

The promoter activity of the 5-HTT (SLC6A4/5HTT-LPR) genelocated on 17q11.2. Three common polymorphism associated with the transporter have been described: an insertion/deletion in promoter region, a available number tandem repeat in intron 2 and recently 3' untranslated region (UTR) G/T single nucleotid polymorphism (SNP). The 44 base pair promotor polymorphism displays two allelic forms, a long and short variants. The short variants is associated with reduced transcription of the gene and subsequent low functionaş expression of the transporter. The intronic VNTR has three alleles consisting of either 9, 10 or 12 repeats. Promotor activity is modified by sequence elements within the proximal 5' regulatory region, designated the 5-HTT gene-linked polymorphic region (5-HTTLPR). The short

(“s”) allele in the 5-HTTLPR is associated with lower transcriptional efficiency of the promoter compared with the long (“l”) allele [1].

Reduced central serotonergic activity has been implicated in poor impulse regulation and aggressive behaviour in animals, adults and also young children [2, 3]. Over the past 5 years, the interaction between the 5-HTTLPR polymorphism and exposure to stressful life events has been under scrutiny [4,5]. The short (s) allele of an insertion/deletion polymorphism within the promoter region of the serotonin transporter gene (5-HTTLPR) has been reported to interact with social adversity and other environmental factors to increase the risk for behavioural problems [6]. The s allele is associated with exposure to stressful life events [7,8], and social adversity [9]. This allele interacts with an adverse childhood environment to increase the risk for violent conduct in young adults. 5-HTTLRP genotype moderates the influence of stressful life events, [7]. Inattention symptoms were more strongly associated with internalizing symptoms, academic difficulties, and neurocognitive weaknesses [10].

The aim of this research was the description of an association between alleles of the 5-HTTLPR (serotonin transporter length polymorphic region), a functional polymorphism within the promoter sequence of the serotonin transporter receptor gene and environmental factors. The 14 events included financial, housing, health, and relationship stressors. We tested this G X E hypothesis among students of Compulsory Preparatory School undergraduate students. This representative 250 young adults birth has been assessed at ages 18,19, 20, and 21. We calculated probabilities for depression across all combinations of levels of exposure to TLE by 5HTTLPR genotype (s/s, l/s or l/l).

Reid identified four basic perceptual style preferences: visual (for reading, charts), auditory (for instance, lectures and audiotapes), kinesthetic (such as physical activity), and tactile (such as building models or performing laboratory experiments). They included the dimensions of group versus individual learning preferences to develop the well-known perceptual learning style preference questionnaire. Reid et al. [11] suggested that although stylistic preferences are relatively stable, students need to be adaptable, since studies have shown that “the ability of students to employ multiple learning styles results in greater classroom success”. In this report, we explored the relationship between the 5-HTTLPR alleles and learning styles in Turkish undergraduate students.

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II. MATERIALS AND METHODS

A. Samples Review Stage

This study was performed with undergraduate students of Compulsory Preparatory School. Ethical approval for this study was obtained from the Ethics Committee. Study participation was voluntary and based on written consent forms. A total of 250 Turkish undergraduate students between the ages of 18 and 21 years.

B. Social demographic form

The social demographic form was developed to investigate the socio-demographic characteristics of students [12].

C. Independent measures

Social distress was measured using the threatening life events (TLE) list. The list includes serious life events, such as the death of a parent, spouse or child, the death of another relative, the onset of a serious illness or accident affecting a relative, a marital separation, the ending of a friendship or relationship, a serious problem with a close friend, neighbor or relative, a financial crisis, or the theft or loss of an item of personal value. Subjects were asked whether any of these events had occurred within the last 6 months. For the purposes of analysis, we divided participants into three levels of exposure to threatening life events (TLEs): Having had no TLE, having had just one TLE or having had two or more TLEs within the previous 6-month period before the interview [13].

D. 5-HTTLPR genotype assays

For 5-HTTLPR genotype assays, amplification of genomic DNA was performed using 50 ng of DNA, the forward primer sequence was 50-GGC GTT GCC GCT CTG AATGC-30 and the reverse primer was 50-GAG GGA CTG AGC TGG ACA ACC AC-30, 250 mM each of dATP, dCTP, dGTP and dTTP, 1.5mM MgCl₂, 50mM KCl, 10mM Tris-HCl and 0.3 units of DNA polymerase in a total volume of 25 ml. Samples were amplified for an initial cycle of 8 min at 95°C followed by 35 cycles each consisting of 30 s at 95°C, 30 s at 62°C and 1 min at 72°C. After amplification, genotypes were resolved by a 2% agarose gel electrophoresis and ethidium bromide staining. [4,14].

The VNTR polymorphism (primer pair 5' GTCAGTATCACAGGCTGCGAG3' and 5'TGTTCTTAGTCTTACGCCAGTG3') amplification was performed with an initial denaturation at 94°C, for 4.5 min followed by 35 cycles with denaturation at 94 °C for 30 s, annealing at 61 °C for 30 s and extension at 72 °C for 30 s. PCR product were visualised on 2.5 agarose gel [15].

E. Learning style questionnaire

The learning style data in this study were collected using the "Perceptual Learning Style Questionnaire" originally developed by Reid et al. in 1987 [11]. The questionnaire consisted of three sections: background information questions, the instructions and the statements related to learning style preferences. The questionnaire included sets of five statements arranged randomly on six different learning styles: visual, auditory, kinesthetic, tactile, group learning, and individual. The statements in the scale were rated based on the five-point Likert Scale (strongly

agree, agree, not sure, disagree and strongly disagree). The reliability of the questionnaire was confirmed by Yilmaz in 2004 [16].

F. Statistical Analysis

Statistical analysis were performed with SPSS 15.0 software (SPSS Inc., Chicago, IL, USA). Both parametric and non-parametric tests were used for evaluation of the data. The difference in allele and genotype frequencies among the students was determined using the chi-square test.

III. RESULTS

A. Independent variables frequencies

Demographic and genotypic data on the sample are provided in Table 1. Summarizing, about half of the percipitant had reported at least one TLE and the other half at least two or more TLE. The most of participants had the l allele, whereas the rest had the s allele.

Table. 1 Descriptive characteristics of participants. Frequencies of independent variants

Socio-demographic variables:

Gender

Female 120 (48%)

Male 130 (52%).

Mean age: 19 ± 8.72

Threatening life experiences: n= 200 Allele variant

No TLE: 39 (17%) with (s/s), (l/s); 211 (84%) with (l/l)

1 TLE: 77 (33%) with (s/s), (l/s); 173 (69%) with (l/l)

2 TLE: 111 (48%) with (l/s), (l/l); 139 (55%) with (s/s)

PCR product were visualised on 2% agaros gel: a short allele of 482 bp and a long allele of 525 bp. 9-repeat allele of 247, a 10 repeat allele of 262 bp and 12 repeat allele of 305 bp for VNTR polymorphism.

Both male and female carry the short 5-HTTLPR allele. Frequencies of short (s/s) and long alleles (l/l and l/s/) of 5-HTTLPR were mean: 17% and mean: 83%, respectively. Two-way ANOVA for attachment disorganization revealed a main effect of 5-HTTLPR, p = 0.06. (Table2).

Table. 2 Frequencies of 5-HTTLPR gene polymorphism at inclinical samples

Allel Frequencies	Male (%) n:130	Female (%) n:120
Long (l/l)	37	33
Long (l/s)	40	48
Short (s/s)	23	19

The 5-HTTLPR polymorphism significantly modified the risk effect for depression conferred by an increasing level of exposure to TLE (Table 1 and Table 2). The interaction reached a higher level of significance when the TLE effect on depression in s/s genotype carriers was compared with the other two genotypes combined (l/l or l/s) (Table 2). Among

s/s individuals minimal levels of exposure to TLEs confer a degree of risk for depression.

B. Independent variables frequencies

Data analysis was performed by analyzing each item in the perceptual learning style questionnaire. The means for six learning styles were calculated individually. Averaged mean and standard deviation were calculated to determine the perceptual learning style preferences of undergraduate students.

Table. 3 Average mean scores of perceptual learning styles

Learning Style	5-HTTLPR genotypes	\bar{X}	SD
Visual	l/l	3.70	0.58
Auditory	s/s (%36),	3.80	0.50
	l/s	3.56	0.55
Kinesthetic	s/s (%54),	3.85	0.60
	l/s	3.44	0.57
Tactile	l/l	3.50	0.76
Group	l/l	3.41	0.88
Individual	s/s (10 %),	3.32	0.89
	l/s	3.44	0.75

The averaged mean score of each learning style was determined. The means and SDs of participant’s responses are provided in Table 3. The mean score of learning style was found to be \bar{X} : 3:85, which was the highest mean among the styles. Second language learners with the (s/s) genotype preferred kinesthetic \bar{X} : 3:85, auditory \bar{X} : 3:80 and individual \bar{X} : 3:34 learning methods. Undergraduate students containing l/l genotype showed the most visual learning style \bar{X} : 3:70 also preferred tactile \bar{X} : 3:50 and group \bar{X} : 3:41 learning styles for second language learning.

IV. DISCUSSION

The 5-HTTLPR s/s genotype and exposure to increasing numbers of TLEs. It is the first study to examine genetic-environment interaction in a homogeneous Turkish population. Caspi et al., 2003 [7] reported an interaction of 5-HTTLPR genotype with a linear progression of exposure to life events, possibly because their measure of stressful life-events included a wide range of situations. Our results show a somewhat better model fit after adjustment for potential confounders, such as gender and age. The relationship between gender and this particular gene-environment interaction is puzzling as some studies have reported it as valid for both sexes, whereas others suggest an effect only in women or even an inverse effect in men [17]. Associations with dopaminergic genes have also been found in other disorders which are frequently comorbid

with ADHD, such as learning disabilities [18], antisocial behavior [19] and autism [11, 20].

Males were affected by living in public housing rather than in own owned homes and by living with separated parents, females were affected by traumatic conflicts within the family. The responses of males and females carrying the short 5-HTTLPR allele to environmental stress factors go in opposite directions. Thus, whereas females tend to develop depressive symptoms, males seem to be protected from depression. Both the molecular and the psychosocial mechanisms may differ between boys and girls [21,22].

Reid stated that learning style can be seen as “an individual’s nature, habitual, and preferred way(s) of absorbing, processing, and retaining new information” (p.viii), and divided perceptual learning styles as visual/auditory, kinesthetic/tactile, and group/individual. [23,24]. We explored individual differences in behavioral attributes, disease susceptibility and learning styles. In this study, we observed an association between learning disabilities and the dopaminergic system. Learners with the s/s genotype preferred kinesthetic learning styles while learners with the l/l genotype preferred visual learning styles (Table 3). This result shows that students frequently prefer kinesthetic learning activities in class. Young adults with the s/s genotype showed significantly lower scores for visual learning. The individual learning style showed the lowest mean among the learning styles : 3:32, suggesting that students occasionally prefer to study and learn individually. The immediate recall scores under auditory ordered conditions are suggestive of better recall than under visual conditions. These young adults showed a significant loss of information over time [25]. Additionally, these young adults were unable to remember what was learned after a short period of time. To ensure that these students retain information, practice and repetition should be emphasized. Stress and/or anxiety may influence cognitive processes negatively, and stress experienced by these students may exacerbate their learning difficulties [26-29].

ABBREVIATIONS

TLE: Threatening Life Events, ADHD: Attention Deficit Hyperactivity Disorder, PCR: Polimeraze Chain Reaction VNTR: Variable Number Tandem Repeat

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