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ORIGINAL PAPER

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Association of the s allele of the 5-HTTLPR with neuroticism-related traits and temperaments in a psychiatrically healthy population

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Abstract *Introduction* Research concerning the genetic background of traits, temperaments and psychiatric disorders has been rapidly expanding. One of the most frequently studied genetic polymorphisms in the background of psychological and psychiatric phenomena is the 5-HTTLPR polymorphism of the serotonin transporter gene which has earlier been found to be associated with neuroticism and neuroticism-related traits and disorders. However, both the neuroticism trait and psychiatric disorders are complex and composed of several subfacets. The aim of our study was to investigate the association of the 5-HTTLPR polymorphism with several smaller, distinct and better characterisable phenomena related to the neuroticism trait. *Methods* 169 healthy females participated in the study. All participants completed the Buss–Durkee Hostility Inventory (BDHI), the State-Trait Anxiety Inventory (STAI), The Zung Self-rating Depression Scale (ZSDS), the Beck Hopelessness Scale, the SCL-51, the Temperament and Character Inventory (TCI) and the Temperament Evaluation of Memphis, Pisa, Paris and San Diego (TEMPS-A) questionnaire. All subjects were genotyped for the 5-HTTLPR using PCR. Data were analysed with ANOVA and MANCOVA with age as a covariate. *Results* We found that the presence of the s allele was significantly associated with anxiety, depression, hopelessness, guilt, hostility, aggression, presence of neurotic symptoms, self-directedness and affective temperaments carrying a depressive component even when controlling for age. *Conclusions* Our study is the first that confirms that traits and characteristics related to neuroticism, such as increased anxiety, depression, hopelessness, somatization, feeling of guilt, hostility,

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aggression, lack of self-directedness and affective temperament are consistently and independently associated with the 5-HTTLPR polymorphism of the serotonin transporter gene. Our study therefore suggests that neuroticism can be considered a unified construct not only from a phenotypical but also from a genetic point of view and 5HTTLPR can be considered one component of its polygenic background. Our results thus yield further insight into the role of the 5-HTTLPR in the background of neuroticism and neuroticism-related psychiatric disorders.

Key words 5-HTTLPR · polymorphism · neuroticism · affective temperaments

Introduction

Research concerning the genetic background of psychiatric disorders and healthy psychological characteristics has been rapidly increasing and expanding in the past decade. One of the first breakthroughs of this research was the initial description of an association between the s allele of the 5-HTTLPR (serotonin transporter length polymorphic region), a functional polymorphism within the promoter sequence of the serotonin transporter gene and the neuroticism personality trait [39]. Several studies have since been carried out concerning this polymorphism, and although some of the results are contradictory, it has been shown that the s allele of the 5-HTTLPR is associated with both affective [11, 12, 18] and anxiety disorders [13] on one hand, and subthreshold depression [25] and anxiety [26], and affective temperaments carrying a depressive component [27] within a healthy population on the other. However, both psychiatric disorders and the neuroticism trait are very complex and multifaceted phenomena and therefore it cannot be established whether these as a whole or only one or more components of them carry the above genetic association. Therefore it is more reasonable to focus research on more restricted, smaller, distinct, better characterisable psychological traits and phenomena related to neuroticism and affective and anxiety disorders and investigate their relationship with the s allele in order to gain a deeper insight into the role of the 5-HTTLPR polymorphism in the background of affective and neuroticism related-traits and temperaments, and neuroticism-related disorders. The association of the 5HTTLPR with neuroticism and neuroticism-related traits has been described in several studies [21, 33, 39, 42, 45, 52], however, fewer or no such studies were carried out concerning the relationship of this polymorphism with traits and characteristics that are components of neuroticism. Although neuroticism is defined as a tendency to experience negative emotional states, accompanied by heightened anxiety or depression, angerl and guilt, as well as increased tendency for somatization of psychological problems [16, 19, 43], no study before has consistently examined the association of the 5HTTLPR polymorphism and such components related to neuroticism as depression, anxiety, hopelessness, somatization, interpersonal sensitivity, obsessive-compulsive symptoms, aggressive traits, traits and temperaments of the TCI, and affective temperaments to establish whether all facets of neuroticism share a role in the association with the 5HTTLPR.

Neuroticism is conceived as a predisposition or tendency to experience negative emotional states as well as anxiety, depression, anger, guilt and hostility and a tendency for somatization [19, 43]. The aim of our study was to investigate the association of the 5-HTTLPR s allele with several neuroticism-related traits, temperaments and characteristics within a psychiatrically healthy population.

Methods

169 unrelated Hungarian women of Caucasian origin participated in the study. All participants were healthy volunteers aged between 18–64 years. The participants were recruited between 2004–2006 from university students and workers at the National Institute for Psychiatry and Neurology, Budapest, Hungary. All participants underwent thorough neurological and psychological/psychiatric testing and were investigated using M.I.N.I. International Neuropsychiatric interview [8, 53] and subjects with any disorders meeting DSM-IV Axis I criteria were excluded from the study. None of the participants took any psychiatric medications. The study was approved by the Scientific and Research Ethics Committee of Scientific Health Council in charge of genetic experimentation with human subjects. All subjects gave informed consent before participating in the study.

All subjects completed the Spielberger State-Trait Anxiety Inventory (STAI) [55, 56], the Zung Self-rating Depression Scale (ZSDS) [54, 59], the Buss–Durkee Hostility Inventory (BDHI) [15, 47], the 51-item Symptom Checklist (SCL-51) [20, 29], the Beck Hopelessness Scale [9, 48], the Temperament and Character Inventory (TCI) [17, 49], and the Temperament Evaluation of Memphis, Pisa, Paris and San Diego (TEMPS-A) questionnaire [4, 7, 50, 51].

In two parallel independent samples we measured the correlation of the above scales with measures of Neuroticism. Data in these studies were collected between 2005–2006. These samples were recruited at universities and from the workers at the National Institute for Psychiatry and Neurology, Budapest, Hungary. In a sample of $n = 931$ we measured the correlation between the Big Five Inventory (BFI 44) [32] Neuroticism Scale and the ZSDS, BDHI, SCL-51, Beck Hopelessness Scale and TEMPS-A. In a sample of $n = 267$ we measured the correlation between the EPQ [23, 40] Neuroticism Scale and the TCI scales.

5-HTTLPR genotypes were determined using polymerase chain reaction (PCR). PCR amplification of 5-HTTLPR was performed on genomic DNA extracted from buccal cells [57], and 5-HTTLPR genotypes were identified as previously reported [31].

All statistical analyses were carried out using Statistica 7.0 for windows. The data were analyzed in two separate ways, according to genotype grouping (ss, sl and ll genotypes; additive model), and according to phenotype groups (subjects carrying and not carrying the s allele; dominant model). Test scores of the three genotype groups and the two phenotype groups were compared using ANOVA, and in a second step, association of significant scores with genotype and phenotype were investigated in a MANCOVA model using age as a covariate. 0.05 was accepted as level of significance. Deviations from the Hardy–Weinberg equilibrium were calculated for our sample.

We computed correlations between the scales we used in our study using Pearson correlations. We also used Pearson correlation to establish the correlations between the scales we used and EPQ-Neuroticism and BFI-Neuroticism subscales in the independent samples.

Results

The frequency of the s allele in our study population was 38.76% which parallels the results of earlier studies and is representative of the Caucasian population [39]. The frequency of sl, ll and ss genotypes were 49.11%, 36.69%, and 14.20%, respectively. The distribution of genotypes in our population followed the Hardy-Weinberg equilibrium ($\chi^2 = 0.6722$, df = 2, $P = 0.7146$). Mean age of the participants was 32.13 ± 0.90 years. There was no significant difference in age between subjects in the three genotype groups ($F = 1.0023$, $P = 0.3692$) and in the two phenotype groups ($t = 1.2093$, $P = 0.2282$)

Investigation of the association of genotype (ss, sl and ll groups) with psychometric test scores

In the first step, we compared test scores in the three genotype groups using ANOVA (Table 1). There were significant differences in the test scores between the three genotype groups in case of the STAI trait anxiety, ZSDS, ZSDS physical-vegetative subscale,

Table 1 Mean, SE values, and analysis of variance table for psychometric scores associated with genotypes ss ($n = 24$), sl ($n = 83$) and ll ($n = 62$), with the two phenotype groups (subjects carrying the s allele vs subjects not carrying the s allele) and the correlation of the scales with measures of Neuroticism

	Comparisons according to genotype groups					Comparisons according to presence of s allele				Correlation with neuroticism scale
	ss ($n = 24$) mean ± SE	sl ($n = 83$) mean ± SE	ll ($n = 62$) mean ± SE	F	P	Subjects carrying the s allele ($n = 107$) mean ± SE	Subjects not carrying the s allele ($n = 62$) mean ± SE	F	P	r vs N
STAI										
STAI state	39.88 ± 2.14	37.28 ± 1.05	34.77 ± 1.20	2.68	0.072	37.86 ± 0.95	34.77 ± 1.20	4.00	**0.047**	0.45
STAI trait	43.29 ± 2.02	40.46 ± 1.03	38.00 ± 1.11	3.08	**0.049**	41.09 ± 0.92	38.00 ± 1.11	4.38	**0.038**	0.64
ZSDS										
ZSDS	37.96 ± 1.20	37.63 ± 0.74	34.42 ± 0.74	5.38	**0.006**	37.70 ± 0.63	34.42 ± 0.74	10.77	**0.001**	0.54
ZSDS phys-vegetative	14.50 ± 0.52	14.37 ± 0.29	13.19 ± 0.35	4.11	**0.018**	14.40 ± 0.25	13.19 ± 0.35	8.23	**0.005**	0.41
Hopelessness	4.17 ± 0.42	4.87 ± 0.32	3.74 ± 0.27	3.54	**0.031**	4.71 ± 0.27	3.74 ± 0.27	5.65	**0.019**	0.31
BDHI										
Assault	1.71 ± 0.38	1.65 ± 0.19	1.71 ± 0.21	0.02	0.976	1.66 ± 0.17	1.71 ± 0.21	0.03	0.868	0.17
Indirect aggression	3.75 ± 0.38	3.92 ± 0.17	3.58 ± 0.24	0.66	0.517	3.88 ± 0.16	3.58 ± 0.24	1.16	0.283	0.36
Irritability	4.92 ± 0.35	5.46 ± 0.19	4.94 ± 0.20	2.13	0.122	5.34 ± 0.17	4.94 ± 0.20	2.27	0.134	0.39
Negativism	2.04 ± 0.33	2.24 ± 0.15	1.85 ± 0.16	1.50	0.226	2.20 ± 0.13	1.85 ± 0.16	2.59	0.109	0.21
Resentment	1.38 ± 0.26	1.87 ± 0.24	1.35 ± 0.18	1.64	0.197	1.76 ± 0.19	1.35 ± 0.18	1.91	0.168	0.42
Suspicion	2.17 ± 0.45	2.13 ± 0.20	1.66 ± 0.21	1.32	0.270	2.14 ± 0.19	1.66 ± 0.21	2.65	0.105	0.28
Verbal aggression	7.13 ± 0.57	7.34 ± 0.27	6.66 ± 0.32	1.27	0.285	7.29 ± 0.25	6.66 ± 0.32	2.42	0.122	0.28
Guilt	3.96 ± 0.32	3.54 ± 0.18	3.11 ± 0.19	2.92	0.056	3.64 ± 0.15	3.11 ± 0.19	4.49	**0.036**	0.39
Aggression index	4.50 ± 0.26	4.79 ± 0.14	4.53 ± 0.15	1.02	0.364	4.73 ± 0.12	4.53 ± 0.15	1.02	0.314	0.41
Hostility index	3.33 ± 0.33	3.59 ± 0.17	3.05 ± 0.17	2.27	0.107	3.53 ± 0.15	3.05 ± 0.17	4.00	**0.047**	0.43
Global aggression	25.75 ± 1.75	26.94 ± 0.91	23.74 ± 1.04	2.63	0.075	26.67 ± 0.81	23.74 ± 1.04	4.89	**0.028**	0.51
SCL51										
Somatization	7.58 ± 0.97	9.23 ± 0.78	6.71 ± 0.55	3.30	**0.039**	8.86 ± 0.65	6.71 ± 0.55	5.15	**0.024**	0.60
Anxiety	7.50 ± 1.20	5.90 ± 0.44	4.73 ± 0.52	3.69	**0.027**	6.26 ± 0.44	4.73 ± 0.52	4.84	**0.029**	0.60
Depression	5.04 ± 0.81	4.39 ± 0.36	3.26 ± 0.35	3.44	**0.034**	4.53 ± 0.33	3.26 ± 0.35	6.12	**0.014**	0.49
Obsessive-compulsive	8.13 ± 1.32	5.95 ± 0.48	4.47 ± 0.49	5.76	**0.004**	6.44 ± 0.48	4.47 ± 0.49	7.17	**0.008**	0.47
Interpersonal sensitivity	5.46 ± 0.96	4.58 ± 0.37	3.50 ± 0.35	3.45	**0.034**	4.78 ± 0.36	3.50 ± 0.35	5.62	**0.019**	0.55
Total	33.71 ± 4.82	30.05 ± 2.01	22.66 ± 1.88	4.46	**0.013**	30.87 ± 1.89	22.66 ± 1.88	8.17	**0.005**	0.62
TCI										
Novelty seeking	18.71 ± 1.14	19.73 ± 0.69	19.00 ± 0.71	0.42	0.656	19.50 ± 0.59	19.00 ± 0.71	0.29	0.594	0.03
Harm avoidance	16.79 ± 1.32	16.71 ± 0.73	15.69 ± 0.71	0.54	0.585	16.73 ± 0.64	15.69 ± 0.71	1.08	0.301	0.40
Reward dependence	17.25 ± 0.61	17.18 ± 0.34	17.03 ± 0.45	0.05	0.947	17.20 ± 0.29	17.03 ± 0.45	0.10	0.751	−0.06
Persistence	4.50 ± 0.37	4.11 ± 0.21	3.85 ± 0.23	1.09	0.337	4.20 ± 0.18	3.85 ± 0.23	1.35	0.247	0.23
Self directedness	30.21 ± 1.21	30.48 ± 0.60	32.73 ± 0.65	3.62	**0.029**	30.42 ± 0.53	32.73 ± 0.65	7.23	**0.008**	−0.49
Cooperativeness	34.67 ± 0.57	33.04 ± 0.61	34.06 ± 0.61	1.34	0.265	33.40 ± 0.50	34.06 ± 0.61	0.69	0.408	−0.40
Self transcendence	16.08 ± 1.58	15.07 ± 0.80	13.69 ± 1.05	1.00	0.368	15.30 ± 0.72	13.69 ± 1.05	1.70	0.194	0.21
TEMPS-A										
Depressive	7.96 ± 0.63	7.72 ± 0.38	5.81 ± 0.33	7.85	**0.001**	7.78 ± 0.33	5.81 ± 0.33	15.67	**0.000**	0.50
Cyclothymic	6.08 ± 0.86	7.11 ± 0.47	4.47 ± 0.40	8.07	**0.000**	6.88 ± 0.41	4.47 ± 0.40	14.84	**0.000**	0.54
Hyperthymic	9.33 ± 0.70	10.08 ± 0.39	10.85 ± 0.57	1.50	0.227	9.92 ± 0.34	10.85 ± 0.57	2.30	0.131	−0.27
Irritable	4.83 ± 0.75	5.27 ± 0.39	3.35 ± 0.36	5.99	**0.003**	5.17 ± 0.35	3.35 ± 0.36	11.71	**0.001**	0.54
Anxious	9.42 ± 1.00	8.69 ± 0.57	6.15 ± 0.57	6.26	**0.002**	8.85 ± 0.49	6.15 ± 0.57	12.14	**0.001**	0.64

$P < 0.05$ is shown in bold

BDHI Buss–Durkee Hostility Inventory, *TCI* Temperament and Character Inventory, *SCL 51* Symptom Distress Checklist, *TEMPS-A* Temperament Evaluation of Memphis, Pisa, Paris and San Diego

Table 2 Significant post hoc LSD tests for significantly different psychometric scores in the three genotype groups (ss, sl and ll)

	sl-ll *P*	ss-ll *P*	ss-sl *P*
STAI trait		0.0183	
ZSDS	0.0027	0.0203	
ZSDS physical-vegetative	0.0087	0.0416	
Hopelessness	0.0093		
SCL 51 somatization	0.0123		
SCL 51 anxiety		0.0088	
SCL 51 depression	0.0391	0.0229	
SCL 51 obsessive		0.0011	0.0417
SCL 51 interpersonal sensitivity		0.0167	
SCL 51 total	0.0156	0.0116	
Self directedness	0.0141		
TEMPS depressive	0.0003	0.0047	
TEMPS cyclothymic	0.0001		
TEMPS irritable	0.0008		
TEMPS anxious	0.0022	0.0058	

hopelessness, SCL-51 somatization, SCL-51 anxiety, SCL-51 depression, SCL-51 obsessive-compulsive scale, SCL-51 interpersonal sensitivity, SCL-51 total, TCI self directedness, TEMPS depressive, TEMPS cyclothymic, TEMPS irritable and TEMPS anxious temperaments. Mean values and standard errors in case of each scale are shown in Table 1. Results of post hoc LSD tests are shown in Table 2. Post hoc analysis also indicates that all but one significant differences were observable between the ss and ll, and sl and ll genotypes. In contrast, the only significant difference between ss and sl genotypes was found in case of SCL-51 obsessive-compulsive subscale (Table 2).

In the next step, scales with significantly different scores in the three genotype groups were further investigated using MANCOVA with age as a covariate. The genotype grouping had a significant effect on the scores of the psychometric scales when controlling for age (wilks lambda = 0.7280, $P = 0.00003$). All scales with a significant difference in the ANOVA model retained their significant difference with respect to genotype grouping when controlling for age.

Investigation of the association of the presence of the s allele (subjects carrying vs subjects not carrying the s allele) with psychometric test scores

We also analyzed psychometric test scores in our sample with grouping according to the presence of the s allele. We compared test scores of subjects carrying and not carrying the s allele with ANOVA. There was a significant difference between the two groups on the STAI state, STAI trait, ZSDS, ZSDS physical-vegetative subscale, hopelessness, BDHI guilt, BDHI hostility, BDHI global aggression, SCL-51 somatization, SCL-51 anxiety, SCL-51 depression, SCL-51 obsessive compulsive, SCL-51 interpersonal sensitivity, SCL-51 total, TCI self-directedness, TEMPS depressive, TEMPS cyclothymic, TEMPS irritable and TEMPS anxious scales (Table 1). Mean and SE values of psychometric scores in the two groups are shown in Table 1.

In the next step, we investigated scales with a significant difference using a MANCOVA model with age as a covariate. The presence of the s allele had a significant effect on the scores of the psychometric scales when controlling for age (wilk's lambda = 0.7708, $P = 0.0108$). All scales with a significant difference in the MANCOVA model showed a significant difference even when controlling for age except for the BDHI hostility scale where a strong tendency was observable.

Correlation between the scales and correlations with measures of Neuroticism

Within our sample we computed the intercorrelations between the scales. These results are available upon request from the authors. All correlations were significant. We obtained strong correlations only between subscales measuring the same construct (SCL anxiety and STAI scales, ZSDS and SCL depression scale). We found moderately strong correlations between some subscales within the SCL 51 scale. All other correlations were weak to moderate. We also computed the correlation between the scales we used with accepted and widely used Neuroticism scales in two independent samples. Correlations are presented in Table 1.

Discussion

Several studies have been carried out to investigate the association of neuroticism as a global construct with the s a allele of the 5-HTTLPR. Neuroticism, however, is composed of several different, seemingly independent factors. The identification of those associated with and those independent of 5-HT function is missing. In our research our aim was to identify those subfacets of neuroticism which are related to the s allele of the 5HTTLPR polymorphism, using several questionnaires covering all major components of neuroticism in one study carried out in a healthy female population without any DSM-IV axis I psychiatric disorders. We chose to carry out our investigations in a psychiatrically healthy sample in order to avoid psychopathology of depressive and anxiety disorders to interfere with the neuroticism trait in the possible association with the serotonin transporter and also to avoid the higher frequency of the s allele observable in depressed and anxiety disorder patients to contaminate our results. Our objective was to establish the relationship between subfacets of neuroticism and the 5HTTLPR polymorphism in the background of the healthy personality.

It has been described that the 5-HTTLPR polymorphism is associated with the neuroticism trait [39] and disorders related to increased neuroticism scores such as anxiety and affective disorders [11–13, 18]. Both psychiatric disorders and the neuroticism trait, however, are very complex in their nature, therefore it warranted further investigation to identify smaller, more distinct and better characterisable traits and characteristics that are related to neuroticism and describe the relationship of these with the 5-HTTLPR s allele in order to gain deeper insight to the relationship of the 5-HTTLPR and neuroticism, and neuroticism-related psychiatric disorders. Our study revealed that several traits and characteristics associated with the neuroticism trait are associated with the 5-HTTLPR independently on their own. We have found that increased state and trait anxiety (as indicated by the STAI state and trait anxiety scores) and anxiety in general (as indicated by the SCL-51 anxiety score), increased depression (as indicated by the SCL-51 depression score and the ZSDS score), somatization (as indicated by the SCL-51 somatization score and the ZSDS physical-vegetative subscale score), interpersonal sensitivity (as indicated by the SCL-51 interpersonal sensitivity score), obsessive-compulsive symptoms (as indicated by the SCL-51 obsessive-compulsive score), an increased general tendency to manifest neurotic symptoms (as indicated by the increased SCL-51 total score), increased feeling of guilt (as indicated by the increased BDHI guilt score), hostility (as indicated by the increased BDHI hostility index) and global aggression (as indicated by the increased BDHI global aggression index), and a decreased score on the self-directedness scale of the TCI is significantly associated with the presence of the s allele. Like in our previous study [27], we also found that subjects carrying the s allele scored significantly higher on those four affective temperament subscales of the TEMPS-A which by definition carry a depressive component, (depressive, cyclothymic, anxious and irritable) but not on the hyperthymic temperament subscale. Our study therefore supports the association of neuroticism with the 5-HTTLPR s allele showing that individual traits and characteristics which are related to, and incorporated by the neuroticism trait are also independently associated with this polymorphism. Contrary to our expectations, we found no significant association between 5HTTLPR genotype allele and harm avoidance, which is considered strongly related to neuroticism [22]. Earlier studies, however, also yielded contradicting results on the association between harm avoidance and the s allele [52].

Neuroticism can be defined as a tendency to experience negative emotional states and feelings of anxiety, depression, hostility, anger, guilt, as well as increased tendency for somatization of psychological symptoms [19, 43]. Neuroticism is conceived as general emotional lability and associated with a poorer reaction to stress. People characterized by higher neuroticism are more prone to experience negative affects like sadness, anger, shame as well as having a poorer control over urges and impulses and show excessive concern over their physical functioning [19, 41]. In general, neuroticism is conceived as a predisposition to experience psychological distress [19]. Neuroticism reflects emotionality, emotional reactivity and affective lability [24] and increased neuroticism scores has been found to be related to anxiety and affective disorders [35, 37]. It has been established earlier that neuroticism as a trait is associated with the s allele of the 5-HTTLPR [39, 52], however, based on this association it could have not been determined whether all of the above characteristics related to neuroticism are also associated with the s allele.

In our study we examined the above characteristics separately in their association with this polymorphism and confirmed their significant relationship with the s allele. Therefore, our results support that neuroticism is a unified construct both from a clinical and phenotypical, and also from a genetic point of view, and 5-HTTLPR can be considered one component of its polygenic background. Neuroticism as a general construct can be considered as a trait or temperament that predisposes to several forms of depressive and anxiety disorders as well as for some pathological personality reactions, and can be contrasted to hyperthymia.

Our results concerning the association of state anxiety, trait anxiety, depression, and affective temperaments other than hyperthymic are the replication of our earlier findings [25–27], but with a greater sample size. The significant association of the 5-HTTLPR s allele and the symptom subscales of the SCL-51 is in line with our expectation based on the well-known association of 5HTTLPR and neuroticism, however, this time we found significant association in case of all major symptoms associated with neurotic symptom formation. A significant association with the 5-HTTLPR was described for depressive disorder, anxiety disorders, and obsessive compulsive disorders [11–13, 18], our results, however, show that subthreshold and subclinical neurotic symptomatology in a healthy population is also significantly associated with the 5HTTLPR. The association of the somatization subscale of the SCL-51 with the s allele supports our earlier results on the significant association between 5-HTTLPR and physical-vegetative symptoms of depression [27], which we also replicated in this study.

Our study is the first to describe a significant association between Hopelessness and the 5-HTTTLPR. It is an important finding, because it has been reported before that this polymorphism is associated with suicidal behaviour [58], and it has also been established that hopelessness is a major predictor of suicidality [44]. Our study provides a

further link between hopelessness, suicidal behaviour and the 5-HTTLPR.

The relationship between the s allele and subscales of the Buss–Durkee Hostility Inventory are in line with our understanding of the role of the serotonergic system in aggression [38] and also with the relationship between neuroticism and feelings of guilt and hostility [19, 43]. In one study a significant association between the s allele and childhood aggression has been described [10]. Studies so far, however, did not investigate the relationship of aggressive traits with the 5-HTTLR. Our results indicate that aggressive traits and characteristics, such as guilt, hostility and global aggression are associated with the 5-HTTLPR polymorphism.

As for the TCI scales, we could not replicate earlier findings concerning the association of the s allele with harm avoidance [22]. In contrast, we found that the presence of the s allele is related to a lower score on the self-directedness scale. Although according to earlier studies neuroticism is related most strongly to harm avoidance among the TCI dimensions [22], lower scores on the self-directedness scale and its subscales in case of subjects carrying the s allele (data not shown), associated with a blaming, helpless, unreliable attitude, the inability to define, set and pursue meaningful goals, poor resourcefulness and unrealistic behaviour [17], correspond to the general maladaptation characteristic of neuroticism.

Our results also indicate that affective temperaments carrying more or less of a depressive component are the most strongly associated with the presence of the s allele of the 5-HTTLPR. Affective temperaments are defined as the subaffective, subclinical manifestation of affective disorders and when present in a dominant form they indicate an increased risk for the manifestation of major unipolar and bipolar mood disorders [1–3] and dominant affective temperaments have also been described to be more frequent among mood disorder patients [6, 14, 30, 36]. While neuroticism is a global construct which subsumes, among others, such traits as anxiousness, depressiveness, and mood lability [5, 24, 46], the depressive, cyclothymic, hyperthymic, irritable and anxious subscales of the recently developed TEMPS-A (Temperament Evaluation of Memphis, Pisa, Paris and San Diego- Autoquestionnaire version) [4] more specifically and individually measure each of the foregoing trait dimensions. As in our previous study [27], we again found that of all affective temperament scales, cyclothymic temperament is most strongly associated with the presence of the s allele. Moreover, of all the scales and subscales studied, cyclothymic temperament showed the strongest association with this polymorphism.

We must also mention that in our analyses according to genotype grouping, we almost exclusively detected significant differences between the ss and ll and sl and ll groups, the only significant difference between ss and sl groups occurred in case of the SCL-51 obsessive compulsive subscale. This is in line with and confirms former evidence on the dominant character of the s allele [28, 39].

According to more recent research, neuroticism is 40–50% heritable and is reasonably stable across adult life. About 55% of genetic risk for major depression is shared by neuroticism [34] suggesting that there may be common genetic factors predisposing to depression, neuroticism, and anxiety, therefore several genetic studies incorporate depressive and anxiety symptoms into a single phenotype. However, both our previous [27] and present findings show that hyperthymic personality features (hyperthymic temperament as measured by TEMPS-A) are outside of the frame of the neurotic cluster and may be protective against the trait and state characteristics related to depression and anxiety. Hyperthymic temperament has been consistently shown to be orthogonal to the other affective temperaments in all TEMPS-A language versions [5]. Deeper understanding of traits, temperaments and characteristics associated with (or contrasted to) neuroticism and their genetic association thus sheds more light on the factors and processes in the background and development of neuroticism-related traits and affective and anxiety disorders.

We computed intercorrelations between our scales in our sample and we also computed the correlations of the scales we used with well accepted measures of neuroticism in order to avoid our results being distorted by carrying out comparisons with such scales that would be highly intercorrelated. Our results indicated that intercorrelation between the scales was moderate. Not taking into consideration the correlations between the subscales within one scale, and within different scales measuring the same trait or symptom (e.g. different anxiety subscales) we observed the highest correlations between the STAI trait anxiety and ZSDS ($r = 0.74$). Similarly, correlations of our scales with the neuroticism scales were moderate, the highest correlation was obtained in case of the STAI trait anxiety scale ($r = 0.644$). These data indicate that our results are not artifacts resulting from a high correlation between our scales and the neuroticism trait. It is worth mentioning, however, that the questionnaires whose correlations with neuroticism are 0.4 or higher associate with the serotonin transporter genotype, whereas scales correlating less than 0.4 show virtually show no association with the transporter genotype. This indicates that although our results are not due to measuring the same neuroticism construct under several names, all the scales showing association with the s allele are related to neuroticism and highlight one aspect of it. This relationship between the correlations with the Neuroticism scales and the association with the s allele is also apparent in case of the TCI scales, where 5-HTTLPR is only associated with the self-directedness scale, which is

the only scale within the TCI which has a correlation with neuroticism above 0.4. This is also an interesting new finding which contrasts earlier results stating that among the TCI scales harm avoidance has the highest correlation with Neuroticism [17]. Both our correlation data and the fact that only Self Directedness but not harm avoidance is associated with the s allele argue against this.

Besides confirming the association of the 5-HTTLPR polymorphism of the serotonin transporter gene with such components of neuroticism as increased depression and anxiety as well as affective temperaments, our study has yielded several new results. We established that 5-HTTLPR is related to subthreshold symptoms related to neurotic disorders, hopelessness, such aggressive characteristics as guilt, hostility and global aggression, and the self-directedness dimension in the Cloninger personality model. Besides the confirmation of our previous results and our several important new findings, a major result of our study is that we managed to confirm an association between the 5-HTTLPR polymorphism of the serotonin transporter gene and neuroticism by showing that all major components related to neuroticism are also independently and on their own associated with this polymorphism and thus established that neuroticism can be considered a unified construct not only symptomatically, but also from a phenotypical and genetic point of view, keeping in mind that neuroticism has a polygenic and multifactorial background, with the environment, other genes, and also the interactions of these playing a role in its development and manifestation.

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