Abstract

Background: Increasing evidence supports the notion of a continuum between affective temperaments and major mood disorders, suggesting that these temperament types represent the subclinical manifestations of affective disorders and often present an increased vulnerability for these diseases.

Methods: The Hungarian rendition of the full-scale 110-item version of the TEMPS-A questionnaire and 5HTTLPR genotype was investigated in a sample of 139 unrelated Caucasian females with no current or lifetime Axis I psychiatric disorders.

Results: A significant association was found between the s allele and the TEMPS scores of the depressive, anxious, irritable, and particularly the cyclothymic temperaments; no such association emerged with respect to the hyperthymic temperament.

Limitation: The database is entirely female. Given that the hyperthymic type predominates in males, our results could have been different if men were included in our sample.

Conclusions: Our results are in good agreement with earlier studies reporting a strong association between the s allele of the 5HTTLPR and major as well as subthreshold forms of depression, and extend this association to the normative temperament level. Indeed, these temperaments might best be regarded as proximate behavioural endophenotypes. Our data raise the provocative possibility that the genetic potential for mood episodes lies in these temperaments. Further studies are needed to delineate the role of gender in the associations under consideration, as well as to investigate the genetic background of the hyperthymia–mania part of the affective spectrum. Given that affective temperaments are widely distributed in the general population, the strategy employed by us is of potential public health significance in terms of detecting individuals in the community at risk for affective spectrum disorders.

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Keywords: Depression; Serotonin transporter; Affective temperaments; TEMPS-A; Endophenotype; Genetics
1. Introduction

In support of the clinical view of Kraepelin (1921) and Kretschmer (1936), recent clinical follow-up investigations as well as familial–genetic, biological, and treatment–response studies have demonstrated that there is a continuum between Cyclothymia–Bipolar II–Bipolar I disorder (Akiskal et al., 1977; Akiskal et al., 1995; Kochman et al., 2005), and the same is true for the subsyndromal depression–minor depression/dysthymia–unipolar major depression spectrum (Akiskal et al., 1978; Akiskal et al., 1980; Rihmer et al., 1983; Rihmer and Szádóczky, 1993; Rihmer, 1990, 1999; Judd and Akiskal, 2000; Lewinsohn et al., 2003). The results also suggest that the specific different affective temperament-types (hyperthymic, cyclothymic, depressive, irritable, anxious) are the subaffective/embryonic (trait-related) manifestations and frequently the precursors of the major depressive and bipolar and unipolar mood disorders (Akiskal and Akiskal, 1992; Akiskal, 1995, 1996; Akiskal and Pinto, 1999; Kochman et al., 2005).

There is plausible evidence that central serotonergic function is dysregulated in a substantial part of patients with unipolar and bipolar major depressive episode (Goodwin and Jamison, 1990; Den Boer et al., 2001), and recently a significant association between the s allele of the serotonin transporter gene and unipolar and bipolar major depression has been also reported by some (Bellivier et al., 1998; Hauser et al., 2003; Caspi et al., 2003; Lotrich and Pollock, 2004), but not all (Willis-Owen et al., 2005) authors.

Most recently, we found a significant connection between the s allele of the serotonin transporter gene and subthreshold depressive symptoms in 128 females without any lifetime DSM-IV Axis I diagnoses (Gonda et al., 2005), providing support of a subthreshold–minor–major depression continuum (Akiskal, 1994; Judd and Akiskal, 2000; Rihmer, 1990) from a molecular genetic point of view.

Considering the familial (possibly genetic) basis for the hyperthymic and cyclothymic temperaments in the genesis of bipolar disorder (Kraepelin, 1921; Chiaroni et al., 2005; Evans et al., 2005; Kesebir et al., 2005), and postulating the same for the unipolar depressive spectrum (Akiskal, 1994), the aim of our present study was to examine whether the genetic vulnerability for depression, as reflected in the serotonin transporter gene 5HTTLPR polymorphism, could be retraced through the major–minor–subthreshold continuum of depression all the way to the temperamental level.

2. Method

2.1. Subjects

139 unrelated females of Caucasian origin participated in the study. The age of the participants was 18–62 years, the mean age was 31.39±1.0279 years. All subjects went through thorough neurological and psychiatric screening. Subjects with any neurological and current or lifetime Axis I psychiatric disorders according to the DSM-IV (American Psychiatric Association, 1994) criteria were excluded. The study protocol was approved by the local ethics committee for experimentation on humans and every subject gave informed consent before participating in the study. All subjects completed the Hungarian version (Rózsa et al., in press) of the original, 110-item TEMPS-A questionnaire (Akiskal and Akiskal, 2005) and were genotyped for the 5HTTLPR polymorphism.

2.2. Genotyping

Polymerase chain reaction (PCR) amplification of 5HTTLPR was performed on genomic DNA extracted from white blood cells. The 5HTTLPR genotypes were identified as previously reported (Heils et al., 1996; Juhasz et al., 2003a,b). Primers for 5HTTLPR were 5′-GGCGTTGCCCCCTCTGATGC-3′ (STPR5) and 5′-GGAGGAGCTGAGTGGACACCAC-3′ (STPR3).

Table 1

<table>
<thead>
<tr>
<th>Subscale</th>
<th>SS effect</th>
<th>df effect</th>
<th>MS effect</th>
<th>SS error</th>
<th>df error</th>
<th>MS error</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depressive</td>
<td>37.9482</td>
<td>1</td>
<td>37.9482</td>
<td>1305.879</td>
<td>137</td>
<td>9.53196</td>
<td>3.981157</td>
<td>0.0480*</td>
</tr>
<tr>
<td>Cyclothymic</td>
<td>72.8207</td>
<td>1</td>
<td>72.8207</td>
<td>2073.784</td>
<td>137</td>
<td>15.13711</td>
<td>4.810742</td>
<td>0.0299*</td>
</tr>
<tr>
<td>Hyperthymic</td>
<td>5.3081</td>
<td>1</td>
<td>5.3081</td>
<td>2202.519</td>
<td>137</td>
<td>16.07678</td>
<td>0.330172</td>
<td>0.5665</td>
</tr>
<tr>
<td>Irritable</td>
<td>46.3714</td>
<td>1</td>
<td>46.3714</td>
<td>1586.578</td>
<td>137</td>
<td>11.58086</td>
<td>4.004139</td>
<td>0.0474*</td>
</tr>
<tr>
<td>Anxious</td>
<td>103.5899</td>
<td>1</td>
<td>103.5899</td>
<td>3583.115</td>
<td>137</td>
<td>26.15413</td>
<td>3.96074</td>
<td>0.0486*</td>
</tr>
</tbody>
</table>

Significant effects are denoted by *. 

This primer pair amplifies two types of fragments: a 484 bp short or a 528 bp long allele. PCR was performed in a volume of 25 μL containing 50 ng of genomic template, 0.1 μM of each primer, 2.5 mM deoxyribonucleotides, 10 mM tris–HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl2, 0.01% bovine albumin, and 1 U of Taq DNA polymerase (Eurogenetech). The cycling conditions were as follows: (1) initial denaturation at 94 °C for 5 min; (2) 35 cycles of amplification: denaturation at 95 °C for 30 s, annealing at 65 °C for 30 s, and synthesis at 72 °C for 60 s; (3) final extension at 72 °C for 10 min. 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. Fragment sizes were determined by comparison with molecular length standards (100 bp ladder, Invitrogen).

2.3. Measures

The temperament measure (TEMPS-A) is a new instrument. The original clinical version was developed in Memphis (Akiskal and Mallya, 1987; Akiskal and Akiskal, 1992) and validated in Interview form (TEMPS-I) in Pisa (Akiskal et al., 1998), and ultimately in Autoquestionnaire version, The Temperament Evaluation of Memphis, Pisa, Paris and San Diego (TEMPS-A [Akiskal and Akiskal, 2005]). In the English version, it exists in longer clinical (Akiskal et al., 2005a) and shorter research (Akiskal et al., 2005b) formats. The current Hungarian version is the complete, unabridged TEMPS-A (Rózsa et al., in press). The questionnaire measures affective temperaments in five scales: depressive, cyclothymic, hyperthymic, irritable and anxious.

2.4. Statistical analyses

All statistical analyses were carried out using Statistica 6.0 for Windows. Analysis of variance was used to determine the difference of the test scores between the three different genotype groups and the two phenotype groups. Honest Significant Distance Tests (Tukey HSD) were used for post hoc comparisons. 0.05 was accepted as the level of significance. Mean ± SEM of the data are presented.

3. Results

Frequency of the s allele in our sample was 38.1%, which corresponds to the results of previous studies and is representative of the Caucasian population (Lesch et al., 1996; Juhasz et al., 2003a,b). The distribution of genotypes in our study population followed the Hardy–Weinberg equilibrium ($\chi^2 = 0.0089$, $p > 0.95$).

To identify the effect of the 5HTTLPR polymorphism on affective temperaments, we analysed our sample in two different ways. First, we compared the scores of the two possible phenotypes, that is, subjects carrying the s allele (i.e., ss and sl subjects) and subjects not carrying the s allele (ll subjects). The rationale for this comparison is that in some studies the two alleles are considered to have a dominant–recessive type of relationship with the s allele being dominant (Heils et al., 1996). Then, as a second step, we compared the scores of the three genotype groups (ss, sl and ll) as well.

<table>
<thead>
<tr>
<th>S allele</th>
<th>Mean score (±SE)</th>
<th>Depressive</th>
<th>Cyclothymic</th>
<th>Hyperthymic</th>
<th>Irritable</th>
<th>Anxious</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects without s allele</td>
<td>52</td>
<td>6.1730±0.3682</td>
<td>4.5961±0.4751</td>
<td>10.4038±0.6154</td>
<td>3.4615±0.4258</td>
<td>6.3654±0.6585</td>
</tr>
<tr>
<td>Subjects with s allele</td>
<td>87</td>
<td>7.2529±0.3557</td>
<td>a 6.0920±0.4440</td>
<td>a 10.0000±0.4001</td>
<td>a 4.6552±0.3844</td>
<td>a 8.1494±0.5702</td>
</tr>
</tbody>
</table>

* Higher score compared to subjects not carrying the s allele, $p < 0.05$.

Table 3

<table>
<thead>
<tr>
<th></th>
<th>SS effect</th>
<th>df effect</th>
<th>MS effect</th>
<th>SS error</th>
<th>df error</th>
<th>MS error</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depressive</td>
<td>23.9120</td>
<td>2</td>
<td>11.9560</td>
<td>1319.915</td>
<td>136</td>
<td>9.7053</td>
<td>1.2320</td>
<td>0.2950</td>
</tr>
<tr>
<td>Cyclothymic</td>
<td>116.1506</td>
<td>2</td>
<td>58.0753</td>
<td>2030.454</td>
<td>136</td>
<td>14.9298</td>
<td>3.8899</td>
<td>0.0228*</td>
</tr>
<tr>
<td>Hyperthymic</td>
<td>8.8509</td>
<td>2</td>
<td>4.4254</td>
<td>2198.976</td>
<td>136</td>
<td>16.1689</td>
<td>0.2737</td>
<td>0.7610</td>
</tr>
<tr>
<td>Irritable</td>
<td>61.9505</td>
<td>2</td>
<td>30.9752</td>
<td>1570.999</td>
<td>136</td>
<td>11.5515</td>
<td>2.6815</td>
<td>0.0721</td>
</tr>
<tr>
<td>Anxious</td>
<td>89.4552</td>
<td>2</td>
<td>44.7277</td>
<td>3597.250</td>
<td>136</td>
<td>26.4503</td>
<td>1.6910</td>
<td>0.1882</td>
</tr>
</tbody>
</table>

Significant effects are denoted by *.

We found a significant association between the phenotype groups and the TEMPS scores in case of four of the five affective temperaments studied (Tables 1 and 2). Subjects carrying the s allele scored significantly higher in all four of these temperaments (depressed, cyclothymic, irritable, anxious) (Tables 1 and 2). No significant difference between the two phenotype groups was found for the hyperthymic temperament (Tables 1 and 2).

When the 3 genotype groups were compared, a significant difference between the 3 groups emerged on the scale measuring the cyclothymic temperament (Tables 3 and 4). Post hoc HSD test revealed that there is a significant difference between the ss and sl groups \( (p = 0.0080) \). A trend \( (p = 0.0721) \) was observed in case of the irritable temperament, with post hoc HSD test indicating that subjects with sl genotype had a significantly higher score than subjects with ll genotype \( (p = 0.0227) \).

### 4. Discussion

#### 4.1. 5HTTLPR polymorphism and affective temperaments conceived as behavioural endophenotypes

The 5HTTLPR is a well-described functional polymorphism, with its position and function well-known and widely investigated. Earlier studies both in vivo and in vitro concluded that this polymorphism has specific effects on the function of the 5HT transporter protein. Lesch et al. (1996) found that the basal activity of the long variant of the gene promoter is more than twice as that of the short variant of the promoter. Similarly, the uptake of serotonin in cells homozygous for the 1 form of the promoter polymorphism was approximately two times that in cells carrying 1 or 2 copies of the s variant. Furthermore, the activity of the ss and sl variants appears to be similar and similarly different from the ll variant, which suggests that the polymorphism has a dominant–recessive effect (Lesch et al., 1996). Our Hungarian group has earlier found that the 5HTTLPR genotype has a significant effect on platelet 5HT concentration in a healthy population and also in migraineurs in a headache free period (Juhasz et al., 2003a). The validity of the human study is further supported by the fact that the correlation was described in a well-defined sample where the platelet 5HT concentration was measured in the same, namely mid-follicular phase of the menstrual cycle in case of all subjects.

In line with earlier reports on the strong association between the s allele of the 5HTTLPR polymorphism of the serotonin transporter gene and major depression (Bellivier et al., 1998; Hauser et al., 2003; Lotrich and Pollock, 2004), as well as subthreshold depression (Gonda et al., 2005), our present findings suggest the same connection regarding the depressive component of personality even at the normative temperament level. The increased vulnerability for depression after stressful life events among people carrying the s allele has also been demonstrated in a prospective-longitudinal study (Caspi et al., 2003). Overall these data and considerations suggest that the affective temperaments under investigation are best conceived as behavioural endophenotypes. For further discussion on this conceptual framework the reader is referred to Akiskal (1995), Niculescu and Akiskal (2001) and Kelsoe (2003).

The fact that all but the hyperthymic type predominates in males (see, for instance, Placidi et al., 1998) and Akiskal et al. (2005a) – have shown that mood-labile depressive (and possibly irritable), temperaments aggregate into a “super factor” of cyclothymia which is distinct from the hyperthymic “super factor”. Whenever the anxious subscale of TEMPS-A was studied, its cognitive subscale strongly correlated with that of the depressive (Erfurth et al., 2005; Vahip et al., 2005; Karam et al., 2005). In other words, psychometric aggregation of temperaments (the depressive, anxious, irritable and cyclothymic) finds molecular genetic support in their association with the s allele of 5HTTLPR.

We wish to point out, nonetheless, a possible limitation of our sample: given that the hyperthymic type predominates in males (see, for instance, Placidi et al., 1998) – and our analyses used a database which is

### Table 4

TEMPS-A scores according to genotype: subscale scores of subjects within the three different genotype groups (ss, sl and ll)

<table>
<thead>
<tr>
<th>SHTT</th>
<th>Mean score (±SE)</th>
<th>N</th>
<th>Depressive</th>
<th>Cyclothymic</th>
<th>Hyperthymic</th>
<th>Irritable</th>
<th>Anxious</th>
</tr>
</thead>
<tbody>
<tr>
<td>sl</td>
<td></td>
<td>66</td>
<td>7.1818±0.4269</td>
<td>6.4848±0.5325</td>
<td>10.0601±0.4747</td>
<td>4.8788±0.4527</td>
<td>7.9091±0.6686</td>
</tr>
<tr>
<td>ll</td>
<td></td>
<td>53</td>
<td>6.3208±0.3902</td>
<td>4.5660±0.4670</td>
<td>10.4340±0.6045</td>
<td>3.4340±0.4186</td>
<td>6.5094±0.6618</td>
</tr>
<tr>
<td>ss</td>
<td></td>
<td>20</td>
<td>7.1500±0.5585</td>
<td>4.9500±0.7486</td>
<td>9.7000±0.7715</td>
<td>4.0500±0.7415</td>
<td>8.6500±1.1151</td>
</tr>
</tbody>
</table>

* Higher score compared to subjects with ll genotype, \( p < 0.05 \).
entirely female – our results could have been different if men had been included in our sample. On the other hand, our data cohere with earlier studies which, with one exception, have related the s allele to neuroticism (Deary et al., 1999; Flory et al., 2005; Munafò et al., 2005; Willis-Owen et al., 2005). We submit that our data are more cogent, because “neuroticism” is a global construct which subsumes, among others, such traits as anxiousness, depressiveness, and mood lability (Eysenck, 1987). The TEMPS-A scales more specifically and individually measure each of the foregoing trait dimensions. This new temperament measure – particularly its anxious subscale – has the potential to identify specific cognitive traits which distinguish healthy relatives of affected probands from general population controls unselected for affective illness familiarity (Evans et al., 2005). Moreover, in the latter San Diego study, the “hyperthymic” type stood apart from other affective temperaments as possibly “protective” against affective breakdowns.

4.2. Future perspectives

The findings of the present study – within the overall thrust of the reviewed data on psychometric studies of affective temperaments and quantitative trait-genetic and molecular genetic measures – are encouraging in showing a reasonable degree of concordance. The present analyses included the full-scale of anxious traits. As already mentioned earlier in this discussion, previous work has shown that the cognitive subscale of the anxious cluster correlates highly with the depressive type (Erfurth et al., 2005; Vahip et al., 2005; Karam et al., 2005); a somatic anxious subscale has emerged as distinct. Future studies should examine the specificity of the newly derived anxious–depressive scale with respect to the s allele of this polymorphism. Further refinements in the other affective temperament measures, and 5HTTLPR analyses based on subsets of affective traits rather than full-scale individual temperaments, might yield more specific characterization of traits that increase vulnerability to clinical episodes.

Research is also needed to characterize the possible genetic components of the hyperthymia–mania trait–state continuum. Some human personality traits, such as reward dependence and novelty seeking, have been found to be significantly related to hyperthymic and cyclothymic traits (Akiskal et al., 2005a,b; Maremmani et al., 2005). Since it has been reported that reward dependence and novelty seeking were connected to chromosomal loci encoding for the serotonin 2C receptor and dopamine-2 or dopamine-3 receptor proteins (Ebstein et al., 1997; Staner et al., 1998), these genes could be good candidates to characterize the possible molecular genetic basis of the hyperthymia–mania spectrum. To search for the genetic background of the central dopaminergic system could be particularly promising in this respect, because it is believed that (hypo)mania is characterized by increased activity of central dopaminergic pathways (Silverstone, 1985; Goodwin and Jamison, 1990; Kujawa and Nemeroff, 2000).

5. Conclusions

Overall, our findings open the potential to identify within the community those with temperamentnal inclination to melancholy (e.g. anxious–depressive) and mood instability (cyclothymic) 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 (see 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 (see 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. Obviously, much work lies ahead toward such an ambitious objective.

Acknowledgements

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