Variants of the Serotonin Transporter Gene and NEO-PI-R Neuroticism: No Association in the BLSA and SardiNIA Samples

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The polymorphism in the serotonin transporter gene promoter region (5-HTTLPR) is by far the most studied variant hypothesized to influence Neuroticism-related personality traits. The results of previous studies have been mixed and appear moderated by the personality questionnaire used. Studies that used the TCI to assess Harm Avoidance or the EPQ to assess Neuroticism have found no association with the 5-HTTLPR. However, studies that used the NEO-PI-R or related instruments (NEO-PI, NEO-FFI) to measure Neuroticism have found some evidence of association. This study examines the association of variants in the serotonin transporter gene in a sample from a genetically isolated population within Sardinia (Italy) that is several times larger than previous samples that used the NEO-PI-R (N = 3,913). The association was also tested in a sample (N = 548) from the Baltimore Longitudinal Study of Aging (BLSA), in which repeated NEO-PI-R assessments were obtained. In the SardiNIA sample, we found no significant association of the 5-HTTLPR genotypes with Neuroticism or its facets (Anxiety, Angry-Hostility, Depression, Self-Consciousness, Impulsiveness, and Vulnerability). In the BLSA sample, we found lower scores on Neuroticism traits for the heterozygous group, which is inconsistent with previous studies. We also examined eight SNPs in the SardiNIA (N = 3,972) and nine SNPs in the BLSA (N = 1,182) that map within or near the serotonin transporter gene (SLC6A4), and found no association. Along with other large studies that used different phenotypic measures and found no association, this study substantially increases the evidence against a link between 5-HTT variants and Neuroticism-related traits.
serotonin uptake in lymphoblasts [Lesch et al., 1996]. In a sample of 505 individuals, Lesch et al. [1996] found that the NEO-PI-R Neuroticism was significantly associated with the 5-HTTLPR: individuals with the SS or SL genotypes had higher scores on Neuroticism than individuals with the LL genotype. Lesch et al. estimated that the polymorphism accounted for 3–4% of total variation and 7–9% of inherited variance of anxiety-related personality traits. Although the mechanism of action of the SSRIs is complex and do not simply fit hypotheses such as the “serotonin depletion” for depression [Lacasse and Leo, 2005], the association between the less active S allele and Neuroticism remains counterintuitive [Lesch et al., 1996; Arbelle et al., 2003; but see Anorge et al., 2004]. Given that the SSRI therapeutic action is through blocking reuptake of serotonin, one would expect the less efficient S allele (which presumably reuptakes less serotonin) to be associated with lower Neuroticism.

Furthermore, the original report was replicated in a number of later studies [Ricketts et al., 1998; Murakami et al., 1999; Greenberg et al., 2000; Sen et al., 2004b], but others failed to find an association using general population [Ball et al., 1997; Ebstein et al., 1997; Jorm et al., 1998; Herbst et al., 2000; Willis-Owen et al., 2005; Middeldorp et al., 2007; Munafo et al., 2008b] or clinical samples [Gelernter et al., 1998; Mazzanti et al., 1998], and some found an association in the opposite direction [Jorm et al., 2000; Van Gestel et al., 2002; Arbelle et al., 2003; Brummett et al., 2003]. Inconsistent findings have been attributed to the use of admixed populations, but the true impact of population stratification in the field of molecular psychiatry is questionable [Hutchison et al., 2004; Gardner et al., 2008]. Several meta-analyses have summarized the results [Sen et al., 2004a; Schinka et al., 2004; Munafo et al., 2008b], and found no association when considering the entire set of studies. However, the choice of personality scale used was a significant moderating variable: In studies that used the Neuroticism scale of the NEO-PI-R, a small (d ~ 0.2) but statistically significant effect was found. No effect was found when personality was assessed using the Harm Avoidance scale of the TCI or the Neuroticism scale of the EPQ. Another meta-analyses [Munafo et al., 2005b] reported a moderating effect in the opposite direction, but this might be due to coding errors [Munafo et al., 2003b].

The results of meta-analyses should be considered with caution because of the uncontrolled differences among studies, publication biases, unknown moderating variables, and other confounding factors. As argued by Munafo et al. [2005b] “Meta-analyses are therefore by no means perfect [. . .]. Very large, well-designed primary studies remain the most reliable way of obtaining reproduceable results.” To date, the only large studies (~4,000 subjects) well-powered to detect small genetic effect did not support the hypothesis that the 5-HTTLPR is associated with Neuroticism, as measured with the EPQ [Willis-Owen et al., 2005], or Harm Avoidance [Munafo et al., 2008b]. However, it is possible that when the phenotype is assessed with the NEO-PI-R an association between the 5-HTTLPR and Neuroticism could be found. To address this hypothesis, we examined in a large sample (~4,000 subjects) whether personality traits assessed with the NEO-PI-R are associated with the 5-HTTLPR and other variants in the serotonin transporter gene. This sample is part of the SardiNIA project [Pilia et al., 2006], a multidisciplinary study that assessed multiple traits and performed a genome-wide association scan in a homogeneous sample from a founder population. Furthermore, we tested the association of the 5-HTTLPR and other variants in a sample from the Baltimore Longitudinal Study of Aging (BLSA). In the BLSA, most subjects have been assessed with the NEO-PI-R at multiple visits. Longitudinal studies have shown that the use of multiple measures yields larger estimates of heritability compared to studies based on single report [Kendler et al., 1993; Riemann et al., 1997]. Aggregating data across multiple occasions should produce more robust results, less dependent on state-specific effects. Although the BLSA sample with 5-HTTLPR genotype is relatively small, the sample size of 548 individuals has a power higher than 0.80, at significance level $P = 0.01$ two-tailed, to detect the differences on Neuroticism reported in recent meta-analyses ($d \sim 0.2$) [Schinka et al., 2004; Sen et al., 2004a; Munafo et al., 2008b]. In addition to the 5-HTTLPR, in both samples we examine a number of single nucleotide polymorphisms (SNPs) that map in the SLC6A4 gene region for association with the Neuroticism related traits. These SNPs have not been routinely examined in previous studies and most are not in linkage disequilibrium with the 5-HTTLPR, thus providing independent association tests of other 5-HTT gene regions with Neuroticism-related traits [Strug et al., 2008].

**METHOD**

**Sample Description: SardiNIA**

We recruited 6,148 individuals, about 62% of the population aged 14–102 years, from a cluster of four towns in the Lanusei Valley [Pilia et al., 2006]. The cohort includes ~700 connected pedigree and over 30,000 relative pairs. Subjects are native-born, and at least 95% are known to have all grandparents born in the same province [Pilia et al., 2006]. Valid personality data were obtained from 5,669 subjects at their first assessment, of whom 3,913 were successfully genotyped for the 5-HTTLPR, and 3,972 were part of the genome-wide association (GWA) scan. Genotyped individuals did not differ on Neuroticism or its facets from those who were not genotyped. The sample includes 57% women with age range from 14 to 90 (Mean = 42.5, SD = 16.7), and 43% men with age range 14–94 (Mean = 42.8, SD = 17.2). More demographic information on the sample has been reported elsewhere [Pilia et al., 2006; Costa et al., 2007]. The project was approved by institutional review boards in Italy and the USA.

**Sample Description: BLSA**

We genotyped the 5-HTTLPR of 548 community-dwelling volunteers enrolled in the BLSA. This sample includes 51% women; age at first visit ranged from 20 to 87 (M = 52.9, SD = 12.5); and the ethnic composition was 86% White non-Hispanic, 11% African American, and 3% other. Nine SNPs that map within or close to the SLC6A4 gene were genotyped as part of a GWA scan in a sample of 1,182 BLSA participants with personality data. This sample includes 48% women; age at first visit ranged from 20 to 93 (M = 57.3, SD = 15.5); and the ethnic composition was 71% White non-Hispanic, 23% African American, and 6% other. Personality traits were assessed from 1989 to 2008, for a total of 4,807 assessments. The number of NEO-PI-R assessments ranged from 1 to 13 times
frequencies were 42% for the s allele and 58% for the l allele, and by ultraviolet light after ethidium bromide staining. Allele frequencies were separated by electrophoresis in a 2% agarose gel and visualized under UV transillumination. The PCR products were generated as described by Lesch et al. [1996]. The PCR system used was the MegaBACE Genetic Profiler Software v1.5 (Amersham Biosciences). PEDSTATS was used for additional quality control, and four individuals were excluded from the analyses for Mendelian incompatibilities. Allele frequencies were in Hardy-Weinberg equilibrium (P > 0.05).

**Genotyping: 5-HTTLPR**

DNA was extracted from whole blood by standard techniques. In the SardiNIA sample, the 5-HTTLPR genotypes were determined using the MegaBACE 1000 fluorescence-based genotyping methodology. Genotypes were scored using the MegaBACE Genetic Profiler Software v1.5 (Amersham Biosciences). PEDSTATS was used for additional quality control, and four individuals were excluded from the analyses for Mendelian incompatibilities. Allele frequencies were 49% for the s allele and 51% for the l allele, and the genotype frequencies were in Hardy-Weinberg equilibrium (P > 0.05).

In the BLSA sample, the 5-HTTLPR genotypes were determined by polymerase chain reaction (PCR) amplification with reaction mix and cycling conditions as described by Lesch et al. [1996]. The PCR products were separated by electrophoresis in a 2% agarose gel and visualized by ultraviolet light after ethidium bromide staining. Allele frequencies were 42% for the s allele and 58% for the l allele, and the genotype frequencies were in Hardy-Weinberg equilibrium (P > 0.05).

**Results**

**SardiNIA**

Analysis of variance controlling for age and sex found no mean level differences between the 5-HTTLPR genotypes groups (SS, SL, LL) on Neuroticism or any of the six Neuroticism facets (Table I). No evidence of association was found when the analyses were conducted grouping individuals with one or two S alleles (P > 0.05). Analyses that did not control for age and sex produced the same results, and analyses within gender indicate no association in either the men or women subsamples (see Fig. 2). Furthermore, in this sample of related individuals, we performed association test
that take advantage of the family relatedness (MERLIN) (Chen and Abecasis, 2007), but again we found no effect of the 5-HTTLPR on Neuroticism or its facets (last column of Table I).

We examined eight SNPs that map within or near the 5-HTT gene (SLC6A4). Table II presents the association results of Neuroticism and its facets with each of the eight SNPs. None of the SNPs reached statistical significance for Neuroticism or any of the six facets.

**BLSA**

Analysis of variance controlling for age and sex found significant mean level differences on Neuroticism, Anxiety, Depression, and Vulnerability between the 5-HTTLPR genotype groups in the BLSA sample (see Table III). Post hoc test indicate that the heterozygous (SL) group scored lower than the LL on Neuroticism and its facets, but there were no differences between the two homozygous groups.

**TABLE I. NEO-PI-R Neuroticism Scores in 5HTTLPR Genotypes Groups From the SardiNIA Sample**

<table>
<thead>
<tr>
<th>Personality traits</th>
<th>SS (n = 967)</th>
<th>SL (n = 1,920)</th>
<th>LL (n = 1,026)</th>
<th>d</th>
<th>MERLIN, P</th>
</tr>
</thead>
<tbody>
<tr>
<td>N: Neuroticism</td>
<td>56.5 (0.28)</td>
<td>56.4 (0.20)</td>
<td>56.4 (0.27)</td>
<td>0.01</td>
<td>0.80</td>
</tr>
<tr>
<td>N1: Anxiety</td>
<td>57.0 (0.27)</td>
<td>56.8 (0.19)</td>
<td>57.4 (0.27)</td>
<td>-0.05</td>
<td>0.20</td>
</tr>
<tr>
<td>N2: Angry hostility</td>
<td>54.1 (0.30)</td>
<td>53.9 (0.21)</td>
<td>53.6 (0.29)</td>
<td>0.04</td>
<td>0.33</td>
</tr>
<tr>
<td>N3: Depression</td>
<td>54.8 (0.30)</td>
<td>54.8 (0.21)</td>
<td>55.0 (0.29)</td>
<td>-0.03</td>
<td>0.99</td>
</tr>
<tr>
<td>N4: Self consciousness</td>
<td>52.7 (0.32)</td>
<td>52.5 (0.22)</td>
<td>52.2 (0.31)</td>
<td>0.04</td>
<td>0.30</td>
</tr>
<tr>
<td>N5: Impulsiveness</td>
<td>47.8 (0.28)</td>
<td>48.0 (0.20)</td>
<td>48.0 (0.27)</td>
<td>-0.01</td>
<td>0.29</td>
</tr>
<tr>
<td>N6: Vulnerability</td>
<td>57.1 (0.33)</td>
<td>57.2 (0.23)</td>
<td>57.7 (0.32)</td>
<td>-0.05</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Mean personality scores for the genotypes are adjusted for age and sex differences. Standard errors are reported in parentheses. The measure of effect size d, is computed as the difference in mean personality traits between genotype groups (SS and SL vs. LL) divided by the standard deviation. None of the effect was significant (P < 0.05).
Analyses that grouped the SS and SL groups found that individuals with those genotypes scored lower on Neuroticism related traits, an effect opposite to those of previous studies [Lesch et al., 1996]. The results were essentially the same when the analyses included as covariates the eigenvalues derived from principal components analysis to correct for population stratification [Price et al., 2006]. None of these weak effects in the full BLSA sample (Table IV) was confirmed when the association analyses were limited to the European-American BLSA subgroup (n = 844).

DISCUSSION

We examined whether variants in the serotonin transporter are associated with Neuroticism-related traits in a large sample from a homogeneous population and in a longitudinal study. Using multiple analytic methods we found no association of the S allele of the 5-HTTLPR with higher scores on anxiety, depression, or other Neuroticism facets. This finding is consistent with two previous large scale studies [Willis-Owen et al., 2005; Munafò et al., 2008b] that adopted different measure of Neuroticism, namely the EPQ and TCI. However, meta-analytic studies have suggested that the personality instrument used could be an influential moderator. Meta-analyses indicate that the 5-HTTLPR had an effect on Neuroticism only when the trait was assessed with the NEO-PI-R. Using the NEO-PI-R in a large homogeneous sample and in a longitudinal study, our results undermine the meta-analytic finding, and reject any substantial effect of the 5-HTTLPR on Neuroticism-related traits regardless of which questionnaire was used. Furthermore, we found no evidence of association between other SNPs within or near the 5-HTT gene and measures of Neuroticism. These SNPs are not linked to the 5-HTTLPR but are part of haplotype blocks in the gene region. The null results from our two samples suggest that the common variants we examined are unlikely to be related to Neuroticism traits. It should be noted that the SNPs we genotyped did not fully cover the SLC6A4 gene region. For example, we did not genotype the A/G variant within the 5-HTTLPR repeat region (Lg), which may influence gene expression, but is unrelated to Neuroticism [Gunthert et al., 2007; Wachleski et al., 2008].

From the first report of a main effect of the 5-HTTLPR on anxiety-related traits, a large number of studies have explored the association of this variant in clinical samples, in neuroimaging studies, and in gene–gene and gene–environment interaction studies. Most of these studies are based on small samples, and the remained significant after Bonferroni correction for multiple tests. The results were essentially the same when the analyses included as covariates the eigenvalues derived from principal components analysis to correct for population stratification [Price et al., 2006]. None of these weak effects in the full BLSA sample (Table IV) was confirmed when the association analyses were limited to the European-American BLSA subgroup (n = 844).

**FIG. 2. Mean Neuroticism score for the 5-HTTLPR genotypes in men and women. 5-HTTLPR = 5-hydroxytryptamine-linked polymorphic region. Mean Neuroticism scores are adjusted for age differences.**

We examined nine SNPs that map within or near the 5-HTT gene (SLC6A4). Table IV presents the association results of Neuroticism and its facets with each of the nine SNPs. As shown on Table IV, there are some associations for two SNPs (rs8071667 and rs140700) with Neuroticism and some facets, but none of these effects remained significant after Bonferroni correction for multiple tests. The results were essentially the same when the analyses included as covariates the eigenvalues derived from principal components analysis to correct for population stratification [Price et al., 2006]. None of these weak effects in the full BLSA sample (Table IV) was confirmed when the association analyses were limited to the European-American BLSA subgroup (n = 844).

**TABLE II. Association of Neuroticism Traits With SNPs in the 5-HTT Gene [SLC6A4] Region in the Sardinia Sample**

<table>
<thead>
<tr>
<th>SNPs</th>
<th>Position</th>
<th>Gene</th>
<th>N (P)</th>
<th>N1 (P)</th>
<th>N2 (P)</th>
<th>N3 (P)</th>
<th>N4 (P)</th>
<th>N5 (P)</th>
<th>N6 (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1906451</td>
<td>25539605</td>
<td>CCDC55</td>
<td>0.68</td>
<td>0.84</td>
<td>0.33</td>
<td>0.86</td>
<td>0.51</td>
<td>0.10</td>
<td>0.43</td>
</tr>
<tr>
<td>rs4325622</td>
<td>25550601</td>
<td>SLC6A4</td>
<td>0.71</td>
<td>0.83</td>
<td>0.34</td>
<td>0.89</td>
<td>0.57</td>
<td>0.10</td>
<td>0.46</td>
</tr>
<tr>
<td>rs8076005</td>
<td>25571336</td>
<td>SLC6A4</td>
<td>0.33</td>
<td>0.35</td>
<td>0.44</td>
<td>0.77</td>
<td>0.55</td>
<td>0.77</td>
<td>0.27</td>
</tr>
<tr>
<td>rs11080122</td>
<td>25571461</td>
<td>SLC6A4</td>
<td>0.30</td>
<td>0.30</td>
<td>0.43</td>
<td>0.71</td>
<td>0.79</td>
<td>0.50</td>
<td>0.27</td>
</tr>
<tr>
<td>rs2020939</td>
<td>25574858</td>
<td>SLC6A4</td>
<td>0.86</td>
<td>0.53</td>
<td>0.39</td>
<td>0.85</td>
<td>0.99</td>
<td>0.15</td>
<td>0.58</td>
</tr>
<tr>
<td>rs2020936</td>
<td>25574940</td>
<td>SLC6A4</td>
<td>0.33</td>
<td>0.29</td>
<td>0.50</td>
<td>0.75</td>
<td>0.52</td>
<td>0.81</td>
<td>0.30</td>
</tr>
<tr>
<td>rs1487971</td>
<td>25596879</td>
<td>—</td>
<td>0.67</td>
<td>0.15</td>
<td>0.05</td>
<td>0.76</td>
<td>0.86</td>
<td>0.08</td>
<td>0.76</td>
</tr>
<tr>
<td>rs7223821</td>
<td>25603446</td>
<td>BLMH</td>
<td>0.91</td>
<td>0.20</td>
<td>0.14</td>
<td>0.62</td>
<td>0.75</td>
<td>0.13</td>
<td>0.61</td>
</tr>
</tbody>
</table>

n = 3,972. N, Neuroticism; N1, anxiety; N2, angry hostility; N3, depression; N4, self consciousness; N5, impulsiveness; N6, vulnerability.
The results from this literature are generally mixed [Munafo et al., 2008a]. Considering the results of the current and two other large scale studies [Willis-Owen et al., 2005; Munafo et al., 2008b] that reject a main effect of the 5-HTTLPR on Neuroticism-related phenotypes, even greater caution is required in interpreting the results of studies with small sample sizes that examined complex phenotypes (e.g., amygdala reactivity or response to SSRIs) or complex interactions.

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