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The serotonin transporter gene and peer-rated neuroticism

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POLYMORPHISMS in the serotonin transporter gene (5HTT) have been reported to be associated with neuroticism (emotionality) and with depression. A recent report of an association between 5HTT and neuroticism involved unselected samples and self-report questionnaires.[1] We attempted to extend these findings using a selected extremes design and peer ratings. From a sample of 2085 individuals, each assessed on neuroticism by two independent peers, we selected 52 individuals from the top 5% and 54 individuals from the bottom 5%. No association was found for either a functional 44 bp insertion/deletion polymorphism in 5HTT regulatory sequence (5HTTLPR) or for a non-functional variable number tandem repeat 5HTT polymorphism.

Key words: Depression; Neuroticism; Polymorphism; Risk factors; Serotonin transporter

Introduction

There is increasing interest in using candidate gene allelic association strategies to identify quantitative trait loci (QTLs) for quantitative dimensions as well as qualitative disorders.[2,3] QTLs are genes of varying effect size in multiple gene systems that contribute additively and interchangeably to a quantitative trait. A QTL perspective raises the possibility that there may be no genes for disorders *per se* – the same genetic factors may link the normal and the abnormal. This implies that the genetic origins of the extreme ends of dimensions of variation may be quantitatively, not qualitatively, different from the rest of the distribution.

For psychiatric disorders, especially common disorders such as anxiety and depression, the QTL perspective suggests that genes for psychopathology can be found by looking for genes for personality. A watershed for QTL research in this area was the simultaneous publication of two reports showing significant allelic association between the personality trait of novelty seeking and a functional polymorphism in the dopamine D4 receptor (DRD4).[4,5] In samples of unselected individuals using self-report questionnaires, individuals with longer repeats (6–8 repeats) had higher novelty seeking scores than individuals with shorter repeats (2–5 repeats). This led to the hypothesis that disorders related to the dimension of novelty seeking would also show an association with the long-repeat alleles of DRD4, an hypothesis confirmed for attention deficit-hyperactivity disorder,[6] opioid dependence,[7] Tourette syndrome,[8] and with the short-repeat alleles for depression.[9]

One of the groups who first reported the association between DRD4 and novelty seeking has recently reported that a functional polymorphism in regulatory sequence for the serotonin transporter gene (5HTT) is associated with the personality trait of neuroticism as well as symptoms of anxiety and depression using a self-report questionnaire.[1] The 5-HTT-linked promoter region (5-HTTLPR) has a 44 bp insertion/deletion polymorphism: the short-form allele (i.e. the deletion) reduces transcriptional efficiency of the promoter and results in decreased serotonin transporter expression.

In two samples, individuals with one or two copies of the short-form allele had higher neuroticism, anxiety and depression scores than individuals homozygous for the long-form allele. In addition, a within-family analysis of sibling pairs discordant for 5-HTTLPR genotype confirmed the association in that the sibling with the short-form allele had higher neuroticism scores than their siblings homozygous for the long-form allele. 5-HTTLPR was reported to explain about 4% of the total variance in these personality traits and was not associated with other major domains of personality.

Because neuroticism is thought to represent a genetic vulnerability to depressive disorder,[10] it is reasonable to hypothesize that 5-HTT is also associated with depressive disorder. A recent report supports this hypothesis. The short 5-HTTLPR allele was significantly more prevalent in a combined group of patients with unipolar and bipolar disease than in controls.[11]

A nonfunctional variable number tandem repeat (VNTR) polymorphism in intron 2 of the serotonin transporter gene was also associated with susceptibility to major depression in a Scottish sample.[12] Perhaps because the sample was small and the associated allele involving nine repeats was rare, the association was not replicated in subsequent reports from Germany[13] and Japan.[14] Two other large studies including unipolar and bipolar depression patients also failed to replicate the 9-repeat association for unipolar depression, although both reported an increased frequency of the 12-repeat allele among patients with bipolar disease.[15,16]

The aim of the present study was to follow up on these QTL results, extending the research in two novel directions. First, we focused on selected extremes of neuroticism rather than unselected samples. From a sample of 2085 individuals, we selected 52 individuals with the highest neuroticism scores and 54 individuals with the lowest neuroticism scores (only one member of a twin pair) from the top and bottom 5% of the distribution, approximately 2 s.d. above and below the mean. It can be shown that this selected extremes design provides power comparable to genotyping an unselected sample of 500 or more individuals depending on allelic frequency and mode of transmission. The power and efficiency of a selected extremes design for allelic association is analogous to the advantages of QTL linkage designs that select high and low extreme concordant and discordant members of sibling pairs.[17]

Second, rather than relying on self-report questionnaires alone, each individual was also rated by two independent peers. Combining the ratings by the two peers provides a more reliable and more objective assessment of neuroticism. A recent twin analysis of this sample showed that peer reports are highly heritable.[18]

We hypothesized that the power of the selected extremes QTL design and the high heritability and objectivity of peer reports would provide even stronger associations between the serotonin transporter gene and neuroticism than the association reported by Lesch *et al.*[1]

Subjects and Methods

Subjects were 2085 adult German twins between 14 and 80 years old (mean = 33.0 ± 13.4 years) participating in a twin study of personality. Among the personality inventories administered was the German version of the NEO-FFI,[19,20] which includes a measure of neuroticism. A peer report version of the NEO-FFI was created by changing the first-person form of the self-report version to the third-person form. Subjects were instructed to complete the self-report version of the NEO-FFI and to give the peer report versions to two peers who knew them very well. Subjects returned self-reports and peer-reports by mail; the latter were sealed by the peers in separate envelopes. Peers were mostly friends (62%), relatives (16%), spouses (10%) and colleagues (9%) who had known the participants for 11.1 years on average (s.d. 10.5 years). Most of the peers (82%) judged their acquaintance with the target person as 'very good' or 'good'. Very few (1%) indicated that they had 'little' or 'very little' knowledge about the target person.

Internal consistency of the neuroticism scale was 0.85 for self-ratings and 0.85 for peer ratings. The Spearman–Brown corrected correlation between the two peers' ratings was 0.63 and the correlation between self-ratings and peer-ratings was 0.55. High and low neuroticism subjects were selected from the top and bottom 5% of the distribution of neuroticism scores created by combining the ratings of the two peers.

DNA was obtained by cheek scrapings sent through the post using a procedure described else-

Table 1. Allelic and genotypic frequencies for two serotonin transporter markers in high and low neuroticism groups

Group	Allelic frequency				Genotypic frequency			
5HTTLPR	S	L			SS	SL	LL	
High neuroticism	0.365	0.635			0.077	0.577	0.346	
Low neuroticism	0.380	0.620			0.185	0.389	0.426	
5HTT VNTR	9	10	12	9–10	9–12	10–10	10–12	12–12
High neuroticism	0.000	0.412	0.588	0.000	0.000	0.157	0.510	0.333
Low neuroticism	0.020	0.363	0.618	0.020	0.020	0.157	0.392	0.412

5HTTLPR, serotonin transporter gene-linked promoter region; 5HTT VNTR, serotonin transporter gene variable number tandom repeat; S, short-form allele; L, long-form allele.

where.[21] The average DNA yield was 38 μg, although the range was wide, from 3.2 μg to 108 μg, and three samples contained no detectable DNA.

The 5HTTLPR marker was genotyped following the procedures described by Lesch *et al.*[1] The VNTR marker was analysed using the polymerase chain reaction method described by Ogilvie *et al.*[12] The fragments were separated by agarose gel electrophoresis using 4% gels and visualized by ethidium bromide staining.

Allelic and genotypic distributions were analysed using the χ^2 statistic.

Results

Allelic and genotypic distributions for the two 5HTT markers for the group selected for high neuroticism and the group selected for low neuroticism are shown in Table 1. We tested the hypothesis that the short-form allele of the 5HTTLPR polymorphism is more prevalent in the high neuroticism group. No allelic association was found. The allelic frequency of the short-form (S) allele was 36.5% in the high neuroticism group and 38.0% in the low neuroticism group ($\chi^2 = 0.1$, df = 1, $p = 0.83$). The genotypic frequency differences approached statistical significance ($\chi^2 = 4.7$, df = 2, $p = 0.09$) but for an odd reason: although the heterozygote (SL) frequencies were in the expected direction for the high and low groups (57.7% *vs* 38.9%), with more S alleles in the high neuroticism group, the homozygote (SS) frequencies were in the opposite direction (7.7% *vs* 18.5%), with more S alleles in the low neuroticism group. Considering genotypes with the short allele (SS + SL) or without the short allele (LL), there was a weak trend in the direction reported by Lesch *et al*[1] (65.4% *vs* 57.4%) but this was far from statistical significance ($\chi^2 = 0.7$, df = 1, $p = 0.40$).

As 29 individuals in the high peer-rated neuroticism group were also in the top 10% on self-reported neuroticism and 21 individuals in the low peer-rated neuroticism group were also in the bottom 10% on self-reported neuroticism, these two subgroups were analysed separately. Again, no association was found. S frequencies were 30% in the high self-reported neuroticism group and 40% in the low self-reported neuroticism group. Furthermore, the results did not differ when males and females were considered separately.

We also tested the hypothesis that neuroticism is associated with greater prevalence of the 12-repeat allele of the VNTR polymorphism in the 5HTT gene, which appears to be associated with bipolar depression.[15,16] As reported by others,[16] the VNTR polymorphism shows significant linkage disequilibrium with the 5HTTLPR polymorphism ($\chi^2 = 16.4$, df = 1, $p < 0.0001$). However, the VNTR marker is not redundant with the 5HTTLPR marker. For example, although 12 of 14 SS individuals had 12–12 genotypes, 16 of 52 LS individuals also had 12–12 genotypes.

For the VNTR polymorphism, the allelic frequency of the 12-repeat allele was 58.8% in the high neuroticism group and 61.8% in the low neuroticism group ($\chi^2 = 2.4$, df = 2, $p = 0.30$; Table 1). The genotypic frequencies for the two groups did not differ significantly ($\chi^2 = 3.2$, df = 4, $p = 0.52$). Similar results were obtained for those individuals high and low on self-reported neuroticism and for males and females.

Discussion

The results of our selected extremes design using peer ratings of neuroticism do not support the recent report of an association between a functional polymorphism in 5HTTLPR and neuroticism.[1] Furthermore, an intronic VNTR polymorphism in 5HTT, which is in linkage disequilibrium with 5HTTLPR and has been reported to be associated with depression,[11] also shows no significant allelic or genotypic association with neuroticism in our sample.

The present study is the first QTL research of personality to employ peer ratings rather than self-report ratings, which increases the objectivity of personality assessment.[18] It is unlikely that differences between our results and the recent report of an association between self-reported neuroticism and 5HTTLPR is due to our use of peer ratings because similar results emerged when we focused on those subjects who were high and low on self-reported neuroticism.

The previous report of an association between 5HTTLPR and neuroticism, similar to other reports of QTL associations with personality,[4,5] was based on unselected samples of a few hundred individuals. The present study was the first to use a selected extremes design. From a sample of 2085, individuals were selected from the top and bottom 5% of the distribution (~±2 s.d). As noted earlier, depending on allelic frequency and mode of transmission, this selected extremes design with about 50 subjects in the high and low groups selected from a sample of 2000 individuals provides power comparable to genotyping an unselected sample of 500 or more individuals. However, the validity of the selected extremes design depends on the fundamental QTL assumption that the high extreme of a quantitative trait represents the quantitative extreme of QTLs that contribute to variation throughout the distribution. If this assumption is not correct, this could be responsible for differences between our results and the previous report of an

association with 5HTTLPR which used an unselected sample. That is, these differing results could be reconciled if 5HTTLPR is associated with the normal distribution of an unselected sample but not with the extremes of the distribution. In other words, different genes may be associated with variation in the normal range and with the extremes of these dimensions. For example, one might hypothesize that there is a greater contribution of genetic factors to individual differences in the normal range than to high or low group membership. In order to explore this issue, we used a quantitative genetic technique called DF extremes analysis,[22,23] which addresses this issue by assessing heritability of the high and low extremes and comparing it to the heritability of individual differences throughout the distribution. DF extremes analysis indicated that the high and low extremes (with 5% or 10% cut-offs) were at least as heritable as individual differences throughout the distribution. In other words, this analysis suggests that the same level of genetic influences are involved both in individual differences and extreme high and low group membership. However, this quantitative genetic evidence is only suggestive and the definitive test will come from large-scale studies that can directly compare associations in the normal range and extremes.

Nonetheless, even though the heritabilities of the high and low extremes of neuroticism appear to be similar to the heritability of individual differences in an unselected sample, it remains a possibility that a particular gene such as 5HTTLPR might be associated with normal variation but not with extremes. We cannot test this hypothesis directly because we have DNA only from those individuals selected from the top and bottom 5% of our sample of 2085 individuals. However, the study that reported an association between neuroticism and 5HTTLPR[1] could be used to explore this issue by comparing allelic and genotypic frequencies for the 25 individuals in the top and bottom 5% of the combined sample of 505 unselected individuals.

Conclusion

The functional 5HTTLPR polymorphism and the intronic VNTR polymorphism in the 5HTT gene are not associated with high *vs* low peer-rated or self-rated neuroticism. Further work is needed to investigate the possibility that 5HTT may be associated with normal variation in neuroticism but not with the high and low extremes of the dimension. The high heritability of neuroticism and its high genetic correlation with depression and anxiety suggest that neuroticism is a good target for additional candidate gene QTL association research.

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General Summary
Genes that are associated with normal variation in personality are beginning to be identified. Neuroticism (emotionality) is an especially important personality trait because it represents a genetic vulnerability to depression. Neuroticism has recently been reported to be associated in an unselected sample with the serotonin transporter gene, which contributes to neural pathways involved in depression. In the present study, we used a selected extremes design in which individuals were selected from the top 5% and bottom 5% of distribution of 2085 individuals. Each individual's neuroticism was rated by two peers who knew them well. For two DNA markers in the serotonin transporter gene, the high and low peer-rated neuroticism groups did not differ in their frequencies of these polymorphisms, nor did they differ for high and low self-reported neuroticism. Nonetheless, it is possible that the serotonin transporter gene is associated with normal variation in neuroticism but not with the high and low extremes of the dimension.