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No Association of the Serotonin Transporter Polymorphisms 5-HTTLPR and rs25531 with Schizophrenia or Neurocognition

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The 5-HTTLPR is a 43 base pair insertion-deletion polymorphism in the promoter region of the serotonin transporter (*SLC6A4*), and contributes to the regulation of the expression of *SLC6A4* (Hu and others 2006; Lesch and others 1996). The serotonin transporter is a membrane bound protein which transports serotonin from synapses into pre-synaptic neurons. The 5-HTTLPR has been heavily studied in relation to many psychiatric diseases, endophenotypes, and drug response phenotypes. A single nucleotide polymorphism rs25531 (A/G) within the polymorphic 5-HTTLPR has been shown to alter expression of *SLC6A4* (Hu and others 2006). Many prior studies have thus incorrectly considered this triallelic polymorphism as biallelic (i.e., 5-HTTLPR*L_A, L_G, and S as opposed to 5-HTTLPR*L or S). In light of this discovery and the prevalence of rs25531 in certain ancestry groups, it is possible that previous studies may have been confounded by the previously unknown variant. Therefore, we tested 5-HTTLPR and rs25531 for association with chronic schizophrenia, cognitive phenotypes related to schizophrenia, and symptom status.

We genotyped the 5-HTTLPR and rs25531 in 738 cases (74% male, 26% female; 57% European, 29% African, and 14% other ancestry) from the CATIE study, a double-blinded randomized clinical trial of typical and atypical anti-psychotics under controlled conditions with follow-up for as long as 18 months, and in which all subjects provided informed consent under IRB approvals (Lieberman 2006; Sullivan and others 2008). Briefly, inclusion criteria required a definite DSM-IV diagnosis of schizophrenia, previous antipsychotic treatment, age 18-65 years, clinical decision that oral medication was appropriate, adequate decisional capacity and provision of written informed consent. The CATIE study exclusion criteria were multiple, but the overall intent was to ensure that participation was safe and clinically reasonable (Stroup and others 2003). A neurocognitive battery assessed aspects of

neurocognitive function that are severely impaired in schizophrenia (Keefe and others 2006). Controls were from the Molecular Genetics of Schizophrenia Study (PI Dr Pablo Gejman) and volunteers were screened for the absence of psychotic illness as described elsewhere (Sullivan and others 2008). There were 733 control subjects (67% male, 33% female; 56% European ancestry, 30% African, 14% other), group-matched to cases by age, sex, and self-reported race. Cases and controls in this report were also studied in a genome-wide association study (Sullivan and others 2008).

The 5-HTTLPR polymorphism and rs25531 were genotyped using a restriction length polymorphism based assay (Supplemental Methods). Briefly, a PCR reaction of the promoter region that spans the 5-HTTLPR was performed and 5 μ L of each PCR product was digested with *HpaII* restriction enzyme (which cleaves the PCR product at rs25531*G). After digestion, the product was analyzed on a 3730 DNA analyzer (Applied Biosystems). The fragment pattern allowed both polymorphisms to be analyzed simultaneously. Two technicians independently called all genotypes by visual observation of peak sizes using GeneMapper software (Applied Biosystems) with reference to explicit standards. Any discrepancies were resolved via review by both technicians. Duplicate samples were included on each plate, and technicians were blinded to all phenotypes and to duplicate identities. Statistical analysis was conducted using logistic regression (dependent variable schizophrenia case/control status) and multiple regression (neurocognitive and treatment response dependent variables, cases only). Independent variables were the composite 5-HTTLPR/rs25531 genotype plus the covariates indicated in the Table S1. Based on empirical expression data (Hu and others 2006), composite 5-HTTLPR/rs25531 alleles were coded 0 (low expression, S or L_G) or 1 (high expression, L_A) with composite genotypes coded as 0, 1, or 2 and analyzed as a 1 df additive test. Population stratification effects were handled in two ways, by stratified analyses and by inclusion of principal components from genome-wide SNP as covariates (Sullivan and others 2008). We estimated that additive genotypic relative risks of 1.23 (80% power) or 1.27 (90% power) for association with schizophrenia were detectable with a liberal type 1 error of 0.05.

Genotyping results

Of 50 pairs of duplicated samples, there was 1 disagreement (0.02), and genotype calls were generated for 1465 of 1471 subjects (0.996). Composite 5-HTTLPR/rs25531 genotypes had low levels of linkage disequilibrium with GWAS SNPs in this region ($r^2 < 0.3$ in subjects of European and African ancestry). Table S1 shows composite 5-HTTLPR/rs25531 genotypes by ancestry; 13 subjects had one or more “XL” alleles (Delbruck and others 1997) of uncertain functional significance and were excluded. Table S2 shows the effects of considering this triallelic polymorphism as biallelic: misclassification proportions are substantial and vary with self-reported ancestry (African 0.417, European 0.153, and other 0.165).

Statistical analysis

Genotypes were available for 1452 subjects, 728 cases and 724 controls. In a logistic regression with case/control status as dependent variable and 7 ancestry-informative principal components as covariates, the effect of 5-HTTLPR/rs25531 composite genotype was not significant (odds ratio=1.008, 95% CI 0.868-1.172, $p=0.91$). Similar results were obtained for stratified analyses for subjects of self-reported African (odds ratio=1.092, 95% CI 0.829-1.438, $p=0.53$) and European ancestry (odds ratio=1.052, 95% CI 0.871-1.271, $p=0.60$).

In CATIE cases only, we analyzed whether 5-HTTLPR/rs25531 composite genotype was associated with neurocognitive impairment. Using the statistical model and covariates detailed elsewhere (Sullivan and others 2007), we found no significant association of the 5-HTTLPR/rs25531 composite genotype with an omnibus measure of neurocognition ($p=0.21$) or with working memory ($p=0.32$) at study baseline. Similarly, there was no association with PANSS positive ($p=0.67$) or negative symptoms ($p=0.46$) at study baseline (covariates age, sex, and 7 principal components).

In conclusion, we were unable to identify association of the triallelic 5-HTTLPR with schizophrenia, neurocognition, or core psychotic symptoms – even at levels of significance unadjusted for multiple comparisons. We note that strong effects of this genetic variant on stressful life events and major depression has recently been excluded (Risch and others 2009). We cannot exclude the possibility that other variants in the serotonin transporter are of etiological relevance.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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