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Brief report

Genetic variants within the serotonin transporter associated with familial risk for major depression

Ardesheer Talati [a,b,*], Guia Guffanti [a,b], Zagaa Odgerel [a,b], Iuliana Ionita-Laza [c], Heli Malm [f], Andre Sourander [a,f], Alan S. Brown [a,b,d,e], Priya J. Wickramaratne [a,b,c,e], Jay A. Gingrich [a,e], Myrna M. Weissman [a,b,d,e]

[a] Department of Psychiatry, Columbia University Medical Center, New York, NY, USA
[b] Division of Epidemiology, New York State Psychiatric Institute, New York, NY, USA
[c] Department of Biostatistics, Mailman School of Public Health, Columbia University, New York, USA
[d] Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, USA
[e] Sackler Institute for Developmental Psychobiology, Columbia University, New York, NY, USA
[f] Department of Child Psychiatry, University of Turku, Turku, Finland

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ABSTRACT

The role of the serotonin transporter promoter linked polymorphism (5HTTLPR) in depression, despite much research, remains unclear. Most studies compare persons with and without depression to each other. We show offspring at high ($N=192$) as compared to low ($N=101$) familial risk for major depressive disorder were almost four times as likely to have two copies of the short allele at 5HTTLPR, suggesting that incorporation of family history could be helpful in identifying genetic differences.

1. Introduction

The serotonin transporter gene (*SLC6A4*) is among the most extensively studied genes in the psychiatric literature (Caspi et al., 2010). A polymorphism identified within its promoter serotonin transporter linked polymorphic region (5HTTLPR) has received particular focus as it occurs primarily as either a shorter (14-repeats) or longer (16 repeats) sequence, associated, respectively, with lower and higher transcriptional efficiency (Clarke et al., 2010; Lesch et al., 1996). How 5HTTLPR contributes to depression remains unclear, and is complicated by several factors, including identification of additional intermediary-length variants (Nakamura et al., 2000), evidence for modulation of 5HTTLPR by other neighboring polymorphisms (Hu et al., 2006; Murdoch et al., 2013), identification of gene effects only in the presence of environmental stressors (Caspi et al., 2003; Uher et al., 2011), and substantial allelic variations by race (Murdoch et al., 2013). Given this, coupled with the heterogeneity of depression itself, it is unsurprising that even meta-analyses have yielded inconsistent patterns (Munafo et al., 2008; Risch et al., 2009).

Most studies of *SLC6A4* have compared persons with and without major depressive disorder (MDD) directly to each other (i.e., case-control design) (McGrath et al., 2013). Given that depression runs in families (Sullivan et al., 2000), however, genetic variation associated with MDD might be expected among *offspring* of depressed parents, even if the offspring do not express the disorder. *Sampling by familial risk for rather than presence of a psychiatric outcome could provide a complementary approach for identifying genetic candidates in environments that are more homogenous in familial loadings and enriched for the outcome*. We use a multi-generational family study of MDD (Weissman et al., 2006; Weissman et al., 2005) to explore this question, hypothesizing that individuals at high- as compared to low-familial risk for the disorder will have higher prevalence of 5HTTLPR risk variants. We classify risk as (1) the presence of shorter (S) allele at 5HTTLPR or (2) the presence of the longer (L) allele in conjunction with a G

* Correspondence to: Columbia University & New York State Psychiatric Institute, 1051 Riverside Drive, Room 2428, Unit 24, New York, NY 10032, USA. Tel.: +1 646.774.6421.
E-mail addresses: talatia@nyspi.columbia.edu, at2071@columbia.edu (A. Talati), Guffant@nyspi.columbia.edu (G. Guffanti), Odgerel@nyspi.columbia.edu (Z. Odgerel), ii2135@columbia.edu (I. Ionita-Laza), Heli.malm@hus.fi (H. Malm), Andsou@utu.fi (A. Sourander), asb11@columbia.edu (A.S. Brown), wickramp@nyspi.columbia.edu (P.J. Wickramaratne), jag46@columbia.edu (J.A. Gingrich), mmw3@columbia.edu (M.M. Weissman).

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allele at a neighboring polymorphism, rs25531 (hereon, "5HTTLPR–rs25531 haplotype") which alters the transcription-factor binding and is thought to result in an under-expressing phenotype akin to the S allele (Hu et al., 2006; Murdoch et al., 2013).

2. Methods

2.1. Sample

The sample has been previously described in numerous prior publications (Weissman et al., 2006; Weissman et al., 2005). Briefly, the study began in 1982 with the simultaneous recruitment of two groups of probands; one with moderate-to-severe MDD with functional impairment from psychiatric clinics in New Haven, CT; the other with no lifetime illness, from the same community. All probands were European, primarily Southern Italian. Their biological children and subsequently grandchildren were followed prospectively over time, with those of the depressed probands forming the "high-risk" ($N=192$) and those of the non-depressed probands, the "low risk" ($N=101$) groups (Weissman et al., 2006; Weissman et al., 2005). Diagnoses were assessed across six longitudinal waves using the age-appropriate version of the semi-structured Schedule for Affective Disorders and Schizophrenia (Kaufman et al., 1997; Mannuzza et al., 1986). The Institutional review board at New York State Psychiatric Institute/Columbia University approved all procedures, and informed consent was obtained.

2.2. Genotyping

DNA was extracted from saliva collected using Oragene DNA Self Collection Kit following standard manufacturer protocol (Oragene Genotek, Ontario, Canada). The region encompassing 5-HTTLPR and rs25531 polymorphisms was amplified with primers; FORWARD: 5′TCCTCCGCTTTGGCGCCTCTTCC-3′; REVERSE: 5′-TGGGGGTT GCAGGGGAGATCCTG-3′ via a polymerase chain reaction in multiplex master mix (Qiagen, Calif., USA). Amplicon was resolved on a 2.3% UltraPure™ Agarose (Invitrogen, Carlsbad, CA), and visualized under the UV transilluminator. Here, 512 bp and 469 bp bands were called as L and S allele at 5-HTTLPR respectively. For rs25531, amplicon was digested with restriction endonuclease MspI (New England Biolabs® Inc., Boston, MA, USA), and the product resolved in a 2.9% UltraPure Agarose (Invitrogen) and visualized under the UV transilluminator. Digested fragments of 402 bp were called as G at rs25531. Parallel analysis of amplicon and restriction fragment products allowed us to determine a phase of the 5-HTTLPR/rs25531 haplotype in each individual. Genotype calling was blind to subject familial risk group or MDD status.

2.3. Analyses

Only biological descendants of probands with (high-risk group, $n=192$) or without (low-risk, $n=101$) MDD were included (spouses and non-biological relatives were not included). However, quality control procedures were conducted on the full sample to allow for Mendelian errors detection, using the Famtypes software (Baldacara et al., 2008) and PLINK (Purcell et al., 2007). The main analyses (Table 1), were performed with Generalized Estimation Equation (GEE) models in the GWAF R package (Chen and Yang, 2010). We chose family-based association tests rather than transmission disequilibrium tests (TDTs) to allow inclusion of family data considered non-informative by TDT (i.e. transmission from homozygote parents). Each pedigree is treated as a cluster, with independence working correlation matrix used in the robust variance estimator. For both 5HTTLPR and 5HTTLPR–rs25531, we performed two main association tests, one to compare genotype differences by familial risk status, the other by MDD status. For 5HTTLPR, the S allele was classified low-functioning. For 5HTTLPR–rs25531, it has been shown that the G allele at rs25531 diminishes SLC6A4 transcription efficiency, and that a GL haplotype has lower transcription rate than the AL haplotype. We thus coded the four possible haplotypes to reflect the corresponding functionality: SA, SG, LG, as low-functioning, and LA as high-functioning. Each offspring could have two low-functioning alleles, two high-functioning alleles, or one of each.

3. Results

There were no deviations from HWE in the proband generation in either the high-or low-risk families. Age, gender, and overall family size and structure did not vary significantly between the high- and low-risk families.

3.1. 5HTTLPR

Overall distributions of the LL/SL/SS genotypes were significantly different by risk group [32/51/17% in high-risk, 28/67/5% in low-risk; $\chi^2=9.46$, $p=0.009$] (Table 1). A recessive model best explained the data, with high-risk offspring 3–4 times more likely to have both copies of the short allele (SS) (OR=3.9, $p=0.02$). Interestingly, rates of SS in the high-risk group were similar to those reported in other European ancestry populations (Clarke et al., 2010), but significantly lower in the low-risk group (see Section 4). Associations also remained significant following alternative classifications of familial risk using parental (instead of proband) depression status (genotype model: $\chi^2=6.64$, $p=0.03$) or proportion of family members affected ($\chi^2=5.36$, $p=0.009$). 5HTTLPR was not directly associated with lifetime MDD [the direction is suggestive though of the shorter variants being the risk conferring alleles (Table 1, OR-dominant model=1.9, $p=0.06$)].

3.2. 5HTTLPR–rs25531

5HTTLPR–rs25531 was not significantly associated with familial risk. However, offspring with two low-functional variants had higher rates of MDD than those with one or none under the additive model (OR=1.7, $p=0.02$). This association remained significant following adjustment for age and gender (OR=1.6, $p=0.05$), and marginally significant after further adjusting for familial risk (OR=1.6, $p=0.07$).

4. Discussion

Offspring at high- familial risk for major depression were more likely to carry two copies of the variants allele at 5HTTLPR; an under-expressing haplotype generated by coupling 5HTTLPR with a neighboring polymorphism (rs25531) that modulates transcriptional efficiency (Hu et al., 2006; Murdoch et al., 2013) predicted whether the offspring developed MDD under an additive model.

Given that prevalence of SS in European-ancestry populations is ~17% (Clarke et al., 2010; Lesch et al., 1996), the numbers suggest that the low-risk group is depleted of SS rather than the high-risk group being enriched for it. Having two copies is associated with increased stress sensitivity (Kendler et al., 2005). Absence of SS may protect against psychobiological responses to stressful events, and is consistent with the lower rates of depressive and anxiety disorders in the low-risk group (Weissman et al., 2006; Weissman et al., 2005). Although we cannot elucidate *why* the low-risk group deviates from population-expected frequencies, it should be noted that probands in the low-risk group were selected to have no history of any psychopathology and thus may not be reflective of population-based controls (Talati et al., 2008). The low-risk group also had higher rates of the heterozygous (SL) genotypes [67% observed, vs 47% expected], which has been shown to be advantageous via increasing fitness and flexibility to adapt (Cools and Robbins, 2004; Gosso et al., 2008).

4.1. Limitations

The sample is small, and results are preliminary. Furthermore, because of the low prevalence of SS in the low-risk group, we could not test further gene-by-environment interactions. Third, even though shorter variants are in general associated with greater risk, patterns do not completely converge. For example, only having both copies of the shorter variants is associated with familial risk, but there is an additive risk of the alleles for the presence of depression. Whether these differences have etiological