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Gender Moderates the Relationship Between Mania Spectrum and Serotonin Transporter Polymorphisms in Depression

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Abstract

The short (s) variant of the serotonin transporter gene linked functional polymorphic region (5-HTTLPR) is associated with depression. Stressful life events, gender, and race have been shown to moderate this association. Because features of mania/hypomania seem to constitute an indicator of higher severity of depression, we examined the relationship between 5-HTTLPR genotype and symptoms of mania-hypomania spectrum occurring over the lifetime in patients with major depression. The possible moderating role of gender in this relationship was taken into account. Two hundred twenty-two patients with unipolar major depression were genotyped for 5-HTTLPR and nine other representative polymorphisms, and were administered the Mood Spectrum Questionnaire, Lifetime Version (MOODS-SR). The manic-hypomanic (MH) component score was used for analysis. Using a linear model of the MH score as a function of genotypes and gender, controlling for age, severity of depression, and site, we found significant effects of gender ($F = 8.003$, $df = 1$, $P = 0.005$), of the interaction gender $\times$ genotype ($F = 4.505$, $df = 2$, $P = 0.012$), and of the baseline Hamilton score ($F = 5.404$, $df = 1$, $P = 0.021$), non-significant effects of genotype ($F = 1.298$, $df = 2$, $P = 0.275$), age ($F = 0.310$, $df = 1$, $P = 0.578$) site ($F = 0.504$, $df = 1$, $P = 0.479$). Significant associations were also detected at three other SNPs. The association between the manic/hypomanic component of the MOODS-SR and the polymorphisms of the 5-HTTLPR is moderated by gender. This finding is intriguing from a clinical point of view because women with unipolar disorder and the “ss” genotype seem to constitute a sub-group with higher severity of depression. These results should be considered tentative pending replication in other samples.

Keywords

serotonin transporter polymorphisms; mood spectrum; gender; mania; hypomania; depression

INTRODUCTION

Serotonergic neurotransmission is a modulator of emotional behavior, therefore the serotonin transporter gene (5-HTT, SLC6A4) represents a promising candidate in studies of mood disorders. 5-HTT mRNA is encoded by a single gene, mapped to chromosome 17q11.1-q12 [Ramamoorthy et al., 1993]. The 5-HTT protein product modulates the reuptake of serotonin from the synaptic cleft, and may represent the prime target for SSRIs.

A 44-base pair insertion/deletion polymorphism in the promoter region of the human 5-HTT gene produces a biallelic polymorphism designated 5-HTTLPR, with a long allele (l) and a short allele (s). The basal transcriptional activity of the long variant (l allele) is more than twice that of the short form (s allele) and differences in 5-HTT mRNA synthesis result in different 5-HTT expressions, and 5-HT cellular uptake [Heils et al., 1996; Lesch et al., 1996]. Recently, additional variants of the “l” allele were found and designated as “LA” and “LG” [Nakamura et al., 2000; Hu et al., 2004; Parsey et al., 2006]. Thus, 5-HTTLPR is essentially a triallelic locus. As the low activity “LG” allele is relatively infrequent (~10% in Caucasian samples), in practice only the “l” and “s” alleles have been considered in most studies.

These variants have been also associated as neurochemical substrates for SSRI response in late-life depression, which seems to be stronger and faster among “ll” homozygous than heterozygous and “ss” homozygous [Pollock et al., 2000], poor response to SSRIs in midlife depression [Smeraldi et al., 1998; Rausch et al., 2002], adverse effects to citalopram treatment [Hu et al., 2007], increased activation in the right amygdala in response to fearful stimuli [Hariri et al., 2002], increased vulnerability to late life depression secondary to factors such as bereavement and medical illness [Smith et al., 2004], more depressive symptoms, diagnosable depression, and suicidality in relation to stressful life events [Caspi et al., 2003; Kendler et al., 2005]. Taken together, these studies suggests that individuals carrying specific genotypes of 5-HTTLPR are more prone to poor treatment outcome and more vulnerable to depression.

Evidence on the effects of gender in the association between depressive symptoms and 5-HTTLPR is limited [Lesch et al., 1996; Deary et al., 1999; Hamer et al., 1999; Gonda et al., 2005; Jacobs et al., 2006]. The two studies that have specifically addressed this issue suggest that: (1) the responses of males and females carrying the short 5-HTTLPR allele to environmental stress factors go in the opposite direction; whereas females tend to develop depressive symptoms, males seem to be protected from depression [Sjoberg et al., 2006]. (2) for females, the s allele, combined with caregiving stress or low childhood SES, is associated with higher depression scores than in participants in the non-stressor group and those with the long (l) allele; whereas, in males, the l allele, combined with a stressor, is associated with higher depression scores as compared to those in the non-stressor group and those with the s allele [Brummett et al., 2008].

Previous studies have indicated that the lifetime experience of manic/hypomanic symptoms in patients with unipolar depression is associated with clinical indicators of greater depression severity [Cassano et al., 2004]. We have operationalized the concept of mood spectrum—the broad array of manifestations of a disorder, including its core and most severe symptoms, as well as a range of more subtle features related to the core condition—via an instrument that assesses lifetime mood features. The MOODS [Fagiolini et al., 1999] includes, in addition to DSM-IV criterion symptoms for mania/hypomania, those associated features, atypical symptoms and behavioral traits that may present at any point in the lifespan irrespective of whether they reach the threshold for a manic/hypomanic episode. The relationship between the lifetime manic-hypomanic spectrum symptoms and a total of ten polymorphisms across the serotonin transporter gene (<http://www.ncbi.nlm.nih.gov/>) was examined in those participants in a study entitled “Depression: the Search for Treatment-relevant Phenotypes” [Frank et al., 2008] for whom DNA was available, taking into account the possible moderating role of gender. We hypothesized that patients with higher levels of mania/hypomania spectrum constitute an endophenotype with more severe depression and that scores in males and females carrying the same genotype would differ across polymorphisms.

MATERIALS AND METHODS

Subjects

The study sample consists of 222 patients with unipolar depression recruited between February 2002, and March 2007 at the outpatient clinics of the Departments of Psychiatry of the Universities of Pisa and Pittsburgh. in the framework of a study designed to identify the mediators and moderators of treatment outcome of depression.

Inclusion criteria were: age between 18 and 66, ability and willingness to give informed consent, currently in an episode of non-psychotic major depression as defined by the Structured Clinical Interview for Axis I DSM-IV Disorders (SCID), and by a rating of 15 on the 17-item Hamilton Rating Scale for Depression (HRSD17), and not currently receiving effective treatment. Subjects with suicidal ideation were eligible as long as outpatient treatment was deemed safe.

Exclusion criteria were a history of manic or hypomanic episodes, schizophrenia or schizoaffective disorder, current primary diagnosis of eating disorders, drug and/or alcohol dependence or abuse, current psychosis, antisocial personality disorder, organic affective syndrome, and any uncontrolled medical illness.

The IRB of the University of Pittsburgh and the Ethics Committee of the University of Pisa approved study procedures. Patients signed a written informed consent to participation after receiving an explanation of the study aims and having an opportunity to ask questions.

Assessments

The mood spectrum self-report [MOODS-SR, Dell’Osso et al., 2002] is a questionnaire developed simultaneously in English and Italian and designed to assess manic and depressive symptoms, traits and lifestyles that characterize the “temperamental” affective dysregulation that make up both fully syndromal and sub-threshold mood disturbances. The latter include symptoms that are either isolated or clustered in time and temperamental traits that may be present throughout an individual’s lifetime. The MOODS-SR consists of 161 items coded as present or absent for at least 3–5 days in the subject’s lifetime. Items are organized into manic-hypomanic (62 items), depressive (63 items), and rhythmicity/vegetative function components (29 items). The score on the manic-hypomanic component is the count of positive items endorsed and ranges from 0 to 62. The instrument has excellent reliability and validity [Fagiolini et al., 1999; Dell’Osso et al., 2002, www.spectrum-project.org].

Blood collection and DNA extraction

Italian sample (N= 63)—Venous blood samples (6 ml) were collected directly in two EDTA vacutainer tubes (3 ml, purple top).

Genomic DNA was extracted from peripheral blood cells using Qia AMP blood kits (Qiagen, Hilden, Germany) as described by the manufacturer. The amount of DNA for each sample was determined by measuring the optical density at 260/280 nm wavelength using a spectrophotometer. DNA samples from Pisa were frozen at –20°C prior to shipment to Pittsburgh.

Pittsburgh sample (N= 159)—Genomic DNA was extracted from venous blood samples using the phenol chloroform method (n = 99), as well as the QIAamp 96 DNA Blood Kit (Qiagen, Inc., Valencia, CA) (n = 60).

Polymorphism selection—The selection of polymorphisms is described elsewhere [Mansour et al., 2005]. Briefly, we evaluated linkage disequilibrium (LD) across single nucleotide polymorphisms (SNPs) identified by other workers who re-sequenced 5-HTT [Kim et al., 2002]. LD is defined as the non-random association of variants at linked loci [Jorde, 2000]. A variable number of tandem repeats (VNTR) polymorphism is also known to exist in the second intron of 5-HTT. The VNTR was not genotyped as it is known to be in strong LD with the 5-HTTLPR polymorphism [Kirov et al., 1999].

Genotyping Procedures

5-HTTLPR variants (l and s) were identified using a polymerase chain reaction (PCR) based assay as described [Heils et al., 1996; Mansour et al., 2005]. Genotype assays for the SNPs were based on multiplex PCR followed by single base extension analysis (SnaPShot, Applied Biosystems Inc., Foster City, CA). All allele calls were re-checked independently. All genetic data were double-checked to guard against data entry errors. In case of any discrepancy, samples were re-typed. All genotype distributions were in Hardy–Weinberg equilibrium (HWE).

Statistical Analyses

Using as a basis for our analyses the approach suggested by Waldman [2005], we employed a general linear model to test the hypothesis that allelic variation of 5-HTTLPR is associated with manic–hypomanic (MH) spectrum score and is moderated by gender. Gender (coded as 0 = female, 1 = male) and genotype (ll, ls, ss) were included in the model together with the interaction gender $\times$ genotype, age, HRSD baseline score and site. The same linear model was used to examine the relationship of the MH score with the genotypes of nine SNP near 5-HTTLPR and gender.

We also evaluated LD across the polymorphisms in our samples. We used a scaled measure, namely r^2 to estimate the LD [Barrett et al., 2005]. We used software based on a hierarchical clustering procedure called “H-clust” that enables selection of “tag SNPs,” that is, SNPs that can be used to successfully predict unmeasured SNPs over a selected region and thus can be said to represent polymorphism in that region [Rinaldo et al., 2005].

RESULTS

Demographic and clinical characteristics of participants—159 recruited at Pittsburgh and 63 at Pisa—are provided in Table I. Participants had a moderate level of depression (mean HRSD = 19.6, SD = 4.2). The frequency distribution of the genotypes was ll (33.3%), ls (45.0%) and ss (21.7%) and did not differ by gender (females: ll (33.3%), ls (47.6%) and ss (19.0%); males: ll (33.3%), ls (40.0%) and ss (26.7%), $\chi^2 = 1.97$, df = 2, $P = 0.379$). All genotype distributions were in HWE. The allele frequencies and the position of the genotyped polymorphisms are reported in Table II.

Haploview software [Barrett et al., 2005] revealed modest LD across all polymorphisms, with stronger LD among two sets of polymorphisms; the first composed of six SNPs, (rs140701, rs2020942, rs6354, rs2020939, rs2020937, rs2066713) and the second composed of three other SNPs (rs2020935, rs2020934, and rs2020933). 5-HTTLPR is in significant LD with rs2020934 (Fig. 1).

Psychometric Properties of the Manic–Hypomanic (MH) Component

The mean MH score was 20.5 [SD = 10.1]. Compared to normal controls [Balestrieri et al., 2006], scores in the present sample were significantly increased (mean MH score in controls = 10.8 [SD = 8.3], $N = 102$, t -test = 8.47, df = 322, $P < 0.001$). The internal consistency of

the scale, measured by Cronbach's alpha, was 0.915, denoting high reliability of this measure.

Relationship Between 5-HTTLPR Genotypes, Gender and MH Score

Using a linear model of the MH score as a function of genotypes and gender, controlling for age, severity of depression, and site, we found significant effects of gender ($F = 8.003$, $df = 1$, $P = 0.005$), of the interaction gender $\times$ genotype ($F = 4.505$, $df = 2$, $P = 0.012$), and of baseline Hamilton score ($F = 5.404$, $df = 1$, $P = 0.021$) non-significant effects of genotype ($F = 1.298$, $df = 2$, $P = 0.275$), age ($F = 0.310$, $df = 1$, $P = 0.578$) site ($F = 0.504$, $df = 1$, $P = 0.479$). The interaction between gender and genotype is depicted in Figure 2. This interaction suggests that the relationship between 5-HTTLPR genotypes and MH score differs by gender. Specifically, in women with "ll" and "ls" genotypes the MH score was significantly lower compared with that of men with the same genotypes and in women with "ss" genotype the MH score did not differ from that of men.

The model was also fit for each of the nine SNPs near the 5-HTTLPR. Results are depicted in Figure 3. Although the graphs consistently suggest a gender $\times$ genotype interaction, this interaction was significant at $P < 0.05$ only for rs2020942, rs2066713, and rs2020934.

DISCUSSION

The present study, based on clinically referred patients with unipolar depression, indicates that gender moderates the association between the 5-HTTLPR genotypes and the lifetime experience of manic-hypomanic symptoms. This association was replicated in three other SNPs from a set of ten representative polymorphisms genotyped across 5-HTT.

A recent report [Gonda et al., 2006] showed a relationship between the s allele and the Temperament Evaluation of Memphis, Pisa, Paris, and San Diego (TEMPS) questionnaire scores of depressive, cyclothymic, irritable, and anxious temperaments in white females without axis-I disorders. However, the study was based on a sample including only females and could not examine the moderating effect of gender. Although TEMPS scores and the MH component overlap only partially in content, the convergence of the two studies suggests that MH can be regarded as a potential behavioral endophenotype. In fact, it meets several of the criteria proposed by Gottesman and Gould [2003] for the selection of valid and useful endophenotypes that underlie psychiatric disorders and that can be used in molecular genetic studies. First, it is derived from a psychometrically sound measure with an approximately normal distribution and high internal consistency. Second, it is related to the disorder of interest because scores are significantly higher in patients with depression compared with controls; third, it is associated with the 5-HTTLPR polymorphisms (in females only), above and beyond the severity of the disorder. In fact, our analyses indicate that even after adjusting for baseline HDRS scores, the association of the polymorphism with the MH scores remains significant. Lastly, the potential confounding effect of age on mania/hypomania score was controlled for and proved to be non-significant. However, it should be acknowledged that two important criteria of Gottesman and Gould (co-segregation within families and heritability) could not be demonstrated in the present study, and requires an ad hoc investigation.

Our findings have potential implications for clinical practice. Several studies have emphasized that patients with unipolar depression and hyperthymic or cyclothymic temperaments are more difficult to treat [Akiskal et al., 1989; Cassano et al., 1989, 1992], and constitute a subgroup with more severe depression [Cassano et al., 2004]. In the study on which the present report is based, eight patients experienced treatment-emergent hypomania and were terminated from the study [Benvenuti et al., 2008]. Of these, four were

genotyped for 5-HTTLPR. Two women who developed hypomania had the “ss” genotype and one man the “ll” genotype. Although this evidence is very limited, the hypothesis of a differential risk of hypomania during antidepressant treatment in women and men carrying specific 5-HTTLPR polymorphisms is intriguing. The ability to identify these groups could allow clinicians to proceed with antidepressant use with greater confidence.

We also detected similar, nominally significant associations at three other SNPs. Associations are consistent with the LD patterns in our sample (Fig. 2). Note that rs2020942 and rs2066713 are in strong LD. There is modest LD between these SNPs and rs2020934, which in turn is in strong LD with 5-HTTLPR. These consistent patterns reinforce the association with 5-HTTLPR, the only polymorphism with known functional impact in the set that was genotyped and suggest that the association at 5-HTTLPR cannot be attributed to genotyping errors.

LIMITATIONS

The possible role of ethnicity could not be explored because the ethnic background of the participants is 97% Caucasian. We did not genotype rare polymorphisms, as our sample would have limited power to detect associations at such variants. The study participants represent a constrained group, all of whom had moderately severe non-psychotic major depression. Future studies are needed to show whether this association holds for minor depression or for very severely depressed inpatients.

Lastly, because of the novelty of the phenotype, and the multiple tests (multiple SNPs), there is some risk of false positives and replication of our results is warranted.

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References

- Akiskal HS, Cassano GB, Muzetti L, Perugi G, Tundo A, Mignani V. Psychopathology, temperament, and past course in primary major depressions. 1. Review of evidence for a bipolar spectrum. *Psychopathology*. 1989; 22(5):268–277. [PubMed: 2690170]
- Balestrieri M, Rucci P, Sbrana A, Ravani L, Benvenuti A, Gonnelli C, Dell’Osso L, Cassano GB. Lifetime rhythmicity and mania as correlates of suicidal ideation and attempts in mood disorders. *Compr Psychiatry*. 2006; 47:334–341. [PubMed: 16905394]

- Barrett JC, Fry B, Maller J, Daly MJ. Haploview: Analysis and visualization of LD and haplotype maps. *Bioinformatics*. 2005; 21(2):263–265. Epub 2004 Aug 05. [PubMed: 15297300]
- Benvenuti A, Rucci P, Miniati M, Papasogli A, Fagiolini A, Cassano GB, Swartz H, Frank E. Treatment-emergent mania/hypomania in unipolar patients. *Bipolar Disord*. 2008; 10(6):726–732. [PubMed: 18837867]
- Brummett BH, Boyle SH, Siegler IC, Kuhn CM, Ashley-Koch A, Jonassaint CR, Zuchner S, Collins A, Williams RB. Effects of environmental stress and gender on associations among symptoms of depression and the serotonin transporter gene linked polymorphic region (5-HTTLPR). *Behav Genet*. 2008; 38(1):34–43. [PubMed: 17955359]
- Caspi A, Sugden K, Moffitt TE, Taylor A, Craig IW, Harrington H, McClay J, Mill J, Martin J, Braithwaite A, Poulton R. Influence of life stress on depression: Moderation by a polymorphism in the 5-HTT gene. *Science*. 2003; 301:386–389. [PubMed: 12869766]
- Cassano GB, Akiskal HS, Musetti L, Perugi G, Soriani A, Mignani V. Psychopathology, temperament, and past course in primary major depressions. 2. Toward a redefinition of bipolarity with a new semi-structured interview for depression. *Psychopathology*. 1989; 22(5):278–288. [PubMed: 2602525]
- Cassano GB, Akiskal HS, Savino M, Musetti L, Perugi G. Proposed subtypes of bipolar II and related disorders: With hypomanic episodes (or cyclothymia) and with hyperthymic temperament. *J Affect Disord*. 1992; 26(2):127–140. [PubMed: 1447430]
- Cassano GB, Rucci P, Frank E, Fagiolini A, Dell'Osso L, Shear MK, Kupfer DJ. The mood spectrum in unipolar and bipolar disorder: Arguments for a unitary approach. *Am J Psychiatry*. 2004; 161:1264–1269. [PubMed: 15229060]
- Deary IJ, Battersby S, Whiteman MC, Connor JM, Fowkes FG, Harman A. Neuroticism and polymorphisms in the serotonin transporter gene. *Psychol Med*. 1999; 29:735–739. [PubMed: 10405095]
- Dell'Osso L, Armani A, Rucci P, Frank E, Fagiolini A, Corretti G, Shear MD, Grochocinski VJ, Maser JD, Endicott J, Cassano GB. Measuring mood spectrum: Comparison of interview (SCI MOODS) and self-report (MOODS-SR) instrument. *Compr Psychiatry*. 2002; 43:69–73. [PubMed: 11788923]
- Fagiolini A, Dell'Osso L, Pini S, Armani A, Bouanani S, Rucci P, Cassano GB, Endicott J, Maser J, Shear MK, Grochocinski VJ, Frank E. Validity and reliability of a new instrument for assessing mood symptomatology: The Structured Clinical Interview for Mood Spectrum (SCI-MOODS). *Int J Methods Psychiatr Res*. 1999; 8:71–82.
- Frank E, Cassano GB, Rucci P, Fagiolini A, Maggi L, Kraemer HC, Kupfer DJ, Pollock B, Bies R, Nimgaonkar V, Pilkonis P, Shear MK, Grochocinski VJ, Scocco P, Battenfield J, Forgiione RN. Addressing the challenges of a cross-national investigation: Lessons from the Pittsburgh-Pisa Study of Treatment-Relevant Phenotypes of Unipolar Depression. *Clin Trials*. 2008; 5(3):253–261. [PubMed: 18559415]
- Gonda X, Juhasz G, Laszik A, Rihmer Z, Bagdy G. Subthreshold depression is linked to the functional polymorphism of the 5HT transporter gene. *J Affect Disorders*. 2005; 87:291–297. [PubMed: 16002148]
- Gonda X, Rihmer Z, Zsombok T, Bagdy G, Akiskal KK, Akiskal HS. The 5HTTLPR polymorphism of the serotonin transporter gene is associated with affective temperaments as measured by TEMPS-A. *J Affect Disord*. 2006; 91:125–131. [PubMed: 16464506]
- Gottesman II, Gould TD. The endophenotype concept in psychiatry: Etymology and strategic intentions. *Am J Psychiatry*. 2003; 160:636–645. [PubMed: 12668349]
- Hamer DH, Greenberg BD, Sabol SZ, Murphy DL. Role of the serotonin transporter gene in temperament and character. *J Person Disord*. 1999; 13:312–327.
- Hariri AR, Mattay VS, Tessitore A, Kolachana B, Fera F, Goldman D, Egan MF, Weinberger DR. Serotonin transporter genetic variation and the response of the human amygdala. *Science*. 2002; 297:400–403. [PubMed: 12130784]
- Heils A, Teufel A, Petri S, Stober G, Riederer P, Bengel D, Lesch KP. Allelic variation of human serotonin transporter gene expression. *J Neurochem*. 1996; 66:2621–2624. [PubMed: 8632190]

- Hu X, Zhu G, Lipsky R, Goldman D. HTTLPR allele expression is codominant, correlating with gene effects on fMRI and SPECT imaging intermediate phenotypes, and behavior. *Biological Psychiatry*. 2004; 55:191S.
- Hu XZ, Rush AJ, Charney D, Wilson AF, Sorant AJ, Papanicolaou GJ, Fava M, Trivedi MH, Wisniewski SR, Laje G, Paddock S, McMahon FJ, Manji H, Lipsky RH. Association between a functional serotonin transporter promoter polymorphism and citalopram treatment in adult outpatients with major depression. *Arch Gen Psychiatry*. 2007; 64(7):783–792. [PubMed: 17606812]
- Jacobs N, Kenis G, Peeters F, Derom C, Vlietinck R, van Os J. Stress-related negative affectivity and genetically altered serotonin transporter function. *Arch Gen Psychiatry*. 2006; 63:989–996. [PubMed: 16953001]
- Jorde LB. Linkage disequilibrium and the search for complex disease genes. *Genome Res*. 2000; 10(10):1435–1444. [PubMed: 11042143]
- Kendler KS, Kuhn JW, Vittum J, Prescott CA, Riley B. The interaction of stressful life events and a serotonin transporter polymorphism in the prediction of episodes of major depression: A replication. *Arch Gen Psychiatry*. 2005; 62:529–535. [PubMed: 15867106]
- Kim SJ, Cox N, Courchesne R, Lord C, Corsello C, Akshoomoff N, Guter S, Leventhal BL, Courchesne E, Cook EH Jr. Transmission disequilibrium mapping at the serotonin transporter gene (SLC6A4) region in autistic disorder. *Mol Psychiatry*. 2002; 7(3):278–288. [PubMed: 11920155]
- Kirov G, Rees M, Jones I, MacCandless F, Owen MJ, Craddock N. Bipolar disorder and the serotonin transporter gene: A family-based association study. *Psychol Med*. 1999; 29(5):1249–1254. [PubMed: 10576317]
- Lesch KP, Bengel D, Heils A, Sabol SZ, Greenberg BD, Petri S, Benjamin J, Muller CR, Hamer DH, Murphy DL. Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science*. 1996; 274:1527–1531. [PubMed: 8929413]
- Mansour HA, Talkowski ME, Wood J, et al. Serotonin gene polymorphisms and bipolar I disorder: Focus on the serotonin transporter. *Ann Med*. 2005; 37:590–602. [PubMed: 16338761]
- Nakamura M, Ueno S, Sano A, Tanabe H. The human serotonin transporter gene linked polymorphism (5-HTTLPR) shows ten novel allelic variants. *Mol Psychiatry*. 2000; 5:32–38. [PubMed: 10673766]
- Parsey RV, Hastings RS, Oquendo MA, et al. Effect of a triallelic functional polymorphism of the serotonin-transporter-linked promoter region on expression of serotonin transporter in the human brain. *Am J Psychiatry*. 2006; 163:48–51. [PubMed: 16390888]
- Pollock BG, Ferrell RE, Mulsant BH, Mazumdar S, Miller M, Sweet RA, Davis S, Kirshner MA, Houck PR, Stack JA, Reynolds CF, Kupfer DJ. Allelic variation in the serotonin transporter promoter affects onset of paroxetine treatment response in late-life depression. *Neuropsychopharmacology*. 2000; 23(5):587–590. [PubMed: 11027924]
- Ramamoorthy S, Bauman AL, Moore KR, Han H, Yang-Feng T, Chang AS, Ganapathy V, Blakely RD. Antidepressant- and cocaine-sensitive human serotonin transporter: Molecular cloning, expression, and chromosomal localization. *Proc Natl Acad Sci USA*. 1993; 90:2542–2546. [PubMed: 7681602]
- Rausch JL, Johnson ME, Fei Y, Li J, Shendarkar N, Hobby HM, Ganapathy V, Leibach FH. Initial conditions of serotonin transporter kinetics and genotype: Influence on SSRI treatment trial outcome. *Biol Psychiatry*. 2002; 51:723–732. [PubMed: 11983186]
- Rinaldo A, Bacanu SA, et al. Characterization of multilocus linkage disequilibrium. *Genet Epidemiol*. 2005; 28:193–206. [PubMed: 15637716]
- Sjoberg RL, Nilsson KW, Nordquist N, Ohrvik J, Leppert J, Lindstrom L, Orelund L. Development of depression: Sex and the interaction between environment and a promoter polymorphism of the serotonin transporter gene. *Int J Neuropsychopharmacol*. 2006; 9:443–449. [PubMed: 16212676]
- Smeraldi E, Zanardi R, Benedetti F, Di Bella D, Perez J, Catalano M. Polymorphism within the promoter of the serotonin transporter gene and antidepressant efficacy of fluvoxamine. *Mol Psychiatry*. 1998; 3(6):508–511. [PubMed: 9857976]

- Smith GS, Lotrich FE, Malhotra AK, Lee AT, Ma Y, Kramer E, Gregersen PK, Eidelberg D, Pollock BG. Effects of serotonin transporter promoter polymorphisms on serotonin function. *Neuropsychopharmacology*. 2004; 29(12):2226–2234. [PubMed: 15354180]
- Waldman ID. Statistical approaches to complex endophenotypes: Evaluating neurophysiological endophenotypes for attention-deficit hyperactivity disorder. *Biol Psychiatry*. 2005; 57:1347–1356. [PubMed: 15950007]

FIG. 1.

Linkage disequilibrium (LD) patterns at 5-HTT gene using samples from study participants from Pisa and Pittsburgh. Haplotype blocks were defined with the HAPLOVIEW program [Barrett et al., 2005]. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

FIG. 2.
Manic-hypomanic (MH) component score as a function of gender and 5-HTT genotypes:
estimated means and standard errors from ANOVA model.

FIG. 3.

Interactions between gender and genotypes of nine SNPs on the manic-hypomanic (MH) component score: estimated means and standard errors from ANOVA model. The P -value of the interaction is indicated in parenthesis. From left to right: rs140701 ($P = 0.073$), rs2020942 ($P = 0.023$), rs6354 ($P = 0.745$), rs2020939 ($P = 0.079$), rs2020937 ($P = 0.074$), rs2066713 ($P = 0.044$), rs2020935 ($P = 0.680$), rs2020934 ($P = 0.013$), rs202093.

TABLE I**Demographic and Clinical Characteristics of Study Participants**

	Total (N = 222)
Age, mean (SD)	38.5 (12.0)
Female, N (%)	147 (66.2)
Educational level (years)	14.5 (3.2)
Married or living with partner (%)	37.4
Working status (%)	
Employed	66.2
Unemployed	11.7
Homemaker	6.8
Other	15.3
Caucasian ethnicity (%)	97.2
Depressive component (MOODS-SR)	35.6 (11.5)
Manic component (MOODS-SR)	20.5 (11.1)
Total MOODS	70.6 (22.1)
17-HDRS score at baseline	19.6 (4.2)

TABLE II

Allelic Frequency of Polymorphisms Genotyped Across the 5-HTT Gene

#	Marker	Allele	Genomic location (bp)	Frequency of less common allele
1	rs140701	C/T	3275525	0.422
2	rs2020942	G/A	3283907	0.359
3	rs6354	G/T	3286891	0.212
4	rs2020939	G/A	3287725	0.410
5	rs2020937	A/T	3287750	0.351
6	rs2066713	G/A	3288658	0.353
7	rs2020935	A/T	3298448	0.098
8	rs2020934	C/T	3298453	0.479
9	rs2020933	A/T	3298748	0.102
10	5-HTTLPR	L/S	3301301–3301344	0.442