Extensive Genotyping of the BDNF and NTRK2 Genes Define Protective Haplotypes Against Obsessive-Compulsive Disorder

Pino Alonso, Mónica Gratacós, José M. Menchón, Jerónimo Saiz-Ruiz, Cinto Segalàs, Enrique Baca-García, Javier Labad, José Fernández-Piqueras, Eva Real, Concepción Vaquero, Mercedes Pérez, Helen Dolengevich, Juan R. González, Mónica Bayés, Rafael de Cid, Julio Vallejo, and Xavier Estivill

Background: Family, twin and molecular studies provide increasing evidence for the importance of genetic factors in obsessive-compulsive disorder (OCD). Recent work suggests that brain-derived neurotrophic factor (BDNF) may be involved in OCD pathophysiology. We used a linkage disequilibrium (LD)-mapping approach to investigate the role that BDNF and its specific receptor neurotrophic tyrosine kinase receptor type 2 (NTRK2) may play in increasing susceptibility to OCD.

Methods: Eight tag single nucleotide polymorphisms (tagSNPs) covering the BDNF gene region and 46 tagSNPs in the NTRK2 region were genotyped in 215 OCD patients and 342 control subjects. Single nucleotide polymorphism association and haplotype analysis were performed. The possible relationship between genetic factors and clinical characteristics including age of OCD onset, tic disorders, clinical dimensions, and family history of OCD were investigated.

Results: Haplotype analysis revealed a significant association between OCD and a five-marker protective haplotype located toward the 5′ of the BDNF gene (odds ratio [OR] = 80; 95% confidence interval [CI] = .69 – .92; permutation p value = .006) containing the functional valine (Val)66-to-methionine (Met) variant. A significant association between a NTRK2 intronic SNP (rs2378672) and OCD was identified (p < .0001) in female patients under an additive model. A protective haplotype located in intron 19 of NTRK2 was also associated with OCD (OR = .76; 95% CI = .66 – .87; permutation p value = .001).

Conclusions: These findings support a role for the BDNF/NTRK2 signaling pathway in genetic susceptibility to OCD.

Key Words: Association, BDNF, haplotype, NTRK2, obsessive-compulsive disorder, tagSNPs

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symptom dimensions, or family history of OCD. To test this hypothesis, we performed a case-control study through an extensive linkage disequilibrium (LD)-mapping approach to examine both genes and analyzed the possible relationship between their variants and OCD clinical characteristics.

Methods and Materials

Subjects

Two hundred fifteen consecutive Spanish Caucasian outpatients with OCD (103 men and 112 women) were included in the study. Obsessive-compulsive disorder subjects were recruited from the OCD Clinics of Bellvitge University Hospital (Barcelona, Spain) (n = 132) and the Ramón y Cajal University Hospital (Madrid, Spain) (n = 83) between 2003 and 2005. All patients met DSM-IV criteria for OCD (14) and had had OCD symptoms for at least 1 year. Diagnoses were independently assigned by two psychiatrists with extensive clinical experience in OCD, who separately interviewed the patients using the Structured Clinical Interview for DSM-IV Axis I Disorders-Clinician Version (SCID-CV) (15). Exclusion criteria were a past or present history of psychoactive substance abuse, a history of psychotic disorders, age under 18 or over 65 years, mental retardation, and severe organic or neurological pathology except tic disorder. Comorbidity with other DSM-IV Axis I disorders was not considered an exclusion criterion, provided that OCD was the primary reason for seeking medical assistance. During the selection period, 283 outpatients were assessed and fulfilled DSM-IV criteria for OCD. Of these patients, 56 were ruled out in accordance with the exclusion criteria and 12 refused to take part in the study.

The control group consisted of 342 unrelated Caucasian subjects (202 men and 140 women, mean age: 39.8 years), who were recruited from a group of blood donors and were not psychiatrically screened.

Written informed consent was obtained from each subject after complete description of the study, which was approved by both hospitals’ ethics committees.

Clinical assessment included information on age at onset of OCD, defined as the age when symptoms became a significant source of distress and interfered with the patient’s social functioning. We decided to consider 10 years old as a reasonable threshold to define the early-onset subgroup of patients (16). As only 12.0% of our patients reported onset before this age, we repeated our analysis using 17 years old as a less restrictive age of onset (17) and defined three groups (below 10 years, between 10 and 17 years, and after 17 years old). Many OCD patients suffer from obsessions and compulsions years before they meet diagnostic criteria for OCD. To take this into account, we considered age of appearance of any obsessive symptom remembered by the patient or a family member, and defined groups of early versus late subclinical OCD onset. A diagnosis of subclinical OCD implied that the subject met all criteria for definite OCD except that symptoms were reported to occur for less than 1 hour a day or were not reported as causing interference or distress. Presence of subclinical obsessive symptoms before 10 years old was reported by 17.3% of our patients, between 10 and 17 years old by 34.6%, and after 17 years old by 48.1%. A clinician-administered version of the Yale-Brown Obsessive-Compulsive Scale (Y-BOCS) (18) and the 21-item Hamilton Depression Rating Scale (HDRS) (19) were used to assess the severity of obsessive-compulsive and depressive symptoms, respectively. The clinician-administered version of the Y-BOCS Symptom Checklist (18) was employed to ascertain scores on five previously identified symptom dimensions: symmetry/ordering, hoarding, contamination/cleaning, aggression/checking, and sexual/religious obsessions, classified as absent, past, or present (20). Presence of family psychiatric history in first-degree relatives was established according to the Family History Research Diagnostic Criteria (FH-RDC) (21). Diagnostic information was obtained on 670 first-degree relatives (on average, 3.1 relatives per proband). Direct interview was possible in 474 of them. For those first-degree relatives who were unavailable for assessment or were unwilling to be assessed, information was gathered from two knowledgeable informants. In these cases, relatives were considered affected if they had received a formal diagnosis by a psychiatrist, had a history of psychiatric hospitalization, or had been maintained on diagnosis-specific medication.

Tag Single Nucleotide Polymorphism Selection

From the HapMap Project dataset, we used genotypes from public release 16 (Phase 1 data freeze, Single Nucleotide Polymorphism database [dbSNP] b124), corresponding to the 60 individuals from 30 Centre d’Etude du Polymorphisme Humain (CEPH) trios of European descent (http://www.hapmap.org). From the gene location, we extended the search for 5 to 10 kilobases (kb) upstream and downstream of BDNF and NTRK2 genes. Only SNPs with a unique mapping location on the National Center for Biotechnology Information (NCBI) B34 assembly and a minor allele frequency (MAF) higher than 10% were considered for further analysis. In the BDNF region (RefSeq: NM_170731; chromosome 11: 27629852-27717072, NCBI B34 assembly), covering 87.2 kb, genotypes for 27 SNPs were available. Twenty-one of these had a MAF > 10% (average spaced 4.4 kb). In the NTRK2 gene region, covering 887 kb (RefSeq: NM_006180; chromosome 9: 8272497-83111512, NCBI B34 assembly), information from 238 SNPs was available. Two hundred six of these had a MAF > 10% (average spaced 1.6 kb). Bins of common SNPs in strong LD, as defined by an r² higher than .85, were identified within both datasets by use of HapMap-LDSelect-Processor. This uses the LD Select method (22) to process HapMap genotype dump format data corresponding to the locus region. Eight tag single nucleotide polymorphisms (tagSNPs) were selected to cover all bins in the case of BDNF, including a nonsynonymous variant in the coding exon (rs6265; corresponding to the functional Val66Met amino acid change). Forty-six tagSNPs were selected to cover all bins in the case of NTRK2 (see Figure 1 and Supplements 1 and 2 for a graphic representation of selected variants for BDNF and NTRK2, respectively, and Supplement 3 for SNP details). Information about SNPlex (Applied Biosystems, Foster City, California) design, quality control of genotypes, and analysis of population stratification is provided in Supplement 4.

Sample Power Calculation

First, we computed power calculations using the Genetic Power Calculator (http://pngu.mgh.harvard.edu/~purcell/gpc/). Thus, we determined that the case-control sample had 93% power for detecting a risk allele with 10% frequency and a dominant genotype relative risk of 2.0. To assess the power for detecting association due to LD with a causal loci, we also carried out power calculations for an indirect association study that uses tagSNPs (23). We estimated that our study was able to detect a susceptibility locus (80% power) with an odds ratio (OR) of 2.0 if the MAF is .15 and an OR of 1.7 if the MAF is .3, assuming a codominant effect at an unobserved locus, an alpha (α) value of
.05, and $r^2 = .85$ for the ability of haplotypes to predict the allele count at the causal locus.

**Statistical Analysis**

Departure from Hardy-Weinberg equilibrium (HWE) for all the biallelic SNP markers was tested using a chi-square test ($1 \times df$). For individual SNP association analyses, genotype frequencies were assessed by means of multivariate methods based on logistic regression analyses and analyzed under codominant, dominant, recessive, overdominant, and additive models. The best model was selected using the Akaike information criteria. We estimated the crude OR and 95% confidence intervals (95% CI). These analyses were performed using the SNPassoc R package (24). To avoid false-positive results due to multiple testing, we applied the Bonferroni correction for 54 independent loci genotyped. Significant $p$ values were raised to $p = .000$. The robustness of these individual findings was investigated by a bootstrap analysis (25). We repeated the selection procedure on 300 bootstrap datasets generated from the original sample and recorded the number of times that a particular association was statistically significant (at Bonferroni significance level) between each polymorphism and the phenotype.

Multinomial regression was used to assess the association between cases, control subjects, and SNPs when cases were categorized by clinical status: symptom dimensions, tic disorders, family history of OCD, and comorbid affective disorders.

Linkage disequilibrium between polymorphisms and haplotype block structures was evaluated by Haploview software version 3.2 (http://www.broad.mit.edu/mpg/haploview). Haplotype blocks were generated by the algorithm of four-gamete rules (26). For the haplotype estimations, we used a sliding window technique using also the haplo.stats R package. Furthermore, the permutation procedure was used to estimate the significance of the best result (1000 permutations). Haplotypes with frequencies lower than 1% were excluded. Interaction effects between SNPs and haplotypes of BDNF and NTRK2 genes were tested using the likelihood ratio test.

**Results**

The demographic and clinical characteristics of the patients and control subjects are summarized in Table 1. Patients from Barcelona and Madrid did not differ significantly in terms of either sociodemographic data or OCD features (data not shown). A higher proportion of male subjects was detected in the control group ($\chi^2 = .01$), and control subjects were significantly older than OCD patients ($t = -5.75; df = 548; p = .01$).

**Single-SNP Association Analysis**

The genotype distribution was in Hardy-Weinberg equilibrium both in control subjects and patients (alpha = .01) (Supplement 3).

No significant differences in genotype distributions for BDNF were detected between OCD patients and control subjects under the five different inheritance models tested (Figure 2). The possibility that the lack of association was due to the confounding effect of sex was ruled out by a stratified analysis.

When we analyzed NTRK2, four SNPs showed nominal $p$-values lower than .05: rs1201363 in 5' upstream of the gene; rs1439050 in intron 4; rs11140783 in intron 14, and rs11795386 and rs2378672, both in intron 19. After Bonferroni correction, only rs2378672 remained significant ($p < .0001$) (Figure 2). A stratified analysis by sex revealed that significance was restricted to the female subgroup under an additive model (Table 2). The association of SNP rs2378672 and OCD remains statistically significant according to the bootstrap $p$-values ($p < .0009$).

Comparisons considering age at onset of OCD detected no significant differences regarding family history of OCD, gender, or OCD severity between patients with early- and late-onset of the disorder. We could not replicate Hall et al.’s (11) finding in any of the onset subgroups of OCD or subclinical OCD in the BDNF gene and found no positive signals in the NTRK2 gene (data not shown). Likewise, we could not find any evidence of the association of BDNF or NTRK2 polymorphisms with other clinical variables, including symptom dimensions, tic disorders, and comorbid affective disorders.

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**Table 1.** Demographic and clinical characteristics of the patients and control subjects.

**Table 2.** Association of SNP rs2378672 with OCD.

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**Figure 1.** (A) Scaled diagram showing the BDNF gene structure. The single coding exon is shown as a black box. Noncoding exons and 3'UTR region are shown in grey boxes. Estrogen response element is shown with an arrow and the NGF protein domain is shown above the gene structure. TagSNPs genotyped in the genomic region in our study are shown, and markers with an asterisk correspond to those SNPs previously genotyped in OCD patients in other studies (11,12). (B) Scaled diagram showing the NTRK2 gene structure. The coding exons are shown as black boxes. Noncoding exons are shown as grey boxes. The protein domains are shown above the gene structure as grey lines. TagSNPs genotyped in the genomic region in our study are shown. The grey bars with arrows indicate the genomic region where the significant haplotypes were identified in the study. NGF, nerve growth factor; OCD, obsessive-compulsive disorder; tagSNP, tag single nucleotide polymorphisms; UTR, untranslated region.
When comparing genotype distributions among patients according to the presence of family history of OCD, a significant difference emerged in SNP rs6265, corresponding to the functional Val66Met variant, under an overdominant model \((p = .008)\). Patients with family history of OCD were more frequently heterozygous for this SNP than patients without this family background. No significant differences in genotype distribution for \(NTRK2\) polymorphisms were detected among patients according to this criterion.

### Haplotype Association Analysis

In \(BDNF\), we identified two blocks with high LD (Supplement 2), a short one of 1 kb and a large block of 79 kb containing the functional Val66Met variant (rs6265). The results from the sliding window analyses in \(BDNF\) pointed to a five-marker region located toward the 5’ of the gene (Supplement 5). The association test with the common reconstructed haplotypes showed that a significant haplotype was associated with OCD \((OR = .80; 95\% CI = .69–.92; permutation p value = .006)\). Thus, this composite haplotype confers a protective effect and contains the Val allele of the functional Val66Met variant (Table 3).

In \(NTRK2\), we identified 11 blocks with high LD (Supplement 1), which ranged in size from 2 to 29 kb. Single nucleotide polymorphism rs2378672, significant in the univariate analysis and associated with a protective effect, was located in block 10, spanning 29 kb, and located in intron 19 of the gene. We further used a sliding window approach (Supplement 6) and performed the case-control haplotype association analysis of this particular block in patients and control subjects as shown in Table 4. A specific haplotype \((G-C-G-G-A-C-A)\) was found to be protective \((OR = .21; 95\% CI = .09–.49; permutation p value = .006)\).

### Interaction Analysis

The interaction effects between \(BDNF\) and \(NTRK2\) were assessed for all possible SNP combinations under an additive model. No significant interaction was detected either between SNPs or between the protective haplotypes of the two genes (Figure 3).
Discussion

The aim of the present study was to explore whether genetic variants at the *BDNF* and/or *NTRK2* loci constitute risk factors for OCD. While there have been two previous studies of specific variants in *BDNF*, this is, to our knowledge, the first attempt to evaluate common nucleotide variation across the genomic region that encompasses both *BDNF* and its receptor *NTRK2*.

We could not find any single-marker association between the *BDNF* SNPs under investigation and OCD. However, haplotype analysis revealed a significant association between OCD and a five-marker protective composite haplotype. This haplotype extends 73 kb upstream of exon 1 and includes the functional Val66Met variant (rs6265, located in exon 6). This variant, located in the 5’ pro-BDNF sequence, affects intracellular trafficking and secretion of BDNF. Studies assessing the involvement of the Val66Met variant in mental disorders have produced conflicting results. However, a recent meta-analysis suggests that this association is confined to substance-related disorders, eating disorders, and schizophrenia (27). The BDNF Met variant has been associated with smaller hippocampal and occipital lobar gray matter volumes and poor medial temporal lobe-related memory performance both in schizophrenic patients and healthy subjects (28). Studies in animal models recently reported that transgenic BDNF+/−Met and BDNFPm/Met mice show a deficit in activity-dependent release of BDNFMet from neurons and a decrease in dendritic arbor complexity. Moreover, BDNFPm/Met mice display increases in anxiety-related behaviors that did not respond to fluoxetine (13). So, the Val allele may exert some kind of protective effect against certain pathologies such as psychosis or adult OCD, while being associated with increased genetic risk for other mental illnesses. In fact, it has been postulated that the Val allele specifically affects the liability to childhood psychiatric pathologies including juvenile-onset mood disorders (29), attention-deficit/hyperactivity disorder (ADHD) (30), or early-onset OCD (11). Moreover, our inability to detect a single-marker association between the Val66Met polymorphism and OCD indicates that not only this SNP but other genetic variants in tight LD with it are important in configuring an extended functional haplotype, implicated in the susceptibility to OCD.

With respect to *NTRK2*, we found that SNP rs2378672, located in intron 19, was significantly associated with the OCD phenotype in the subgroup of female patients. Gene deletion studies in mice reveal that defects in NTRK2 signaling can have detrimental effects.
effects on the viability and differentiation of neocortical neurons (31) and increase impaired learning behavior and inappropriate coping responses when facing complex and/or stressful learning paradigms (32). NTRK2 contains an extracellular domain consisting of three tandem leucine-rich motifs flanked by two cysteine clusters and two immunoglobulin (Ig)-like domains, a single transmembrane segment and an intracellular domain possessing a tyrosine-kinase domain. The positive susceptibility region that we identified lies in intron 19, located in the region that contains the exons coding for the tyrosine kinase domain of the protein (exons 17 to 21). We investigated the putative functional effects of rs2378672 through the interactive web-based SNP analysis tool Pupasuite (http://pupasuite.bioinfo.cipf.es/). The results show that rs2378672 lies in a mouse conserved region but does not

Table 2. Stratified Analysis Covariation by Sex of the NTRK2 TagSNP rs2378672 in Patients with OCD

<table>
<thead>
<tr>
<th></th>
<th>Control Subjects n (%)</th>
<th>Patients n (%)</th>
<th>OR (95% CI)</th>
<th>p Value</th>
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<tr>
<td>AA</td>
<td>170 (88.54)</td>
<td>99 (96.12)</td>
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<td>.05</td>
<td>381.9</td>
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<td>4 (3.88)</td>
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<tr>
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<td>1 (.52)</td>
<td>0 (.00)</td>
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<td>.02</td>
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<td>340.6</td>
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<td>1 (.75)</td>
<td>0 (.00)</td>
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<tr>
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<td>112 (45.7)</td>
<td>.17 (.05–.59)</td>
<td>.0007b</td>
<td>330.3</td>
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AIC, Akaike Information Criterion; CI, confidence interval; OCD, obsessive-compulsive disorder; OR, odds ratio.
aAIC, Akaike Information Criterion, which attempts to find the minimal model that correctly explains the data.
bSignificant value at p < .0009 after Bonferroni correction for 54 independent comparisons.

effects on the viability and differentiation of neocortical neurons (31) and increase impaired learning behavior and inappropriate coping responses when facing complex and/or stressful learning paradigms (32). NTRK2 contains an extracellular domain consisting of three tandem leucine-rich motifs flanked by two cysteine clusters and two immunoglobulin (Ig)-like domains, a single transmembrane segment and an intracellular domain possessing a tyrosine-kinase domain. The positive susceptibility region that we identified lies in intron 19, located in the region that contains the exons coding for the tyrosine kinase domain of the protein (exons 17 to 21). We investigated the putative functional effects of rs2378672 through the interactive web-based SNP analysis tool Pupasuite (http://pupasuite.bioinfo.cipf.es/). The results show that rs2378672 lies in a mouse conserved region but does not

Table 3. Haplotype Association Results for a Five-Marker Window Across BDNF in OCD Patients

<table>
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<tr>
<th>Haplotype</th>
<th>TagSNP</th>
<th>Frequency</th>
<th>OR 95% CI</th>
<th>p Valuea</th>
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<td>Referent</td>
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<td>.94 (.86–1.02)</td>
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<td>1.10 (.99–1.23)</td>
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<tr>
<td>Rare (&lt;1%)</td>
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<td>.02</td>
<td>.85 (.67–1.08)</td>
<td>.18</td>
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</table>

Odds ratios (OR) and confidence intervals (CI) for tagSNP haplotypes using the most common haplotype (haplotype 1) as referent are shown. BDNF, brain-derived neurotrophic factor; CI, confidence interval; OCD, obsessive-compulsive disorder; OR, odds ratio; tagSNP, tag single nucleotide polymorphism.
aSignificant value adjusted covariation by sex.
bSignificant value obtained in the permutation analysis.

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alter splicing points that constitute the splicing signal, nor does it disrupt the suggested regulatory regions for controlling gene expression. The overall LD pattern in the region is low. Even when the most recent HapMap data (Phase III, release 22; April 2007) are considered, no tightly linked markers are associated to rs2378672 ( \( \chi^2 < 5 \)). This suggests that, in the absence of a direct causal implication of this SNP, the observed association of the intron 19 polymorphism should be in linkage disequilibrium with another unraveled functionally important sequence variant adjacent to the investigated NTRK2 gene region, which may be population specific. Thus, confirmation of the association should be corroborated by resequencing of the region to characterize population specific. Therefore, women who inherit certain NTRK2 polymorphisms may somehow lose part of this estrogen protective effect, which, together with other as yet unknown genetic factors, may influence BDNF expression, resulting in an increased susceptibility to OCD. Nevertheless, our results must be considered with caution since the significant difference on gender distribution between OCD patients and control subjects in our sample may have influenced this specific outcome.

We were not able to detect any significant association between BDNF or NTRK2 variants and age at onset of OCD. Although greater familial loading for OCD has been associated with early onset of the disorder, other studies have described, as in our case, a lack of any difference between adults with early- and late-onset OCD in terms of their family histories of obsessive illness (40). It has recently been emphasized that although familial aggregation is largely concentrated in families with early-onset OCD probands, between 43% and 66% of these early-onset patients are not familial cases (16,41). So, age of onset does not appear to be a sufficient clinical index for individualizing the familial character of OCD and its genetic basis.

Since BDNF has been postulated to contribute to genetic risk to affective disorders, the most frequent comorbid condition in OCD, we decided to analyze our results considering the presence of comorbid affective diagnosis. Our negative results suggest that the influence of BDNF and NTRK2 on OCD susceptibility is not related to comorbid affective disorders. Similar analyses considering comorbid eating disorders would have been very interesting but could not be performed due to the limited number of patients who met both conditions.

Significant genetic differences emerged when considering familiarity of OCD. Our results for the association between the heterozygosity of the Val66Met variant and family OCD may suggest a case of positive molecular heterosis related to family forms of the disorder. Molecular heterosis occurs when subjects heterozygous for a polymorphism associated with a single gene show a significantly greater or lesser effect for a quantitative or dichotomous trait than homozygotes. Examples of molecular heterosis are quite frequent in humans and have been described in genes of the serotonergic and dopaminergic pathways involved in the pathophysiology of several psychiatric disorders (42). Brain-derived neurotrophic factor is known to exist as a behaviors compared with male SERT \( \times \) BDNF-deficient mice, suggesting that estrogen availability exerts a protective effect against the monoamine depletion associated with these genetic deficiencies (39). Therefore, women who inherit certain NTRK2 polymorphisms may somehow lose part of this estrogen protective effect, which, together with other as yet unknown genetic factors, may influence BDNF expression, resulting in an increased susceptibility to OCD. Nevertheless, our results must be considered with caution since the significant difference on gender distribution between OCD patients and control subjects in our sample may have influenced this specific outcome.

Two previous reports have also shown the positive association of the NTRK2 gene with psychiatric disorders. In the first one, a mutational screening in eating disorders patients found that two three-marker haplotypes were associated with purging anorexia nervosa and bulimia nervosa (33). In the second study, a family-based analysis showed a positive association between allelic variants of the NTRK2 gene and three nicotine dependence measures (34). Interestingly, involvement of NTRK2 in the development of eating disorders reported by Ribases et al. (33) appears to depend not only on its direct participation in food intake and body weight regulation, but also on modulation of harm avoidance, a personality trait highly correlated with OCD (35). Further studies should clarify whether the contribution of NTRK2 to the shared genetic susceptibility between OCD and eating disorders depends on its influence on genetic variants of personality dimensions commonly associated with both disorders.

The gender-selective nature of the association between NTRK2 polymorphisms and OCD is congruent with the previously reported sexually dimorphic pattern of genetic susceptibility to OCD (8,36). Although published results are highly controversial (6,37,38), our results suggest that this dimorphic pattern may extend to genes outside the monoamine pathways. Estrogens have recently been shown to modify BDNF influence in the brain serotonergic system—the neurotransmitter pathway most clearly related to OCD—via its NTRK2 receptor. Female serotonin transporter (SERT) and BDNF-deficient mice show significantly lesser reductions in serotonin concentration in the hypothalamus and other brain regions and no increase in anxiety-like

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### Table 4. Haplotype Combination of the NTRK2 LD Block Containing rs2378672, Significant in the Univariate Analysis, in OCD Patients

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>rs10512159</th>
<th>rs1948308</th>
<th>rs2378672</th>
<th>rs1387926</th>
<th>rs1387924</th>
<th>rs3739570</th>
<th>rs1490403</th>
<th>Frequency</th>
<th>OR</th>
<th>CI 95%</th>
<th>p Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1</td>
<td>G</td>
<td>T</td>
<td>A</td>
<td>G</td>
<td>C</td>
<td>C</td>
<td>T</td>
<td>.42</td>
<td>Referent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H2</td>
<td>A</td>
<td>T</td>
<td>A</td>
<td>G</td>
<td>C</td>
<td>C</td>
<td>T</td>
<td>.16</td>
<td>.81</td>
<td>(0.52–1.25)</td>
<td>.33</td>
</tr>
<tr>
<td>H3</td>
<td>C</td>
<td>C</td>
<td>A</td>
<td>A</td>
<td>C</td>
<td>C</td>
<td>T</td>
<td>.12</td>
<td>.95</td>
<td>(0.63–1.44)</td>
<td>.82</td>
</tr>
<tr>
<td>H4</td>
<td>G</td>
<td>C</td>
<td>A</td>
<td>G</td>
<td>A</td>
<td>C</td>
<td>T</td>
<td>.12</td>
<td>1.03</td>
<td>(0.69–1.45)</td>
<td>.89</td>
</tr>
<tr>
<td>H5</td>
<td>C</td>
<td>C</td>
<td>A</td>
<td>G</td>
<td>C</td>
<td>A</td>
<td>T</td>
<td>.01</td>
<td>1.33</td>
<td>(0.47–3.79)</td>
<td>.59</td>
</tr>
<tr>
<td>H6</td>
<td>G</td>
<td>C</td>
<td>A</td>
<td>G</td>
<td>C</td>
<td>C</td>
<td>T</td>
<td>.04</td>
<td>.59</td>
<td>(0.29–1.20)</td>
<td>.14</td>
</tr>
<tr>
<td>H7</td>
<td>G</td>
<td>C</td>
<td>A</td>
<td>G</td>
<td>C</td>
<td>C</td>
<td>T</td>
<td>.07</td>
<td>.89</td>
<td>(0.54–1.47)</td>
<td>.65</td>
</tr>
<tr>
<td>H8</td>
<td>G</td>
<td>C</td>
<td>G</td>
<td>G</td>
<td>A</td>
<td>C</td>
<td>A</td>
<td>.05</td>
<td>.21</td>
<td>(0.09–0.49)</td>
<td>.0003 (0.0006)</td>
</tr>
<tr>
<td>Rare (&lt;1%)</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>.014</td>
<td>.68</td>
<td>(0.38–1.23)</td>
<td>.20</td>
</tr>
</tbody>
</table>

Odds ratios (OR) and confidence intervals (CI) for tagSNP haplotypes using the most common haplotype (haplotype 1) as referent are shown. CI, confidence interval; LD, linkage disequilibrium; OCD, obsessive-compulsive disorder; OR, odds ratio; tagSNP, tag single nucleotide polymorphism.

* p value adjusted covariation by sex.

* p value obtained in the permutation analysis.
tightly associated homodimer, which is required for high affinity binding to its dimeric receptor (43). In this particular case, the two structurally different BDNF subunits may distort the ability of the dimer to interact with the NTRK2 receptor.

Published studies suggest that genetic factors may be specially implicated in susceptibility to certain clinical obsessive-compulsive dimensions, including aggressive/sexual/religious (44), symmetry/ordering (44), and, in particular, hoarding symptoms (36,45). Although we tested for such an association in the case of BDNF and NTRK2 polymorphisms, we were not able to detect any significant genetic variant. Hoarding, symmetry/ordering, and sexual/religious obsessions and compulsions were present in less than one third of our sample; therefore, our negative results may be related to a type II error. Recent work in the OCD field has led to the development of the Dimensional Yale-Brown Obsessive-Compulsive Scale (DY-BOCS), an instrument that permits measurement of the presence and severity of obsessive-compulsive symptoms within distinct dimensions (46). The use of a dimensional approach of this kind may constitute a useful tool for analyzing the genetic basis of the different obsessive-compulsive symptoms, avoiding their consideration as categorically excluding subtypes. Alternatively, our negative results may suggest that BDNF and NTRK2 polymorphisms increase overall susceptibility to OCD, while other coexisting genetic or environmental factors determine the specific pattern of presentation of the disorder.

Some of the shortcomings of the study include the fact that our patients were recruited from specialized OCD clinics and may not be representative of the community. The information regarding age of onset was obtained retrospectively, introducing the possibility that patient recall may have been inaccurate. As in all case-control designs, population stratification may constitute a confounding factor. On one hand, the Spanish population is

Figure 3. Analysis of interaction (epistasis) between SNPs at BDNF and NTRK2 in obsessive-compulsive disorder patients using an additive model. The plot contains the p values obtained from different likelihood ratio tests. Different colors indicate different statistical significant levels. The diagonal contains the p values from likelihood ratio test for the crude effect of each SNP. The upper triangle in matrix contains the p values for the interaction log-likelihood ratio test. Finally, the lower triangle contains the p values from LRT comparing the two-SNP additive likelihood with the best of the single-SNP models. LRT, likelihood ratio test; SNP, single nucleotide polymorphism.
highly homogeneous (47), and on the other, structure convincingly identified only one population stratum among cases and control subjects. Nevertheless, subtle or weak admixture might have not been identified with the limited number of genotyped ancestry informative markers (AIMs) and thus cannot be completely discarded. Finally, we used a group of psychiatrically unscreened blood donors as the control group, which reduces the power to detect associations. Moskvina et al. (48) have recently concluded that for real-world situations in complex genetics, the use of unscreened control subjects is potentially cost-effective and can be considered for disorders with population prevalence lower than 20%. So, since OCD has a population lifetime risk of approximately 1%, the use of unscreened control subjects, although not ideal, can be considered to have a negligible effect on power.

In sum, our results add further evidence to the complex pattern of inheritance of OCD and suggest that BDNF/NTRK2 signaling may be one of the molecular pathways involved in its pathogenesis. Additional studies that examine the role of the genes involved in the neurotrophic systems and their interaction with other neurotransmitter pathways or hormonal factors are required to determine whether variant alleles at these loci might play an etiologic role in the expression of OCD. Further dissection of the samples using clinical phenotypes may also help to disentangle the genetic heterogeneity of OCD. Finally, possible protective BDNF and NTRK2 allelic combinations against OCD should be identified and their effect on other neurotransmitter systems explored, since they could constitute a promising pharmacotherapy target.

PA and MG contributed equally to this article.

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phism in patients with schizophrenia and healthy volunteers. *Arch Gen Psychiatry* 63:731–740.


