Genetic susceptibility to obsessive-compulsive hoarding: the contribution of neurotrophic tyrosine kinase receptor type 3 gene

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Recent work suggests that neurotrophic factors may contribute to the genetic susceptibility to obsessive-compulsive disorder (OCD). Among other clinical dimensions, the presence of hoarding obsessions and compulsions has been shown to be correlated with a number of clinical and neuroimaging findings, as well as with a different pattern of genetic inheritance. We used a linkage disequilibrium (LD)-mapping approach to investigate whether neurotrophic tyrosine kinase receptor type 3 (NTRK3), the high-affinity receptor of neurotrophin 3 (NT-3), plays a role in increasing susceptibility to hoarding in OCD. We performed an association study of 52 single nucleotide polymorphisms (tagSNPs) covering the whole NTRK3 gene in a sample comprising 120 OCD patients and 342 controls. Single nucleotide polymorphism association and haplotype analysis were performed. Thirty-six of our patients (30%) exhibited significant hoarding obsessions and compulsions. A significant association of two SNPs in the 3′ downstream region of NTRK3 gene and obsessive-compulsive hoarding was identified: rs1017412 [odds ratio (OR) = 2.16; \( P = 0.001 \)] and rs7176429 (OR = 2.78; \( P = 0.0001 \)), although only the latter remained significant after Bonferroni correction. Although the haplotype analysis did not show significant results, a more extended block of LD in the OCD hoarders with respect to the control group was observed, suggesting a lower haplotype diversity in these individuals. Our findings suggest that NTRK3 may contribute to the genetic susceptibility to hoarding in OCD and may constitute an interesting gene to focus on in studies of the genetic basis of obsessive-compulsive hoarding.

Keywords: association, hoarding, haplotype, NTRK3, obsessive-compulsive disorder, tagSNP

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Hoarding behavior is a complex phenomenon described in nonclinical populations and in a variety of clinical contexts: organic conditions such as primary degenerative dementia, mental retardation or bilateral and left damage to the orbitofrontal cortex; and psychiatric pathologies including schizophrenia, autism, eating disorders and obsessive-compulsive personality disorder (Saxena 2007). Besides these clinical conditions, approximately 18–42% of those suffering from obsessive-compulsive disorder (OCD) report hoarding obsessions and saving compulsions (Wheaton et al. 2008). Compared with nonhoarding patients with OCD, obsessive hoarders have more severe illness (Lochner et al. 2006, Samuels et al. 2007b, Wheaton et al. 2008), poorer insight into their condition (Samuels et al. 2007b), higher scores on anxiety and depression inventories (Frost & Gross 1993), a higher prevalence of personality disorders (Frost et al. 2000), more severe family and social disability (Frost et al. 2000) and lower global functioning (Saxena et al. 2002). Recent work suggests that the hoarding syndrome may constitute a neurobiologically distinct variant of OCD with specific clinical and neuroanatomical correlates as well as a different pattern of genetic inheritance (Saxena 2007). Hoarding obsessions and compulsions showed the highest familiarity in a recent linkage analysis of multigenerational families (Mathews et al. 2007) as well as in two studies of OCD-affected sibling pairs, especially for the female probands (Chacon et al. 2007; Hasler et al. 2007). An association has been reported between the hoarding phenotype and a recessive pattern of inheritance (Leckman et al. 2003). Genetic markers on chromosomes 4q34-35, 5q35.2-35.3 and 17q25 have been associated with hoarding in sibling pairs with...
Tourette’s syndrome (Zhang et al. 2002), and extensive linkage was found to chromosome 14 (D14S558) in OCD families with two or more hoarding relatives (Samuels et al. 2007a). Finally, a preponderance of methionine/methionine (met/met) genotypes and met (L/L) allele for the COMT Val158Met polymorphism has been reported in OCD hoarders (Lochner et al. 2005).

Hoarding and saving compulsions have been strongly associated with poor response to selective serotonin reuptake inhibitors (Cullen et al. 2007), suggesting that neuro-transmission pathways other than serotoninergic route may be involved in its pathogenesis. Our group has recently reported the association between a protective haplotype in brain-derived neurotrophic factor and its specific receptor, the neurotrophic tyrosine kinase receptor type 2 (NTRK2) and adult OCD, supporting the involvement of neurotrophic factors in the genetic susceptibility to OCD (Alonso et al. 2008). Nevertheless, no significant differences between obsessive-compulsive symptom dimensions on these specific genetic markers were detected. The neurotrophic tyrosine kinase receptor type 3 (NTRK3) is the high-affinity receptor of neurotrophin-3 (NT-3), another member of the neurotrophin family, implicated in the proliferation and differentiation of neurons during embryonic development and in their growth and survival in the adult nervous system (Beltaifa et al. 2005). Altered expression of NTRK3 has been recently described to contribute to the genetic susceptibility to anxiety-like behavior in mice (Dierssen et al. 2006). A reduction in NTRK3 mRNA in the dorso lateral prefrontal cortex (DLPFC) has been described in schizophrenic patients (Weickert et al. 2005), and NTRK3 immuno-reactivity in the parietal cortex has been reported to be reduced in patients with Alzheimer’s disease (Savaskan et al. 2000), two conditions that frequently exhibit hoarding behaviors. In humans, immunoreactivity for NTRK3 has been detected in a majority of dopaminergic neurons of the substantia nigra pars compacta and the mesocortical dopaminergic system (Lacroix et al. 1998), and it has been suggested that impaired signaling of NT-3 through its specific receptor may compromise the integrity of dopaminergic neurons (von Bohlen and Halbach et al. 2005). The dopamine system appears to play an important role in hoarding behavior because animal studies have consistently shown that food hoarding is stimulated by dopamine agonist and reduced by lesions of dopaminergic pathways mainly of the prefrontal cortex and the mesocortical dopaminergic system (Lacroix et al. 1998). Based on these findings, we hypothesized that NTRK3 may contribute to genetic vulnerability to hoarding in OCD and tested this hypothesis through a case–control study using an extensive linkage disequilibrium (LD)-mapping approach.

Method

Subjects
A total of 120 consecutive Spanish Caucasian outpatients with OCD (64 men and 56 women), recruited from the OCD Clinic and Research Unit of Bellvitge Hospital (Barcelona, Spain) between 2004 and 2006, were included in the study. All patients met Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria for OCD (American Psychiatric Association 1994) and had had OCD symptoms for at least 1 year. Diagnosis was independently assigned by two psychiatrists with extensive clinical experience in OCD who interviewed the patients separately using the Structured Clinical Interview for DSM-IV Axis I Disorders – Clinician Version (First et al. 1997). Exclusion criteria were age under 18 or over 65 years, mental retardation assessed by the Wechsler Adult Intelligence Scale (WAIS)-III, any other lifetime DSM-IV Axis I comorbid disorder and severe organic or neurological pathology including Gilles de la Tourette or other chronic tic disorders. During the selection period, 221 outpatients were assessed and fulfilled DSM-IV criteria for OCD. Of these patients, 92 were ruled out in accordance with the exclusion criteria (above all, the presence of another lifetime comorbid Axis I diagnosis) and 9 refused to take part in the study.

The control group consisted of 342 unrelated Caucasian subjects (202 males and 140 females, mean age of 39.8 years), who were recruited from a group of blood donors and who were not psychiatrically screened. Written informed consent was obtained from each subject after a full description of the study, which was approved by the hospital’s ethical committee.

A clinician administered version of the Yale-Brown Obsessive-Compulsive Scale (Y-BOCS) (Goodman et al. 1989) and the 21-item Hamilton Depression Rating Scale (HDRS) (Hamilton, 1960) were used to assess the severity of obsessive-compulsive and depressive symptoms, respectively. The Y-BOCS Symptom Checklist (SC) (Goodman et al. 1989) was employed to ascertain scores on the following symptom dimensions: contamination/cleaning, aggressive/checking, symmetry/ordering, sexual/religious and hoarding. So as to restrict the study to patients with clinically significant hoarding symptoms, OCD patients who gave affirmative answers on the Y-BOCS items related to hoarding were considered hoarders only if they spent at least 1 h a day on hoarding-related activities and reported at least moderate to severe distress and impairment secondary to hoarding. This clinical information was obtained through direct interviews of the patients, applying the first three questions of the Y-BOCS to hoarding behavior (time spent per day, anxiety or distress and impairment). Whenever possible, this information was confirmed by direct interviews with relatives living with the patient.

Tag single nucleotide polymorphism selection

From the HapMap project data set, we used genotypes from public release 16 (Phase I data freeze, dbSNP b124), corresponding to the 60 individuals from 30 Centre d’Etude du Polyiomorphisme Humain (CEPH) trios of European descent (http://www.hapmap.org). From the gene location, we extended the search for 5–10 kb upstream and downstream NTRK3 gene. Only SNPs with a unique mapping location on the NCBI B34 assembly and a minor allele frequency (MAF) higher than 10% were considered for further analysis. In the NTRK3 region, the LD select algorithm (Carlson et al. 2004), which partitions the SNPs of a given region into “bins”. In a given bin, there is one SNP that has a pairwise r² exceeding a user-specific threshold. Each of the tagSNPs in a particular bin can be used to represent the allelic variation of SNPs within each bin and is a candidate for genotyping in a larger sample. In our study, we identified bins of common SNPs in strong LD, as defined by an r² higher than 0.85, from the HapMap genotype dump format data corresponding to the NTRK3 region in European ancestry trios. Fifty-two tagSNPs were finally selected by the LD method to cover all bins in the NTRK3 genomic region (Table S1 and Figure S1).

SNPlex design and method

TagSNPs were genotyped using the SNPlex Genotyping System. SNPs that passed the design rules for the development of good assays were selected. The high-throughput genotyping assays were performed at the CeGen genotyping facilities, in the Barcelona Node (Centro Nacional de Genotipo, Genoma España). We followed the
Quality control of genotypes

All genotyping was performed blind with respect to phenotype. In addition to internal positive and negative controls provided by ABI for the SNPlex procedure, we analyzed the genotype concordance and the Mendelian inheritance in six samples corresponding to two HapMap reference trios: samples NA10860, NA10861, NA11992, NA11993, NA11994 and NA11995 (family numbers CEPH131 and CEPH132). Genotype concordance was tested using SNPassor (http://www.CEGEN.org) and Mendelian inheritance was tested using HAPLOVIEW software (version 3.2; Barrett et al. 2005).

Genotyping for population admixture

To detect population admixture, we chose 48 anonymous unlinked SNPs derived from a panel of 52 Ancestral Informative Markers reported to be polymorphic in European, Asian and African populations (Sanchez et al. 2006). We used the computer program STRUCTURE 2.2 (Pritchard et al. 2000) to identify clusters of genetically similar individuals. An admixture model with correlated frequencies was used, using five putative K values (1 to 5). Analysis was performed both with and without prior population information.

Only the SNPs in Hardy-Weinberg equilibrium (HWE), with a genotyping rate >90% and an $I^2 < 0.1$, were retained for analysis from our data. In total, 37 SNPs of 48 were used for these analyses. Using the STRUCTURE program, no allelic differences were observed and we obtained the highest log likelihood scores when the number of populations was set at 1.

Sample power calculation

We computed post hoc power calculations using the Genetic Power Calculator (http://pngu.mgh.harvard.edu/~purcell/gpc/). Thus, we determined that the case–control sample had 98.6% power of detecting a risk allele with 10% frequency and a dominant genotype relative risk of 2.8 if the disease prevalence is 0.3% (considering the population lifetime risk of approximately 1% for OCD and that obsessive–compulsive hoarding is usually present in nearly one-third of these patients).

Statistical methods

Clinical and demographic data

Continuous independent data were compared between controls and both groups of OCD patients with one-way analysis of variance (ANOVA) and Tukey b post hoc corrections. Categorical data were compared between the three groups with chi-square test. On comparing hoarding and nonhoarding OCD patients on demographic features and clinical characteristics, the chi-square test for categorical variables and Student’s t-test for continuous variables were used.

Genetic data

The effects of NTRK3 tagSNPs on hoarding were examined with multinomial logistic regression, controlling for sex (Hosmer & Lemeshow 2000). The comparisons were performed between OCD hoarders and nonhoarders using controls as the base category or reference point. The associations are expressed in terms of risk ratios. Multinomial regressions were also performed for all the other described OCD clinical symptom dimensions to assess the possible association between them and NTRK3 SNPs. The analysis entailed multiple comparisons across SNPs, which might have inflated the chances of type I error and suggested untrue associations. This bias was controlled with a Bonferroni correction taking into account 52 independent SNPs. We also used the max-statistic to correct for the five inheritance models tested (co-dominant, dominant, recessive, overdominant and log additive), which showed that the most effective number of tests was 2.2 (González et al. 2008). Therefore, associations that were statistically significant at the 0.05 and 0.01 levels are not reported here because they could be viewed as tentative, and the experiment-wide significance was set to a P value of 0.0009.

To characterize the LD pattern, we estimated $D^2$ and $r^2$ values for all pairs of SNPs within the genomic region of NTRK3 using an expectation maximization (EM) algorithm as implemented in the HAPLOVIEW computer program (http://www.broad.mit.edu/mpg/haploview) (Barrett et al. 2005). Regions of strong LD (haplotype blocks) were defined using the confidence interval method of Gabriel et al. (2002).

To estimate the association of the haplotype and the hoarding phenotype, the frequency of haplotypes was estimated in the combined populations of hoarding patients and controls using the EM algorithm, as implemented in the HAPLOVIEW software. For the analysis, we examined haplotypes whose frequency was higher than 1% so as to exclude the many haplotypes that were not present in the sample and hence creating the possibility of a rank-deficient regression matrix.

Results

The demographic and clinical characteristics of both groups of OCD patients are summarized in Table 1. Controls were significantly older than patients in both OCD groups ($F = 799.8, df = 2,459, P < 0.001$), but they did not significantly differ from them on gender distribution. Thirty-six of our patients (30%) exhibited significant hoarding obsessions and compulsions. All hoarding subjects had other obsessions and compulsions in addition to hoarding. In 6.6% of the patients ($n = 8$), hoarding was the main obsessive symptom, while in the remaining 23.3% ($n = 28$) hoarding obsessions and compulsions were clinically significant but not the patient’s foremost obsessive concern. Hoarders exhibited more severe OCD symptomatology according to Y-BOCS scores and were more frequently male than nonhoarders. They were also more often diagnosed as suffering from a personality disorder, mainly a schizotypal one, and reported more symmetry and ordering obsessions and compulsions.

Single SNP and haplotype association analysis

The complete list of the 52 tagSNPs used in the study, their chromosomal locations, allele frequencies and genotyping rates are presented in Table S1.

The genotype distribution was in Hardy-Weinberg equilibrium both in controls and patients ($x^2 = 0.01$). No significant difference in the distribution of alleles or genotypes was detected between controls and OCD subjects considered as a whole (data not shown).

A significant association of two SNPs in the 3’ downstream region of NTRK3 gene and obsessive–compulsive hoarding was identified: rs1017412 ($P = 0.001$) and rs7176429
Although only the later remained significant after Bonferroni correction (Tables 2 and 3) (see Table S2 and Figure S2 with the P values for the rest of the genotyped SNPs on the hoarding group). Thus, heterozygous patients for this last SNP showed a 2.8-fold increased risk of presenting hoarding symptoms compared with nonvariant homozygous control individuals. This effect was not observed in the non-hoarder group. No evidence of an association of NTRK3 polymorphisms with any other OCD symptom dimension was detected (data not shown).

To check whether the haplotype structure differed in controls and hoarding subjects, we independently derived the haplotype block structure in a 75-kb region near the 3’ region of the NTRK3 gene that contains the two consecutive and nominally positive SNPs detected in the single-variant analysis (namely rs1017412 and rs7176429). For this particular region, which contains 12 tagSNPs, four blocks were defined in the control group (7, 6, 5 and 14 kb) and only two in the hoarder group (7 and 12 kb). A detailed composition of the haploblocks for each group is summarized in Fig. 1. In spite of the slight differences in the distribution of LD across this region, the block structure that contains the two nominally positive SNPs did not differ between hoarders and controls. Finally, we performed a haplotype-based analysis using a structured approach to test for associations based on the LD pattern of this region. None of the haplotype distributions differed in the hoarding group and the controls in any of the identified blocks (P > 0.05) (Table 4).

### Table 1: Sociodemographic and clinical characteristics of hoarding and nonhoarding obsessive–compulsive patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>OCD nonhoarders (n = 94)</th>
<th>OCD hoarders (n = 36)</th>
<th>t</th>
<th>P</th>
<th>95% confidence intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>33.7 ± 10.1</td>
<td>31.0 ± 10.0</td>
<td>1.3</td>
<td>0.1</td>
<td>-1.2 to 6.6</td>
</tr>
<tr>
<td>Age at OCD onset, years</td>
<td>20.5 ± 8.5</td>
<td>18.0 ± 7.1</td>
<td>1.3</td>
<td>0.1</td>
<td>-1.0 to 5.3</td>
</tr>
<tr>
<td>Y-BOCS score</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Global</td>
<td>26.8 ± 5.3</td>
<td>30.3 ± 4.4</td>
<td>-3.4</td>
<td>0.001</td>
<td>-5.5 to -1.5</td>
</tr>
<tr>
<td>Obsessions</td>
<td>13.6 ± 2.7</td>
<td>15.2 ± 2.2</td>
<td>-3.2</td>
<td>0.002</td>
<td>-2.6 to -0.6</td>
</tr>
<tr>
<td>Compulsions</td>
<td>13.2 ± 3.6</td>
<td>15.0 ± 2.2</td>
<td>-2.8</td>
<td>0.005</td>
<td>-3.1 to -0.5</td>
</tr>
<tr>
<td>Basal HDRS score</td>
<td>14.1 ± 5.7</td>
<td>15.0 ± 5.0</td>
<td>-0.7</td>
<td>0.4</td>
<td>-2.9 to 1.3</td>
</tr>
</tbody>
</table>

### Table 2: Multinomial risk ratios of the effect of SNPs on hoarding, corrected by sex, considering control individuals as a reference

<table>
<thead>
<tr>
<th>OR (P value)</th>
<th>OCD hoarders</th>
<th>OCD nonhoarders</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1017412</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>CT</td>
<td>2.16 (0.001)</td>
<td>0.47 (0.35)</td>
</tr>
<tr>
<td>TT</td>
<td>0.82 (0.03)</td>
<td>0.68 (0.27)</td>
</tr>
<tr>
<td>rs7176429</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>GT</td>
<td>2.78 (0.0001*)</td>
<td>0.47 (0.06)</td>
</tr>
<tr>
<td>TT</td>
<td>1.93 (0.03)</td>
<td>0.59 (0.20)</td>
</tr>
</tbody>
</table>

*Significant after Bonferroni correction (52 SNPs and 2.2 models tested; P < 0.00043).

To check whether the haplotype structure differed in controls and hoarding subjects, we independently derived the haplotype block structure in a 75-kb region near the 3’ region of the NTRK3 gene that contains the two consecutive and nominally positive SNPs detected in the single-variant analysis (namely rs1017412 and rs7176429). For this particular region, which contains 12 tagSNPs, four blocks were defined in the control group (7, 6, 5 and 14 kb) and only two in the hoarder group (7 and 12 kb). A detailed composition of the haploblocks for each group is summarized in Fig. 1. In spite of the slight differences in the distribution of LD across this region, the block structure that contains the two nominally positive SNPs did not differ between hoarders and controls.

Finally, we performed a haplotype-based analysis using a structured approach to test for associations based on the LD pattern of this region. None of the haplotype distributions differed in the hoarding group and the controls in any of the identified blocks (P > 0.05) (Table 4).

### Discussion

Current theories hold that OCD does not constitute a unitary nosological entity, but a multidimensional and etiologically heterogeneous condition. So, there is increasing interest in identifying clinically meaningful phenotypes that might be more homogeneous to facilitate genetic, neurobiological and outcome studies. Among strategies of phenotype description, the dimensional approach has provided promising leads for neuroimaging, genetic and treatment response studies (Mataix-Cols et al. 2005). Hoarding obsessions and compulsions have been consistently replicated as

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Table 1: Sociodemographic and clinical characteristics of hoarding and nonhoarding obsessive–compulsive patients

Table 2: Multinomial risk ratios of the effect of SNPs on hoarding, corrected by sex, considering control individuals as a reference

Table 3: Genotyping details of the two SNPs associated with hoarding in OCD patients and controls

<table>
<thead>
<tr>
<th>SNP</th>
<th>MAF (%)</th>
<th>Genotyping rate (%)</th>
<th>HWE (P value)</th>
<th>Genotype</th>
<th>Nonhoarders, n (%)</th>
<th>Hoarders, n (%)</th>
<th>Controls, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1017412</td>
<td>31.9</td>
<td>96.2</td>
<td>0.17</td>
<td>TT</td>
<td>26 (31.3)</td>
<td>22 (62.9)</td>
<td>155 (46.5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CT</td>
<td>52 (62.6)</td>
<td>10 (28.6)</td>
<td>146 (43.8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CC</td>
<td>5 (6.0)</td>
<td>3 (8.6)</td>
<td>32 (9.6)</td>
</tr>
<tr>
<td>rs7176429</td>
<td>33.7</td>
<td>96.2</td>
<td>0.46</td>
<td>GG</td>
<td>21 (25.3)</td>
<td>22 (62.9)</td>
<td>153 (45.9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>GT</td>
<td>53 (63.8)</td>
<td>10 (28.6)</td>
<td>145 (43.5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TT</td>
<td>9 (10.8)</td>
<td>3 (8.6)</td>
<td>35 (10.5)</td>
</tr>
</tbody>
</table>

HWE, Hardy-Weinberg equilibrium.

Figure 1: Scaled diagram showing the NTRK3 gene structure surrounding its 3’ end, where a positive association was detected. Coding exons are shown as black boxes. Non-coding exons and 3’ UTR region are shown in gray boxes. The tyrosine kinase protein domain is shown as a black line above the gene structure. The relative location of the TagSNPs genotyped in this genomic region is also shown. Below the diagram, there are the LD in this region of the gene obtained from the genotyped SNPs in controls (upper panel) and hoarder patients (lower panel), determined and visualized using the program HAPLOVIEW. The boxes in black indicate the high-LD blocks, with pairwise D’ >0.8. Block size and interblock distances are indicated.


5
Table 4: Haplotype distribution in hoarding subjects and controls

<table>
<thead>
<tr>
<th>Haplotype*</th>
<th>Hoarders (n = 34)</th>
<th>Controls (n = 342)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>0.647</td>
<td>0.627</td>
<td>0.74</td>
</tr>
<tr>
<td>CT</td>
<td>0.324</td>
<td>0.316</td>
<td>0.89</td>
</tr>
<tr>
<td>CT</td>
<td>0.029</td>
<td>0.057</td>
<td>0.34</td>
</tr>
<tr>
<td>Block 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TCC</td>
<td>0.588</td>
<td>0.655</td>
<td>0.27</td>
</tr>
<tr>
<td>CTT</td>
<td>0.294</td>
<td>0.249</td>
<td>0.42</td>
</tr>
<tr>
<td>CCC</td>
<td>0.103</td>
<td>0.064</td>
<td>0.23</td>
</tr>
<tr>
<td>CTC</td>
<td>0.000</td>
<td>0.026</td>
<td>0.18</td>
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<tr>
<td>Block 3</td>
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<td></td>
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<tr>
<td>TC</td>
<td>0.515</td>
<td>0.524</td>
<td>0.88</td>
</tr>
<tr>
<td>CT</td>
<td>0.382</td>
<td>0.359</td>
<td>0.70</td>
</tr>
<tr>
<td>CC</td>
<td>0.103</td>
<td>0.115</td>
<td>0.76</td>
</tr>
<tr>
<td>Block 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TA</td>
<td>0.779</td>
<td>0.793</td>
<td>0.79</td>
</tr>
<tr>
<td>AG</td>
<td>0.162</td>
<td>0.137</td>
<td>0.57</td>
</tr>
<tr>
<td>AA</td>
<td>0.093</td>
<td>0.067</td>
<td>0.81</td>
</tr>
</tbody>
</table>

*Inferred haplotype in blocks defined by confidence interval block partition algorithm. Block 1, rs3743165 and rs10117412; block 2, rs1369430, rs11638486 and rs1435403; block 3, rs3903308 and rs1347424; block 4, rs1836592 and rs3784434.

A valid and reliable dimensional construct (Mataix-Cols et al. 2005). In addition, it has been shown to be correlated with a number of neuroimaging findings (Mataix-Cols et al. 2004; Saxena et al. 2004), specific clinical characteristics (Frost & Gross, 1993, Frost et al. 2000, Lochner et al. 2005, Wheaton et al. 2008) and treatment response (Cullen et al. 2007; Saxena et al. 2002). Nevertheless, it has been in the search for genetic susceptibility factors to OCD where the consideration of the hoarding dimension has proved to be especially useful (Leckman et al. 2003; Samuels et al. 2007a; Zhang et al. 2002).

Thirty per cent of our patients had significant hoarding obsessions and compulsions, a finding in agreement with previously reported rates of hoarding behaviors in OCD subjects. As in earlier studies (Lochner et al. 2005; Samuels et al. 2007b; Wheaton et al. 2008), the severity of obsessive symptoms measured by the Y-BOCS was greater in hoarders. They were more likely to have associated symmetry and ordering compulsions, a phenomenon also previously described (Hasler et al. 2007; Samuels et al. 2002; Samuels et al. 2007b). Finally, as other authors have reported (Frost et al. 2000; Samuels et al. 2007b), hoarders also had a greater prevalence of personality disorders, especially of schizotypal type.

Our results suggest that NTRK3 loci may contribute to the genetic susceptibility to obsessive–compulsive hoarding with the single-marker association study indicating two nominally associated SNPs in the 3' downstream region of the gene. According to the public HapMap database, this region has a high degree of LD in Caucasian population. When we compared the LD pattern of this region in our sample set, we observed a more extended block of LD in the hoarder group, suggesting lower haplotype diversity in these individuals. These results must, however, be interpreted with caution because block estimation is highly dependent on marker saturation and, given the small sample size, this difference in spatial patterns of LD can only be taken as indicative and not as statistically proven. Besides, the block structure of the particular region that contains the two nominally positive SNPs did not differ between hoarders and controls, and the single SNP that underwent Bonferroni correction (rs7176429) is located in a lower recombination region between two adjacent blocks. This suggests a need of a more subtle analysis of the surrounding region that might possibly identify haplotypes carrying other unscorced, relatively rare SNPs and markers accounting for a functional role, because this genomic region contains genomic sequences for the tyrosine kinase domain of the protein, encoded by the exons 13–18.

However, the finding of a heterozygote status (or over-dominant effect) of the significant SNP genotype in the hoarding phenotype raises an interesting question. Although this may constitute a statistical artifact because of our small sample size, one may wonder whether two structurally different NTRK3 subunits might result in a distorted ability of the dimer to interact with the NTF3 ligand.

From a functional perspective, NTRK3 is required for dendritic maturation and synaptogenesis, as shown in the knockout mice that have reduced axonal arborization and synaptic density in the hippocampus (Martinez et al. 1998). NTRK3 mRNA has been localized in all layers of the human prefrontal cortex (Beltaifa et al. 2005) and animal models show that signaling through NTRK2 and NTRK3 is also important for the maintenance of the catecholaminergic innervation of two limbic key regions, the hippocampus and amygdala (von Bohlen und Halbach & Minichiello 2006). Activity in mesial prefrontal structures, basically the anterior cingulate and the frontal pole, appears to be necessary for regulating the tendency to collect that originates primarily in subcortical bioregulatory nuclei, including the ventral segmental area, lateral hypothalamus, nucleus accumbens, hippocampus, amygdala and thalamus (Anderson et al. 2005). OCD hoarders show a unique pattern of lower metabolism in the posterior cingulate cortex, the cuneus, the dorsal anterior cingulate gyrus and the thalamus (Saxena et al. 2004) as well as greater activation in left precentral gyrus and right orbitofrontal cortex than controls (Mataix-Cols et al. 2004). So, orbitofrontal, cingulate and other limbic structures appear to be important in the development of obsessive–compulsive hoarding behaviors. It is tempting to speculate whether altered function of neurotrophic factors in prefrontal or limbic regions such as the hippocampus or the amygdala, might contribute to the development of hoarding obsessions and compulsions in OCD patients.

This is, to our knowledge, the first attempt to study the implication of neurotrophic factors in obsessive–compulsive hoarding covering the whole genomic region of NTRK3 gene. Nevertheless, several limitations of this study should be mentioned. Our sample size is limited, which reduces the power of the analysis to detect significant associations and may have influenced, at least partially, our negative findings in
the haplotype study. We decided to consider only pure OCD patients without any other comorbid Axis I disorder, although this significantly reduced our sample size, to avoid the possible bias derived from comorbid affective or anxiety disorders, psychiatric conditions in which the involvement of neurotrophic factors has been postulated. What is more, the best assessment of hoarding behaviors in OCD is still a highly controversial point. The Y-BOCS-SC, the gold standard for assessing OCD symptoms, contains only two questions that tap hoarding thoughts and behaviors, which may not be sufficient to capture hoarding symptoms accurately. For their part, recently developed self-report measures of hoarding, such as the Saving Inventory-Revised (SI-R) (Frost et al. 2004), may be compromised by the reduced insight hoarders often demonstrate with regard to their behavior. We tried to overcome these difficulties by using a strict definition of hoarding, which combined both the Y-BOCS-SC hoarding items and clinical criteria. One important issue is that our results are only applicable to hoarding behaviors that appear in the context of OCD and cannot be generalized to compulsive hoarders who do not have other OCD symptoms and who may represent a separate though related OCD spectrum disorder. As in all case-control designs, population stratification may constitute a confounding factor, although the results of the structured association analysis make the admixture issue unlikely. Finally, we used a group of psychiatrically unscreened blood donors as the control group, which reduces the power to detect associations. Nevertheless, Moskvina et al. (2005) recently concluded that for real-world situations that tap hoarding thoughts and behaviors, which may contribute to the development of hoarding symptoms in unscreened controls, although not ideal, can be considered to be present in nearly one-third of these patients, the use of unscreened controls, although not ideal, can be considered to have a negligible effect on power.

In summary, our results suggest that neurotrophic factors may contribute to the development of hoarding symptoms in OCD and that NTRK3 may be of particular use in studies of the genetic basis of obsessive–compulsive hoarding. These findings must nevertheless be considered as preliminary until further confirmation is obtained in independent samples or in family studies. Our results support the hypothesis that the consideration of clinical dimensions such as hoarding in defining more homogeneous OCD phenotypes may constitute a useful tool in the search for the neurobiological basis of the disorder.

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Supplementary material
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Figure S1. Scaled diagram showing the NTRK3 genomic
region covered in the present study with the relative location of
the genotyped tagSNPs. Below the region, the linkage disequilibrium
(r2) and block structure of the gene obtained from the
genotyped SNPs in control subjects as determined and
visualized using the program haplview. The boxes in black
indicate the high-LD blocks, with pairwise D’ >0.8. Block
size and interblock distances are indicated.
Figure S2. Plot of the –log10 P values for the multinomial
models of the effect of all genotyped SNPs on hoarding
(corrected by sex). The statistically significant association at
Bonferroni corrected level (red dashed line) is also indicated.
Table S1. Summary of the tagSNPs genotyped in the
NTRK3 genomic region.
Table S2. P values deduced from the multinomial models
of the effect of all genotyped SNPs on hoarding, corrected by sex.
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