Association of distinct allelic haplotypes of DISC1 with psychotic and bipolar spectrum disorders and with underlying cognitive impairments

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Bipolar disorder (BPD) and schizophrenia (SCZ) have at least a partially convergent aetiology and thus may share genetic susceptibility loci. Multiple lines of evidence emphasize the role of disrupted-in-schizophrenia-1 (DISC1) gene in psychotic disorders such as SCZ. We monitored the association of allelic variants of translin-associated factor X (TSNAX)/DISC1 gene cluster using 13 single-nucleotide polymorphisms (SNPs) in 723 members of 179 Finnish BPD families. Consistent with an earlier finding in Finnish SCZ families, the haplotype T-A of rs751229 and rs3738401 at the 5′ end of DISC1 was over-transmitted to males with psychotic disorder ($P = 0.008$; for an extended haplotype $P = 0.0007$ with both genders). Haplotypes at the 3′ end of DISC1 associated with bipolar spectrum disorder ($P = 0.0002$ for an under-transmitted haplotype T-T of rs821616 and rs1411771, for an extended haplotype $P = 0.0001$), as did a two-SNP risk haplotype at the 5′ end of TSNAX ($P = 0.007$). The risk haplotype for psychotic disorder also associated to perseverations ($P = 0.035$; for rs751229 alone $P = 0.0012$), and a protective haplotype G-T-G with rs1655285 in addition to auditory attention ($P = 0.0059$). The 3′ end variants associated with several cognitive traits, with the most robust signal for rs821616 and verbal fluency and rs980989 and psychomotor processing speed ($P = 0.011$ for both). These results support involvement of DISC1 in the genetic aetiology of BPD and suggest that its distinct variants contribute to variation in the dimensional features of psychotic and bipolar spectrum disorders. Finding of alternative associating haplotypes in the same set of BPD families gives evidence for allelic heterogeneity within DISC1, eventually leading to heterogeneity in the clinical outcome as well.

INTRODUCTION

The disrupted-in-schizophrenia-1 gene (DISC1) encodes for a multifunctional protein that plays important roles in neurodevelopment and synaptic modulations by interacting with several proteins (1,2). It is highly expressed in the central nervous system, particularly in the hippocampus and the cerebral cortex (3–5). The genomic locus was originally identified and linked with a range of psychiatric disorders in a large Scottish pedigree (6,7). Members of that large family carried a balanced (1,11) (q42.1;q14.3) translocation that segregated with major psychiatric disorders, including schizophrenia (SCZ), bipolar disorder (BPD) and recurrent major depression (7). The translocation directly disrupts two genes, DISC1 and DISC2 at 1q42 (8–10). A 4-bp frame-shift deletion

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in exon 12 of DISC1 segregated with SCZ and schizoaffective disorder (SA) in a small American pedigree (11), although in a study of 655 affected and 694 control Caucasian individuals the frame-shift deletion was found only in two control individuals (12). Linkage and association studies between the DISC1 locus and SCZ were replicated in other study samples originating from America (European- and African-Americans), Taiwan, Finland, Scotland and China (13–21), but not from Japan (13,14). In the Finnish population, linkage to the DISC1 locus was reported in two independent SCZ sample sets (15,16). A common two single-nucleotide polymorphism (SNP) haplotype assigned as HEP3 (rs751229 and rs3738401), spanning an average 62-kb region from intron 1 to exon 2 of DISC1 (Fig. 1), was over-transmitted to affected males when the population frequency was taken into account (17,18) and was associated with potential cognitive endophenotypes (19) of SCZ, such as poor visual working memory and visual attention (18).

Although representing separate disease entities with specific diagnostic criteria, BPD and SCZ share several clinical features and neuropsychological impairments, and can be found to be co-segregated in many pedigrees (20–22). Both disorders are highly heritable and characterized by young ages at onset and a lifelong course. While psychosis is a primary diagnostic criterion for SCZ, a great part of cases with BPD also have intermittent psychotic symptoms (23–26), and cognitive impairments related to the disease susceptibility have been proposed for both disorders. Concerning BPD, the strongest evidence for impairments has been obtained for executive functioning and verbal memory. These impairments have been found both in the patients and in their unaffected family members (27–29), and the same functions have been found to be impaired in SCZ families as well (30). There is an overlapping incidence of primary thought and mood disorders within pedigrees as found, for example, in the DISC1 translocation family with cases of SCZ, BPD and recurrent major depression (6,7,10). Finally, there is one distinct diagnostic category that comprises a bridge between these conditions: SA disorder, with diagnostic requirement of both psychotic illness and a concurrent depressive or a manic episode, and with relatively high rates of both SCZ and mood disorder in relatives of probands (31).

In recent years, the evidence for linkage and association of BPD with DISC1 has been accumulating (32–38). Overtransmission of a risk haplotype to affected BPD females, who also showed lower levels of DISC1 mRNA expressions in their lymphoblasts, was reported in a recent family-based association study (33), and evidence for linkage of SA disorder with 1q42 was observed in a family-based sample from the UK and Ireland (39).

With the assumption that BPD and SCZ have at least a partially convergent genetic background, we looked for an association of DISC1, previously established as a susceptibility gene for SCZ in Finland, in the bipolar family sample of the same genetically homogenous population. We also analyzed SNPs in the translin-associated factor X (TNSAX) gene, located immediately upstream from DISC1. To catch the gamut of the causality between the genes and the clinical outcome, we focused on two diagnostic entities: psychotic and bipolar spectrum disorders. In addition, we monitored for potential association of neuropsychological variables, including general intellectual functioning, attention and working memory, verbal learning and memory, and executive functions with the allelic variants of TNSAX and DISC1. Our results provide evidence for an association of distinct allelic variants of TNSAX and DISC1 to psychotic and bipolar spectrum disorders and with related cognitive endophenotypes.

**RESULTS**

### Association of distinct regions of TNSAX/DISC1 with psychotic and bipolar spectrum disorders

We genotyped a total of 13 SNPs across the 600-kb region containing the TNSAX and DISC1 genes in a sample of 179 families of Finnish origin ascertained for BPD (Tables 1 and 2; Fig. 1). We used two diagnostic categories in the association study: psychotic disorders [BPD type I with intermittent psychotic features, psychotic depression, SCZ, SA disorder and psychosis not otherwise specified (NOS)] and bipolar spectrum disorders (BPD type I and II, NOS and cyclothymia) (Table 1). Each variant was first analyzed for an association using the Pseudomarker program (40), followed by testing the allelic transmission of the adjacent two-SNP haplotypes across the TNSAX/DISC1 gene cluster to affected individuals using the program TRANSMIT (41). Considering the previous results of sex-specific association for this genomic region (17,18,32,33), we performed both combined and gender-specific analyses.

With psychotic disorder as an outcome, the analysis of individual SNPs in the complete sample provided only evidence of a trend for an association with rs3738401 in exon 2 ($P = 0.051$, Pseudomarker) (Table 2). In analysis of the adjacent two-SNP haplotypes across the TNSAX/DISC1 gene cluster, no two-SNP haplotype showed global association at significance level of $P < 0.05$ in the complete sample, but several significant associations were detected for males (Table 3). The strongest association for specific haplotypes was obtained with a haplotype at the 5’ end of DISC1, composed of the T and A alleles of rs751229 and rs3738401, respectively. This particular haplotype was over-transmitted to affected males (global $P = 0.012$, for the T-A haplotype $P = 0.008$; $f = 0.08$) (Table 3). The signal for association was found to be derived from both males with or without bipolar spectrum disorder (haplotype-specific $P = 0.066$ for BPD I with psychotic features and $P = 0.062$ for those psychotic individuals that did not overlap with the bipolar spectrum category) (Table 4). Interestingly, exactly the same two-SNP haplotype, designated as “HEP3”, was in an earlier study shown to be over-transmitted to affected males in Finnish SCZ families (17,18). To define the extent of the associated haplotype, the adjacent three-SNP haplotypes of the regions of interest were analyzed. A significant association with psychotic disorder was detected with a rare haplotype composed of the T, A and G alleles of rs751229, rs3738401 and rs1538977, respectively, that was over-transmitted to affected males (haplotype-specific $P = 0.0007$ for both genders and 0.007 for males alone; $f = 0.03$). Another allelic haplotype of the same variants was also over-transmitted to males ($P = 0.001$ for an allele combination C-G-A; $f = 0.17$). A three-SNP haplotype of SNPs rs1655285, rs751229 and
rs3738401, including the variants of HEP3 and in addition one variant at the 5' end, was over-transmitted to males with psychotic disorder ($P = 0.007$ in males for the allele combination G-T-A; $f = 0.07$). Finally, another, common allelic haplotype G-T-G of these three variants was significantly under-transmitted to males ($P = 0.003$; $f = 0.43$). Taken together, these data provide evidence for association of multiple common and relatively rare haplotypes around the HEP3 region with psychotic disorder, particularly in males, in these Finnish families.

Using bipolar spectrum disorder as an outcome, we observed weak evidence for association of rs1655285 at intron 5 of TSNAX with bipolar spectrum disorder ($P = 0.025$, Pseudomarker) (Table 2). In analysis of two-SNP haplotypes by the sliding window (Table 5), a major haplotype containing rs1630250 and rs1615409 within the TSNAX gene was over-transmitted
Table 2. Results from association analysis with SNPs in TSNAX/DISC1 locus using the recessive model of Pseudomarker program in the Finnish families ascertained for BPD

<table>
<thead>
<tr>
<th>SNP</th>
<th>Coding change</th>
<th>Locationb</th>
<th>Major allele</th>
<th>Minor allele</th>
<th>MAF</th>
<th>Psychotic disorder P-valuesa</th>
<th>Bipolar spectrum disorder P-valuesa</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>G</td>
<td>C</td>
<td></td>
<td>0.274</td>
<td>0.160</td>
<td>0.365</td>
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<td>2</td>
<td></td>
<td>C</td>
<td>A</td>
<td></td>
<td>0.650</td>
<td>0.109</td>
<td>0.569</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>G</td>
<td>C</td>
<td></td>
<td>0.080</td>
<td>0.920</td>
<td>0.541</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>T</td>
<td>C</td>
<td></td>
<td>0.414</td>
<td>0.689</td>
<td>0.150</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>A</td>
<td>G</td>
<td></td>
<td>0.134</td>
<td>0.665</td>
<td>0.131</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>A</td>
<td>G</td>
<td></td>
<td>0.279</td>
<td>0.251</td>
<td>0.399</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>A</td>
<td>G</td>
<td></td>
<td>0.374</td>
<td>0.334</td>
<td>0.916</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>C</td>
<td>T</td>
<td></td>
<td>0.121</td>
<td>0.507</td>
<td>0.157</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>G</td>
<td>T</td>
<td></td>
<td>0.306</td>
<td>0.823</td>
<td>0.791</td>
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<tr>
<td>10</td>
<td></td>
<td>A</td>
<td>C</td>
<td></td>
<td>0.238</td>
<td>0.920</td>
<td>0.178</td>
</tr>
<tr>
<td>11</td>
<td></td>
<td>A</td>
<td>T</td>
<td></td>
<td>0.338</td>
<td>0.247</td>
<td>0.655</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td>A</td>
<td>T</td>
<td></td>
<td>0.326</td>
<td>0.233</td>
<td>0.175</td>
</tr>
<tr>
<td>13</td>
<td></td>
<td>A</td>
<td>C</td>
<td></td>
<td>0.121</td>
<td>0.507</td>
<td>0.157</td>
</tr>
</tbody>
</table>

The option ‘association given no linkage’ of the Pseudomarker program was used in the analyses (65).

bThe position of the SNP on chromosome 1 according to May 2004 human reference sequence (NCBI Build 35).

to affected females and males (global \( P = 0.007 \) and for the G-C haplotype \( P = 0.007; \ f = 0.72 \)). The signal for association was found to be mainly derived from the individuals with bipolar spectrum disorder non-overlapping with the psychiatric disorder category (global \( P = 0.0014; \) for females \( P = 0.00001 \) (Table 6). Another haplotype at the 3’ end of DISC1, composed of SNPs rs821616 and rs1411771, was under-transmitted to affected females and males (global \( P = 0.004 \) and haplotype-specific \( P = 0.0002 \) for the allele combination T-T; \( f = 0.24 \)). SNP rs821616 is a non-synonymous variant that causes an amino acid change of serine to cysteine at codon 704, the under-transmitted T-allele corresponding to cysteine and the A allele to serine. An extended allelic haplotype including rs821616, rs1411771 and in addition rs980898, was also under-transmitted to affected males and females (global \( P = 0.021 \) and for the T-T-G haplotype \( P = 0.0001, \ f = 0.24 \). Both cases with or without psychotic disorder contributed to this signal of association (haplotype-specific \( P = 0.015 \) for BPDI with psychotic features and \( P = 0.0012 \) for cases with bipolar spectrum, but no psychotic features) (Table 6). Thus, the data provide consistent evidence for association of haplotypes around the 5’ region of TSNAX and the 3’ region of DISC1 with bipolar spectrum disorder.
Neuropsychological endophenotypes show association with separate regions of DISC1

We tested the association between the DISC1 and TSNAX variants and several quantitative variables derived from neuropsychological tests in 158 individuals from the bipolar family sample (Table 1), using the QTDT program (Table 7 and Supplementary Material, Table S1). The traits were chosen based on previous findings for DISC1 (18), the high heritability estimates for these traits in bipolar families, and their suggested validity as endophenotypes of BPD (42,43).

Analysis of single variants revealed an association between perseverative recall errors and SNP rs751229 that was part of the risk haplotype for psychotic disorder ($P = 0.0012$) (Table 7). In general, the individuals with genotype TT performed slightly better than those with CT or CC, but in the psychotic males with TT genotype, the mean value for making errors was higher than in the rest of the sample in consistency with the results from the association analysis using psychotic disorder as an outcome (Supplementary Material, Table S2). Rs751229 also showed association to long delay recall ($P = 0.0032$), and the same trait was associated with visuospatial abilities ($P = 0.022$ and $P = 0.027$, respectively).

However, better functioning for both traits was observed in cases with CC genotypes, inconsistent with the finding of
Examination of the association of bipolar spectrum disorder to two haplotypes, a haplotype of SNPs rs1630250 and rs1615409, and a haplotype of SNPs rs821616, rs1411771 and rs980898, by dividing the sample to cases with or without psychotic disorder.

<table>
<thead>
<tr>
<th>Sample SNPs</th>
<th>Global P-value $^a$</th>
<th>Individual P-value $^a$</th>
<th>Haplotype</th>
<th>Haplotype frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete ($n = 227$)</td>
<td>1–2</td>
<td>0.007</td>
<td>G-C</td>
<td>0.72</td>
</tr>
<tr>
<td>Females ($n = 112$)</td>
<td>1–2</td>
<td>0.007</td>
<td>G-C</td>
<td>0.72</td>
</tr>
<tr>
<td>Males ($n = 115$)</td>
<td>1–2</td>
<td>0.014</td>
<td>G-C</td>
<td>0.72</td>
</tr>
<tr>
<td>Overlapping between psychotic and BPD spectrum categories ($n = 162$)</td>
<td>1–2</td>
<td>0.015</td>
<td>G-C</td>
<td>0.72</td>
</tr>
<tr>
<td>Females ($n = 81$)</td>
<td>1–2</td>
<td>0.014</td>
<td>G-C</td>
<td>0.72</td>
</tr>
<tr>
<td>Males ($n = 81$)</td>
<td>1–2</td>
<td>0.015</td>
<td>G-C</td>
<td>0.72</td>
</tr>
</tbody>
</table>

$^a$Permutated P-value for each haplotype, no correction for multiple testing.

SNPs $^b$

allele C as a part of the haplotype over-transmitted to individuals affected with BPD spectrum disorder. At the 3’ end of DISC1, verbal fluency and visuospatial ability were associated with rs821616 that was part of the protective haplotype for bipolar spectrum disorder ($P = 0.011$ and $P = 0.021$, respectively). For both traits, better functioning was related to the T allele of rs821616, corresponding to Cys704, which was consistently also part of the protective haplotype for the bipolar spectrum disorder. Rs980989 was associated with several cognitive traits, of which statistically the most significant evidence was obtained for psychomotor processing speed ($P = 0.011$). However, better performance was observed here with the TT or TG genotypes, this finding being inconsistent with our earlier observation of allele G as a part of the protective three-SNP haplotype for bipolar spectrum disorder.

Finally, we selected those haplotypes that had been particularly informative with dichotomous traits for analysis with the quantitative variables derived from neuropsychological tests: the risk haplotype T-A of rs751229 and rs3738401, protective haplotype C-G-A of rs751229, rs3738401 and rs1538977 and protective haplotype G-T-G of rs1655285, rs751229 and rs3738401 for males with psychotic disorder; as well as the risk haplotype G-C of rs1630250 and rs1615409, protective haplotypes T-T of rs821616 and rs1411771 and T-T-G with rs980898 in addition for bipolar spectrum disorder (Supplement Material, Table S3). In consistency with the finding for rs751229 alone, we observed an association of perseverative recall errors to the haplotype T-A of rs751229 and 3738401 ($P = 0.035$), and a trend of association with the haplotype C-G-A of rs751229, rs3738401 and rs1538977 ($P = 0.065$), while the haplotype G-T-G of rs1655285, rs751229 and rs3738401 was associated with auditory attention ($P = 0.0059$). The risk haplotype for bipolar spectrum disorder, composed of alleles G-C of rs1630250 and rs1615409, showed some evidence for association to traits related to general intellectual function, in consistency with the finding for rs1615409 alone ($P = 0.026$ for verbal ability, $P = 0.048$ for psychomotor processing and $P = 0.038$ for visuospatial ability). The analyses with the protective haplotypes for BPD at the 3’ end of DISC1 could not be reliably conducted due to a limited number of informative individuals for these analyses (i.e. offspring with neurocognitive test data and with at least one heterozygote parent), regardless of the fact that both affected and unaffected family members had been systematically assessed without any a priori information of their genotype status.

Table 6. Examination of the association of bipolar spectrum disorder to two haplotypes, a TSNAX haplotype of SNPs rs1630250 and rs1615409, and a DISC1 haplotype of SNPs rs821616, rs1411771 and rs980898, by dividing the sample to cases with or without psychotic disorder.

To summarize, the same variant that was included in the risk haplotype for psychotic disorder, rs751229 at the 5’ end of DISC1, also showed an association to perseverative recall errors and long delay recall, while a protective haplotype G-T-G of rs1655285, rs751229 and rs3738401 was associated to auditory attention. The variants within the haplotypes associated to bipolar spectrum disorder, located at TSNAX or at 3’ end of DISC1, were associated with several neurocognitive traits. The most robust signal for the 3’ end variants of DISC1, rs821616 and rs980989, was obtained with verbal fluency and psychomotor processing speed, respectively. These traits, and in particular psychomotor processing speed,
were considered as strong and valid candidates for endophenotypes of BPD in our previous studies (42,43).

**DISCUSSION**

In the present study, we attempted to determine whether DISC1 played a possible aetiological role in the genetic background of psychotic or bipolar spectrum disorders (i.e. BPD type I with intermittent psychotic features, psychotic depression, SCZ, SA disorder and psychosis NOS, or BPD type I and II, NOS and cyclothymia, respectively), or any related quantitative cognitive traits in Finnish families ascertained for BPD (17,18). We found association of distinct allelic haplotypes with these two groups of disorders and underlying cognitive impairments, suggesting that genetic variation within DISC1 could contribute to variation in the dimensional features of psychotic and bipolar spectrum disorders as well.

We found significant deviations from the expected transmission rates for multiple common and relatively rare haplotypes at the 5’ end of DISC1 in males with psychotic disorder. A two-SNP haplotype T-A of rs751229 and rs3738401 located on the first intron and second exon of DISC1, respectively, was over-transmitted to males with psychotic disorder ($P = 0.008$), similarly to a rare haplotype T-A-G with an additional variant rs1538977 in intron 6 ($P = 0.0007$ for both genders and $P = 0.007$ for males), while a haplotype C-G-A with these same variants was over-transmitted to affected males as well ($P = 0.001$). The current finding on the haplotype T-A of rs751229 and rs3738401 is in consistency with our earlier results for Finnish SCZ families, where an association of the same haplotype, termed ‘HEP3’, was observed to males affected with SCZ spectrum psychosis or severe mood disorder, including BPD (13). In the current study, the signal for association was obtained for males with psychotic disorder irrespective of the presence or absence of bipolar spectrum condition, while a putative association for males with bipolar spectrum disorder was obtained with an extended allelic haplotype with allele G of rs1655285 at the 5’ end. These data provide evidence for a shared aetiology of the diseases along the axis of spectrum of psychosis. This conclusion was further strengthened by finding of association of the risk haplotype T-A of rs751229 and rs3738401 in one, and allele T of rs751229 separately, to a cognitive endophenotype for SCZ: making perseverative (repetitive) errors in the California Verbal Learning Test ($P = 0.035$ and $P = 0.0012$, respectively). Psychotic males that were homozygous for the allele T included in the risk haplotype made more perseverative errors, in consistency with the findings from analysis with the psychotic disorder. Variant rs751299 was also associated to long delay recall ($P = 0.0032$), and another protective haplotype for psychotic disorder, composed of alleles G-T-G of rs1655285, rs751229 and rs3738401, respectively, to auditory attention ($P = 0.0059$). The finding of association of the variants at 5’ end of DISC1 both to psychotic disorder in males, as well as to traits related to verbal learning, memory and attention, might hint for possible mechanisms underlying the
disease liability caused by genetic variation at this particular genomic region. In contrast to the findings for males, however, the evidence for association of psychotic disorder in females at this particular region was scarce. Previous studies also implied similar sex differences for the effect of the DISC1 gene, although it remains unclear which molecular mechanisms underlie this effect (17,18,32,33).

There are now several reports of the association of SCZ or BPD with the region comprising intron 1 and exon 2 of DISC1. Cannon et al. (44) found that a rare haplotype, including variants comprising the HEP3 and two additional SNPs (rs1615409 and rs766288) at the 5' end of DISC1, was overrepresented in a sample of Finnish SCZ twins and also associated with impaired spatial working memory, increased reaction time to visual targets and reduced prefrontal grey matter. The associating allelic variants of the 5' end of DISC1 were found to be rare in both Finnish SCZ and BPD study samples. In another study of a Scottish population, an association with extended four- and eight-marker haplotypes including HEP3 variants (C and G allele at rs751229 and rs3738401, the opposite haplotype of 'HEP3') was established for BPD, particularly in males (32). This is in agreement with our results in which this same C-G haplotype, and also an extended haplotype C-G-A of SNPs rs751229, rs3738401 and rs1538977, were also over-transmitted to affected males with psychosis. Hodgkinson et al. (35) found an association of SA disorder with an under-transmitted haplotype carrying the A allele of SNP rs3738401 in a North American study sample. Thus, while evidence for the importance of this particular region of DISC1 associating to neuropsychological traits accumulates, the definite risk allele is not yet defined (Fig. 1).

In the present study, we found evidence for association of allelic haplotype G-C of rs1630250 and rs1615409 at the 5' end of TSNAX to bipolar spectrum disorder (\(P = 0.007\)). This finding is in consistency with a previous study of a Scottish population, in which an association with extended four-marker haplotypes including the same allelic variants (G-C) was obtained particularly for males with BPD (32). Marker rs1615409 of the TSNAX gene was also related to better performance in verbal ability (\(P = 0.022\)) and in visuospatial ability (\(P = 0.027\)). However, affected individuals with genotype CC performed slightly better than those with AC or AA genotype, which is not consistent with the results obtained for the dichotomously defined bipolar spectrum disorder.

Finally, we found a significant association with several haplotypes at the 3' end of DISC1 to bipolar spectrum disorder. A protective haplotype T-T-G of rs821616, rs14117771 and rs980989, located at exons 11 and 13 of DISC1, was found to be under-transmitted to both males and females with bipolar spectrum disorder (\(P = 0.0001\), irrespective of the presence or absence of psychotic features in the affected individuals. This haplotype includes the T allele of rs821616, corresponding to Cys704, that was also related to better performance in neuropsychological tasks measuring verbal fluency and visuospatial ability in our sample (\(P = 0.011\) and \(P = 0.021\), respectively), in consistency with the protective effect seen for bipolar spectrum disorder. Rs980989 was also associated with several tasks, with the most robust signal obtained for psychomotor processing speed, (\(P = 0.011\), but better performance was observed here with the TT or TG genotypes, inconsistent with results for bipolar spectrum disorder. The relevance of cognitive traits for BPD was evident in our recent study on neuropsychological functioning in Finnish bipolar families (42,43), where psychomotor performance showed high heritability and was found to be among the most promising cognitive endophenotypes for BPD, with impaired performance observed both in affected and unaffected family members and high estimate for additive heritability (43). Thomson et al. (32) previously reported an association of BPD with the three- and four-SNP haplotypes at the same 3' end region of DISC1 to A allele or Ser704 of rs821616, and C allele at rs1411771, particularly in females with BPD, whereas in another study, Cys704 was found to be associated with impaired cognitive ability in healthy aged women (45). Callicott et al. (46) found over-transmission of Ser704 in SCZ, and reduction in hippocampal grey matter volume as well as altering engagement of the hippocampus during cognitive tasks assayed with functional magnetic resonance imaging in healthy individuals with that allele. In another study by Hashimoto et al. (47), a haplotype including the Cys704 was associated with major depressive disorder and with a reduced grey matter volume in the cingulated cortex in healthy subjects. Thus, there is remarkable inconsistency in the finding of associated alleles at this particular locus, and the present findings are in harmony with those by Thomson et al., obtained for BPD (32), and Callicott et al. (46) for SCZ as well as for functional and structural features in healthy subjects. More direct functional relevance for different DISC1 alleles emerges from a recently reported family-based association study showing over-transmission of a 12-SNP haplotype of DISC1 (spanning exons 2–13) to affected BPD females, who also showed lower levels of DISC1 mRNA expression in their lymphoblasts (33). Furthermore, a higher number of symptoms of mania were correlated with lower levels of DISC1 expression (33).

Exons 3-13 of DISC1 encode the putative helical tail that interacts with the nuclear distribution E-like (NDEL1) protein to form a trimolecular complex with lissencephaly 1 (LIS1) protein. Kamiya et al. (2) recently reported that the Cys704 isoform of DISC1 has slightly stronger binding affinity for NDEL1 than does the Ser704 isoform. It is thus tempting to hypothesize that the structural changes interfering in the formation of the DISC1-NDEL1 complex could contribute to the dysfunction of the central nervous system. Exons 1 and 2 of DISC1 encode the putative globular domains that bind and interact with the cytoplasmic microtubules of α-tubulin (1,48,49). There is evidence for intergenic splicing of exons from DISC1 and TSNAX, and four intergenic exons located between these two genes, that results in a transcript encoding a novel TSNAX/DISC1 fusion protein (9). In addition, TSNAX protein forms protein complex with translclin, which may suppress the translation of certain mRNAs by binding to their 3'-UTRs (50–52), through which it is involved in development and function of the central nervous system and therefore, changes affecting TSNAX may affect DISC1. It is of interest that these regions of DISC1 and TSNAX bind and interact with each other or multiple proteins (1,2,48), and thus may contribute to the present findings for the associations of different clinical phenotypes and cognitive functions.
The statistical significance of the obtained results is dimmed by the issue of multiple testing. However, since statistical corrections for that kind of errors are challenging and prone to additional faults, we preferred to show the non-corrected values to allow for full interpretation of the data. Moreover, the strength of the current study comes from the consistency of the findings in regard of the associating allelic haplotypes in the individuals within the two diagnostic groups, as well as from the relatively high harmony for the findings regarding endophenotypes. To summarize, we find that different allelic haplotypes of DISC1 seem to contribute to variation in the dimensional features of psychotic and bipolar spectrum disorders in the Finnish families ascertained for BPD. The variants included in these haplotypes also display associations with different quantitative cognitive traits, hinting of possible mechanisms underlying the disease liability. In order to allow for estimation of the implication of these findings for the general population, however, these variants need to be tested in independent samples of cases and controls and, furthermore, studied in large cohorts with comprehensive data on endophenotypes related to disease liability.

MATERIALS AND METHODS

Study sample

All individuals born between 1940 and 1969 and hospitalized with a diagnosis of BPD were identified from the Finnish National Hospital Discharge Register. The diagnosis of BPD was based on the International Classification of Diseases, version 8 (ICD-8) (53) before 1987 and between 1987 and 1994 the diagnosis was coded according to the criteria of the Diagnostic and Statistical Manual of Mental Disorders, revised third edition (DSM-III-R) and later the fourth edition (DSM-IV) (54). Data on relatives of probands were obtained from the National Population Register. Finnish twin cohorts were used to identify BPD twins (55). The study was approved by the Ministry of Social Affairs and Health and the Ethical Committee of the National Public Health Institute. All patients and family members gave full informed consent. All blood samples were taken in accordance with the Helsinki Declaration and its amendments. The detailed ascertainment strategy and sample collection were described elsewhere (56,57).

The present analyses with the clinical phenotype were performed with a study sample consisting of 723 individuals from 175 nuclear families and from four extended families. This study sample is an extension of the sample previously used in analysis of the linkage in Finnish BPD families (56,57). The largest extended family consisted of four generations with 18 affected subjects. The diagnoses were based on DSM-IV criteria, and were assigned using the Structural Clinical Interview for DSM-IV Axis I Disorders (SCID) (58). The study sample comprised a group of patients with bipolar spectrum disorders, i.e. BPD type I and II, NOS and cyclothymia (n = 227), and a group of patients with psychotic disorders, i.e. BPD type I with intermittent psychotic features, psychotic depression, SCZ, SA disorder and psychosis NOS (n = 251), with an overlap of 162 individuals belonging to both groups (Table 1). In addition, the sample comprised 44 dizygotic twins (with psychotic disorder n = 19 and with bipolar spectrum disorder n = 22) from 29 twin pairs and 6 monozygotic twins (with psychotic disorder n=5 and with BPD n = 5) from 6 twin pairs, from which the detailed ascertainment strategy and sample collection has been described elsewhere (55). In addition, a random sample of 57 anonymous Finnish trios was used as a control sample in the molecular genetic analyses. The composition of the study sample available for analysis is detailed in Table 1.

Neuropsychological assessment

During 1999–2004, 158 family members from 57 nuclear families completed a comprehensive neuropsychological test battery and a detailed clinical interview using the SCID (58). The neuropsychological test battery from which the cognitive traits were obtained has been described elsewhere in detail for both the family sample (43) and for the twin sample (59). The patients were assessed with standardized and internationally well-known neuropsychological measures. Verbal ability was assessed with the vocabulary test from the Wechsler Adult Intelligence Scale-Revised (WAIS-R) (60). Psychomotor processing speed and visuospatial ability were assessed with the digit symbol and block design tests of the WAIS-R, respectively. From the Wechsler Memory Scale-Revised (WMS-R) (61), we included the Digit Span Forward and Backward tests, which measure auditory attention and verbal working memory, respectively, and the Visual Span Forward and Backward tests, which assess visual attention and visual working memory, respectively. From the California Verbal Learning (62), we included the following variables: learning (total recall from trials 1-5), short-delay memory, long-delay memory, recognition memory and perseverative recall errors. Executive functioning was assessed with the Colour-Word Interference Score from the Stroop test (63). Verbal fluency was assessed with the category Fluency (animals) from the Controlled Oral Word Association Test (64), which can be also considered as a measure of executive functioning. The neuropsychological test battery was administered by psychologists or advanced psychiatric nurses extensively trained and supervised with the test battery. Experienced psychologists scored all the tests.

SNP selection and genotyping

DNA was extracted from EDTA-treated blood according to standard procedures (65). The SNP markers were selected from the HapMap database (http://www.hapmap.org/) and also according to studies by Hennah et al. (17) and Thomson et al. (32). The primers for the SNP assays were designed using the SpectroDESIGNER software, versions 1.3 and 2.0 (Sequenom Inc., San Diego, CA, USA). SNP genotyping was performed using the homogenous Mass Extension reaction on the Sequenom MassARRAY System (Sequenom Inc.) and following the manufacturer’s guidelines. The genotypes were analyzed using SpectroTYPER RT 2.0 software (Sequenom Inc.) All genotype profiles were manually reviewed and genotypes of low quality were removed. Duplicate sample errors were not detected among 17 successful duplicate genotypes. SNPs with a genotyping success rate
Statistical analyses

All genotypes were checked for correct Mendelian transmission using PedCheck v.1.1 (66). Mendelian error was observed for a single SNP, rs3738401, in one family. Because the marker genotypes were otherwise of high quality and there were no duplicate errors, the genotypes for that marker were removed throughout the family, and the marker was used for analyses.

Single-marker association analysis was conducted using the Pseudomarker program, which is a joint test for linkage and association and can test for association in a mixture of singletons and extended pedigree structure of families. The ‘association given no linkage’ option of the program was considered in our sample (67). Recessive and dominant analyses were conducted for both status categories.

Haplotype analysis was performed using the TRANSMIT 2.5.4. program (41). The TRANSMIT program is able to test for the transmission of a haplotype even when the phase is unknown and when the parental genotypes are not completely known (41). Allelic haplotypes below a sample frequency of 3% were aggregated and counted as one haplotype during calculation of the global P-value, but were ignored as being too rare as individual haplotypes. TRANSMIT performed 100,000 bootstrap tests for all analyses, from which it too rare as individual haplotypes. TRANSMIT performed 100,000 bootstrap tests for all analyses, from which it was recoded to form biallelic markers in DISC1.

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Conflict of Interest statement. None declared.

REFERENCES


SUPPLEMENTARY MATERIAL

Supplementary Material is available at HMG Online.


