Is the Dysbindin Gene (DTNBP1) a Susceptibility Gene for Schizophrenia?

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Over recent years the gene DTNBP1 (chromosome 6p24-22) has emerged as one of the most promising candidate genes for schizophrenia. In this article, we review the current genetic evidence that implicates DTNBP1 as a schizophrenia-susceptibility gene. While there is now impressive support from genetic association studies, it is important to remain aware that the actual DTNBP1 susceptibility variants have not been identified. While functional analyses have allowed us to speculate their likely function, only when they are identified will we be able to confidently specify the type of altered gene function that is relevant to schizophrenia pathogenesis. This we hope will then open up new vistas for neurobiological research, allowing us to study the exact contribution of DTNBP1 in schizophrenia, its relationships with various aspects of the phenotype, and the potential of epistatic interactions with other genes, as well as functional interactions between the gene products.

Key words: schizophrenia/dysbindin/DTNBP1/gene/association

Introduction: Genetic Evidence Supporting DTNBP1 as a Schizophrenia Susceptibility Locus

Chromosome 6p24-22 is the location of one of the most well established linkages to schizophrenia.1–5 In the absence of very clear positional candidate genes, and even arguably when such genes exist, association analysis using a grid of genetic markers spanning the region is typically the method of choice for progressing from linkage to a causal gene. This was the approach used by Straub and colleagues.6 Using their sample of Irish families in whom linkage was initially observed,1 they were able to identify significant evidence for association (p = .008 to p = .0001) between schizophrenia and several individual markers as well as haplotypes (a series of nucleotides occurring on the same chromosome) that spanned DTNBP1, the gene encoding dystrobrevin binding protein 1, or dysbindin (figure 1).

Given that it was the first reported gene for schizophrenia identified by positional cloning, this initial report was fairly rapidly followed by attempts to replicate these findings in independent samples (figure 1). Schwab and colleagues performed the first such replication attempt, using 78 German and Israeli families (also showing evidence for linkage to 6p) and 127 proband parent trios, mainly from Germany but including a small number of subjects from Hungary.7 Both samples independently gave evidence for both single-marker and haplotypic association, with the combined data sets giving strong evidence (p = .00001) for a 3-marker haplotype. Van Den Bogaert and colleagues studied 5 single-nucleotide polymorphisms (SNPs) in their samples of 142 cases and 272 controls from Poland, Germany, and Sweden.8 While the first 2 samples failed to show any evidence for association, the researchers did identify significant haplotypes (p = .0098) in the Swedish sample that were even more significant in their subset of cases with a family history of schizophrenia (p = .0009). Kirov and colleagues similarly found evidence in favor of dysbindin as a susceptibility gene for schizophrenia in 488 parent proband trios from Bulgaria (minimum p < .001),9 as did Funke et al. in 258 Caucasian cases and 467 controls as well as in 51 Hispanic cases and 32 matched controls.10 Tang and colleagues extended the evidence to samples of non-European origin when they reported significant haplotypic association using a sample of 223 parent proband trios from China (p = .00072),11 as did Numakawa and colleagues in 670 Japanese cases and 588 controls (minimum p = .001).12 Not all studies have used the same genetic markers, but even allowing for this, there is considerable disagreement between studies with respect to the specific markers and haplotypes that have been reported to be associated (table 1). Nevertheless, such a substantial body of supportive evidence for association at a single gene is unprecedented in studies of schizophrenia and is far beyond what can be attributed simply to chance.

All of the above studies used panels of SNPs selected from public databases, and therefore it was not a priori particularly likely that any would actually have a direct...
pathogenic role in the function of \textit{DTNBP1}. Instead, as in the original study, the replication studies relied on the hope that the marker SNPs analyzed were sufficiently close to the true risk variant(s) to be in linkage disequilibrium (LD) with it. LD arises when the population-specific history of recombination events between 2 polymorphic sites has been inadequate to result in allelic independence between the loci, that is, genotypes at each site are to some extent correlated. This means that where an allele contributes to a disease, any correlated allele at an adjacent marker in LD should also be associated indirectly with disease, although whether such an association is detectable depends upon the degree of correlation, the size of the genetic effect, and the sample size examined. It is worth stressing that the degree of correlation between 2 sites may be highly population dependent, as might the size of the contribution of any single variant (due, for example, to differences in frequencies of environmental factors or other risk alleles with which the variant in question interacts). Such factors alone can potentially explain sample-specific patterns of association. Other possible explanations are the existence of multiple risk alleles at an individual gene (known as allelic heterogeneity) with the pattern varying across populations or that some of the positive findings are false positives due to chance, stratification, or genotyping errors. Clearly, false positives can add to the apparent heterogeneity. Moreover, genetic and etiological heterogeneity can greatly affect the power of individual replication studies, which are already compromised by the small contribution to overall disease susceptibility (odds ratio < 2.5) associated with the markers or haplotypes identified thus far. Consequently, it is not unexpected that when using these particular panels of SNPs, some studies failed to show any evidence for association with schizophrenia.\textsuperscript{8, 13–14}

While factors such as these can explain the variability in patterns of LD across studies, and even some failures to replicate, it does not follow that they do so. In an attempt to avoid some of the limitations of LD, we set out to try to identify schizophrenia-susceptibility variants using a direct association approach. Thus, instead of relying on databases, we undertook polymorphism discovery. A disadvantage of that approach is that it is difficult to know when all possible functional domains of a gene have been scanned. Williams and colleagues focused their efforts on regions of the gene thought most likely to harbor functional variants, namely, all the known and predicted exons (specific segments of the gene that are transcribed to give an mRNA gene product) as well as 4 putative alternative promoter regions (specific genomic regions that regulate gene expression).\textsuperscript{14} Moreover, because of the limitations, they supplemented their analyses by seeking evidence for indirect association based upon haplotypes. Three novel SNPs yielded weak evidence for association with schizophrenia; but when included in haplotypes with SNPs from the original study,\textsuperscript{6} 1 novel marker (rs2619538) yielded highly significant evidence for association (minimum global \(p = .00005\)). The evidence for haplotype association appeared to come from 1 common risk haplotype \((p = .013)\), 1 common protective haplotype \((p = .006)\), and 1 uncommon protective haplotype \((p = 7 \times 10^{-7})\). Interestingly, when the same marker was added to an Irish sample that also had not shown evidence for association at the \textit{DTNBP1} gene using original markers,\textsuperscript{13} an identical pattern of association was observed (table 1).\textsuperscript{14}
Table 1. Complex Pattern of Genetic Association at DTNBP1

<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>rs760666</th>
<th>rs2619539</th>
<th>rs3213207</th>
<th>rs1011313</th>
<th>rs2619528</th>
<th>rs2005976</th>
<th>rs760761</th>
<th>rs2619522</th>
<th>rs1018381</th>
<th>rs909706</th>
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<tr>
<td>Straub, Jiang, MacLean, et al., 2002; van den Oord, Sullivan, Jiang, et al., 2003</td>
<td>Irish</td>
<td>C</td>
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<td>Schwab et al., 2003&lt;sup&gt;a&lt;/sup&gt;</td>
<td>German and Israeli</td>
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<td>Van Den Bogaert, Schumacher, Schulze, et al., 2003</td>
<td>Swedish</td>
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<td>Funke, Finn, Plocik, et al., 2004&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Caucasian</td>
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<td>Kirov, Ivanov, Williams, et al., 2004</td>
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<td>Williams, Preece, Morris, et al., 2004</td>
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<td>Morris, McGhee, Schwaiger, et al., 2003; Williams, Preece, Morris, et al., 2004</td>
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<td>Numakawa, Yagasaki, Ishimoto, et al., 2004</td>
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Note: Presented is a selection of the single nucleotide polymorphisms (SNPs) genotyped in each study that has reported a positive association at the DTNBP1 locus to date. SNPs genotyped in each study are highlighted in gray, and the alleles of key SNPs that define the reported putative “risk” haplotypes are given. For the original study by Straub et al.<sup>6</sup> the risk allele of each associated SNP is presented; however, it should be noted that in the subsequent study by van den Oord et al.<sup>22</sup> the risk haplotype was defined by alleles A and C of rs2005976 and rs1018381, respectively.

<sup>a</sup>Only specific haplotype data for the full sample were available.
<sup>b</sup>Despite evidence for allelic association in the Hispanic sample, there was no evidence for haplotypic association.
Functional Effect of DTNBP1 Risk Variants at the Gene Level

Taken together, the data from 14 samples, 11 of which show statistically significant association with schizophrenia (table 1), provide strong evidence that DTNBP1 is a susceptibility gene for schizophrenia; but until the actual susceptibility variants that confer disease risk are identified, it is inevitable that a degree of skepticism about the finding will remain. When the true susceptibility variants are identified, we will also be able to confidently specify at a mechanistic level what type of altered gene function, be it increased normal, reduced normal, or some novel function, is relevant to schizophrenia pathogenesis. Until then we can make some informed speculations.

Most simple genetic disorders are caused by missense and nonsense mutations that change the amino acid sequence of the encoded protein and therefore its function. However, even moderately common polymorphisms of this nature have effectively been excluded by the direct association approach, while unpublished sequence data from a much larger sample of schizophrenics also indicate that the existing association evidence is not caused by the accumulation of rare variants either (R. Straub, personal communication, August 2005).

Another possible mechanism that is generally held (without a large body of empirical data in support) to be particularly important in complex disorder is sequence variation, which affects the amount of the gene expressed. For DTNBP1 this is a genuine possibility, as it has been shown that polymorphisms exist in DTNBP1 that influence its expression in the human brain. Others have shown that dysbindin mRNA and protein levels are decreased in the hippocampal formation and dorsolateral prefrontal cortex of schizophrenics. However, again, until it is functionally tied to specific risk variants, it is possible that reduced expression is not the causal mechanism but, instead, is a response to it, for example, a hyperfunctioning allele. Bray and colleagues recently followed up their original study by examining the expression levels of mRNA from specific haplotypes in individuals heterozygous for those haplotypes. Intriguingly, they were able to demonstrate that the “risk” haplotype identified by the same group was expressed at a lower level than other haplotypes. Their analysis also provides preliminary evidence that the risk and protective haplotypes identified in other sample groups index low and high expression, respectively. These data await independent replication but, if confirmed, suggest low expression as a common mechanism underpinning the diversity of associated haplotypes (table 1).

While we remain aware that other pathogenic mechanisms that influence DTNBP1 function may yet prove to be important, for example, mRNA splicing or up-regulation of a minor transcript, the current evidence suggests strongly that the as-yet-unknown cis-acting variants that reduce DTNBP1 expression are prime candidates for being the true DTNBP1 susceptibility variants. However, there are numerous possible places where polymorphisms influencing expression might be localized, many of which can be distal to the gene and most of which are very difficult to predict. It therefore seems likely that the identification of DTNBP1 cis-acting variants will require resequencing of the entire DTNBP1 gene in a considerable number of affected individuals from each associated sample set. Putative susceptibility variants will then have to have their function confirmed by correlation with low expression in vivo and through direct analyses of function in vitro. Their relevance to schizophrenia will also require retesting by genetic association.

How Altered Dysbindin Function Might Lead to Schizophrenia

Dysbindin is a ubiquitously expressed protein. Although only 2 protein isoforms have been reported (40–51 kDa), bioinformatic analysis (figure 1) and analysis of polymerase chain reaction–amplified products from cDNA (Straub, personal communication, August 2005) suggest that many more mRNAs exist, but whether these encode active proteins remains to be seen. Dysbindin is part of the dystrophin-associated protein complex (DPC), specifically, α- and β-dystrobrevins. The dystrophin complex is found in the sarcolemma of muscle but is also located in postsynaptic densities in a number of brain areas. In the brain, dysbindin expression is thought to be confined to neurons, where it is both pre- and postsynaptic. Its expression is particularly intense in axons and mossy fiber synaptic terminals in the cerebellum and hippocampus, where it appears to be presynaptic. Of possible relevance to schizophrenia, dysbindin is expressed in glutamatergic neurons and synapses of the hippocampus.

Recently, in addition to being a component of the DPC, dysbindin has emerged as a component of the biogenesis of lysosome-related organelles complex 1 (BLOC-1). This came to light when it was shown that Hermansky–Pudlak syndrome (HPS) type 7 can be caused by a nonsense mutation in the DTNBP1 gene. HPS is characterized by oculocutaneous albinism, prolonged bleeding time, and pulmonary fibrosis due to abnormal vesicle trafficking to lysosomes and related organelles such as platelet-dense granules and melanosomes. No major psychiatric manifestations have so far been documented. The sandy mouse is a model of HPS7 and is caused by an in-frame deletion of 2 exons of the murine dysbindin gene. It therefore represents a naturally occurring dysbindin knockout.

At the time of writing how dysbindin’s role in the DPC or BLOC-1 relates, if at all, to the pathogenesis of schizophrenia is unknown. Initially it was speculated that dysbindin may influence schizophrenia risk through
postsynaptic mechanisms.\(^6\) However, the study by Talbot and colleagues\(^7\) reporting a significant reduction in presynaptic dysbindin expression in several hippocampal regions and an inverse relationship between presynaptic dysbindin and the vesicular glutamate transporter (VGlutT-1), suggests the possibility of a mechanism related to glutamate release. This hypothesis is supported circumstantially by the demonstration that knockdown of endogenous dysbindin protein results in reduction of glutamate vesicles and reduced glutamate release. However, whether this is of any relevance to dysbindin’s role in schizophrenia is as yet uncertain. What is certain is that dysbindin and its multiple functions are likely to occupy a prominent role in schizophrenia research over the next few years.

**Conclusions**

The large volume of supportive evidence implicating DTNBP1 as a schizophrenia-susceptibility gene has catapulted both schizophrenia genetics and schizophrenia biology into an exciting phase. However, it is important to emphasize that there remains room for caution. So far, the risk haplotypes do not fully explain the initial linkage findings at 6p24-22 that prompted the follow-up association analysis. While this could suggest that the associated polymorphisms/haplotypes are only weakly correlated with the true pathogenic variants, it is also possible that the linkages reflect multiple risk alleles at each locus or even variation at 2 or more genes. In addition, in order to unambiguously confirm that DTNBP1 is a schizophrenia-susceptibility gene, it is a priority that we isolate its susceptibility variants. The current evidence suggests that these are likely to contribute to disease susceptibility by influencing DTNBP1 expression. However, in light of their unknown location and probable allelic heterogeneity, identifying these susceptibility variants will be a considerable task. Finally, an important lesson is that the initial linkage to 6p24-22 and subsequent identification of DTNBP1 were achieved by exploiting the classical clinical schizophrenia phenotype. This is not to say that when valid, and hopefully genetically less complex, endophenotypes are available the task will not be easier. However, in the meantime, we should acknowledge that the schizophrenia phenotype is of proven utility and can allow genetic studies to deliver crucial insights into the nature of the disorder.

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