Analysis of HapMap tag-SNPs in dysbindin (DTNBP1) reveals evidence of consistent association with schizophrenia

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Abstract

Dystrobrevin binding protein 1 (DTNBP1), or dysbindin, is thought to be critical in regulating the glutamatergic system. While the dopamine pathway is known to be important in the aetiology of schizophrenia, it seems likely that glutamatergic dysfunction can lead to the development of schizophrenia. DTNBP1 is widely expressed in brain, levels are reduced in brains of schizophrenia patients and a *DTNBP1* polymorphism has been associated with reduced brain expression. Despite numerous genetic studies no *DTNBP1* polymorphism has been strongly implicated in schizophrenia aetiology. Using a haplotype block-based gene-tagging approach we genotyped 13 single nucleotide polymorphisms (SNPs) in *DTNBP1* to investigate possible associations with *DTNBP1* and schizophrenia. Four polymorphisms were found to be significantly associated with schizophrenia. The strongest association was found with an A/C SNP in intron seven (rs9370822). Homozygotes for the C-allele of rs9370822 were more than two and a half times as likely to have schizophrenia compared to controls. The other polymorphisms showed much weaker association and are less likely to be biologically significant. These results suggest that *DTNBP1* is a good candidate for schizophrenia risk and rs9370822 is either functionally important or in disequilibrium with a functional SNP, although our observations should be viewed with caution until they are independently replicated.

Keywords: Schizophrenia and psychosis; Genetics; Molecular biology; Polymorphism; Genetic association; Glutamatergic pathway; Dystrobrevin binding protein 1

1. Introduction

Schizophrenia is thought to have a high heritability of between 64- 81% [1-3] but the exact nature of this genetic risk is complex and few of the many targeted research studies have led to significant reproducible genetic associations with schizophrenia. Evidence suggests that the complexity of schizophrenia inheritance is a result of the involvement of multiple loci that act both additively and epistatically [4]. Adding to this is the possibility that environmental factors also interact with certain genotypes.

To date, a number of polymorphisms have been identified that are associated with schizophrenia. Many of these are associated with the dopamine pathway in the brain and are likely to cause over-stimulation of dopamine receptors. Hence the primary site of action of anti-psychotic medication is dopamine receptors. The glutamatergic pathway has also been proposed to modulate the pathogenesis of schizophrenia. The first metabotropic glutamate 2/3 (mGlu2/3) receptor agonistic drug was based on the glutamatergic hypothesis [5] and is the first drug developed not acting as a dopamine antagonist. This hypothesis is based on glutamate hypofunction which ultimately leads to increased sensory flooding and changes in dopamine concentration. Dystrobrevin binding protein 1 (*DTNBP1*), otherwise known as dysbindin, is one of the genes thought to be pivotal in regulating the glutamatergic system.

Through linkage and association studies *DTNBP1* has been extensively investigated but there is conflicting evidence for association with schizophrenia. A meta-analysis identified only a weak association of one *DTNBP1* SNP with schizophrenia, which was not significant after multiple testing [6]. *DTNBP1* was first associated with schizophrenia when linkage studies identified 6p24-p22 as a region of high

schizophrenia susceptibility [7-9]. Later investigations reported associations in Irish pedigrees with eight *DTNBP1* single nucleotide polymorphisms (SNPs) [8]. Associations with six of these polymorphisms were replicated in sib-pair and triad families [10]. Differences were observed in a Chinese population but analysis of the 6p22.3 region still showed strong association with schizophrenia [11]. Associations have also been identified in Caucasian and Hispanic populations [12]. Since these earlier studies several haplotypes have been identified to be associated with schizophrenia, although no common polymorphism or haplotype has been established [13-22]. There are also a number of studies that failed to identify an association between *DTNBP1* and schizophrenia [15,23-27].

The 140 kilobase *DTNBP1* gene contains eight exons and encodes a 40 kDa neuronal protein dystrobrevin binding protein 1 that binds to alpha- and beta-dystrobrevin in muscle and brain [28]. It is found highly concentrated in mossy fibre synaptic terminals in both the cerebellum and hippocampus [28,29] and functional studies have also implicated it in schizophrenia pathogenesis [16,30-33]. A role has been hypothesised in cognitive functioning and memory as well as maintaining glutamatergic neurotransmission [34]. Studies have found widespread *DTNBP1* mRNA in the brain, reduced mRNA expression in dorsolateral prefrontal cortex and hippocampal formation [33] and reduced protein expression of hippocampal formation in schizophrenia patients [29,32]. Reduced *DTNBP1* mRNA expression in cerebral cortex has also been associated with risk haplotypes for schizophrenia [30].

Association and functional studies suggest a role for *DTNBP1* in schizophrenia and dysbindin deficient mice have been shown to provide a good model for schizophrenia

[35-39]. To identify novel polymorphism associations and confirm previous association studies, 13 SNPs in *DTNBP1* that span from the 3'-end to the 5'-end of the gene were selected to mark the haplotype blocks in *DTNBP1* so that all associations could be detected. These 13 SNPs were then genotyped to investigate possible genetic association with schizophrenia.

2. Materials and Methods

2.1 Subjects

The study included 160 schizophrenia patients and 250 unrelated Caucasian controls. Subjects were aged between 18 and 65 years. Diagnostic and Statistical Manual of Mental Disorders-IV (DSM-IV) diagnosis of schizophrenia was confirmed by at least two independent psychiatrists. These patients had never been diagnosed with other psychiatric disorders, including schizoaffective disorder, major depressive episode with psychotic features, or bipolar disorder. No patients were treated with antidepressants, anxiolytic agents, or mood-stabilizing psychotropic medications and all were maintained on a constant dose of antipsychotic medication for a minimum of three weeks. There were 22 females and 138 males in the group diagnosed with schizophrenia with a mean age of 36.2 years (s.d. $\pm$ 12.1 years). The mean age of patients at first diagnosis was 23.4 years (s.d. $\pm$ 7.47 years). They were being treated at the Royal Brisbane and Women's Hospital, The Park Psychiatric Unit, and the Valley Community Psychiatric Centre. The clinical sample was composed of 69 inpatients and 91 outpatients. All patients were administered the Positive and Negative Symptom Scale (PANSS) [40] to assess severity of psychotic features. A history of psychological distress, indicated by past suicidal behaviour, was reported by 74 patients. A total of 121 patients were able to provide information on psychiatric illness among first-degree relatives and 82 of these patients (68%) reported a positive family history of schizophrenia. This clinical sample was a group who had lived with schizophrenia for an average of 13 years since diagnosis continued to experience positive and negative symptoms despite treatment with antipsychotic medication. In addition the group contained a significant proportion of individuals with a relatively severe history and/or a familial risk for psychosis.

The control group consisted of 102 females and 148 males, with a mean age of 36.8 years (s.d. $\pm$ 12.8 years). The control group consisted mostly of medical and nursing staff recruited through hospitals, and university students and academic staff. Formal screening for schizophrenia or other psychological disorders was not undertaken in the control population. As such, the controls represent an unselected control group.

Ethics approval was obtained from all institutions involved.

2.2 Selection of SNPs

Using the International HapMap Project (HapMap) phase II [41], eleven tag-SNPs in *DTNBP1* with a minor allele frequency of 0.15 were selected. Tag-SNPs were identified using the pair-wise option of Tagger with a threshold of $r^2 > 0.8$. Two non-synonymous SNPs reported in The National Center for Biotechnology Information (NCBI) SNP database (dbSNP) were also chosen.

2.3 Genotyping

Oragene kits were used to extract DNA from saliva samples. Samples were genotyped using a homogeneous MassEXTEND (hME) Sequenom assay performed by the Australian Genome Research Facility. The hME assay is based on the annealing of an oligonucleotide primer (hME primer) adjacent to the SNP of interest. The addition of a DNA polymerase along with a mixture of terminator nucleotides allows extension of the hME primer through the polymorphic site and generates allele-specific extension products, each having a unique molecular mass. The resultant masses of the extension products are then analysed by matrix-assisted laser

desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and a genotype is assigned in real time. The hME assay was performed in multiplex with up to 36 reactions in a single well.

2.4 Statistical Analysis

A Pearson's chi-squared test was performed to identify statistical associations between alleles/genotype and schizophrenia status. Odds ratios (OR) were also calculated. Tests were performed on both genotype and allele data. All statistical tests were performed using the COMPARE2 program from the WinPepi suite of epidemiology programs [42]. Hardy-Weinberg equilibrium (HWE) was computed using Utility Programs for Analysis of Genetic Linkage [43]. The analysis of genotypes under a recessive model involved pooling the low-risk homozygotes and the heterozygotes and comparing frequencies with the high-risk homozygotes, i.e. OR > 1. Correction for multiple testing was conducted using the Benjamini-Hochberg method [44].

3. Results

A comprehensive genotype analysis of *DTNBP1* and its association with schizophrenia was undertaken using a haplotype block-based gene-tagging approach. A total of eleven tag-SNPs and two non-synonymous SNPs from NCBI that span from the 3'-end to the 5'-end of the gene were genotyped in a sample of 160 schizophrenia patients and 250 controls. One of the non-synonymous SNPs, rs16876589, was excluded from the final analysis after genotyping revealed no heterogeneity. Three of the remaining 12 SNPs analysed were found to be significantly (p <0.05) associated with schizophrenia at the allele level (Table 1) and a further two SNPs were approaching significance. These included four tag-SNPs; rs1997679, rs9370823, rs9370822, rs4236167 and one non-synonymous SNP identified in NCBI, rs17470454 (Table 1). All of these SNPs were selected for further analysis at the genotype level (Table 2). Genotype frequencies indicated that all polymorphisms were in HWE in both case and control samples, with the exception of rs17470454 which was not in HWE for either group. Data from this SNP was therefore excluded from further analyses. One of the tag-SNPs (rs4236167) was significant at the genotype (p = 0.03) but not at the allele level. The rs1997679 SNP achieved significance at the genotype level when it was analysed using the Mantel-Haenszel test for trend but only rs9370822 was significant at both the allele (p = 0.002) and genotype (p = 0.004) level and it was the only SNP to survive correction for multiple testing (corrected α-values varied between 0.025 to 0.0125, for the two nominally significant SNPs) .

When compared to the low-risk homozygote, the odds ratios of the other two genotypes indicated that a recessive pattern of inheritance for schizophrenia

susceptibility was present for all 4 remaining SNPs, i.e. both the heterozygote OR and the OR for the low-risk homozygote were approximately one (Table 2). All four SNPs were subsequently found to be associated with schizophrenia when analysed under a recessive model by pooling genotypes (Table 3); again, rs9370822 showed the strongest association under a recessive inheritance model. All four associations survived correction for multiple testing (corrected α-values varied between 0.05 and 0.0125, for the four nominally significant SNPs).

In order to evaluate whether there was any sexual dimorphism in the population, the male schizophrenia patients were analysed separately from the female patients. At the allele level, the same four SNPs showed association with disease with slightly improved *p*-values (rs9370822, $p = 0.0006$; rs4236167, $p = 0.017$; rs1997679, $p = 0.023$; rs9370823, $p = 0.036$) although only rs9370822 survived correction for multiple testing. At the genotype level, the same four SNPs were even more clearly associated with schizophrenia when genotypes were analysed independently or under a recessive model (recessive model *p*-values: rs9370822, $p = 0.0005$; rs4236167, $p = 0.002$; rs1997679, $p = 0.006$; rs9370823, $p = 0.009$). The remaining female cases were too small in number to analyse meaningfully.

4. Discussion

Analysis of eleven tag-SNPs and two non-synonymous SNPs in *DTNBP1* revealed associations with schizophrenia at the allele or genotype level for four *DNTBP1* polymorphisms (rs1997679, rs9370823, rs9370822 and rs4236167) in an Australian schizophrenia cohort. The rs1997679, rs9370823 and rs4236167 SNPs have been analysed previously but were not found to be associated with schizophrenia

[24,26,27,45]. The strongest association was found for the rs9370822 SNP which has not previously been tested by other groups. Of the remaining nine SNPs studied in this paper, rs2743857 and rs16876589 have not previously been studied while the remaining seven SNPs have been analysed for association with schizophrenia but did not show association [19,21,24,25,27,45-47].

Although rs9370822 has not been analysed previously by other groups, Duan et al. [48] identified three other SNPs (rs875462, rs760666 and rs7758659), all located in intron seven and flanking rs9370822 that were significantly associated with schizophrenia. We analysed rs7758659 in our study but we observed no association with schizophrenia (allele association $p = 0.91$).

In 2006, Mutsuddi et al. [49] analysed data from six earlier studies that reported association of *DTNBP1* with schizophrenia but they found no consistency between the reported associations with schizophrenia. None of the SNPs reported in our study were analysed by Mutsuddi et al. [49]. A recent case-control study of twelve *DTNBP1* SNPs found one SNP in intron seven (rs1040410) associated with schizophrenia and an associated haplotype involving this SNP and two flanking SNPs in intron seven (rs875462 and rs6926401) [50], further supporting our observation of a strongly associated SNP in intron seven of *DTNBP1*. Another recent study failed to find schizophrenia association with 39 individual tag-SNPs (including rs9476886, rs1997679 and rs3829893) or haplotypes [46]. They did not find rs1997679 to be associated with schizophrenia under any inheritance model although it was showing indications of nominal association with schizophrenia under a partial dominance model. However, we identified a nominally significant association of the rs1997679

SNP with schizophrenia in our cases although the effect was relatively small and could have occurred by chance. We found the SNP to be significant under a T-dominant/C-recessive ($p = 0.019$) and partially-dominant ($p = 0.048$) or additive model.

In a very large study of genetic association with schizophrenia, Sanders et al. [27] examined SNPs in 14 candidate genes, including *DTNBP1*, but they did not identify any association. Interestingly, none of these recent studies analysed rs9370822 which we found to be the most strongly associated with schizophrenia. We found rs9370822 to be significantly associated at both the allele and genotype level and under a T-dominant/C-recessive model. Further support for this being a biologically significant SNP comes from our observation of weaker association displayed by the two flanking SNPs (rs9370823 and rs4236167; Tables 2 and 3) that lie just 6 kb and 11 kb away from rs9370822, respectively and the observations by Duan et al. [48] and Vilella et al. [50] of association with SNPs that flank rs9370822 . At this stage it is uncertain whether this SNP is in linkage disequilibrium with another functional SNP. Even though this is an intronic SNP if it is in disequilibrium with another SNP it is likely to also be in intron 7 as both the flanking SNPs are also in intron 7. As such, it is possible that the functional SNP either affects RNA splicing or gene transcription as no coding or untranslated regions are involved.

Another SNP in the 3′-untranslated region of *DTNBP1* that looked promising as a candidate for schizophrenia etiology was rs1047631. A study found the A allele to be associated with reduced *DTNBP1* mRNA expression in the brains of schizophrenic patients [30]. This result is consistent with a previous study that found the G allele

was associated with an increase in mRNA levels in the pre-frontal cortex of schizophrenia brains [33]. Bray et al. [30] also found the A-allele to be included in a schizophrenia risk haplotype. However, we found no significant association with the rs1047631 SNP and schizophrenia.

Examination of the odds ratios for the genotypes of all associated SNPs in our study suggests that they follow a recessive pattern of inheritance with respect to schizophrenia risk (Table 2). We describe the pattern as recessive because the odds ratio of the heterozygote did not differ from the odds ratio of the homozygote for the allele that was not associated with schizophrenia. This is consistent with a *DTNBP1* functional allele that results in loss or reduction of gene function. It is also consistent with the glutamatergic hypothesis [5] which is based on glutamate hypofunction and with the observation of recessive inheritance of the dysbindin-1 gene deletion in the mouse model of schizophrenia [35]. Interestingly, there is another potential mechanism of action of *DTNBP1* in schizophrenia that also involves loss-of-function or down regulation of *DTNBP1*. It has been shown that damping of *DTNBP1* expression with interfering RNA led to an increase in cell surface DRD2 receptors [51] which is consistent with an over activity of the dopamine pathway in schizophrenia.

Gender differences were also examined in this study as there was an underrepresentation of women in our schizophrenia population. By analysing males separately the same four SNPS were still found to be significantly associated with schizophrenia. However, the *p*-values were improved, indicating possible sexual

dimorphism. Future studies representing a larger female schizophrenia population may explain these gender differences.

5. Conclusions

This study does not rule out population stratification and therefore our results need to be confirmed independently in a Caucasian group as well as defined ethnic groups. Population stratification can produce false positive associations where the underlying structure of the population results in genetic differences in the frequency of variants rather than differences due to the presence of disease susceptibility alleles. The sample size for the schizophrenia population was relatively small and the associations await replication preferably using a larger sample of schizophrenia patients or possibly other disease cohorts with a similar disease etiology. However a *p*-value of 0.002 for the rs9370822 SNP is encouraging using unselected controls as a more significant association is likely to be found using controls screened for lack of a mental illness.

To date, no functional variants of *DTNBP1* have been identified that are associated with schizophrenia. From our study we identified four SNPs that showed some association with schizophrenia. The strongest association with schizophrenia was found with the rs9370822 polymorphism. Though it is intronic, this SNP may still be functional or in disequilibrium with a nearby functional SNP that would also most likely be intronic.

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Author Contributions

Joanne Voisey: Analysed and interpreted data, drafted article, critically revised article and approved final version of article

Christopher D Swagell: Involved in conception and design, critically revised article and approved final version

Ian P Hughes: Involved in conception and design, analysed and interpreted data, critically revised article and approved final version of article

Bruce R Lawford: Involved in conception and design, critically revised article and approved final version

Ross McD Young: Involved in conception and design, critically revised article and approved final version

C Phillip Morris: Analysed and interpreted data, involved in conception and design, critically revised article and approved final version

Conflict of Interest

All authors declare that they have no conflicts of interest.

References

[1] Cardno AG, Marshall EJ, Coid B, Macdonald AM, Ribchester TR, Davies NJ, et al. Heritability estimates for psychotic disorders: the Maudsley twin psychosis series. Arch Gen Psychiatry 1999;56:162-8.

[2] Sullivan PF, Kendler KS, Neale MC. Schizophrenia as a complex trait: evidence from a meta-analysis of twin studies. Arch Gen Psychiatry 2003;60:1187-92.

[3] Lichtenstein P, Yip BH, Bjork C, Pawitan Y, Cannon TD, Sullivan PF, et al. Common genetic determinants of schizophrenia and bipolar disorder in Swedish families: a population-based study. Lancet 2009;373:234-9.

[4] Riley B, Kendler KS. Molecular genetic studies of schizophrenia. Eur J Hum Genet 2006;14:669-80.

[5] Patil ST, Zhang L, Martenyi F, Lowe SL, Jackson KA, Andreev BV, et al. Activation of mGlu2/3 receptors as a new approach to treat schizophrenia: a randomized Phase 2 clinical trial. Nat Med 2007;13:1102-7. Epub 2007 Sep 2.

[6] Li D, He L. Association study between the dystrobrevin binding protein 1 gene (DTNBP1) and schizophrenia: a meta-analysis. Schizophr Res 2007;96:112-8.

[7] Moises HW, Yang L, Kristbjarnarson H, Wiese C, Byerley W, Macciardi F, et al. An international two-stage genome-wide search for schizophrenia susceptibility genes. Nat Genet 1995;11:321-4.

[8] Straub RE, Jiang Y, MacLean CJ, Ma Y, Webb BT, Myakishev MV, et al. Genetic variation in the 6p22.3 gene DTNBP1, the human ortholog of the mouse dysbindin gene, is associated with schizophrenia. Am J Hum Genet 2002;71:337-48.

[9] Wang S, Sun CE, Walczak CA, Ziegle JS, Kipps BR, Goldin LR, et al. Evidence for a susceptibility locus for schizophrenia on chromosome 6pter-p22. Nat Genet 1995;10:41-6.

[10] Schwab SG, Knapp M, Mondabon S, Hallmayer J, Borrmann-Hassenbach M, Albus M, et al. Support for association of schizophrenia with genetic variation in the 6p22.3 gene, dysbindin, in sib-pair families with linkage and in an additional sample of triad families. Am J Hum Genet 2003;72:185-90.

[11] Tang JX, Zhou J, Fan JB, Li XW, Shi YY, Gu NF, et al. Family-based association study of DTNBP1 in 6p22.3 and schizophrenia. Mol Psychiatry 2003;8:717-8.

[12] Funke B, Finn CT, Plocik AM, Lake S, DeRosse P, Kane JM, et al. Association of the DTNBP1 locus with schizophrenia in a U.S. population. Am J Hum Genet 2004;75:891-8.

[13] Fallin MD, Lasseter VK, Avramopoulos D, Nicodemus KK, Wolyniec PS, McGrath JA, et al. Bipolar I disorder and schizophrenia: a 440-single-nucleotide polymorphism screen of 64 candidate genes among Ashkenazi Jewish case-parent trios. Am J Hum Genet 2005;77:918-36.

[14] Kirov G, Ivanov D, Williams NM, Preece A, Nikolov I, Milev R, et al. Strong evidence for association between the dystrobrevin binding protein 1 gene (DTNBP1) and schizophrenia in 488 parent-offspring trios from Bulgaria. Biol Psychiatry 2004;55:971-5.

[15] Li T, Zhang F, Liu X, Sun X, Sham PC, Crombie C, et al. Identifying potential risk haplotypes for schizophrenia at the DTNBP1 locus in Han Chinese and Scottish populations. Mol Psychiatry 2005;10:1037-44.

[16] Numakawa T, Yagasaki Y, Ishimoto T, Okada T, Suzuki T, Iwata N, et al. Evidence of novel neuronal functions of dysbindin, a susceptibility gene for schizophrenia. Hum Mol Genet 2004;13:2699-708.
[17] Tochigi M, Zhang X, Ohashi J, Hibino H, Otowa T, Rogers M, et al. Association study of the dysbindin (DTNBP1) gene in schizophrenia from the Japanese population. Neurosci Res 2006;56:154-8.
[18] Tosato S, Ruggeri M, Bonetto C, Bertani M, Marrella G, Lasalvia A, et al. Association study of dysbindin gene with clinical and outcome measures in a representative cohort of Italian schizophrenic patients. Am J Med Genet B Neuropsychiatr Genet 2007;144B:647-59.
[19] Van Den Bogaert A, Schumacher J, Schulze TG, Otte AC, Ohlraun S, Kovalenko S, et al. The DTNBP1 (dysbindin) gene contributes to schizophrenia, depending on family history of the disease. Am J Hum Genet 2003;73:1438-43.
[20] van den Oord EJ, Sullivan PF, Jiang Y, Walsh D, O'Neill FA, Kendler KS, et al. Identification of a high-risk haplotype for the dystrobrevin binding protein 1 (DTNBP1) gene in the Irish study of high-density schizophrenia families. Mol Psychiatry 2003;8:499-510.
[21] Williams NM, Preece A, Morris DW, Spurlock G, Bray NJ, Stephens M, et al. Identification in 2 independent samples of a novel schizophrenia risk haplotype of the dystrobrevin binding protein gene (DTNBP1). Arch Gen Psychiatry 2004;61:336-44.
[22] Pae CU, Mandelli L, De Ronchi D, Kim JJ, Jun TY, Patkar AA, et al. Dysbindin gene (DTNBP1) and schizophrenia in Korean population. Eur Arch Psychiatry Clin Neurosci 2009;259:137-42.
[23] Datta SR, McQuillin A, Puri V, Choudhury K, Thirumalai S, Lawrence J, et al. Failure to confirm allelic and haplotypic association between markers at the chromosome 6p22.3 dystrobrevin-binding protein 1 (DTNBP1) locus and schizophrenia. Behav Brain Funct 2007;3:50.
[24] Holliday EG, Handoko HY, James MR, McGrath JJ, Nertney DA, Tirupati S, et al. Association study of the dystrobrevin-binding gene with schizophrenia in Australian and Indian samples. Twin Res Hum Genet 2006;9:531-9.
[25] Morris DW, McGhee KA, Schwaiger S, Scully P, Quinn J, Meagher D, et al. No evidence for association of the dysbindin gene [DTNBP1] with schizophrenia in an Irish population-based study. Schizophr Res 2003;60:167-72.
[26] Peters K, Wiltshire S, Henders AK, Dragovic M, Badcock JC, Chandler D, et al. Comprehensive analysis of tagging sequence variants in DTNBP1 shows no association with schizophrenia or with its composite neurocognitive endophenotypes. Am J Med Genet B Neuropsychiatr Genet 2008;147B:1159-66.
[27] Sanders AR, Duan J, Levinson DF, Shi J, He D, Hou C, et al. No significant association of 14 candidate genes with schizophrenia in a large European ancestry sample: implications for psychiatric genetics. Am J Psychiatry 2008;165:497-506.
[28] Benson MA, Newey SE, Martin-Rendon E, Hawkes R, Blake DJ. Dysbindin, a novel coiled-coil-containing protein that interacts with the dystrobrevins in muscle and brain. J Biol Chem 2001;276:24232-41.

[29] Talbot K, Eidem WL, Tinsley CL, Benson MA, Thompson EW, Smith RJ, et al. Dysbindin-1 is reduced in intrinsic, glutamatergic terminals of the hippocampal formation in schizophrenia. J Clin Invest 2004;113:1353-63.
[30] Bray NJ, Preece A, Williams NM, Moskvina V, Buckland PR, Owen MJ, et al. Haplotypes at the dystrobrevin binding protein 1 (DTNBP1) gene locus mediate risk for schizophrenia through reduced DTNBP1 expression. Hum Mol Genet 2005;14:1947-54.
[31] Owen MJ, Williams NM, O'Donovan MC. Dysbindin-1 and schizophrenia: from genetics to neuropathology. J Clin Invest 2004;113:1255-7.
[32] Weickert CS, Rothmond DA, Hyde TM, Kleinman JE, Straub RE. Reduced DTNBP1 (dysbindin-1) mRNA in the hippocampal formation of schizophrenia patients. Schizophr Res 2008;98:105-10.
[33] Weickert CS, Straub RE, McClintock BW, Matsumoto M, Hashimoto R, Hyde TM, et al. Human dysbindin (DTNBP1) gene expression in normal brain and in schizophrenic prefrontal cortex and midbrain. Arch Gen Psychiatry 2004;61:544-55.
[34] Lang UE, Puls I, Muller DJ, Strutz-Seebohm N, Gallinat J. Molecular mechanisms of schizophrenia. Cell Physiol Biochem 2007;20:687-702.
[35] Cox MM, Tucker AM, Tang J, Talbot K, Richer DC, Yeh L, et al. Neurobehavioral abnormalities in the dysbindin-1 mutant, sandy, on a C57BL/6J genetic background. Genes Brain Behav 2009;8:390-7.
[36] Bhardwaj SK, Baharnoori M, Sharif-Askari B, Kamath A, Williams S, Srivastava LK. Behavioral characterization of dysbindin-1 deficient sandy mice. Behav Brain Res 2009;197:435-41.
[37] Feng YQ, Zhou ZY, He X, Wang H, Guo XL, Hao CJ, et al. Dysbindin deficiency in sandy mice causes reduction of snapin and displays behaviors related to schizophrenia. Schizophr Res 2008;106:218-28.
[38] Hattori S, Murotani T, Matsuzaki S, Ishizuka T, Kumamoto N, Takeda M, et al. Behavioral abnormalities and dopamine reductions in sdy mutant mice with a deletion in Dtnbp1, a susceptibility gene for schizophrenia. Biochem Biophys Res Commun 2008;373:298-302.
[39] Takao K, Toyama K, Nakanishi K, Hattori S, Takamura H, Takeda M, et al. Impaired long-term memory retention and working memory in sdy mutant mice with a deletion in Dtnbp1, a susceptibility gene for schizophrenia. Mol Brain 2008;1:11.
[40] Peralta V, Cuesta MJ. Psychometric properties of the positive and negative syndrome scale (PANSS) in schizophrenia. Psychiatry Res 1994;53:31-40.
[41] Frazer KA, Ballinger DG, Cox DR, Hinds DA, Stuve LL, Gibbs RA, et al. A second generation human haplotype map of over 3.1 million SNPs. Nature 2007;449:851-61.
[42] Abramson JH. WINPEPI (PEPI-for-Windows): computer programs for epidemiologists. Epidemiol Perspect Innov 2004;1:6.
[43] Ott J. (1988) Utility programs for analysis of genetic linkage, Program, HWE version 1.10. Columbia University, New York.
[44] Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society, Series B 1995;57:289-300.
[45] Betcheva ET, Mushiroda T, Takahashi A, Kubo M, Karachanak SK, Zaharieva IT, et al. Case-control association study of 59 candidate genes

reveals the DRD2 SNP rs6277 (C957T) as the only susceptibility factor for schizophrenia in the Bulgarian population. J Hum Genet 2009;54:98-107.
[46] Peters K, Wiltshire S, Henders AK, Dragovic M, Badcock JC, Chandler D, et al. Comprehensive analysis of tagging sequence variants in DTNBP1 shows no association with schizophrenia or with its composite neurocognitive endophenotypes. Am J Med Genet Part B 2008;147B:1159-66.
[47] Wood LS, Pickering EH, Dechairo BM. Significant support for DAO as a schizophrenia susceptibility locus: examination of five genes putatively associated with schizophrenia. Biol Psychiatry 2007;61:1195-9.
[48] Duan J, Martinez M, Sanders AR, Hou C, Burrell GJ, Krasner AJ, et al. DTNBP1 (Dystrobrevin binding protein 1) and schizophrenia: association evidence in the 3' end of the gene. Hum Hered 2007;64:97-106.
[49] Mutsuddi M, Morris DW, Waggoner SG, Daly MJ, Scolnick EM, Sklar P. Analysis of high-resolution HapMap of DTNBP1 (Dysbindin) suggests no consistency between reported common variant associations and schizophrenia. Am J Hum Genet 2006;79:903-9.
[50] Vilella E, Costas J, Sanjuan J, Guitart M, De Diego Y, Carracedo A, et al. Association of schizophrenia with DTNBP1 but not with DAO, DAOA, NRG1 and RGS4 nor their genetic interaction. J Psychiatr Res 2008;42:278-88.
[51] Iizuka Y, Sei Y, Weinberger DR, Straub RE. Evidence that the BLOC-1 protein dysbindin modulates dopamine D2 receptor internalization and signaling but not D1 internalization. J Neurosci 2007;27:12390-5.

Table 1
Allele association of 13 *DTNBP1* SNPs with schizophrenia

SNP ID	Position*	SNP details	χ^2	*p*-value†	Odds Ratio	95% CI
rs9476886	15769440	Intron 1 C/T	0.18	0.894	0.98	0.72-1.34
rs1997679	15766884	Intron 1 C/T	3.907	**0.048**	1.37	1.00-1.87
rs2743857	15758475	Intron 3 A/G	0.121	0.728	0.93	0.63-1.38
rs3829893	15723616	Intron 5 A/G	0.618	0.432	0.86	0.60-1.25
rs7758659	15701219	Intron 7 C/T	0.013	0.91	1.02	0.72-1.44
rs9370823	15658637	Intron 7 A/G	3.729	**0.053**	1.38	1.00-1.92
rs9370822	15652715	Intron 7 A/C	9.883	**0.002**	1.61	1.19-2.17
rs4236167	15641930	Intron 7 G/T	3.111	**0.078**	1.30	0.97-1.73
rs16876589	15641476	Exon 8 D214G	Not polymorphic in this study			
rs4712253	15634396	Intron 8 C/T	0.008	0.928	0.99	0.74-1.32
rs742106	15632459	Intron 9 C/T	0.04	0.841	1.03	0.76-1.40
rs17470454	15631427	Exon 10 S272P	5.74	**0.017**	2.03	1.13-3.67
rs1047631	15631080	3'-UTR A/G	2.386	0.122	0.73	0.48-1.09

***** Nucleotide position on the chromosome 6 reference sequence
† *p*-value determined by Pearson's chi-squared test

Table 2

Genotype association of 5 candidate *DTNBP1* SNPs with schizophrenia.

Polymorphism	**Genotype counts (%)**			***p*-value***
rs1997679	TT	CT	CC	
Control	25 (10.8)	111 (47.8)	96 (41.4)	0.060
schizophrenia	15 (9.4)	59 (37.1)	85 (53.5)	0.048†
Odds Ratio‡ (*p*-value)	1.00	0.89 (1.00)	1.48 (0.55)	
rs9370823	AA	AG	GG	
Control	124 (56.9)	84 (38.5)	10 (4.6)	0.057
schizophrenia	78 (50.6)	59 (38.3)	17 (11.0)	0.055†
Odds Ratio‡ (*p*-value)	1.00	1.12 (1.00)	2.70 (0.03)	
rs9370822	AA	AC	CC	
Control	113 (47.9)	101 (42.8)	22 (9.3)	0.004
schizophrenia	58 (37.2)	66 (42.3)	32 (20.5)	0.002†
Odds Ratio‡ (*p*-value)	1.00	1.27 (0.57)	2.83 (0.002)	
rs4236167	CC	CT	TT	
Control	77 (32.4)	124 (52.1)	37 (15.5)	0.032
schizophrenia	46 (30.3)	66 (43.4)	40 (26.3)	0.080†
Odds Ratio‡ (*p*-value)	1.00	0.89 (1.00)	1.81 (0.09)	

* *p*-value determined by Pearson's χ^2 test
† *p*-value determined using the extended Mantel-Haenszel test for trend
‡ OR, with respect to the genotype that was not associated with schizophrenia

Table 3

Genotype association of 5 candidate *DTNBP1* SNPs with schizophrenia under a recessive inheritance model.

Polymorphism	**Genotype counts (%)**		**Odds Ratio (95% CI)**	***p*-value***
rs1997679	TT/CT	CC		
control	136 (58.6)	96 (41.4)	1.63 (1.06-2.49)	0.019
schizophrenia	74 (46.5)	85 (53.5)		
rs9370823	AA/AG	GG		
Control	208 (95.4)	10 (4.6)	2.58 (1.08-6.49)	0.018
schizophrenia	137 (89.0)	17 (11.0)		
rs9370822	AA/AC	CC		
control	214 (90.7)	22 (9.3)	2.51 (1.34-4.74)	0.002
schizophrenia	124 (79.5)	32 (20.5)		
rs4236167	CC/CT	TT		
control	201 (84.5)	37 (15.5)	1.94 (1.14-3.31)	0.009
schizophrenia	112 (73.7)	40 (26.3)		

* *p*-value determined by Pearson's χ^2 test
DTNBP1