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Association of the Dystrobrevin Binding Protein 1 Gene (*DTNBP1*) in a Bipolar Case–Control Study (BACCS)

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Recent studies suggest a degree of overlap in genetic susceptibility across the traditional categories of schizophrenia and bipolar disorder. There is some evidence for an association of the dystrobrevin binding protein 1 gene (*DTNBP1*) with schizophrenia, and, thus, this gene has also become a focus of further investigation in bipolar disorder (BD). The aim of our study is to explore the association of *DTNBP1* with BD and with a sub phenotype, presence/absence of psychotic symptoms, in a sample of 515 patients with BD (ICD10/DSMIV) and 1,316 ethnically matched control subjects recruited from the UK. Seven *DTNBP1* SNPs: rs2743852 (SNP C), rs760761 (P1320), rs1011313 (P1325), rs3213207 (P1635), rs2619539 (P1655), rs16876571 and rs17470454 were investigated using the SNPlex genotyping system and 1 SNP (rs2619522) genotypes were imputed. Association analyses were conducted in a sample of 452 cases and 956 controls. We found significant differences in genotypic and allelic frequencies of rs3213207 and rs760761 of *DTNBP1* between bipolar patients and controls. We also showed a global haplotypic association and an association of a particular haplotype with BD. Our results are consistent with previous studies in term of a general association between *DTNBP1* and bipolar disorder and provide additional evidence that a portion of the genotypic overlap between schizophrenia and bipolar affective disorder is attributable to this gene. © 2008 Wiley-Liss, Inc.

Key words: dysbindin; affective disorder; association; psychosis; haplotype

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INTRODUCTION

Recent studies point to a degree of genetic overlap between schizophrenia and bipolar disorder (BD) [Cardno et al., 2002 and for review, see Craddock et al., 2005]. Genetic linkage and association studies have implicated several candidate genes in schizophrenia, including that encoding dystrobrevin binding protein (or dysbindin, *DTNBP1*) [Straub et al., 2002; Schwab et al., 2003]. A study on schizophrenia and bipolar disorders involving Ashkenazi Jewish case-parent trios showed evidence that several genes, including *DTNBP1*, are associated with the development of both disorders [Fallin et al., 2005]. Moreover, the *DTNBP1* gene is located on chromosome 6p22.3, a region that has received genome-wide significant support in BD [Ewald et al., 2002]. Functional studies have shown that dystrobrevin binding protein plays a role in synaptic glutamate neural transmission in the brain [Benson et al., 2001; Hajek et al., 2005]. And, in addition, DTNBP1 is involved in the modulation of synaptic signal transduction and plasticity which could be associated with bipolar disorder [Straub et al., 2002; Numakawa et al., 2004].

Additional Supporting Information may be found in the online version of this article.

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To date there have been some studies showing association of *DTNPB1* gene polymorphisms with bipolar disorder in Koreans [Joo et al., 2007; Pae et al., 2007] and in the UK population [Breen et al., 2006]. The main limitation of these studies is the small sample size. Raybould et al. [2005] failed to find an association of a 3-locus haplotype of *DTNBP1* gene (rs2619539-rs3213207-rs2619538) in a larger group of patients with BD (N = 726), but found a weak, nominally significant association in a subset of BD patient with psychotic symptoms (N = 133). Therefore, we investigated a possible association of individual SNPs and haplotypes of the *DTNBP1* gene with bipolar disorder in the UK case–control sample.

MATERIALS AND METHODS

Sample

The sample of 515 patients (65.8%—women) (mean age ± SD: 47.99 ± 11.40) of white European parentage was recruited from psychiatric clinics, hospitals, primary care physicians, patient support groups and from volunteers responding to media advertisements. The diagnosis of bipolar I disorder (N = 459) or bipolar II disorder (N = 56) was defined by the Diagnostic and Statistical Manual 4th edition operational criteria (DSMIV). The Schedules for Clinical Assessment in Neuropsychiatry (SCAN) [Wing et al., 1990] and the Operational Criteria Checklist for Psychotic Illness program [McGuffin et al., 1991] were used to assess patients. The age of onset of disease was 21.28 ± 10.48 and was defined as the age of the first depressive or manic episode occurred in a patient life course based on the SCAN interview. Subjects were excluded if they, or a first-degree relative, have ever fulfilled criteria for schizophrenia, if they experienced psychotic symptoms that were mood incongruent or present when there was no evidence for mood disturbance. Additional exclusion criteria included intravenous drug use with a lifetime diagnosis of drug dependency, or if manias occurred solely in relation to, or a consequence of, alcohol or substance abuse/dependence and/or medical illness.

Potential control subjects were recruited from among students and staff working at Kings College London (internal email) and via media advertisement (local newspapers). They were interviewed face to face or by telephone using a modified version of the Past History Schedule [McGuffin et al., 1986], and were included if there was no evidence of past or present clinically significant psychiatric disorder (such as schizophrenia, mania, hypomania, depression). Subjects were also excluded if they scored 10 or above on the Beck Depression Inventory [Beck and Steer, 1984] at the time of interview, or were related to an individual already included in the study. One thousand three hundred and sixteen control subjects (mean age ± SD: 41.70 ± 13.16) were included in the study. As all controls in the present study were screened for the absence of psychiatric disorder, we consider that they are not truly representative of the UK general population where we would expect the rates of any psychiatric disorder to be between 10% and 20%. All enrolled subjects were of white European parentage. The ethnic origin of BACCS cases and controls was defined by self-report based on information available on the origin of their mother/father and grandmothers/grandfathers. The study was approved by the local Research Ethics Committee and informed written consent was obtained from all participants.

Diagnostic Quality Control

Interviewers were all graduate psychologists who received a 1 week training course in the administration and item coding of the SCAN interview by Anne Farmer (AF), a WHO approved trainer. Interviewers then carried out ~10 practice interviews before submitting a complete taped interview with a volunteer subject with bipolar disorder to AF for review before their final approval as trained interviewers. Regular inter rater reliability sessions were held approximately monthly for all raters where each in turn submitted a complete taped interview from a bipolar subject for joint ratings led by AF. Interviewers also produced written transcripts of all interviews as well as brief written vignettes describing the mental status of the subject at interview. Excellent inter rater reliability for diagnosis was achieved (mean across multiple rater pairs kappa = 0.83).

Genotyping

Genomic DNA was extracted by an in-house procedure from bloods or cheek swabs, as described previously [Freeman et al., 1997, 2003]. Genotyping of seven DTNBP1 polymorphisms was carried out using the SNPlex Genotyping System (Applied Biosystems, Foster City, CA). The system uses oligonucleotide ligation, polymerase chain reaction and capillary electrophoresis to analyze bi-allelic single nucleotide polymorphism (SNP) genotypes [Tobler et al., 2005]. In the current study we have examined seven markers of *DTNBP1* gene—rs2743852 (SNP C) in a promoter region, rs760761 (P1320) in intron 3, rs1011313 (P1325) and rs3213207 (P1635) in intron 4, and rs2619539 (P1655) in intron 5—from the reports by Straub et al. [2002] and Williams et al. [2004], and, additionally, we included rs16876571 in intron 9 and rs17470454 in exon 10. Altogether the seven markers cover a region of 141 kb.

Analyzing the raw data was performed using GeneMapper Software v3.7 and Microsoft Office Excel 2003. 1,619 samples were genotyped, and 211 (13%) were excluded from the further analysis based on an 80% threshold for the call rate across the whole SNPs set. As an internal control, two plates (176 samples) were regenotyped with a 100% consistence of the results of *DTNBP1* SNPs.

Statistics

One thousand four hundred and eight samples (452 cases and 956 controls) were available for statistical analysis. To calculate power the PS program was used [Dupont and Plummer, 1990]. To test the deviation from the Hardy–Weinberg Equilibrium (HWE) an exact statistic was calculated with the computer program FINETTI (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl). Genotype and allele frequencies were assessed for association with BD using standard contingency table analysis incorporating chi-squared tests of independence, producing a χ^2 statistic with 1 or 2 degrees of freedom depending on the number of parameters and corresponding p values for allele and genotype distributions, respectively. Risk magnitudes were estimated by calculating odds ratios (OR) with 95% confidence intervals (CI) using Woolf's method.

Haplotype analysis of seven SNPs was conducted using Haploview 4.0 [Barrett et al., 2005] and UNPHASED [Dudbridge, 2003]. In the Haploview analyses samples with call rates less than 50% were

excluded. Using the UNPHASED program rare haplotypes (frequency threshold 0.01 in both in cases and controls) were excluded. UNPHASED uses unconditional logistic regression to perform likelihood ratio tests under a log-linear model of the probability that an allele or haplotype belongs to the case rather than control group. The global null hypothesis is that the odds ratios of all haplotypes are equal between cases and controls. Individual haplotypes were also tested for association by grouping the frequencies of all other haplotypes together.

We also imputed the genotypes for all SNPs available in Hapmap database using IMPUTE program (http://www.stats.ox.ac.uk/~marchini/software/gwas/impute.html) and analyzed the genotypes and allele association of rs2619522 in the BACCS sample. Accuracy over 90% of imputed data makes them appropriate for analyses of genotypes and alleles, but not haplotypes.

To correct for multiple testing in the single marker comparisons for both genotypic and allelic association we applied Bonferroni corrections whereby an α-level of 0.007 was taken as the significant. This was under the conservative assumption that there were seven independent tests (based on the total number of SNPs genotyped). Correction for multiple testing has not been applied to haplotype analyses as these tests can not be considered as independent of tests of alleles or genotypes.

To determine tagging SNPs (tSNPs) for each of the studies investigating *DTNBP1* gene in BD we used Haploview version 4.0 [Barrett et al., 2005] and the Tagger implementation therein [de Bakker et al., 2005].

RESULTS

The results of genotype and allele distributions of the *DTNBP1* markers in the UK Bipolar Case–Control sample (BACCS) are shown in Table I (where the percentage of the missing data for each SNP is also presented). Since there were no significant differences in allele and genotype frequencies between the groups of patients with bipolar II disorder and bipolar I disorder (Table II), we analyzed both groups together. All markers showed genotype distribution consistent with Hardy–Weinberg equilibrium in both cases and controls ($P > 0.05$) (the data are not shown but are available on request). Association analysis of single markers showed significant differences in genotype or allele distribution of two markers between cases and controls: rs760761 (genotypes: $P = 0.0073$, borderline significant after Bonferroni correction; alleles: $P = 0.02$, OR = 1.26, 95%CI 1.03–1.54, lost after Bonferroni correction) and rs3213207 (genotypes: $P = 0.019$, lost after Bonferroni correction; alleles: $P = 0.006$, OR = 1.41, 95%CI 1.10–1.80). Marker rs17470454 showed nominally significant differences in distribution of alleles ($P = 0.048$), but not genotypes. There were no significant differences in allelic or genotypic frequencies of the other four markers genotyped between the bipolar disorder group and control group. The allelic frequencies of the analyzed SNPs in controls were similar to previously reported ones in UK samples [Williams et al., 2004; Breen et al., 2006].

Strong linkage disequilibrium was found across the markers studied in both controls and cases (Fig. 1). We analyzed two-, three-, and four-marker haplotypes. The results of a three SNP sliding window analysis are provided in Table III. One combination of three markers (rs16876571-rs2619539-rs3213207) showed a significant difference in haplotypes distribution between the BD patients and controls (global $P = 0.027$) with a significant overrepresentation of the G-C-G haplotype in bipolar disorder ($P = 0.015$). The four-marker haplotype G-C-G-T (rs16876571-rs2619539-rs3213207-rs760761) was also overrepresented among patients with BD compared to controls ($P = 0.02$).

When we compared the subset of bipolar cases diagnosed with either "manic episode with psychosis" or/and "major depressive disorder (MDD) with psychosis" (N = 36) against controls, we found an allelic association of rs3213207 (P1635) ($P = 0.021$, OR = 2.053, 95% CI 1.103–3.82) and a haplotypic association of the same marker combination (rs16876571-rs2619539-rs3213207) (global $P = 0.044$) as in the total sample.

To make the results of our study comparable with the previously published studies of *DTNBP1* gene and BD (Table IV) we applied the approach used by Mutsuddi et al. [2006] to compare the results of the studies on *DTNBP1* in schizophrenia. We have identified the single marker or multimarker haplotype that best captured the association signal in each study. Using the data available from the HapMap project we determined which SNPs tagged the associated haplotypes reported in the original study. Data on all SNPs, except of rs2005976, were available for as part of the HapMap data release 23a (phase II of the March 2008 on NCBI B36 assembly; dbSNP b 126). However, more complete information LD structure of *DTNBP1* gene is available from the study by Mutsuddi et al. [2006], which shows that rs2005976 is in a strong LD with rs2619522 ($r^2 > 0.8$) (this SNP was not included for the comparative analyses). For each study Table IV shows the haplotypes, and the tSNP(s) for each study are identified by asterisk. Across the five studies six SNPs were required to define all associated alleles. These SNPs are shown on Figure 2. For each of the studies the strongest evidence of association of the following alleles/haplotypes was shown. Raybould et al. [2005]: T-A-C risk haplotype of three markers 1-10-11, and this haplotype can be tagged by these three SNPs. Breen et al. [2006]: G-T-A-A risk haplotype (5-6-7-8), which can be effectively tagged by allele G at SNP 5 (rs2619522). Pae et al. [2007]: T-T-G-C-A protective haplotype (5-6-7-9-10) can be tagged by T-C-A haplotype at SNPs 5-9-10. Joo et al. [2007]: the strongest association is shown for allele T (rs2619522) and allele C (=G) (rs760761), since these SNPs are in strong LD ($r^2 = 1.0$), both associations can be tagged by allele T (rs2619522). In the BACCS four-marker haplotype T-G-C-G (6-10-11-12) can be tagged by these four SNPs, for better comparison with the previous studies we have decided to use rs2619522 (SNP 5) instead of rs760761 (SNP 6), since the both are shown to be in a complete LD.

With the defined associated alleles or haplotypes from each sample we mapped each of these associated alleles or haplotypes onto the CEU sample as a reference to examine all of the studies together. For this analysis we concentrated on the tSNPs from each study, as defined above (SNPs 1, 5, 9, 10, 11, and 13). From these six SNPs we identified eight common haplotypes in the CEU trios and build a possible phylogenetic tree. Figure 2 displays this tree and identifies the eight common CEU haplotypes and their respective frequencies. The ancestral haplotype remains the most common haplotype.

TABLE I. The Results of Association Analysis of Individual SNPs of the DTNBP1 Gene in the BACCS: χ^2 tests are Applied for Comparison of Allele and Genotype Frequencies Between Bipolar Disorder and Control Groups

				BD					Controls				
				Genotypes			Alleles		Genotypes			Alleles	
SNP ID	Chromosomal location (6p22.3)	Alleles (1/2)	Missing (%)	11	12	22	1	2	11	12	22	1	2
rs17470454	15631427	A/G	0.2	1	58	390	60	838	2	89	865	93	1,819
				0.002	0.129	0.869	0.067	0.933	0.002	0.093	0.905	0.049	0.951
χ^2 (P)					4.25 (0.119)		3.92 (0.048)						
rs16876571	15632658	A/G	1.3	1	19	427	21	873	0	28	915	28	1,858
				0.002	0.043	0.955	0.023	0.977	0.000	0.030	0.970	0.015	0.985
χ^2 (P)					3.65 (0.16)		2.62 (0.106)						
rs2619539	15728834	C/G	6.4	108	220	122	436	464	183	436	249	802	934
				0.240	0.489	0.271	0.484	0.516	0.211	0.502	0.287	0.462	0.538
χ^2 (P)					1.51 (0.47)		1.2 (0.273)						
rs3213207	15736081	A/G	1.1	333	107	7	773	121	763	175	7	1,701	189
				0.745	0.239	0.016	0.865	0.135	0.807	0.185	0.007	0.900	0.100
χ^2 (P)					7.96 (0.019)		7.66 (0.006)						
rs1011313	15741411	A/G	0.6	2	83	361	87	805	11	151	792	173	1,735
				0.004	0.186	0.809	0.098	0.902	0.012	0.158	0.830	0.091	0.909
χ^2 (P)					3.19 (0.203)		0.34 (0.559)						
rs760761	15759111	C/T	6.2	254	164	18	672	200	587	257	40	1,431	337
				0.583	0.376	0.041	0.771	0.229	0.664	0.291	0.045	0.809	0.191
χ^2 (P)					9.83 (0.007)		5.41 (0.02)						
rs2743852	15772743	C/G	1.8	343	81	3	767	87	781	162	13	1,724	188
				0.803	0.190	0.007	0.898	0.102	0.817	0.169	0.014	0.902	0.098
χ^2 (P)					1.86 (0.395)		0.08 (0.773)						
rs2619522[a]	15761628	T/G	6.0	259	152	18	670	188	603	252	38	1,458	328
				0.604	0.354	0.044	0.780	0.220	0.675	0.282	0.043	0.816	0.184
					7.21 (0.027)		4.64 (0.031)						

[a]Genotypes have been imputed.

TABLE II. DTNBP1 Allele Distribution in BACCS Bipolar I Disorder (BID) and Bipolar II Disorder (BIID) Groups

Marker	Allele	BID (%)	BIID (%)	χ^2 (*P*)
rs17470454	A/G	54/754 (6.7/93.3)	6/78 (7.1/92.9)	0.026 (0.873)
rs16876571	A/G	19/795 (2.3/97.7)	2/82 (2.4/97.6)	0.001 (0.978)
rs2619539	C/G	394/426 (48.0/52.0)	44/40 (47.6/52.4)	0.573 (0.449)
rs3213207	A/G	707/109 (86.6/13.4)	70/12 (85.4/14.6)	0.104 (0.747)
rs1011313	A/G	80/732 (9.9/90.1)	8/76 (9.5/90.5)	0.009 (0.923)
rs760761	C/T	613/181 (77.2/22.8)	63/19 (76.8/23.2)	0.006 (0.939)
rs2743852	C/G	693/79 (89.8/10.2)	76/8 (90.5/9.5)	0.042 (0.838)

We were then able to map the associated allele or haplotype from each study onto the phylogenetic tree (Fig. 2). The associated allele from the study of Breen et al. [2006] (allele G of SNP 5) maps onto haplotypes 6, 7, and 8 in the CEU data (Fig. 2 from left to right). The allele that tags the protective haplotype in the study of Joo et al. [2007] (allele T at SNP 5) maps onto haplotypes from 1 to 5. The associated risk haplotype from the study of Pae et al. [2007], captured by the haplotype A-G-T at SNPs 11-10-9, maps onto haplotypes from 2 to 5. The associated haplotype from the study of Raybould et al. [2005], captured by the haplotype C-A-T at SNPs 11 -10-1, maps onto haplotype 5. The strongest association signal from the BACCS is tagged by haplotype G-C-G-G at SNPs 13-11-10-5; this maps onto haplotype 6.

DISCUSSION

In our comparatively large, well-characterized, UK white bipolar disorder case–control study we analyzed seven *DTNBP1* SNPs, some of which have previously been reported to be associated with schizophrenia [Straub et al., 2002; van den Oord et al., 2003; Williams et al., 2004] and four of which have been previously been tested for association with bipolar disorder (rs760761, rs1011313, rs3213207, rs2619539) [Raybould et al., 2005; Breen et al., 2006; Joo et al., 2007; Pae et al., 2007]. In addition we examined three SNPs, previously not tested for association with BD. These were "SNP C" (rs2743852), known to be in strong LD with "SNP A," a schizophrenia associated SNP [Williams et al., 2004], rs16876571 (intron 9), and rs17470454 (exon 10).

We found allelic association of two markers, rs3213207 (P1635) (allele G: OR = 1.41, 95%CI 1.10–1.80) and rs760761 (P1320) (allele T: OR = 1.26, 95%CI 1.03–1.54), and bipolar disorder. This is in keeping with previous findings, which are summarized at Table IV. Specifically our finding of association with rs760761 (P1320) in the BACCS is consistent with the previous results by Breen et al. [2006] in a Scottish sample and by Joo et al. [2007] in Koreans. However, the association of rs760761 T allele with BD was

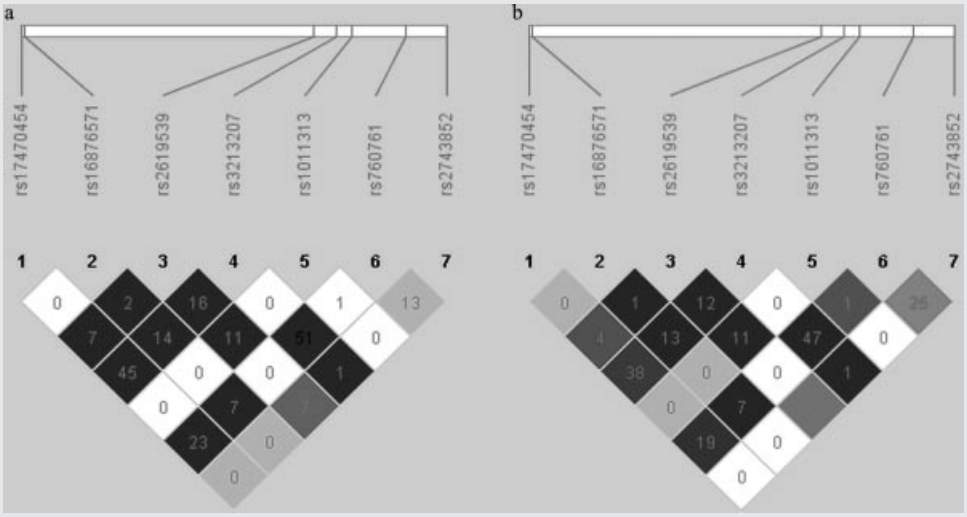

FIG. 1. Linkage disequilibrium (LD) plots of the investigated DTNBP1 SNPs in the BACCS: (a) in a bipolar disorder group, (b) in a control group; pairwise LD (r^2) values (%) are annotated in rhombus, black rhombus corresponds to a high LD between two SNPs (high D′ and high r^2), gray ones to a LD based on high D′ but low r^2 while white ones to a low LD (low D′ and low r^2).

TABLE III. The Results of a Three Marker Haplotype Sliding-Window Analysis of DTNBP1 Gene in BACCS Using UNPHASED Program: Global *P*-Values Show the Differences of Haplotype Distribution Between Cases and Controls, While Individual *P* Values Show the Association of the Most Significant Haplotypes Which can be Seen in the Table Gray Boxes

rs17470454	A				
rs16876571	G	G			
rs2619539	C	C	C		
rs3213207		G	G	G	
rs1011313			G	G	G
rs760761				T	T
rs2743852					G
Global *P*	0.1	0.027	0.09	0.1	0.08
Individual *P*	0.04	0.015	0.011	0.04	0.016

reported both by Breen et al. [2006] and in our BACCS sample, while the association of C allele and BD was reported by Joo et al. [2007].

Although no previous studies found an association between bipolar disorder and rs3213207 (P1635), a Korean study found evidence for a protective haplotype including allele A [Pae et al., 2007]. The same allele was also a part or risk haplotype reported by Raybould et al. [2005].

Raybould et al. [2005], using three SNPs (two of them are P1635 and P1655), reported a nominally significant association of 3-locus haplotype in the subgroup of bipolar cases in whom psychotic features occurred in 50% or more episodes of mood disorder. In contrast, we did not find an association that was specific to bipolar patients with psychotic symptoms rather than bipolar disorder as a whole.

Eight *DTNBP1* SNPs, analyzed in BACCS sample, captured 20 of 134 (14%) alleles at $r^2 = 0.8$ (mean max $r^2 = 0.987$). Across the whole gene area the mean r^2 with the Hapmap SNPs (N = 134) is equal to 0.11. The list of the captured SNPs is provided in supplementary Table I. In comparison with the previously published studies [Raybould et al., 2005; Joo et al., 2007; Pae et al., 2007], which have the capture of 5% (8 SNPs), (8%) 12 SNPS and 7% (10 SNPs) of the gene, correspondingly, we have the better coverage. However, the study by Breen et al. [2006], using 8 SNPs, provides the much better coverage with 60% (86 SNPs) of the gene area. With use of pairwise tagging, a total of 42 SNPs are needed to capture 100% of alleles with $r^2 > 0.8$ (mean $r^2 = 0.975$) across the region covering DTNBP1 [Mutsuddi et al., 2006].

A major difficulty in interpretation of the results from *DTNBP1*-association studies is that the same SNPs have not been genotyped in all studies, which precludes direct comparison of risk alleles and haplotypes. For more comprehensive comparison of our data with previously published results we imputed the genotypes for SNP rs2619522. A SNP rs2619522, previously reported to be within an associated haplotype (G-T-A-A) by Breen et al. [2006], has been also included into analyses by 2 other research groups: by Pae et al. [2007], who reported other allele T to be associated with a low risk of BD, and later by Joo et al. [2007], who did not find the allelic association ($P = 0.078$) but showed significant genotypic association ($P = 0.014$) with overrepresentation of T allele in a BID group (92.4% vs. 88.3% in controls). In our sample based on imputed

TABLE IV. Positive Findings by Case–Control Association Studies of *DTNBP1* Gene and Bipolar Disorder: Gray Boxes Indicate the SNPs Genotyped; Associated Haplotypes in Studies by Raybould, Breen, Pae and BACCS are Presented; Alleles Shown Association in Single Marker Analyses is in Bold

SNP ID		Gene region	Raybould et al. [2005] British	Breen et al. [2006] Scottish	Pae et al. [2007][a] Korean	Joo et al. [2007][c] Korean	BACCS British
(1) rs2619538	SNP A	Promoter	**T***				
(2) rs2743852	SNP C	Promoter					
(3) rs909706	P1583	Intron 1					
(4) rs1018381	P1578	Intron 1					
(5) rs2619522	P1763	Intron 1		G*	T*	**T**[b,*]	**G**[d,*]
(6) rs760761	P1320	Intron 3		**T**	T	**C**[b]	**T**[b]
(7) rs2005976	P1757	Intron 3		**A**	G		
(8) rs2619528	P1765	Intron 3		A			
(9) rs1011313	P1325	Intron 4			C*		
(10) rs3213207	P1635	Intron 4	A*		A*		**G***
(11) rs2619539	P1655	Intron 5	C*				C*
(12) rs760666	P1287	Intron 7					
(13) rs16876571		Intron 9					G*
(14) rs17470454		Exon 10					

*tSNP.
[a]Protective haplotype is reported by Pae et al. [2007].
[b]Allele which is not a part of any haplotype associated.
[c]Information based on genotypes frequencies provided by the authors in the article.
[d]Based on genotypes imputed.

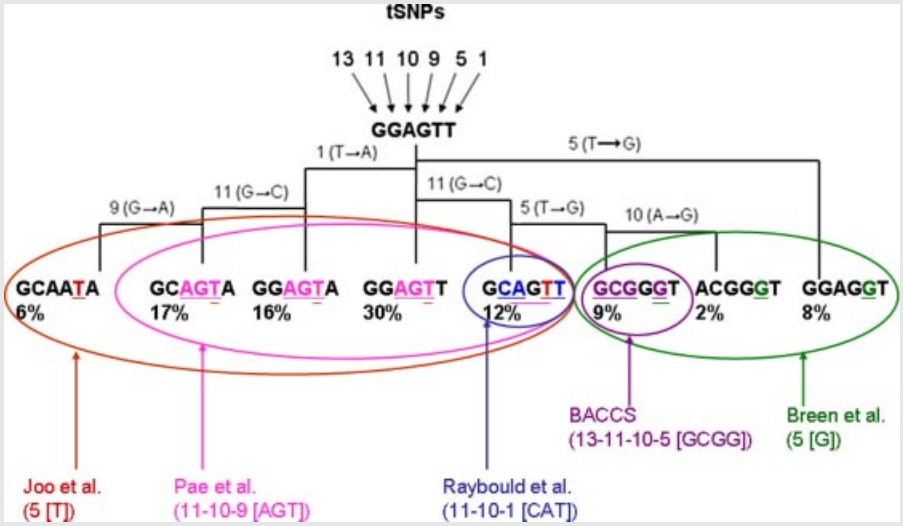

FIG. 2. Phylogenetic tree of the eight common haplotypes derived from tSNPs 1, 5, 9, 10, 11 and 13 (see Table IV) in the CEU sample. Haplotype frequencies are shown at the bottom of the tree. Mutational events are detailed on the horizontal lines of the tree. Each associated allele or haplotype from the five association studies of DTNBP1 and BD, mapped onto the phylogenetic tree. tSNPs are shown in parentheses, and haplotypes are shown in brackets. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

genotypes (Table I) we showed that the same allele G, as it was in a risk haplotype of Breen et al. [2006], is significantly over-represented in a BD group (22.0% vs. 18.4% in controls: $P = 0.031$, OR = 1.25, 95% 1.02–1.53).

Using the approach by Mutsuddi et al. [2006] for cross-study comparison of the association of *DTNBP1* gene and schizophrenia, we were able to identify the marker(s) that best captured the association signal in each of association studies of DTNBP1 gene in BD (Table IV). We have demonstrated that each common *DTNBP1* haplotype is tagged by the association signal of at least one study and there is some agreement across studies. The biggest overlapping region of five haplotypes are captured by signals from studies of Joo et al. [2007] and Pae et al. [2007], and one of these 5 is also tagged by signal from study of Raybould et al. [2005]. Our study has tagged the haplotype which is also one of 3 possible haplotypes captured by association signal reported by Breen et al. [2006]. In BACCS we have been able to demonstrated the significant association of the allele G of the rs2619522 as well as haplotype G-C-G-G with BD, the same allele that tagged the risk haplotype in the study by Breen et al. [2006], this is highly likely that in both samples the same haplotype would be a risk one.

Furthermore, the actual *DTNBP1* variants that confer susceptibility have not yet been identified. It is unlikely that any of the SNPs so far associated with BD or schizophrenia have a direct pathogenic role, and it is more likely that the association is due to linkage disequilibrium (LD) with the true risk variant(s). LD is known to be highly population dependent that can affect the reproducibility of results in association studies. However, another possibility is that the relevant functional variants do not affect the protein sequence but rather the pattern of splicing or protein expression levels. The

TABLE V. Power (%) of BACCS Sample (Case/Control = 452/956) to Detect Significant Association (One-Sided $\alpha = 0.05$) by Effect Size and Allele Frequency for Predisposing Allele

	Frequency of susceptibility allele in controls						
Allelic odds ratio	**1%**	**5%**	**10%**	**20%**	**30%**	**40%**	**50%**
1.1	6	7	8	11	12	13	13
1.2	7	12	18	27	32	35	36
1.3	9	20	32	49	58	63	63
1.4	12	30	49	70	80	83	84
1.5	15	42	65	86	92	94	94
2.0	35	87	99	100	100	100	100
4.0	92	100	100	100	100	100	100

causative variants may, therefore, be found in non coding regions. In this respect, P1635 and P1320, in the introns of the *DTNBP1*, which are within the alternative promoter regions, may be worth further exploration.

Because of the non uniformity of *DTNBP1* SNPs across studies it becomes debatable as to what constitutes a replication. We, therefore, took a conservative approach in our single marker analyses of requiring that either genotypic or allelic association ''significance'' should withstand Bonferroni correction. This is almost certainly over stringent given that of the two SNPs that we found were positively associated with BD. One has been previously reported and the other is in LD with markers previously associated with BD. In addition, as Figure 1 shows, there is a degree of LD across the region spanned by our seven SNPs, so that the seven single marker test of association are not independent. We can, therefore, be reasonably confident that our findings do not reflect type I errors. On the other hand, we cannot be as confident that we have avoided type II errors. We have calculated the power of BACCS sample for a range of allele frequencies and allelic odds ratios (Table V). For example, in our sample we would have 94% power (at nominal significance of $P < 0.05$) to detect an allelic association with a SNP with a minor allele frequency of 0.46–0.48 (as in case of rs2619539) and an odds ratio of 1.5, but the power is decreased to 35% if the OR were 1.2. The power of our sample is lower for SNPs with a rare allele, for example rs16876571, where it is around 20%.

The associations we have identified are unlikely to have arisen as a result of any admixture within our investigated groups, in part because the genotype and allele frequencies of all analyzed SNPs are congruent with the data for CEU population and for the studies published previously; secondly, all our subjects are of white European parentage that also reduces this possibility. The problem will also be definitively resolved by genotyping a large genomic control panel of markers across BACCS data sets in future.

In conclusion, our results are consistent with previous studies in term of a general association between the *DTNBP1* and bipolar disorder. They also provide additional molecular genetic evidence that a portion of the genotypic overlap between schizophrenia and bipolar affective disorder is attributable to this gene.

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REFERENCES

Barrett JC, Fry B, Maller J, Daly MJ. 2005. Haploview: Analysis and visualization of LD and haplotype maps. Bioinformatics 21(2):263–265 [Epub 2004 August 5].

Beck AT, Steer RA. 1984. Internal consistencies of the original and revised Beck Depression Inventory. J Clin Psychol 40(6):1365–1367.

Benson MA, Newey SE, Martin-Rendon E, Hawkes R, Blake DJ. 2001. Dysbindin, a novel coiled-coil-containing protein that interacts with the dystrobrevins in muscle and brain. J Biol Chem 276(26):24232–24241 [Epub 2001 April 20].

Breen G, Prata D, Osborne S, Munro J, Sinclair M, Li T, Staddon S, Dempster D, Sainz R, Arroyo B, et al. 2006. Association of the dysbindin gene with bipolar affective disorder. Am J Psychiatry 163(9):1636–1638.

Cardno AG, Rijsdijk FV, Sham PC, Murray RM, McGuffin P. 2002. A twin study of genetic relationships between psychotic symptoms. Am J Psychiatry 159(4):539–545.

Craddock N, O'Donovan MC, Owen MJ. 2005. The genetics of schizophrenia and bipolar disorder: Dissecting psychosis. J Med Genet 42(3): 193–204.

de Bakker PI, Yelensky R, Pe'er I, Gabriel SB, Daly MJ, Altshuler D. 2005. Efficiency and power in genetic association studies. Nat Genet 37(11):1217–1223.

Dudbridge F. 2003. Pedigree disequilibrium tests for multilocus haplotypes. Genet Epidemiol 25(2):115–121.

Dupont WD, Plummer WD Jr. 1990. Power and sample size calculations. A review and computer program. Control Clin Trials 11(2):116–128.

Ewald H, Flint T, Kruse TA, Mors O. 2002. A genome-wide scan shows significant linkage between bipolar disorder and chromosome 12q24.3 and suggestive linkage to chromosomes 1p22-21, 4p16, 6q14-22, 10q26 and 16p13.3. Mol Psychiatry 7(7):734–744.

Fallin MD, Lasseter VK, Avramopoulos D, Nicodemus KK, Wolyniec PS, McGrath JA, Steel G, Nestadt G, Liang KY, Huganir RL, et al. 2005. 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 77(6):918–936 [Epub 2005 October 28].

Freeman B, Powell J, Ball D, Hill L, Craig I, Plomin R. 1997. DNA by mail: An inexpensive and noninvasive method for collecting DNA samples from widely dispersed populations. Behav Genet 27(3):251–257.

Freeman B, Smith N, Curtis C, Huckett L, Mill J, Craig IW. 2003. DNA from buccal swabs recruited by mail: Evaluation of storage effects on long-term stability and suitability for multiplex polymerase chain reaction genotyping. Behav Genet 33(1):67–72.

Hajek T, Carrey N, Alda M. 2005. Neuroanatomical abnormalities as risk factors for bipolar disorder. Bipolar Disord 7(5):393–403.

Joo EJ, Lee KY, Jeong SH, Chang JS, Ahn YM, Koo YJ, Kim YS. 2007. Dysbindin gene variants are associated with bipolar I disorder in a Korean population. Neurosci Lett 418(3):272–275 [Epub 2007 March 21].

McGuffin P, Katz R, Aldrich J. 1986. Past and present state examination: The assessment of 'lifetime ever' psychopathology. Psychol Med 16(2):461–465.

McGuffin P, Farmer A, Harvey I. 1991. A polydiagnostic application of operational criteria in studies of psychotic illness. Development and reliability of the OPCRIT system. Arch Gen Psychiatry 48(8):764–770.

Mutsuddi M, Morris DW, Waggoner SG, Daly MJ, Scolnick EM, Sklar P. 2006. Analysis of high-resolution HapMap of DTNBP1 (Dysbindin) suggests no consistency between reported common variant associations and schizophrenia. Am J Hum Genet 79(5):903–909.

Numakawa T, Yagasaki Y, Ishimoto T, Okada T, Suzuki T, Iwata N, Ozaki N, Taguchi T, Tatsumi M, Kamijima K, et al. 2004. Evidence of novel neuronal functions of dysbindin, a susceptibility gene for schizophrenia. Hum Mol Genet 13(21):2699–2708 [Epub 2004 September 2].

Pae CU, Serretti A, Mandelli L, Yu HS, Patkar AA, Lee CU, Lee SJ, Jun TY, Lee C, Paik IH, et al. 2007. Effect of 5-haplotype of dysbindin gene

(DTNBP1) polymorphisms for the susceptibility to bipolar I disorder. Am J Med Genet Part B 144B(5):701–703.

Raybould R, Green EK, MacGregor S, Gordon-Smith K, Heron J, Hyde S, Caesar S, Nikolov I, Williams N, Jones L, et al. 2005. Bipolar disorder and polymorphisms in the dysbindin gene (DTNBP1). Biol Psychiatry 57(7):696–701.

Schwab SG, Knapp M, Mondabon S, Hallmayer J, Borrmann-Hassenbach M, Albus M, Lerer B, Rietschel M, Trixler M, Maier W, et al. 2003. 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 72(1):185–190 [Epub 2002 December 9].

Straub RE, Jiang Y, MacLean CJ, Ma Y, Webb BT, Myakishev MV, Harris-Kerr C, Wormley B, Sadek H, Kadambi B, et al. 2002. Genetic variation in the 6p22.3 gene DTNBP1, the human ortholog of the mouse dysbindin gene, is associated with schizophrenia. Am J Hum Genet 71(2):337–348 [Epub 2002 July 3].

Tobler AR, Short S, Andersen MR, Paner TM, Briggs JC, Lambert SM, Wu PP, Wang Y, Spoonde AY, Koehler RT, et al. 2005. The SNPlex genotyping system: A flexible and scalable platform for SNP genotyping. J Biomol Tech 16(4):398–406.

van den Oord EJ, Sullivan PF, Jiang Y, Walsh D, O'Neill FA, Kendler KS, Riley BP. 2003. 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 8(5):499–510.

Williams NM, Preece A, Morris DW, Spurlock G, Bray NJ, Stephens M, Norton N, Williams H, Clement M, Dwyer S, et al. 2004. Identification in 2 independent samples of a novel schizophrenia risk haplotype of the dystrobrevin binding protein gene (DTNBP1). Arch Gen Psychiatry 61(4):336–344.

Wing JK, Babor T, Brugha T, Burke J, Cooper JE, Giel R, Jablenski A, Regier D, Sartorius N. 1990. SCAN. Schedules for clinical assessment in neuropsychiatry. Arch Gen Psychiatry 47(6):589–593.