DTNBP1 (Dystrobrevin Binding Protein 1) and Schizophrenia: Association Evidence in the 3' End of the Gene

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Key Words

Single nucleotide polymorphism · Haplotype · Linkage disequilibrium · Complex disorder · Dystrobrevin binding protein 1 · Schizophrenia · Association

Abstract

Objectives: *Dysbindin* (*DTNBP1*) has been identified as a susceptibility gene for schizophrenia (SZ) through a positional approach. However, a variety of single nucleotide polymorphisms (SNPs) and haplotypes, in different parts of the gene, have been reported to be associated in different samples, and a precise molecular mechanism of disease remains to be defined. We have performed an association study with two well-characterized family samples not previously investigated at the *DTNBP1* locus. **Methods:** We examined 646 subjects in 136 families with SZ, largely of European ancestry (EA), genotyping 26 SNPs in *DTNBP1*. **Results:** Three correlated markers (rs875462, rs760666, and rs7758659) at the 3' region of *DTNBP1* showed evidence for association to SZ ($p = 0.004$), observed in both the EA ($p = 0.031$) and the African American (AA) subset ($p = 0.045$) with the same over-transmitted allele. The most significant haplotype in our study was rs7758659-rs3213207 (global $p = 0.0015$), with rs3213207 being the most frequently reported associated

marker in previous studies. A non-conservative missense variant (Pro272Ser) in the 3' region of *DTNBP1* that may impair *DTNBP1* function was more common in SZ probands (8.2%) than in founders (5%) and in dbSNP (2.1%), but did not reach statistical significance. **Conclusion:** Our results provide evidence for an association of SZ with SNPs at the 3' end of *DTNBP1* in the samples studied.

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Introduction

A locus for SZ in chromosome 6p24-p22 has been supported by some [1–4] but not all linkage studies [5–7]. Straub et al. reported four SNPs from exons 1–5 of the 140 kb *DTNBP1* (at 6p22.3), and several 3-marker haplotypes ($p = 0.008$ – 0.0001), to be associated with SZ [8]. In vitro functional studies of *DTNBP1* have suggested that *DTNBP1* may influence exocytotic glutamate release presynaptically [9]. Patients with SZ were reported to have decreased *DTNBP1* in the glutamatergic terminal of the hippocampus and dorsolateral prefrontal cortex [10], and risk haplotypes for SZ were also shown to be associated with reduced *DTNBP1* expression in human cerebral cortex [11]. Convergent effects of several putative SZ suscep-

Table 1. Complex results from previous association studies on *DTNBP1*^a

Markers ^b	SNP	Distance to next marker (kb) ^c	Origin of the samples studied	Irish	UK/Irish	Scottish	German and Israeli	Polish	Bulgarian	Swedish	Australian	Canadian	US-EA	US-Hispanic	US-AA	African	Indian South	Chinese Han	Japanese	Korean
rs1474588	P1730	42.9	C/G	ns																Joo 2006: 194 cases vs. 351 controls
rs760659	P1283	18.5	G/A	ns																Tochigi 2006: 314 cases vs. 314 controls
rs1047631	P3230	1.4	A/G		ns															Numakawa 2004: 670 cases vs. 588 controls
rs742106	P1328	23	C/T	ns				ns												Li 2005 ⁵ : 638 nuclear families
rs1040410		25.6	C/T		ns			ns												Tang 2003 ³ : 233 trios
rs742105	P1333	13.1	G/A	0.0099 (A)	ns						ns									Holliday 2006: 197 families
rs12524251	P3236	3	T/C		ns															Hall 2004: 233 families
rs760666	P1287	31.7	C/T	ns	ns			ns												Funke 2004: 215 cases vs. 74 controls
rs2619539	P1655	6.7	C/G	0.0059 (G)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	Funke 2004: 51 cases vs. 32 controls
rs16876738	P2381	0.2	G/C																	Gornick 2005 ^h : 102 families
rs12525702		0.3	G/A																	Wood 2006 ^g : 451 cases vs. 291 controls
rs3213207	P1635	1.1	A/G	9E-05 (G)	ns	ns	0.0052 (A)	ns	9E-04 (A)	ns	ns	ns	ns	ns	ns	ns	ns	0.02 (A)	0.001 (G)	Hall 2004: 210 trios
rs2619542		4.2	G/A	ns																Funke 2004: 258 cases vs. 467 controls
rs1011313	P1325	0.2	G/A	ns	ns	ns	0.0092 (G)	ns	ns	0.032 (A)	ns	ns	ns	ns	ns	ns	ns	ns	ns	De Luca 2005: 117 families (106 EA, 2 AA and 9Asian)
rs6924627	P3762	0.6	G/A																	Holliday 2006: 41 families and 194 cases vs. 180 controls
rs2619550		15.6	C/G	ns																Van Den Bogaert 2003: 142 cases vs. 272 controls
rs2619528	P1765	1	G/A	ns	0.013 (A)	ns	0.014 (G)	ns	ns		ns	0.017 (A)	0.017 (A)	0.017 (A)	ns	ns	ns	0.002 (G)	ns	Kirov 2004 ⁱ : 488 trios
rs2005976	P1757	0.3	G/A	ns	0.012 (A)	ns	ns	ns	0.0013 (G)	ns										Van Den Bogaert 2003: 294 cases vs. 113 controls
rs760761	P1320	2.5	C/T	0.0004 (T)	0.016 (T)	ns	7E-04 (C)	ns	ns	ns	ns	ns	ns	0.035 (T)	ns	ns	ns	0.027 (T)	ns	Fallin 2005: 274 Ashkenazi Jewish trios
rs2619522	P1763	3.4	T/G	ns	ns	ns	0.03 (T)	ns			ns	0.017 (G)	0.024 (G)	0.024 (G)	ns	ns	ns	0.022 (G)	ns	Van Den Bogaert 2003: 418 cases vs. 285 controls
rs1018381	P1578	1.1	C/T	ns	ns	ns	ns	ns	ns	ns	ns	0.003 (T)		ns	ns	ns	ns	ns	ns	Schwab 2003: 203 families
rs1474605	P1792	0.7	A/G		0.003 (G)						ns									Li 2005 ⁴ : 580 cases and 620 controls
rs1997679	P1795	2	C/T	ns							ns									Williams 2004: 708 cases vs. 711 controls

DTNBP1 Association with Schizophrenia

Hum Hered 2007;64:97–106

Hum Hered 2007;64:97-106 99

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Fig. 1. Alternative transcripts for *DTNBP1* and relative positions of the tested SNPs in this study. Exons are numbered in the order of the typical 10-exon transcript variant (NM_032122). 1a, 6a, b, 7a–c indicate cryptic exons. 1-ex, 3-ex, 6-ex, 8-ex, 9-ex, and 10-ex represent exons with variable boundaries. All the information for 15 transcripts is taken from AceView, an integrated view of hu-

man genes reconstructed by co-alignment of all publicly available mRNAs and expressed sequence tags (ESTs) on the genome sequence, updated on November 29, 2004 (www.ncbi.nlm.nih.gov/IEB/Research/Acembly/). Positively associated SNPs are indicated by an asterisk.

Table 2. FBAT results for *DTNBP1* coding variants

SNP ^a	Genomic position ^b	Exon ^c	Nucleotide changes ^d	Amino acid changes	Source ^e		MAF ^f		FBAT p value
					dbSNP	Li et al., 2003	dbSNP	founders	
rs9476887	15,770,870	1-ex	C/T	Pro/Leu	+		N/A	NT	N/A
N/A	A162G	4	G/A	Arg/Arg		+	N/A	NT	N/A
N/A	C307T	5	C/T	Gln/Stop		+	N/A	NT	N/A
rs6926401	15,693,988	7a	C/A	Pro/His	+		N/A	0.06	0.90
rs16876589	15,641,476	8	G/A	Gly/Asp	+		0.005	0.00	N/A
rs16876573	15,632,677	9-ex	A/G	Pro/Pro	+		0.062	0.04	0.23
rs16876571	15,632,658	9-ex	C/T	His/Tyr	+		0.028	0.03	0.09
rs16876569	15,632,640	9-ex	G/A	Ala/Thr	+		0.006	0.004	1.00
rs17470454	15,631,427	10	C/T	Pro/Ser	+	+	0.021	0.05	0.53
N/A	A874G	10	A/G	Arg/Gly		+	N/A	0.02	0.88

^a rs number is listed first, and any other names follow from Li et al. [51].

^b Position on UCSC Human Genome draft May 2004 freeze.

^c Exon number named in the order of typical 10-exon transcript shown in figure 1 (NM_032122); -ex means extended exon; 7a is a cryptic exon.

^d Listed as major allele/minor allele on the minus strand.

^e Source of SNP is dbSNP and/or Li et al. [51] as indicated by a plus sign.

^f MAF = Minor allele frequency. Allele frequency is taken from dbSNP or from the founders of our family sample.

N/A = Not available; NT = not tested because of failure of the genotyping assay design.

Materials and Methods

Subjects and Phenotyping

Our total sample is comprised of 136 families ascertained from all over the US of EA (72%) and AA (18%) descent (online suppl. table 1, www.karger.com/doi10.1159/000101961). This sample was approximately equally derived from two collections, the NIMH-IRP (Intramural Research Program, also known as the Clinical Neurogenetics or CNG sample) [34, 35] and the NIMH-GI (Genetics Initiative – Part I) [36]. This combined set

contained 319 genotyped individuals diagnosed as SZ (n = 273) or schizoaffective disorder (n = 46) via the criteria of the Diagnostic and Statistical Manual of Mental Disorders, third edition, revised (DSM-III-R) [37]. Besides the affected probands (n = 136), most of the additional genotyped individuals were siblings (n = 258), parents (n = 207), or other relatives (n = 45) of the probands; genotyping these additional individuals, 183 of whom were themselves affected, made the sample more informative for the family-based association testing. Our group and others have used these families in previous linkage studies, although no suggestive or significant

linkage evidence was found for 6p [34, 38, 39]. There is no overlap with any other US sample used in previous association studies with *DTNBPI* and SZ. The Institutional Review Board of the Evanston Northwestern Healthcare Research Institute approved the study.

As estimated by TDT-PC (transmission disequilibrium test – power calculator) software [40], our sample has about 87% power to detect a locus with a relative risk (RR) of 2 at a significance level of 5%; but power drops to 44% for an RR of 1.4 [41].

SNP Selection and Genotyping

A total of 26 SNPs in *DTNBPI* were genotyped by either TaqMan or SNPlex. Out of 10 previously reported as associated SNPs (table 1), 9 SNPs were successfully genotyped (rs742105, rs2619539, rs3213207, rs1011313, rs2619528, rs2619522, rs760761, rs1018381, and rs2619538); rs2005976 was not selected since it is tagged by the genotyped rs2619522 with $r^2 = 0.94$ [8]. To increase map coverage, we typed 3 previously known (but not associated) SNPs (rs742106, rs760666, and rs909706), and 7 other previously untested SNPs (rs742102, rs875462, rs10949305, rs2743553, rs7758659, rs2743865, and rs742208). We also genotyped 7 out of 10 coding SNPs (3 failed assay designs; SNP-flanking DNA sequences were drawn from the UCSC Genome draft, genome.ucsc.edu; May 2004 freeze) (table 2). Thirteen SNPs (rs17470454, rs1094305, rs742105, rs7758659, rs1018381, rs16876589, rs6926401, rs16876573, rs16876571, rs16876569, A874G, rs760761, and rs2619538) were genotyped with TaqMan on an Applied Biosystems (ABI) Prism 7900 instrument. Thirteen other SNPs (rs742102, rs742106, rs875462, rs2743553, rs760666, rs2619539, rs2743865, rs3213207, rs1011313, rs2619528, rs2619522, rs909706, and rs742208) were genotyped with SNPlex following standard procedures.

Genotyping Cleaning

Genotypes were checked for Mendelian inconsistencies and unlikely recombinants via MERLIN [42]. All Mendelian inconsistencies were removed by zeroing out the genotypes for the involved individuals in that family for that SNP. For the genotypes that were flagged as ‘unlikely’ recombinants ($p < 0.01$), we manually checked the raw genotyping traces to search for questionable genotypes (e.g., obviously external to the genotype clusters, very low signal intensity, etc.) and questionable genotypes were zeroed. The average genotyping completion rate was 98.4% (95.7–99.4%). The total genotyping error rate was 0.54%, including 15 Mendelian errors and 55 unlikely recombinations out of 12,911 non-zero genotypes. Detected error rates for individual SNPs ranged from 0.16 to 1.30%. All the genotyping errors were blanked. Given our high genotyping completion rate and low genotyping error rates, we did not attempt second-pass genotyping for these zeroed errors. All the markers were in Hardy-Weinberg equilibrium.

Inter-Marker LD Analysis

LD between SNPs was estimated with Haploview 3.0 [43] using the genotypes from unrelated founders. The standard D' , LOD, and r^2 were derived in the EA and AA subsets separately. HapMap genotype data were downloaded from HapMap (www.hapmap.org).

Association Analysis

For association analyses, we used FBAT v1.5.5 [44, 45]. Alleles and haplotypes were tested for association if there were at least 5

informative families; in our data this corresponds to testing alleles and haplotypes with frequencies over 3%. We limited the number of multi-locus systems tested by using a stepwise procedure, and limited the number of multi-locus tests to the combinations including the SNP with highest single Z score value.

Results

Initially, 20 SNPs in –140 kb of the genomic sequence of *DTNBPI* were analyzed (all the missense SNPs, except for rs17470454, were analyzed separately because of their low minor allele frequency, MAF). Consistent with previous studies [8, 13, 17], our LD analyses in EA founders showed only one long LD block (defined by $D' > 0.80$ and $\text{LOD} > 2$, about 122 kb from rs875462 to rs909706) spanning almost the whole *DTNBPI* gene. The r^2 estimates were variable (fig. 2); grouping SNPs with $r^2 > 0.8$ [46] yielded 7 bins (A–G) [47] of high LD, where any SNP can be a proxy ($r^2 > 0.8$) of all remaining SNPs within the bin (note that one SNP that is not tagged by other SNPs constitutes a one SNP bin). The bins often overlapped (fig. 2b). Bin B spans approximately 110 kb, encompassing rs1094305, rs2743553, rs2743865, and rs1018381. In total, 12 out of the 20 analyzed SNPs were tag SNPs.

The LD patterns and allele frequencies of the tested markers were similar in EA founders and in AA founders, although LD was weaker in AAs (data not shown). We have performed association analyses in the EA and AA subsets separately, in addition to a combined analysis. Single-locus FBAT results for the 12 tag SNPs ($r^2 < 0.8$) are summarized in table 3. We found evidence for association with allele G of rs7758659 on the 3' end of *DTNBPI* in both the EA subset ($p = 0.031$) and the smaller AA subset ($p = 0.045$). Since the over-transmitted alleles for all the tested SNPs were the same in EA and AA, we also performed association tests in the combined sample. Only rs7758659 remained associated after Bonferroni correction ($p = 0.004$; $p = 0.048$ when corrected by 12, the number of tag SNPs). Because rs7758659 tags two other SNPs, rs875462 and rs760666 (bin A with $r^2 > 0.94$; fig. 2b), the association is also present (and with similar significance) with each of these SNPs (online suppl. table 2).

We performed haplotypic analyses using the 12 tag SNPs, starting by analyzing all two-marker systems anchored with rs7758659 (table 4). In the EA subset, 3 out of the 11 two-marker combinations gave global p values < 0.05 . The most significant two-marker system was rs7758659-rs2619522 (global $p = 0.016$). In the AA sub-

Fig. 2. LD structure of *DTNBP1* in EA founders. **a** Pair wise LD structure is shown as matrix according to D' and LOD at the bottom. Genomic positions are according to the NCBI Build 35 of the human genome assembly (genome.ucsc.edu; May 2004 freeze).

We generated the figure by the LocusView program (www.broad.mit.edu/mpg/locusview/). **b** r^2 among different markers. Within the long LD block (D'), seven LD bins measured by r^2 are indicated with A to G.

Table 3. FBAT results for 12 *DTNBP1* tag SNPs^a

Markers ^b	Distance ^c	SNP	Allele	All families			EA Subset			AA Subset		
				Freq.	Z score	p value	Freq.	Z score	p value	Freq.	Z score	p value
rs742102	6,815	C/T	T	0.04	1.07	0.28	0.03	1.01	0.31	0.03	N/A	N/A
rs17470454	1,032	C/T	C	0.95	0.67	0.5	0.93	0.63	0.53	0.98	N/A	N/A
rs742106	P1328 68,760	C/T	T	0.31	0.26	0.79	0.33	0.64	0.52	0.20	-0.27	0.79
rs7758659	27,615	G/A	G	0.81	2.84	0.004	0.77	2.16	0.03	0.92	2.00	0.045
rs2619539	P1655 5,448	C/G	G	0.4	0.55	0.58	0.44	0.43	0.67	0.45	0.343	0.73
rs2743865	1,799	C/T	T	0.17	0.87	0.38	0.1	0.47	0.64	0.10	0.47	0.64
rs3213207	P1635 5,330	A/G	A	0.9	0.55	0.58	0.87	0.64	0.52	0.98	N/A	N/A
rs1011313	P1325 20,217	G/A	A	0.09	0.14	0.89	0.1	-0.31	0.76	0.02	N/A	N/A
rs2619522	P1763 7,222	T/G	T	0.72	0.22	0.83	0.77	0.82	0.41	0.51	-0.32	0.75
rs909706	P1583 4,338	A/G	G	0.31	1.33	0.18	0.32	1.43	0.15	0.20	-0.62	0.53
rs2619538	SNP A 3,452	A/T	T	0.43	0.58	0.56	0.42	1.09	0.27	0.37	-0.61	0.54
rs742208	N/A	T/C	C	0.17	1.00	0.32	0.11	-0.12	0.90	0.43	1.35	0.18

^a Results are shown in the whole sample, and EA and AA subsets. N/A = Not available due to the small number of AA families; SNP = nucleotide changes listed as major allele/minor allele. Allele nucleotides were converted to a unified format by ensuring they were from the minus strand. Nominal p value and associated allele are shown. Freq. = Frequency of the more often transmitted allele. Significant SNP (rs7758659) row is bold.

^b rs numbers are in the first column, and alias if any in the second column. Markers are in the order from the 3' to the 5' flanking regions.

^c Distance to next marker in base pairs (bp). The position for the first marker is nucleotide 15,624,612 in the UCSC May 2004 freeze of chromosome 6.

Table 4. FBAT results for two-marker haplotypes anchoring with rs7758659 in *DTNBPI*^a

Markers	Haplotype	All families				EA subset			
		frequency	Z score	p value	global p	frequency	Z score	p value	global p
rs742102-rs7758659	C-G	0.78	2.33	0.020	0.029	0.76	1.82	0.068	0.15
rs17470454-rs7758659	C-G	0.75	3.13	0.0018	0.0081	0.71	2.50	0.012	0.047
rs742106-rs7758659	C-G	0.51	1.86	0.063	0.017	0.46	0.97	0.33	0.12
rs7758659-rs2619539	G-C	0.37	1.80	0.071	0.009	0.30	1.52	0.13	0.067
rs7758659-rs2743865	G-C	0.65	1.98	0.047	0.013	0.69	1.74	0.082	0.11
rs7758659-rs3213207	G-A	0.69	3.28	0.0010	0.0015	0.64	2.49	0.013	0.031
rs7758659-rs1011313	G-G	0.72	2.27	0.023	0.017	0.68	2.15	0.032	0.066
rs7758659-rs2619522	G-T	0.53	2.59	0.010	0.0073	0.54	2.72	0.0065	0.016
rs7758659-rs909706	G-G	0.30	1.52	0.13	0.018	0.32	1.46	0.14	0.12
rs7758659-rs2619538	G-T	0.38	1.19	0.23	0.067	0.38	1.29	0.20	0.24
rs7758659-rs742208	G-T	0.64	1.58	0.11	0.073	0.68	1.49	0.14	0.36

^a Only haplotypes anchoring with rs7758659 were tested, and only the most over-transmitted haplotypes are listed. All the significant haplotypes contain allele G of rs7758659, consistent with the result of single marker analyses.

set, only one two-marker system, rs7758659-rs3213207, showed nominal significance (global $p = 0.02$; the corresponding global p value in the EA families was 0.03). In the combined dataset, 9 of the 11 two-marker combinations gave global p values <0.05 . The most significant two-marker haplotype was found with rs7758659-rs3213207 (global $p = 0.0015$; the only two-marker combination with higher significance than rs7758659, $p = 0.004$, by itself). rs3213207 (also known as P1635 [1]) is the most frequently reported associated SNP, albeit with the associated alleles being different across those studies (table 1). Haplotypic analyses with a three-marker system anchored to rs7758659-rs3213207 did not increase significance (data not shown). We have also extended haplotypic analysis to 7 markers from rs875462 to rs909706 (tag SNPs: rs7758659, rs2619539, rs2743865, rs3213207, rs1011313, rs2619522, and rs909706; each representing one bin, A-G). Ninety-eight percent of the haplotypes were represented by only 6 common (frequency $>5\%$) haplotypes. None of those 6 common haplotypes yielded a global p value or haplotypic p value <0.05 , with haplotype G-C-C-A-A-T-A (frequency = 20%) having the lowest p value of 0.08.

Finally, we tested association with the coding SNPs in *DTNBPI* (not included in haplotype analysis due to low MAF). None of the 7 genotyped coding SNPs showed association with SZ (p values >0.09 ; table 2) or co-segregated with disease. We also searched for aggregations of missense SNPs within families, but found no evidence of this. However, it is worth noting that the MAF of

rs17470454 (C814T; Pro272Ser) was higher in founders in our samples (5%) than that in dbSNP (2%); interestingly, the MAF in EA probands was found to be even higher (8.2%) than in founders, though no transmission distortion was found ($p = 0.53$; table 2).

Discussion

We have studied 26 SNPs in *DTNBPI*, including 6 missense variants. Our results support an association of SZ with three highly correlated markers in the 3' region of *DTNBPI* (rs875462, rs760666, and rs7758659). The association was observed in both EA and AA subsets with the same over-transmitted allele (G) at rs7758659. rs875462 and rs7758659 were previously untested, while rs760666 (or P1287 [8]) had been previously tested, but no evidence for association was reported (table 1). Interestingly, rs7758659 is near the boundary of exon 7 and intron 7 (being 49 bp away from exon 7), suggesting the possibility that it might affect splicing.

Association Evidence for SNPs Located at the 3' Region of the *DTNBPI* Gene

The four initially reported associated markers [8] and an associated haplotype that spans a promoter SNP, rs2619538 (SNP A) [19], are all located in the 5' region of the *DTNBPI*. All other replication studies limited their tests to regions in the 5' portion of the gene, as it has been speculated that the potential causative polymorphism(s)

lie in the 5' region of *DTNBP1*. With a more comprehensive set of markers than in previous studies, we did not observe evidence of association with SNPs located in the 5' region of the gene; instead, we found evidence for association of SZ with SNPs located in the 3' region of *DTNBP1*. However, it may not be possible to define the location of a true disease variant in a long region with strong LD (as in *DTNBP1*) [48]. One could argue that rs875462, rs760666, and rs7758659 that were found associated to SZ in our study are, in fact, in LD with some other untested variants in the 5' region of the gene. On the other hand, it is also conceivable that associated markers located in the 5' region of the gene may actually reflect associations with causative loci in the 3' region. Indeed, the LD pattern of *DTNBP1* allows for this possibility (fig. 2b). For instance, rs1018381 in the 5' region of the gene has been reported to be associated with SZ [15, 20], but this SNP is highly correlated ($r^2 > 0.90$) with three other SNPs in bin B (fig. 2b), two of which (rs1094305 and rs2743553) are in the 3' region of *DTNBP1*.

Missense Variants in DTNBP1 May Still Confer Susceptibility to SZ

We have tested 7 *DTNBP1* coding variants, but none of them showed association with SZ (table 2). It is nonetheless worth noting that some of these missense SNPs are in LD with some previously reported associated SNPs: rs3213207 (in bin D) was found in our data moderately correlated ($r^2 = 0.52$) to rs17470454 (C814T; Pro272Ser; not tested in any of the previous studies). The MAF of rs17470454 was higher in SZ probands than in founders and dbSNP, albeit not reaching statistical significance. The substitution of a proline for a serine at rs17470454 is predicted by PolyPhen (tux.embl-heidelberg.de/ramensky/index.shtml) to be a non-conservative change with potential for impairing *DTNBP1* function [49]. Therefore, rs17470454 might (together with other still unrecognized

functional SNPs) underlie the previously observed association between *DTNBP1* and SZ [8, 9, 13, 17, 18, 23].

In conclusion, our results provide evidence for an association of SNPs at the 3' end of *DTNBP1* and SZ in both EA and AA samples, but not at the 5' region of *DTNBP1*. However, our sample has limited statistical power and some true loci might not have been detected. Furthermore, in the absence of a molecular hypothesis that can provide a clear mechanistic explanation, association tests should be considered 'indirect', and this severely constrains the statistical power. Though we genotyped a denser marker map than other studies (table 1), the SNP set we have used only captures 76% of all the common SNPs of *DTNBP1* (MAF > 0.05) currently available in HapMap Phase II (release #20) at $r^2 > 0.8$ (data not shown). A total of 42 SNPs (based on all the known variants in *DTNBP1*) would be needed to capture all alleles at *DTNBP1* locus [31]. A critical experiment would require a large sample with sufficient statistical power and genotyping of a comprehensive and sufficiently dense SNP set.

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Supplementary information is available at *Human Heredity's* website.

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