

Genetic Variation in the 6p22.3 Gene *DTNBP1*, the Human Ortholog of the Mouse Dysbindin Gene, Is Associated with Schizophrenia

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Prior evidence has supported the existence of multiple susceptibility genes for schizophrenia. Multipoint linkage analysis of the 270 Irish high-density pedigrees that we have studied, as well as results from several other samples, suggest that at least one such gene is located in region 6p24-21. In the present study, family-based association analysis of 36 simple sequence-length-polymorphism markers and of 17 SNP markers implicated two regions, separated by ~7 Mb. The first region, and the focus of this report, is 6p22.3. In this region, single-nucleotide polymorphisms within the 140-kb gene *DTNBP1* (dystrobrevin-binding protein 1, or dysbindin) are strongly associated with schizophrenia. Uncorrected, empirical *P* values produced by the program TRANSMIT were significant ($P < .01$) for a number of individual SNP markers, and most remained significant when the data were restricted to include only one affected offspring per nuclear family per extended pedigree; multiple three-marker haplotypes were highly significant ($P = .008-.0001$) under the restricted conditions. The pattern of linkage disequilibrium is consistent with the presence of more than one susceptibility allele, but this important issue is unresolved. The number of markers tested in the adjacent genes, all of which are negative, is not sufficient to rule out the possibility that the dysbindin gene is not the actual susceptibility gene, but this possibility appears to be very unlikely. We conclude that further investigation of dysbindin is warranted.

Introduction

Although genetic factors are known to be important in the etiology of schizophrenia (Gottesman and Shields 1982; Kendler 2000), unambiguous identification of the important susceptibility genes for this common and debilitating condition has not yet been accomplished. Various causes for this barrier to progress, both established and speculated, have been amply discussed, and, in recognition of the magnitude of the problem, improvements on various fronts have been initiated; these include (a) dramatic expansion of sample sizes by inclusion of triad and case-control studies and (b) a renewed emphasis on additional biologically based intermediate phenotypes.

As has been detailed in dozens of extensive reviews (Karayiorgou and Gogos 1997; Tsuang et al. 1999; Riley and McGuffin 2000; Baron 2001; Bray and Owen 2001), after several well-publicized false leads, many

plausible candidate regions for schizophrenia are now under intense scrutiny; these include regions 1q21-22, 1q32-42, 5q21-34, 6p24-21, 6q13-26, 8p22-21, 10p15-11, 13q14-32, 15q13-15, and 22q11-13. After the initial reports (Straub et al. 1995; Wang et al. 1995), chromosome 6p (locus SCZD3 [MIM 600511]) has had support from a number of follow-up studies, with markers in a variety of locations showing at least some degree of positive evidence. It has been difficult, though, to distinguish true positives from false positives, because of the large number of markers tested under many genetic and phenotypic models using many samples, most of which were only of moderate size, that were ascertained under different conditions. In addition, it is still not clear to what extent (if any) a positive marker or multipoint peak >20 cM from the original should be interpreted as a “replication.” Statistical significance alone is clearly neither a sufficient nor a particularly well-chosen metric (Morton 1998), since (1) very significant results (e.g., a heterogeneity LOD score [H-LOD] >4) are not necessarily true and (2) supposedly weaker results (e.g., H-LOD 1.5–3) have led to gene identification and so must not be prematurely discounted. Independent replication attempts, although theoretically a powerful tool, are actually only rarely

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performed, because of numerous inherent and fundamental differences between studies (Straub et al. 1996; Vieland 2001).

On 6p, the markers positive for schizophrenia are distributed widely over the 25 Mb between D6S296 and D6S291. They do appear, however, to be concentrated within four subregions: 6p25-24, near D6S296/D6S309 (Antonarakis et al. 1995; Maziade et al. 1997, 2001; Hovatta et al. 1998; Lindholm et al. 1999; Bailer et al. 2000; Hwu et al. 2000); 6p24, near D6S940/D6S470 (Schizophrenia Linkage Collaborative Group for Chromosomes 3, 6 and 8 1996); 6p23-22, near D6S260 (Turcecki et al. 1997; Hovatta et al. 1998; Hwu et al. 2000); and 6p21, both proximal to (Arolt et al. 1996) and within (Wright et al. 2001) the locus for human leukocyte antigen. The sample studied by Schwab et al. (1995) shows positive markers and multiple multipoint peaks from 6p24 through 6p21 (Schwab et al. 2000), as does the Irish high-density sample that we have studied. In addition, there have been association studies of *SCA1* (on 6p22) (Wang et al. 1996; Pujana et al. 1997; Joo et al. 1999) and of *NOTCH4* (Wei and Hemmings 2000; Imai et al. 2001; McGinnis et al. 2001; Sklar et al. 2001; Ujike et al. 2001).

In two previous reports from the Irish Study of High Density Schizophrenia Families (ISHDSF), we have presented evidence in support of the linkage of region 6p24-21 to schizophrenia and schizophrenia-related disorders (Straub et al. 1995, 2002). In the present study, we describe the results of family-based association analysis of simple sequence-length polymorphism (SSLP) markers, additional genotyping of SNPs in 6p22, and analysis of SNP haplotypes. We find that genetic variation in the gene for *DTNBP1*, which is the human ortholog of mouse dysbindin, is associated with schizophrenia and related phenotypes.

Subjects and Methods

Pedigree Ascertainment and Diagnostic Assessment

A detailed description of the ISHDSF has been published elsewhere (Kendler et al. 1996). The sample used here contains 270 families and 1,425 genotyped individuals (Straub et al. 2002). The main diagnostic instruments utilized in the fieldwork were the Structured Interview for DSM-III-R Diagnosis (Spitzer et al. 1992) and the Structured Interview for Schizotypy (Kendler et al. 1989). Diagnosis was based on DSM-III-R criteria and all available information (personal history, hospital record, and family-history report), by individuals blind to knowledge of genotypes and to psychopathology of relatives. This research was approved by the Ethical Review Boards of Virginia Commonwealth University, the Health Research Board (Dublin), and Queen's University (Belfast). The diagnostic definitions are as follows.

Narrow: categories D1–D2 (625 affected individuals, 565 of whom were genotyped).—These categories comprise the “core schizophrenic phenotypes,” which include schizophrenia, poor-outcome schizoaffective disorder, and simple schizophrenia (Kendler et al. 1994).

Intermediate: categories D1–D5 (804 affected individuals, 691 of whom were genotyped).—These categories comprise a restricted definition of the schizophrenia spectrum, which includes only disorders that repeatedly have been shown to coaggregate in families with narrowly defined schizophrenia (Kendler and Diehl 1993); to the “narrow” definition, the three additional categories (i.e., D3–D5) add schizotypal personality disorder and all other nonaffective psychotic disorders (i.e., schizophreniform disorder, delusional disorder, atypical psychosis, and good-outcome schizoaffective disorder).

Broad: categories D1–D8 (888 affected individuals, 744 of whom were genotyped).—These categories comprise a broad definition of the schizophrenia spectrum, which includes all disorders that aggregate significantly in relatives of schizophrenic probands in the Roscommon Family Study (Kendler et al. 1993a, 1993b, 1993c), an epidemiologic, case-controlled family study conducted, in parallel, in the west of Ireland; to the “intermediate” definition, the three additional categories (i.e., D6–D8) add mood-incongruent and mood-congruent psychotic affective illness, and paranoid, avoidant, and schizoid personality disorder.

Very broad: categories D1–D9 (1,172 affected individuals, 952 of whom were genotyped).—These categories comprise all psychiatric disorders; to the “broad” definition, the additional category (i.e., D9) adds all other psychiatric disorders (e.g., nonpsychotic affective disorders, anxiety disorders, alcoholism, and other non-schizophrenia-spectrum personality disorders).

SNP Identification

SNPs were identified by sequencing of amplicons from pools of 6–12 unrelated affected individuals from families showing a linkage signal in 6p22 but not in 6p24. We sequenced in both directions, using ABI 377 sequencers (Perkin Elmer Biosystems) and dye-terminator chemistry. Many of the novel SNPs that we discovered were assigned NCBI reference identification numbers (see the NCBI Single Nucleotide Polymorphism Web site) after being submitted to NCBI by other laboratories, and these “rs” numbers are shown in table 2. Information on SNP P1635 can be accessed via its NCBI “Assay ID” (i.e., “ss” number), which is 4473786.

SNP Genotyping

The fluorescence-polarization template-directed incorporation method (Chen and Kwok 1999; Chen et al. 1999), which is a single-base extension with allele-spe-

cific ddNTP termination, was used, with minor modifications (Sullivan et al. 2001).

Statistical Analysis

The GENEHUNTER multipoint methods and a graph of the results are contained in our genome-scan report (Straub et al. 2002). The Penetrance-Additive model is an intermediate, heterozygotic genetic model that is additive on the penetrance scale. For example, for the phenotypic model using categories D1–D2, we set AA to 0.55, Aa to 0.275, and aa to 0.0006, with a phenocopy proportion of 0.10 and a schizophrenia-allele frequency of 0.0098. The statistical methods used in the present study are as follows. SIMWALK2 (Weeks et al. 1995; Sobel and Lange 1996) was used for detection of errors in the data. Before data cleaning, the recombinant profiles produced by SIMWALK haplotyping showed 72 single recombinants, 104 double recombinants, and 4 quadruple recombinants. There were 26,337 genotypes that were checked, and SIMWALK flagged 324 recombinants that had a $>.25$ posterior probability of being due to genotyping error; for this group of 324 recombinants, the probability was $>.5$ for 196 and $>.75$ for 119. As a rule, only those recombinants for which the probability is $>.90$ are highly likely to be true errors, so, to be conservative, we eliminated the 119 recombinants (0.45% of all genotypes) having probabilities $>.75$. Version 2.5 (April 1999) of the program TRANSMIT (Clayton 1999; Clayton and Jones 1999; Dudbridge et al. 2000) was used for family-based transmission/disequilibrium test (TDT) analysis. In all TRANSMIT runs, the program performed $\geq 100,000$ replicates, and, from these, derived the P values empirically. For both the pairwise analysis of SSLPs and the haplotype analysis, to protect against misleading results due to rare alleles or haplotypes, the command-line switches (i.e., flags) “-agg3” and “-c3” were used to aggregate all alleles or haplotypes with frequencies $<.03$ before haplotype construction. For some tests, the switches “-1” and “-nomf” were used together, to simultaneously restrict the analysis to one affected offspring per nuclear family per extended pedigree, each chosen at random by the program. To assess the effect of the particular (random) choices that were made, we ran each test 20 times, and we present here the median P value. For the most significant results, we also performed an additional 50 runs, and the median was consistently the same as that for the initial 20 runs. We note that, given the linkage evidence, there is an increased a priori probability that small P values represent true positive results. Since the markers are tightly linked, the P values may be highly correlated, and thus a simple Bonferroni correction is unduly conservative, and we report only these empirical P values as determined by TRANSMIT. In our Irish data set, only 60 families have both parents of the largest sibship ge-

notyped, another 90 families have one parent genotyped, and the remaining 120 families have neither parent genotyped. Thus, a program such as TRANSMIT, which is “vertical” in that it attempts to reconstruct the missing parental information, is more powerful (see Cervino and Hill 2000) than either (a) programs such as SIBASSOC and STDT, which utilize only information within sibships, or (b) the traditional TDT, which relies heavily on the usually scarce heterozygous parents. The standardized (i.e., Lewontin [1988]) measure of linkage disequilibrium (LD), denoted as “ D' ,” and the measure d^2 were both calculated from the TRANSMIT haplotype frequencies for alleles at all possible pairs of SNP loci. MEGA2 (Mukhopadhyay et al. 1999), version 2.2, was used to calculate allele and genotype frequencies and to test for departure from Hardy-Weinberg equilibrium, in affected individuals, in unaffected individuals, and in the two groups combined.

Results

The results from GENEHUNTER multipoint analysis (see Straub et al. 2002) of data from 36 SSLP markers are shown in table 1. In both the H-LOD (shown) and nonparametric linkage (NPL) (not shown) results, there are two predominant, sharp peaks, one at ~ 8 cM (in 6p24.3, near D6S940) and another at ~ 17 cM (in 6p22, near D6S260). A third peak, smaller than these two, is present near D6S422 (in 6p22, distal to HLA). For the two larger peaks, the H-LODs are greatest when the broad-definition diagnostic categories (i.e., D1–D8) are used. Whether these peaks indicate the presence of multiple susceptibility loci or are statistical artifacts could not be determined on the basis of the linkage data alone. In an attempt to narrow the target region(s), family-based TDT analysis of the 36 SSLPs was performed, by TRANSMIT (Clayton 1999; Clayton and Jones 1999; Dudbridge et al. 2000). In the first analysis (denoted “All”), data from all individuals in all families were used. The second analysis, designed to mimic aspects of the traditional TDT test, used data from only one affected offspring per nuclear family per extended pedigree. We did not run simple TDT or haplotype relative-risk analysis, since, for family structures such as those in the ISHDSF, each has the considerable disadvantage of discarding valuable information (Cervino and Hill 2000). For all tests, the program generates empirical P values by simulation. Under the first analysis (i.e., All), of the 36 SSLPs, only the 6p24.3 marker D6S940 showed a global P value $<.01$. The P values were .033, .023, .016, and .008, for diagnostic categories D1–D2, D1–D5, D1–D8, and D1–D9, respectively. The adjacent SSLPs, GATA23E10 (892 kb distal) and D6S470 (182 kb proximal), were both negative. For D6S940, when the analysis was performed on the restricted data set without aggregation of rare alleles, the P values were .024, .114,

Table 1

GENEHUNTER Multipoint Linkage Results

REGION	MARKER NAME	LOCUS	LOCATION		MULTIPOINT H-LOD			
			cM	Mb ^a	D1–D2	D1–D5	D1–D8	D1–D9
	GATA3H05	D6S477	0	7.19	.10	.55	1.12	.05
	F13A1	F13A1	1.8	7.20	.15	.79	1.40	.14
	D6S309	D6S309	4.9	9.28	.26	1.02	1.40	.28
	D6S277	D6S277	6	9.56	.22	.99	1.44	.31
	D6S263	D6S263	6.01	9.60	.33	1.20	1.50	.34
	MS236	D6S226	6.02	9.64	.34	1.05	1.33	.29
	D6S296F	D6S296	6.03	9.91	.33	1.05	1.33	.28
	D6S410	D6S410	6.33	10.05	.27	1.08	1.39	.34
6p24.3	GATA23E10		7.53	10.54	.46	1.50	1.90	.45
6p24.3	UT6077F	D6S940	7.73	11.44	.43	1.29	1.69	.42
6p24.3	D6S470L	D6S470	8.03	11.62	.82	1.75	2.15	.46
6p24.3	AFMc031yd9		8.04	11.68	.46	1.36	1.75	.44
	ATA33A11	D6S1263	12.34	14.56	.23	.64	1.08	.31
	ATA50C05	D6S2434	14.04	15.83	.33	.54	1.02	.27
	D6S443	D6S443	14.34	15.87	.33	.59	1.00	.29
	B337WG9L	D6S1653	14.54	16.33	.33	.56	.95	.31
	D6S259	D6S259	15.74	16.62	.49	1.16	1.60	.79
	a244wf5	D6S1578	16.04	16.90	.56	1.33	1.78	.79
6p22.3	D6S260F	D6S260	16.64	17.85	.67	1.60	2.22	.90
6p22.3	AFM321tc5	D6S1676	17.34	18.53	.64	1.55	2.08	.91
6p22.3	AFM189ye3		17.35	18.53	.64	1.56	2.08	.91
6p22.3	AFMb008wb5	D6S1605	18.05	18.76	.59	1.81	2.20	1.07
	SCA1 (CAG)	SCA1	18.25	18.77	.32	1.45	1.68	.98
	SCA78a		18.35	18.78	.26	1.33	1.56	.90
	D6S288	D6S288	18.36	18.84	.30	1.42	1.66	.96
	a288zg9	D6S1584	19.16	19.16	.05	1.05	1.02	.43
	TW9		19.17	19.18	.05	1.04	1.01	.38
	D6S274L	D6S274	19.18	19.20	.04	1.00	.99	.36
	a219zd9	D6S1567	20.28	19.94	.00	.41	.56	.07
	D6S285	D6S285	20.78	21.43	-.01	1.01	1.15	.20
	D6S422L	D6S422	21.98	23.23	.00	1.12	1.55	.38
	D6S299	D6S299	28.08	26.78	-.01	.24	.68	.59
	D6S276	D6S276	29.18	28.26	-.01	.16	.57	.51
	MFD61	D6S105	29.28	31.42	.01	.21	.65	.48
	D6S273	D6S273	32.58	35.32	.07	.21	.78	.56
	D6S291	D6S291	34.08	43.02	.04	.13	.52	.32

NOTE.—The two primary peaks are boxed.

^a Chromosomal location in UCSC August 2001 draft assembly of the genome (see the UCSC Human Genome Project Working Draft Web site).

.08, and .08, respectively. With both the restricted data set and aggregation of alleles having frequency $\leq 3\%$, all four tests were negative. This LD finding in region 6p24.3 was not pursued further in the present study.

On the basis of these previously published linkage results, we investigated region 6p22 in further detail, by sequencing to find SNPs, genotyping, and analysis using both case-control and family-based TDT designs. We first concentrated on the 250-kb interval between D6S1676 and the CAG repeat, in gene *SCA1*. We tested a total of 34 SNPs in subsets of individuals ($n = 47$ –300), in an “internal case-control” design. The most highly selected group of cases (one per family) consisted of affected individuals in categories D1–D2 who were

selected from those families that, during those separate GENEHUNTER runs (results not shown), yielded positive H-LOD/NPL scores with the 16 SSLP markers in 6p23–22 (D6S1653–D6S422) but did *not* do so with the adjacent set of 15 SSLP markers in 6p25–22 (D6S477–D6S443). The control group comprised unaffected individuals who were unrelated (i.e., married-in) to the affected individuals in their families and were chosen from families that produced negative results across the entire region. For these 34 SNPs, no sizable or regionally consistent differences in allele frequencies were observed (data not shown).

On the basis of these negative results, we then focused on the 670-kb interval between D6S260 and D6S1676,

Table 2
Markers and Map of 6p22

Gene (Exon Size)	Marker (NCBI Number ^a)	Polymorphism	Frequency ^b	Chromosomal Location ^c (Nucleotide Position)	Distance from D6S260 (bp)	Size of Interval (bp)
<i>Jumonji</i>	D6S260	SSLP	NA	17854849–17855203
	P1730 (rs1474588)	G/C	.330	17912083	56,880	56,880
	P1283 (rs760659)	G/A	.196	17954963	99,760	42,880
Dysbindin gene: ^d						
Exon 10 (413 bp)				17973412–17973824		
Exon 9 (144 bp)				17975131–17975274		
Exon 8 (156 bp)	P1328 (rs742106)	C/T	.376	17974854	119,651	19,891
				17983845–17984000		
Exon 7 (23 bp)	P1333 (rs742105)	C/T	.480	18023448	168,245	48,594
	P1287 (rs760666)	C/T	.246	18039495	184,292	16,047
Exon 6 (133 bp)				18043664–18043686		
Exon 5 (133 bp)	P1655 (rs2619539)	G/C	.537	18065872–18066004	216,026	31,734
				18077948–18078080		
Exon 4 (61 bp)	P1635	G/A	.102	18078476	223,273	7,247
	P1325 (rs1011313)	T/C	.088	18083806	228,603	5,330
Exon 3 (51 bp)				18088349–18088409		
	P1765 (rs2619528)	A/G	.167	18100203	245,000	16,397
	P1757 (rs2005976)	A/G	.167	18101176	245,973	973
Exon 2 (54 bp)	P1320 (rs760761)	C/T	.178	18101506	246,303	330
Exon 1 (160 bp)				18101918–18101968		
				18102692–18102745		
	P1763 (rs2619522)	C/A	.155	18104023	248,820	2,517
	P1578 (rs1018381)	C/T	.076	18107444	252,241	3,421
Unknown	P1583 (rs909706)	C/T	.377	18111245	256,042	3,801
				18113419–18113578		
	P1586 (rs885773)	A/G	.093	18119860	264,657	8,615
	P1294 (rs441539)	A/G	.380	18215012	359,809	95,152
	P1140 (rs1000117)	C/A	.301	18324363	469,160	109,351
	D6S1676	SSLP	NA	18525332–18525609	670,406	201,246
	AFM189YE3	SSLP	NA	18534500–18534767	679,564	9,158

^a Reference identification number for each SNP, from the dbSNP database (see the NCBI Single Nucleotide Polymorphism Web site).

^b Of the second allele shown. NA = not applicable.

^c From the UCSC August 2001 draft assembly of the genome (see the UCSC Human Genome Project Working Draft Web site).

^d Exons and their genomic locations are based on transcript BC011912.

the two SSLP markers located atop the 6p22 multipoint-linkage peak. We identified SNPs by database searches and by sequencing of pools of the highly selected cases.

Table 2 shows the map locations and marker characteristics for the 3 SSLPs and 17 SNPs in 6p22 that were tested in the entire sample. The markers are presented in chromosomal order, with D6S260 telomeric. The first three markers in the table are in the gene *Jumonji*, the next 12 SNPs are in the gene *DTNBP1* (i.e., the dysbindin gene), and the next 5 markers are proximal to the dysbindin gene. Markers P1655, P1635, and P1765, as well many others that we identified but did not test in the entire sample, were first detected by sequencing of pools of affected individuals, as described above. All SNPs tested in dysbindin are intronic, except P1328, which is in the 3' UTR of transcript AL136637. Analysis using multiple splice-site prediction programs (Mount 2000)

showed that none of the SNPs are predicted to affect splicing, but this must be tested experimentally.

The pairwise TRANSMIT results are shown in table 3. When all individuals are included in the analysis, the pattern of significance varies somewhat across diagnostic categories, with maximal evidence for a given marker usually occurring when either categories D1–D5 or categories D1–D8 are used. One clear exception to this pattern, however, is SNP P1320. Except for the most centromeric marker, the SSLP AFM189YE3, the strongest evidence was never found for the very-broad definition (i.e., categories D1–D9), which includes psychiatric disorders not considered to be within the schizophrenia spectrum. When the analysis was restricted to one affected offspring per nuclear family per extended pedigree, the median *P* value of 20 runs was not significant for markers P1333, P1655, P1325, markers that reached

Table 3**Pairwise TRANSMIT Results**

GENE AND MARKER (NCBI NUMBER)	OVERTRANSMITTED ALLELE (FREQUENCY)	TRANSMIT <i>P</i> VALUE ^a							
		All Individuals				One Affected Offspring per Nuclear Family per Extended Pedigree ^b			
		D1–D2	D1–D5	D1–D8	D1–D9	D1–D2	D1–D5	D1–D8	D1–D9
<i>Jumonji:</i>									
D6S260		.193	.096	.018	.077	.579	.333	.341	.355
P1730 (rs1474588)		.403	.743	.487	.187	.499	.242	.635	.605
P1283 (rs760659)		.536	.797	.831	.411	.616	.535	.552	.626
<i>Dysbindin:</i>									
P1328 (rs742106)		.127	.125	.275	.256	.502	.332	.467	.436
P1333 (rs742105)	T (.480)	.0099	.0036	.0027	.019	.533	.507	.504	.559
P1287 (rs760666)		.769	.603	.597	.954	.717	.638	.615	.578
P1655 (rs2619539)	G (.463)	.0059	.001	.0008	.014	.558	.504	.465	.544
P1635	A (.102)	.00009	.00008	.00004	.024	.006	.0066	.027	.114
P1325 (rs1011313)	C (.088)	.174	.0377	.0104	.024	.562	.399	.512	.628
P1765 (rs2619528)	G (.167)	.109	.036	.095	.738	.0466	.171	.158	.561
P1757 (rs2005976)	G (.167)	.091	.037	.065	.960	.0078	.0366	.039	.157
P1320 (rs760761)	T (.178)	.0004	.020	.172	.929	.0029	.107	.127	.526
P1763 (rs2619522)	A (.155)	.202	.101	.130	.604	.0465	.229	.392	.677
P1578 (rs1018381)		.762	.738	.488	.176	.473	.824	.553	.705
P1583 (rs909706)		.969	.308	.299	.244	.282	.558	.645	.469
Unknown:									
P1586 (rs885773)		.478	.378	.226	.065	.293	.489	.495	.501
P1294 (rs441539)		.018	.049	.051	.103	.071	.117	.146	.414
P1140 (rs1000117)		.316	.137	.193	.078	.436	.604	.364	.604
D6S1676		.617	.359	.371	.354	.166	.218	.195	.090
AFM189YE3		.644	.086	.544	.036	.580	.695	.696	.623

^a Generated by simulation.

^b Median of 20 runs. Values <.05 are in boldface italic.

significance when all individuals were included. None of the markers were positive when categories D1–D9 were used. All of the markers in the entire sample were in Hardy-Weinberg equilibrium. To test the possibility that the positive TDT results were artifactual, due, in some way, to transmission distortion, for P1635 we recoded the affected individuals as unknown and the unaffected individuals as affected. No transmission distortion was observed.

We also analyzed two-, three-, and four-marker haplotypes for the 17 SNPs and for the 2 flanking SSLPs. Figure 1 shows the results, when diagnostic categories D1–D2 were used, for all of the three-marker haplotypes tested. The “global” *P* value represents the overall significance when the observed versus expected transmissions of all of the haplotypes are considered together. Also shown are the most significant of the overtransmitted haplotypes from that median run, the haplotype frequency, and the associated *P* value. All of the adjacent, overtransmitted three-marker haplotypes are consistent with each other in terms of the SNP alleles represented. Results with the two- and four-marker haplotypes (data not shown) were also in quite good agreement with the results for the pairwise and three-marker haplotypes. One difference between the pairwise results and the haplotype results occurred with marker P1325, for which the C

allele was slightly overtransmitted in all pairwise runs but for which the T allele was contained in all three overtransmitted three-marker haplotypes. The frequency of the most positive haplotypes varied between 0.082 and 0.162, which is consistent with our previous estimates based on linkage results—that <30% of the families segregate susceptibility alleles on 6p.

Table 4 shows the pairwise marker-to-marker LD results; below the diagonal is the standard Lewontin *D'* statistic, and above the diagonal is the *d*² statistic. Note the extremely strong LD (*a*) between P1333 and the 60-kb region bounded by P1287 and P1325 and (*b*) with the region bounded by P1578 and P1586, but not with the markers between these two regions. In contrast, the more proximal marker P1287 is in strong LD with the entire 72-kb region bounded by P1655 and P1583. In addition, P1325 is in strong LD with P1765 and P1757 (17 kb), is not at all in LD with P1320, and is “again” in LD with P1763–P1294 (131 kb). With this complex pattern of LD, neither the number of susceptibility alleles nor their specific location(s) is readily apparent.

Discussion

The picture emerging from detailed studies of the limited number of genes that have been identified in complex

Marker	Interval Length	Haplotypes Overtransmitted to Affecteds																
D6S260	---	ns																
P1730	56,880	ns	ns															
P1283	42,880	ns	ns	ns														
P1328	19,891		ns	ns	ns													
P1333	48,594			ns	ns	ns												
P1287	16,047				ns	ns	C											
P1655	31,734					ns	G	G										
P1635	7,247						A	A	A									
P1325	5,330							A	T	T								
P1765	16,397								G	G	G							
P1757	973									G	T							
P1320	330										G	T	T					
P1763	2,517											A	A	A				
P1578	3,421												C	C	C	ns		
P1583	3,801													C	C	ns		
P1586	8,615														ns	ns		
P1294	95,152														ns	ns		
P1140	109,351														ns	ns		
D6S1676	201,246															ns		
Global (P value):		.63	.42	.17	.18	.24	.062	.004	.038	.025	.0032	.037	.094	.018	.17	.14	.29	.36
Individual Haplotype (P value):		na	na	na	na	na	.0013	.0034	.0006	.0001	.0001	.008	.006	.008	na	na	na	na
Haplotype Frequency:							.097	.084	.089	.162	.145	.139	.082	.086				

DYSBINDIN GENE

Figure 1 TRANSMIT results with three-marker haplotypes. When diagnostic categories D1–D2 and the condition of one affected offspring per nuclear family per extended pedigree are used, a sliding window of three markers was tested, at two-marker overlaps. Below each (most significant) overtransmitted haplotype is the P value for that haplotype, the haplotype frequency, and the global P value (median value of 20 runs), derived by evaluation of the transmissions of all haplotypes at once. SNPs within the dysbindin gene are in boldface and are offset, and the location of the gene is shown below the haplotype frequencies. Interval Length = marker-to-marker distance (in bp); na = instances in which no individual haplotype (either over- or undertransmitted) resulted in $P < .05$.

disorders is one of subtle, rather than striking, relationships between genetic variation, gene function, and increased risk (Risch 2000; Reich and Lander 2001). For example, the role of specific SNPs in the calpain-10 gene that are associated with type 2 diabetes is still unclear, despite extensive efforts directed toward both SNP detection and genotyping in multiple, large samples (Altshuler et al. 2000a; Horikawa et al. 2000; Weiss and Terwilliger 2000; Cox 2001; Evans et al. 2001). Another example from type 2 diabetes is the frequent PPAR- γ Pro12Ala polymorphism, for which a meta-analysis of >3,000 individuals was necessary to resolve apparently conflicting studies (Altshuler et al. 2000b). Likewise, despite extensive efforts, the relationship between the sequence diversity in the APOE gene (Nickerson et al. 2000) and the contribution of this variation to the risk of Alzheimer disease remains incompletely understood (Daly 1998; Martin et al. 2000). Given these well-documented difficulties, the results presented here represent only an initial step toward an understanding of the possible etiologic role that dysbindin plays in schizophrenia. The complex pattern of positive versus negative TDT results that we observed with individual markers may indicate multiple susceptibility alleles (haplotypes), although other explanations are possible; if true, this would again support the expectation of a high degree of allelic heterogeneity in most complex phenotypes (Weiss and Terwilliger 2000). Nevertheless, the central location of the positive SNPs within the 140-kb dysbin-

din gene strongly implicates this gene as a source of the positive linkage signal in this chromosomal region. Our results suggest that the susceptibility haplotypes probably do not extend distally to the neuronally expressed gene *Jumonji*, which directly abuts the dysbindin gene but is transcribed in the opposite direction. Proximally, there are no ESTs or consistently predicted exons for the next few hundred kilobases, but this certainly does not rule out genes being present. It remains a formal possibility that dysbindin itself is not aberrant in schizophrenia but, rather, that variations in it affect expression of nearby genes; and this can be tested.

In view of the recent data showing the punctate, usually 10–100-kb haplotype block structure of the human genome (Daly et al. 2001; Johnson et al. 2001; Reich et al. 2001), confirmation that the dysbindin gene is the sole susceptibility gene in this region may be relatively straightforward, since the most strongly associated markers and haplotypes reside within this gene. It does appear that marker P1635, which we found by sequencing affected individuals from 6p22-linked families but which other groups have not yet deposited into the public databases, is an important boundary. In the pairwise analysis, P1635 is strongly associated with schizophrenia, whereas the marker that is 5.3 kb proximal (i.e., marker P1325) is not. This boundary effect is also reflected in both the haplotype and the marker-to-marker LD results. Although higher marker densities achieved by use of the available (i.e., common) SNPs may be of

Table 4

Pairwise (Marker-to-Marker) LD Results

	PAIRWISE LD RESULT ^a																
	P1730	P1283	P1328	P1333	P1287	P1655	P1635	P1325	P1765	P1757	P1320	P1763	P1578	P1583	P1586	P1294	P1140
P173000	.16	.04	.10	.04	.02	.04	.03	.03	.01	.02	.14	.07	.08	.00	.02
P1283	.1502	.00	.02	.00	.01	.13	.00	.00	.00	.01	.03	.00	.06	.00	.00
P1328	.45	.3822	.05	.19	.00	.00	.02	.01	.00	.02	.01	.21	.01	.01	.00
P1333	.26	.00	.5728	.81	.11	.09	.00	.00	.02	.00	.09	.54	.05	.05	.01
P1287	.79	.54	.49	.9514	.38	.38	.36	.06	.06	.05	.03	.15	.01	.09	.00
P1655	.25	.07	.52	.93	1.0010	.08	.01	.01	.02	.00	.05	.49	.03	.05	.00
P1635	.61	.53	.04	.96	1.00	.8600	.36	.38	.46	.36	.12	.02	.00	.02	.01
P1325	.93	.58	.19	.94	.99	.94	.0830	.28	.00	.30	.15	.14	.01	.05	.00
P1765	.28	.08	.37	.15	1.00	.17	.79	.9992	.66	.87	.35	.09	.26	.03	.00
P1757	.29	.07	.34	.14	.94	.15	.82	.94	.9670	.94	.38	.13	.23	.03	.00
P1320	.14	.06	.10	.28	.95	.26	.93	.00	.85	.8769	.26	.04	.14	.04	.00
P1763	.25	.11	.38	.14	.99	.14	.76	1.00	.96	.99	.9041	.49	.25	.04	.00
P1578	.93	.32	.45	.90	.95	.83	.87	1.00	.93	.97	.82	.9707	.60	.01	.01
P1583	.29	.09	.47	.89	.86	.83	.51	.94	.83	.82	.54	.99	1.0003	.02	.02
P1586	.61	.39	.37	.73	.52	.60	.22	.75	.71	.68	.56	.68	.85	.7401	.01
P1294	.12	.04	.17	.30	.42	.30	.56	.90	.48	.53	.52	.62	.50	.20	.4302
P1140	.17	.00	.07	.14	.16	.09	.38	.20	.01	.01	.07	.01	.22	.16	.22	.16	...

^a For each pair of markers, the standardized D' is shown below the diagonal, and d^2 is shown above the diagonal. D' values >0.8 are in boldface italic.

some utility in finer mapping of the risk alleles, the sobering findings from the haplotype-tagging study by Todd and colleagues (Johnson et al. 2001) suggest that the publicly available SNPs will be inadequate for “mutation” detection, and so saturation resequencing in affected individuals, starting near P1635, is necessary. The SNPs tested are intronic, and, although none is located in a canonical splice site, very little is known about the function that specific intronic sequences have with regard to hnRNA secondary structure, protein binding, stability, and splicing efficiency. Thus, any of these associated SNPs may affect expression, and this possibility has to be investigated directly by testing for differences in transcript structures, quantity, and spatial and developmental distribution between patients and control subjects.

Dysbindin is evolutionarily conserved and is predicted to have a coiled-coil secondary structure (Burkhard et al. 2001) similar to that of the mouse ortholog (Dtnbp1) (Benson et al. 2001), with which it exhibits a high degree of protein-sequence identity. On the basis of EST alignments alone, there appear to be at least five alternative transcripts.

In mouse brain, the dysbindin protein is found in multiple anatomical locations, including axon fibers in the corpus callosum, mossy-fiber terminal fields in the hippocampus and cerebellum, and neuropil areas of the neocortex, hippocampus, and substantia nigra (Benson et al. 2001). Using semiquantitative PCR, we have found that human dysbindin transcripts are expressed in all 24 tissues (e.g., brain, heart, lung, etc.) tested, as well as in all 12 brain regions (frontal lobe, temporal

lobe, cerebellum, hippocampus, substantia nigra, caudate nucleus, amygdala, thalamus, hypothalamus, pons, medulla, and spinal cord) tested (data not shown).

Dysbindin binds to β -dystrobrevin and thus is likely to also be a component of the dystrophin protein complex (DPC) (Mehler 2000; Roberts 2001) found in postsynaptic densities (PSD) (Blake et al. 1999) and elsewhere in brain. In addition to its structural role in neuromuscular synapse formation and maintenance (Grady et al. 2000), the DPC appears to be involved in signal transduction—for example, by regulating nicotinic receptor clustering and by recruiting specific signaling molecules such as neuronal nitric oxide synthase (Bredt 1999; Grady et al. 1999), an enzyme that also interacts with PSD proteins (e.g., PSD-93 and PSD-95) that are involved in *N*-methyl-D-aspartate–receptor clustering. The composition of the DPC in muscle and brain (e.g., content of dystrophin isoforms Dp 427, Dp71, and Dp140) differs and is also heterogeneous within the brain (Moukhles and Carbonetto 2001), but it is almost certain both to exert effects on synaptic function in brain as well and to modulate other receptors. For example, dystrophin is extensively colocalized with postsynaptic gamma-aminobutyric acid (GABA-A)–receptor subtypes in hippocampus, cortex, and cerebellum (Kneusel et al. 1999). Also, Kneusel et al. (1999) have found that, in the mdx (dystrophin deficient) mouse cerebellum and hippocampus, there was a marked reduction in the number (but not in the size) of synaptic GABA-A–receptor clusters, whereas this was not true for the striatum, which does not normally contain dystrophin.

The relationship between genomic variation in dysbindin and the biological processes involved in schizophrenia is entirely speculative. Nevertheless, if dysbindin plays a role in synaptic signaling and plasticity, it would be well positioned to play a pathogenic role. There is abundant neuropathological evidence that synaptic changes are associated with schizophrenia; for example, both a reduced density of dendritic spines on the excitatory pyramidal neurons and reductions in multiple proteins found in afferent terminals in frontal cortex and hippocampus have been reported (Weinberger 1999; Glantz and Lewis 2001). Such changes suggest reduced cortical connectivity and plasticity, which are likely to be primary to the illness. Numerous as they may be, genes coding for synaptic proteins, whether functioning on dendritic spines presynaptically or postsynaptically, are all tenable functional candidate genes (Mirnics et al. 2001). In addition to the still rather diffuse dopaminergic and developmental "hypotheses" of schizophrenia, both the glutamatergic and GABA-ergic systems have been implicated (Goldman-Rakic and Selemon 1997; Benes 2000; Pearlson 2000), and these four areas of investigation already overlap considerably. It is conceivable that defects in dysbindin function could affect more than one of these systems, during development, adulthood, or both.

The intermediate (schizophrenia) phenotypes, of moderately diminished premorbid IQ and related cognitive measures (Elvevag and Goldberg 2000; Weickert et al. 2000), may be an interesting connection between the function of the DPC in synaptic transmission and the observed association between dysbindin and schizophrenia. The nonprogressive, cognitive deficits present in Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) are reflected in significantly lower IQ scores—approximately one-quarter of boys with DMD (whose dystrophin is either missing entirely or functionally mutant) are mildly mentally retarded (Blake and Kroger 2000; Anderson et al. 2002). The impairment is found to be greater on verbal than on nonverbal tests (Moizard et al. 1998) and may be due to disruption of DPC function during embryogenesis, although, as in the case of schizophrenia, the majority of patients with DMD exhibit neither gross nor histological brain abnormalities. Since mutations in dysbindin may also compromise DPC function, this may explain, at least in some small measure, the well-known observation that the average IQ of schizophrenics is lower than that in control subjects. Overall, there appears to be little evidence of comorbidity between schizophrenia and the muscular dystrophies, but this alone would not preclude some overlap in molecular mechanisms. Early reports had described (a) a family in which four of five adult patients with BMD also had schizophrenia or related disorders (Zatz et al. 1993) and, separately, (b) an association between schizo-

phrenia and DMD (Melo et al. 1993), but these results may be anecdotal. Southern blotting of DNA from 94 schizophrenics was used to screen for deletions in exons 1–59 of dystrophin, but no deletions were identified (Lindor et al. 1994). No SNP association studies have been reported for dystrophin or other components of the DPC, such as syntrophins, sarcoglycans, and dystroglycans.

A considerable amount of work will be required in order to confirm and extend our findings of an association between dysbindin and schizophrenia. At present, the possibility that this is a false-positive result, due to any of several possible artifacts, cannot, of course, be formally ruled out. However, given the internal consistency and strength of the results, we think that genetic variation in dysbindin influences the risk of schizophrenia and related schizophrenia-spectrum disorders and, thus, that this gene should be the subject of further inquiry.

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Electronic-Database Information

The accession number and URLs for data presented herein are as follows:

NCBI Single Nucleotide Polymorphism, <http://www.ncbi.nlm.nih.gov/SNP/> (for reference identification numbers for SNPs)
 Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim/> (for SCZD3 [MIM 600511])
 UCSC Human Genome Project Working Draft, <http://genome.ucsc.edu/> (for marker and SNP locations)

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