Report

The *DTNBP1* (Dysbindin) Gene Contributes to Schizophrenia, Depending on Family History of the Disease

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We have investigated the gene for dystrobrevin-binding protein 1 (DTNBP1), or dysbindin, which has been strongly suggested as a positional candidate gene for schizophrenia, in three samples of subjects with schizophrenia and unaffected control subjects of German (418 cases, 285 controls), Polish (294 cases, 113 controls), and Swedish (142 cases, 272 controls) descent. We analyzed five single-nucleotide polymorphisms (P1635, P1325, P1320, P1757, and P1578) and identified significant evidence of association in the Swedish sample but not in those from Germany or Poland. The results in the Swedish sample became even more significant after a separate analysis of those cases with a positive family history of schizophrenia, in whom the five-marker haplotype A-C-A-T-T showed a *P* value of .00009 (3.1% in controls, 17.8% in cases; OR 6.75; *P* = .00153 after Bonferroni correction). Our results suggest that genetic variation in the dysbindin gene is particularly involved in the development of schizophrenia in cases with a familial loading of the disease. This would also explain the difficulty of replicating this association in consecutively ascertained case-control samples, which usually comprise only a small proportion of subjects with a family history of disease.

Chromosome 6p is one of the most consistently replicated susceptibility regions in studies of schizophrenia (locus SCZD3 [MIM 600511]). Initial linkage findings by Straub et al. (1995), Wang et al. (1995), Schwab et al. (1995), Moises et al. (1995), and The Schizophrenia Linkage Collaborative Group for Chromosomes 3, 6, and 8 (1996) were supported by a number of subsequent individual linkage studies and by a recently performed large meta-analysis of all completed schizophrenia genome scans (Lewis et al. 2003).

After their linkage finding in 270 Irish pedigrees with a high frequency of schizophrenia, Straub et al. (2002)

performed linkage disequilibrium (LD) studies in the same sample and identified significant associations between SNPs within the positional candidate gene DTNBP1 (dystrobrevin-binding protein 1, also known as "dysbindin" [MIM 607145]) on 6p22.3 and schizophrenia. None of the associated variants appeared to have a functional effect; all were located in noncoding regions of the gene. The pattern of LD was complex and consistent with the presence of more than one susceptibility allele.

Since then, two replication studies have been published: Schwab et al. (2003) tested six SNPs in two independent parent-offspring trio samples from Germany, one derived from 78 linked affected-sib-pair families, the second representing an independently ascertained sample of 125 trios with a family history of psychiatric disorders. Evidence of association was obtained by single-marker and haplotype analyses. Schwab et al. (2003) found association with alleles different from those re-

ported by Straub et al. (2002). An explanation could be the presence of different susceptibility alleles in the Irish and German populations.

A second study by Morris et al. (2003) tried to replicate the finding by Straub et al. (2002) in a case-control sample of schizophrenia from the Irish population. They tested eight SNPs, five of which were identical to SNPs genotyped by Straub et al. (2002), in 219 Irish schizophrenia cases and 231 controls. No evidence was found to suggest an association between the dysbindin gene and schizophrenia in their sample. This was surprising because intermarker LD of the tested SNPs appeared to confirm that the samples of Straub et al. (2002) and Morris et al. (2003) had been drawn from the same homogeneous population. The major difference between the study by Morris et al. (2003) and those by Straub et al. (2002) and Schwab et al. (2003) was that the sample of Morris et al. was not selected for familiality. The question therefore was whether this result may reflect different genetic mechanisms underlying schizophrenia in individuals derived from high-density families and in individuals not selected for a family history of disease; however, a separate analysis, using the family history–positive cases only, also failed to provide evidence of association (Morris et al. 2003), but definite conclusions were difficult to draw because of the small sample size.

In the present study, we have attempted to perform an independent replication of the results obtained by Straub et al. (2002) and Schwab et al. (2003) in three samples of schizophrenic cases and controls from Germany, Poland, and Sweden, including a total of 854 cases and 670 controls.

The German sample comprised 418 cases and 285 controls, all of German descent. All patients were interviewed by experienced psychiatrists using the Structured Clinical Interview for DSM-IV (Diagnostic and Statistical Manual, 4th edition) Disorders (SCID I) (American Psychiatric Association 1994; First et al. 1997). Lifetime "best estimate" diagnoses, according to DSM-IV criteria (American Psychiatric Association 1994), were based on multiple sources of information, including the SCID I, medical records, and family history. All probands had a schizophrenic ($N = 336$), schizophreniform ($N = 7$), or schizoaffective disorder ($N = 75$). Two hundred nineteen (52.4%) of the cases were female, and 199 (47.6%) were male. The controls included 113 females (39.7%) and 172 males (60.3%).

The Polish sample comprised 294 probands and 113 controls of Polish descent. Phenotype characterization was identical to the procedure in the German probands. The Polish proband sample consisted of 291 individuals with schizophrenic and 3 with schizoaffective disorder; there were 120 (40.8%) females and 174 (59.2%) males.

The control sample comprised 71 (62.8%) females and 42 (37.2%) males.

The Swedish probands ($N = 142$; 54 [38.0%] females, 88 [62.0%] males) were all of Swedish descent. Lifetime "best estimate" diagnoses were based on DSM-III-R criteria (American Psychiatric Association, 1987). All probands had a schizophrenic ($N = 130$), schizophreniform ($N = 5$), or schizoaffective disorder ($N = 7$). There were 272 controls available, 123 (45.2%) females and 149 (54.8%) males.

We genotyped four SNPs that had provided the most significant results in the studies by Straub et al. (2002) (P1635, P1325, P1320, and P1757) and Schwab et al. (2003) (P1635, P1325, and P1320). One additional SNP, P1578, was chosen on the basis of results from a recent follow-up analysis of the Straub et al. (2002) data (van den Oord et al. 2003), in which this SNP was identified as a tag SNP for a high-risk haplotype for schizophrenia.

Because no odds ratio/relative risk information was given by Straub et al. (2002), we could not calculate the power of our sample to replicate their findings. However, Schwab et al. (2003) reported effect sizes for their associated SNPs (P1635, P1325, and P1320) and, on the basis of their figures, we calculated, using the Genetic Power Calculator (Purcell et al. 2003), that the German sample had a power of 0.93 to replicate their finding for P1325, 0.84 for P1320, and 0.76 for P1635 at a nominal significance level of .05, under the assumption that there is no influence of family history of disease. Under the same assumptions, the Polish sample had a power of 0.78, and the Swedish sample a power of 0.54 to replicate the finding for P1325.

SNPs were genotyped using the Pyrosequencing technique (Pyrosequencing), which employs a primer extension method and an indirect bioluminiscent assay of pyrophosphate (PPi) that is naturally released during DNA synthesis. Released PPi is detected by a cascade of enzymatically induced reactions, generating a light pulse that is recorded and displayed as a peak in a pyrogram. Detailed information on PCR primer and extension primer sequences as well as PCR/primer extension conditions can be obtained on request. Marker-trait association analysis was performed with the program COCAPHASE 2.35 (Dudbridge 2002). Using a standard unconditional logistic regression, this software package performs likelihood ratio tests under a log-linear model of the probability that an allele or a haplotype belongs to the case rather than the control group; the expectation maximization (EM) algorithm is used to resolve uncertain haplotypes and provides maximum-likelihood estimates of frequencies. Results for the single-marker analyses were confirmed with the Cochrane-Armitage trend test (Armitage 1955). For the haplotype analysis, we analyzed two-, three-, and four-marker haplotypes from adjacent SNPs in a sliding-window fashion, as well

Table 1

Allele Frequencies of Five SNPs in the Dysbindin Gene in Case-Control Samples of Schizophrenia from Germany, Sweden, and Poland

SNP[a]	dbSNP ID[b]	POLYMORPHISM[c]	GERMAN			POLISH			SWEDISH		
			Cases[d] ($n = 418$)	Controls[d] $n = 285$	P[e]	Cases[c] $n = 294$	Controls[d] $n = 113$	P[e]	Cases[c] $n = 142$	Controls[d] $n = 272$	P[e]
P1635	rs3213207	A/G	.109	.137	.130	.111	.110	1.000	.115	.113	.915
P1325	rs1011313	C/T	.105	.086	.271	.120	.106	.624	.110	.068	.032[f]
P1757	rs2005976	G/A	.202	.240	.122	.177	.137	.261	.208	.169	.178
P1320	rs760761	C/T	.214	.249	.141	.190	.152	.235	.220	.199	.512
P1578	rs1018381	C/T	.072	.078	.678	.053	.027	.124	.085	.061	.213

[a] Described by Straub et al. (2002).

[b] National Center for Biotechnology Information, Single Nucleotide Polymorphism Database.

[c] Second allele is the less frequent allele.

[d] Frequency of the less frequent allele.

[e] Calculated by COCAPHASE.

[f] Underlining indicates difference between cases and controls reached statistical significance. $P = .124$ after adjustment for multiple testing through permutation.

as the complete five-marker haplotype of all analyzed SNPs. Since the EM algorithm does not accurately estimate haplotype frequencies <1% (Fallin and Schork 2000), such haplotypes were excluded. Individuals with missing genotype information at any of the five loci were excluded from the haplotype analysis. For the single-marker analyses, a permutation procedure was used to estimate the significance of the best results, correcting for the five loci tested. For the haplotype analyses, the global null hypotheses that all odds ratios are equal were also tested by permutation, owing to the fact that estimated haplotype frequencies cannot be treated as observed data. Furthermore, the permutation procedure was used to estimate the significance of the best result from the 2-, 3-, and 4-marker sliding window analyses,

correcting for the number of windows tested. The permutation method implemented in COCAPHASE randomly reassigns the "case" and "control" labels in the actual data. Ten thousand permutations were performed in each permutation analysis.

Each of the three samples was analyzed separately, because a comparison of SNP allele frequencies in the controls revealed significant differences for some of the SNPs (German vs. Swedish controls: P1757 [$P = .025$]; German vs. Polish controls: P1757 [$P = .002$], P1320 [$P = .003$], P1578 [$P = .009$]; Polish vs. Swedish controls: P1578 [$P = .046$]). All SNPs were in Hardy-Weinberg equilibrium in both the case and control samples.

None of the five investigated SNPs showed association with schizophrenia in the German and Polish samples

Table 2

Global *P* Values for Haplotype Analysis of Dysbindin SNPs in the Total Sample and Subgroup of Cases with a Family History of Schizophrenia

MARKER HAPLOTYPE[a]	GERMANY		POLAND		SWEDEN	
	All ($n = 418$)	FH+[b] ($n = 56$)	All ($n = 294$)	FH+[b] ($n = 38$)	All ($n = 142$)	FH+[b] ($n = 32$)
1_2	.198	.408	.761	.512	.068	.780
2_3	.149	.870	.526	.897	.014 (.038)[c]	.012
3_4	.197	.798	.608	.523	.427	.020
4_5	.202	.584	.276	.305	.154	.003 (.009)[c]
1_2_3	.225	.421	.295	.385	.021 (.033)[c]	.009
2_3_4	.096	.887	.845	.928	.064	.023
3_4_5	.248	.398	.368	.432	.092	.0004 (.001)[c]
1_2_3_4	.168	.448	.683	.948	.083	.006
2_3_4_5	.113	.506	.656	.933	.033 (.045)[c]	.0009 (.0016)[c]
1_2_3_4_5	.178	.543	.342	.833	.0098	.0009

NOTE.—Significance was calculated by COCAPHASE.

[a] 1 = P1635; 2 = P1325; 3 = P1757; 4 = P1320; and 5 = P1578.

[b] FH+ indicates individuals with family history of schizophrenia.

[c] Underlining indicates difference reached statistical significance. Significance of the best result in the respective sliding-window analysis, corrected for multiple testing by permutation.

Table 3

Allele Frequencies of Five SNPs in the Dysbindin Gene in Case-Control Samples from Germany, Sweden, and Poland with a Family History of Schizophrenia

SNP[a]	dbSNP ID	POLYMORPHISM[b]	GERMAN			POLISH			SWEDISH		
			Cases[c] $n = 56$	Controls[c] $n = 285$	P[d]	Cases[c] $n = 38$	Controls[c] $n = 113$	P[d]	Cases[c] $n = 32$	Controls[c] $n = 272$	P[d]
P1635	rs3213207	A/G	.181	.137	.242	.105	.110	.915	.125	.113	.771
P1325	rs1011313	C/T	.090	.086	.853	.158	.106	.243	.065	.064	.987
P1757	rs2005976	G/A	.259	.240	.677	.197	.137	.226	.297	.169	.018[e]
P1320	rs760761	C/T	.255	.249	.904	.208	.152	.271	.293	.199	.110
P1578	rs1018381	C/T	.054	.078	.343	.079	.027	.063	.156	.061	.013[f]

[a] Described by Straub et al. (2002).
[b] Second allele is the less frequent allele.
[c] Frequency of the less frequent allele.
[d] Calculated by COCAPHASE.
[e] Underlining indicates difference reached statistical significance.
[f] Underlining indicates difference reached statistical significance. $P = .068$ after adjustment for multiple testing through permutation.

in single-marker (table 1) and haplotype analyses (table 2). In the Swedish sample, P1325 reached significance ($P = .032$; table 1). However, the permutation analysis adjusting for the five loci tested revealed a nonsignificant P value of .124. On the other hand, haplotype analyses clearly showed evidence of an association, the most significant haplotype combination being the five-marker haplotype ($P = .0098$; table 2).

In accordance with the hypothesis that a stratification of the samples with regard to a family history of schizophrenia might have a higher chance of replicating the association finding, we performed a separate analysis with the family history–positive subgroups. "Positive family history" was defined as having at least one first- or second-degree relative with schizophrenia. There were 56 (13.4% of the total sample) family history–positive individuals in the German, 38 (12.9%) in the Polish, and 32 (22.5%) in the Swedish sample. No association was found in the German and Polish subsamples. In the Swedish sample, however, frequencies of the rare alleles were significantly higher for P1757 ($P = .018$) and P1578 in the patients with schizophrenia ($P = .013$) (table 3). After adjusting for multiple testing by permutation across the five analyzed markers, the marker with the best result (P1578) did not reach significance ($P = .068$). Conclusive results were obtained from the haplotype analyses. Several haplotypes showed significant global P values, the five-marker haplotype P1635-P1325-P1757-P1320-P1578 showing $P = .0009$ (table 2). This P value remains significant, even after applying a Bonferroni correction for the number of samples (three) and haplotype combinations (four) tested ($P = .0108$). Given this result, we were interested in the contribution of individual haplotypes to the global result. Thus, individual haplotypes were tested for association by grouping all others together and applying the χ^2 test with 1 df. Results of this analysis are

given in table 4. The most significant haplotype was A-C-A-T-T, which occurs at a frequency of 3.1% in the controls, and 17.8% in the cases ($P = .00009$; $P = .00153$ after Bonferroni correction). The possibility has been discussed that, given a population with significant association, an improvement in P value may exist in random subsets of the population (Johnson et al. 2002). To test whether the improvement observed in familial cases could have arisen by chance alone, we randomly chose 32 individuals from the Swedish sample (10,000 replicates). Only 68 replicates showed a P value equal to or smaller than that of the observed subgroup with a family history of schizophrenia, suggesting that our observation is unlikely to be a chance finding ($P = .0068$).

Although the associated haplotype in the Swedish sample is not exactly the same as the ones reported in the Irish (G-C-A-T-C) (van den Oord et al. 2003) and German populations (A-C-X-C-C ["X" is marker P1757, which was not genotyped in that study]) (Schwab et al.

Table 4

Dysbindin Haplotype Frequencies in the Swedish Subsample with Family History of Schizophrenia

Marker Haplotype[a]	Cases ($n = 32$)	Controls ($n = 272$)	P[b]
1_2_3_4_5			
A_C_G_C_C	.643	.753	.09
A_C_A_T_C	.018	.024	.84
A_C_A_T_T	.178	.031	.00009[c]
A_T_G_C_C	.051	.060	.85
G_C_A_T_C	.107	.094	.09

[a] 1 = P1635; 2 = P1325; 3 = P1757; 4 = P1320; 5 = P1578.
[b] Calculated by COCAPHASE.
[c] Underlining indicates difference reached statistical significance.

2003), phylogenetic tree analysis shows a close relationship between the risk haplotype in the Swedish population and the Irish population (data not shown). The risk haplotype A-C-A-T-T has an odds ratio of 6.75 in the Swedish population.

In light of the findings described elsewhere (Straub et al. 2002; Morris et al. 2003; Schwab et al. 2003), our data suggest that genetic variation in the dysbindin gene is particularly involved in the development of schizophrenia in cases with a familial loading of the disease. This would also explain the difficulty of replicating the association in consecutively ascertained case-control samples, which usually comprise only a relatively small proportion of cases with a positive family history of schizophrenia. Morris et al. (2003), for example, stratified their sample with regard to family history of schizophrenia but found no evidence of association. However, owing to the small sample size (65 of 219 cases [29.7% of the original sample size]), they could not make any firm conclusions. If medium-sized case-control samples are subdivided according to family history, the inevitable result is an enormous loss of statistical power. In our study, the German familial subsample ($n = 56$) had a power of only 35% to replicate the effect for marker P1325 reported by Schwab et al. (2003). The Polish subsample ($n = 38$) had a power of 29%, and the Swedish subsample ($n = 32$) had a power of 19%. Given the small power of the subsamples, it is not surprising that we find association in only one of three samples; on the contrary, it meets the expectation.

Interestingly, an effect of family history has also been suggested for the involvement of the neuregulin 1 gene in schizophrenia by Williams et al. (2003), who were able to replicate an association that was originally reported by Stefansson et al. (2002). Williams et al. (2003) found a borderline significant association of a particular haplotype in a large collection of 573 schizophrenia cases and 618 controls ($P = .04$), which became stronger when the subset of 141 cases with a family history of schizophrenia was analyzed ($P = .019$).

Recently, numerous gene identification studies have been following the positional-candidate paradigm, in which initial evidence of a gene locus is obtained from linkage analyses in families loaded with the disease under study. In the subsequent step, association analyses are performed, to eventually identify genetic variants that confer risk of disease. Our results show that it could be crucial for the success of these studies to use association samples enriched for familial cases instead of consecutively ascertained cases, which normally include only a small proportion of individuals with a family history of disease.

Electronic-Database Information

URLs for data presented herein are as follows:

Genetic Power Calculator, http://statgen.iop.kcl.ac.uk/gpc/
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for SCZD3 and DTNBP1)
National Center for Biotechnology Information, Single Nucleotide Polymorphism Database, http://www.ncbi.nlm.nih.gov/SNP/ (for reference identification numbers for SNPs)
UNPHASED Documentation, http://www.hgmp.mrc.ac.uk/⁓fdudbrid/software/unphased/ (for COCAPHASE 2.35)

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