

The *DISC* locus and schizophrenia: evidence from an association study in a central European sample and from a meta-analysis across different European populations

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Association studies, as well as the initial translocation family study, identified the gene *Disrupted-In-Schizophrenia-1 (DISC1)* as a risk factor for schizophrenia. *DISC1* encodes a multifunctional scaffold protein involved in neurodevelopmental processes implicated in the etiology of schizophrenia. The present study explores the contribution of the *DISC* locus to schizophrenia using three different approaches: (i) systematic association mapping aimed at detecting *DISC* risk variants in a schizophrenia sample from a central European population (556 SNPs, $n = 1621$ individuals). In this homogenous sample, a circumscribed *DISC1* interval in intron 9 was significantly associated with schizophrenia in females ($P = 4 \times 10^{-5}$) and contributed most strongly to early-onset cases ($P = 9 \times 10^{-5}$). The odds ratios (ORs) were in the range of 1.46–1.88. (ii) The same sample was used to test for the locus-specific SNP–SNP interaction most recently associated with schizophrenia. Our results confirm the SNP interplay effect between rs1538979 and rs821633 that significantly conferred disease risk in male patients with schizophrenia ($P = 0.016$, OR 1.57). (iii) In order to detect additional schizophrenia variants, a meta-analysis was performed using nine schizophrenia samples from different European populations (50 SNPs, $n = 10\,064$ individuals maximum, $n = 3694$ minimum). We found evidence for a common schizophrenia risk interval within *DISC1* intron 4–6 ($P = 0.002$, OR 1.27). The findings point to a complex association between schizophrenia and *DISC*, including the presence of different risk loci and SNP interplay effects. Furthermore, our phenotype–genotype results—including the consideration of sex-specific effects—highlight the value of homogenous samples in mapping risk genes for schizophrenia in general, and at the *DISC* locus in particular.

INTRODUCTION

Disrupted-In-Schizophrenia-1 (DISC1), MIM 605210) is one of the most frequently implicated risk genes for schizophrenia

(MIM 181500) (1). The *DISC* locus was first discovered through a breakpoint mapping on chromosome 1q42 in a Scottish family with a balanced translocation (1,11) (q42.1;q14.3) that was found to segregate with a wide spectrum of mental

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disorders (2,3). Linkage disequilibrium (LD) studies using different schizophrenia samples were then performed and provided evidence that the *DISC* locus is also involved in more common forms of schizophrenia (4) (Supplementary Material, Table S1). Furthermore, *DISC1* is an interesting gene for neuropsychiatric disorders at the functional level. It encodes a multifunctional scaffold protein involved in neuronal migration, cortical layering and hippocampal formation, all of which are implicated in the etiology of schizophrenia (5). Despite these encouraging findings, the genetic risk variants at the *DISC* locus remain unidentified. In addition, different *DISC* intervals have shown association across studies, and this is complicated by the fact that most studies analyzed different markers and that their coverage often did not suffice to systematically detect LD (Supplementary Material, Table S1). To date, only two studies have performed a locus-specific LD mapping using a sufficient marker grid (6,7).

This study investigated the contribution of the *DISC* locus in a large schizophrenia case-control sample from a central European population ($n = 1621$ individuals). In order to map the schizophrenia risk variants in this homogenous sample as precisely as possible, we performed a systematic LD mapping using 556 variants [121 genotyped, 435 imputed single nucleotide polymorphisms (SNPs)]. In addition, we tested for locus-specific interaction effects by analyzing the marker-marker interplay at the *DISC* locus most recently identified in schizophrenia (7). The study also attempted to identify more common schizophrenia variants at the *DISC* locus across nine case-control samples obtained from different European populations. Notably, this combined analysis (50 markers, $n = 10\,064$ individuals maximum, $n = 3694$ individuals minimum) is the first *DISC* meta-analysis in schizophrenia to date.

RESULTS

LD mapping in the central European schizophrenia sample

Sample homogeneity and haplotype structure. Our initial step was to assess the genetic homogeneity of our cases and controls and—in accordance with previous studies using this sample (8)—the Structure analysis suggested that our sample represented a homogenous population (see Materials and Methods and Supplementary Material).

Next, we explored the genomic architecture at the *DISC* locus. Using the haplotype definition of Gabriel *et al.* (9) and all 556 analyzed markers, we identified a total of 32 different haplotype blocks across the target region (see Materials and Methods, Supplementary Material, Table S2 and Supplementary Material, Fig. S1).

Single-marker association analysis. For the *DISC* LD mapping, we stratified our sample according to gender. This procedure represents an a priori approach, as previous studies have reported sex-specific *DISC* associations in schizophrenia (Supplementary Material, Table S1). In addition, we stratified the patient sample in a *post hoc* analysis for specific phenotypic characteristics that may have represented greater etiological homogeneity (see Materials and Methods). To prevent type-I association errors, we performed 10 000 permutations of each

of the markers and samples (all, female, male and using all phenotypic strata tested in all three samples).

After the permutation, none of the *DISC* markers showed an association in the entire schizophrenia sample or in the male sub-sample (Supplementary Material, Table S2). In contrast, the female sub-sample showed a significant association in a circumscribed *DISC1* intron 9 interval. Alleles of three markers, all belonging to haplotype block 22, were significantly more frequent in female patients with schizophrenia than female controls (P -values between 0.0002–0.0006 and 0.0225–0.0475 corrected) (Table 1, Supplementary Material, Fig. S1). The corresponding odds ratios (ORs) ranged between 1.46 and 1.48. In addition, two genotyped and 17 imputed SNPs within block 22 showed a nominally significant association (Table 1).

The detailed phenotype-genotype analysis revealed that female patients with an early age of onset (EAO; less than 21 years) predominantly contributed to this association. In this phenotypically more homogeneous sample, the genetic effect size increased by an OR of 1.88 at two of three formerly associated markers (P -values of 0.0003 and 0.0312, corrected) (Table 1). The additional strata [lifetime history of depressive symptoms (DEP), and family history of psychiatric illness (FAM)] did not strengthen the association in this sample (Table 1).

Our haplotype block 22 findings are consistent with some previously reported schizophrenia associations. Hodgkinson *et al.* (10) found an association in a European-American schizophrenia sample at all three markers tested in this region (rs1122359: $P = 0.003$; rs999710: $P = 0.003$; rs9432024: $P = 0.019$). We genotyped two of these markers and both showed a significant association in females with schizophrenia in our sample (rs999710: $P = 0.0002$; rs9432024: $P = 0.0013$) (Table 1, Supplementary Material, Table S3). Notably, the same alleles were associated with schizophrenia in both studies. Furthermore, some previous studies that did not use a sex-specific analysis detected no association between schizophrenia and rs999710 (11,12) or rs9432024 (13) (Supplementary Material, Table S3); these findings are consistent with our results, which revealed no association between schizophrenia and these markers in the all-gender analysis (Supplementary Material, Tables S2 and S3).

Supplementary Material, Table S3 lists three additional SNPs—in haplotype blocks 1, 12 and 27—where we and other independent studies observed a disease association in the same direction. However, none of the association signals in our sample were significant after the permutation correction.

Haplotype association analysis. For the multi-marker analysis, we used the haplotype block boundaries defined by Gabriel *et al.* (9) and only individually genotyped markers. All haplotypes that showed significant differences in their case-control distributions are presented in Supplementary Material, Table S4. Consistent with the single-marker analysis, a nine-marker haplotype in block 22—GTCGACTGG—was significantly more frequent in females with schizophrenia than in female controls (41.1% versus 31.1%, $P = 0.00009$) (Table 2).

In addition, the neighboring haplotype block 23 showed an association in females with schizophrenia. The four-marker

Table 1. Single-marker association analysis in female cases and female controls within *DISC1* haplotype block 22

SNP ^a	Position ^b	Inter-bp	MA ^c	Controls ^d	All (<i>n</i> = 254)				EAO (<i>n</i> = 81)			
					Cases ^e	<i>P</i> -value ^f	OR	<i>P</i> p ^g	Case ^e	<i>P</i> -value ^f	OR	<i>P</i> p ^g
rs9432024	230073842		G	0.397	0.313	0.0008	0.69	0.12	0.265	0.0020	0.55	0.3
rs1015101	230074317	475	G	0.363	0.458	0.0002	1.48	0.02	0.519	0.0003	1.88	0.03
rs9432025	230074347	30	C	0.514	0.436	0.0031	0.73	0.36	0.377	0.0020	0.58	0.3
rs9431706	230074383	36	A	0.514	0.436	0.0031	0.73	0.36	0.377	0.0020	0.58	0.3
rs7553949	230074415	32	G	0.514	0.436	0.0031	0.73	0.36	0.377	0.0020	0.58	0.3
rs9432028	230075733	1318	A	0.409	0.333	0.0028	0.72	0.34	0.284	0.0031	0.57	0.38
rs9431708	230076025	292	G	0.514	0.436	0.0031	0.73	0.36	0.377	0.0020	0.58	0.3
rs999710	230077566	1541	T	0.363	0.458	0.0002	1.48	0.02	0.519	0.0003	1.88	0.03
rs7549204	230077793	227	A	0.228	0.186	0.0473	0.77	0.99	0.13	0.0051	0.5	0.52
rs999708	230078214	421	G	0.514	0.436	0.0031	0.73	0.36	0.377	0.0020	0.58	0.3
rs1009587	230078572	358	A	0.364	0.457	0.0003	1.47	0.05	0.519	0.0003	1.87	0.07
rs9431711	230080135	1563	A	0.23	0.183	0.0268	0.75	0.83	0.13	0.0051	0.5	0.31
rs9432040	230080739	604	G	0.514	0.436	0.0031	0.73	0.36	0.377	0.0020	0.58	0.3
rs7535913	230081033	294	T	0.409	0.333	0.0028	0.72	0.34	0.284	0.0031	0.57	0.38
rs2356606	230081663	630	G	0.23	0.186	0.0409	0.77	0.99	0.13	0.0051	0.5	0.52
rs7534369	230082857	1194	A	0.409	0.333	0.0028	0.72	0.34	0.284	0.0031	0.57	0.38
rs12740724	230083249	392	G	0.409	0.333	0.0028	0.72	0.34	0.284	0.0031	0.57	0.38
rs11122357	230083676	427	A	0.409	0.333	0.0028	0.72	0.34	0.284	0.0031	0.57	0.38
rs9431714	230087871	4195	A	0.409	0.333	0.0028	0.72	0.34	0.284	0.0031	0.57	0.38
rs4658890	230090704	2833	G	0.529	0.441	0.0008	0.7	0.07	0.605	0.0018	1.73	0.14
rs4333837	230091773	1069	G	0.33	0.417	0.0006	1.46	0.04	0.469	0.0009	1.79	0.08
rs9659649	230094131	2358	T	0.332	0.285	0.0532	0.8	1	0.235	0.0154	0.62	0.84
rs1407599	230094534	403	T	0.327	0.415	0.0005	1.46	0.08	0.469	0.0006	1.82	0.12
					DEP (<i>n</i> = 127)				FAM (<i>n</i> = 67)			
					Cases ^e	<i>P</i> -value ^f	OR	<i>P</i> p ^g	Cases ^e	<i>P</i> -value ^f	OR	<i>P</i> p ^g
rs9432024	230073842				0.342	0.1372	0.79	1.00	0.331	0.1773	0.77	1.00
rs1015101	230074317				0.445	0.0238	1.40	0.78	0.418	0.2409	1.25	1.00
rs9432025	230074347				0.469	0.2426	0.84	1.00	0.522	0.4757	1.14	1.00
rs9431706	230074383				0.469	0.2426	0.84	1.00	0.522	0.4757	1.14	1.00
rs7553949	230074415				0.469	0.2426	0.84	1.00	0.522	0.4757	1.14	1.00
rs9432028	230075733				0.362	0.1883	0.82	1.00	0.358	0.2689	0.81	1.00
rs9431708	230076025				0.469	0.2426	0.84	1.00	0.522	0.4757	1.14	1.00
rs999710	230077566				0.445	0.0238	1.40	0.78	0.418	0.2409	1.25	1.00
rs7549204	230077793				0.173	0.0626	0.71	1.00	0.172	0.1409	0.7	1.00
rs999708	230078214				0.469	0.2426	0.84	1.00	0.522	0.4757	1.14	1.00
rs1009587	230078572				0.445	0.0264	1.39	0.94	0.418	0.2537	1.24	1.00
rs9431711	230080135				0.173	0.0626	0.71	0.97	0.172	0.1409	0.7	1.00
rs9432040	230080739				0.469	0.2426	0.84	1.00	0.522	0.4757	1.14	1.00
rs7535913	230081033				0.362	0.1883	0.82	1.00	0.358	0.2689	0.81	1.00
rs2356606	230081663				0.173	0.0626	0.71	1.00	0.172	0.1409	0.7	1.00
rs7534369	230082857				0.362	0.1883	0.82	1.00	0.358	0.2689	0.81	1.00
rs12740724	230083249				0.362	0.1883	0.82	1.00	0.358	0.2689	0.81	1.00
rs11122357	230083676				0.362	0.1883	0.82	1.00	0.358	0.2689	0.81	1.00
rs9431714	230087871				0.362	0.1883	0.82	1.00	0.358	0.2689	0.81	1.00
rs4658890	230090704				0.551	0.0249	1.39	0.79	0.522	0.2586	1.24	1.00
rs4333837	230091773				0.409	0.0245	1.40	0.79	0.343	0.7819	1.06	1.00
rs9659649	230094131				0.256	0.0238	0.69	0.93	0.269	0.1473	0.74	1.00
rs1407599	230094534				0.409	0.0176	1.43	0.87	0.343	0.7097	1.08	1.00

Only markers with significant association findings are presented and only *P*-values <0.05 are given in bold. None of the SNPs showed Hardy–Weinberg equilibrium (HWE) deviation in controls (*P* > 0.05).

^aSNP markers in bold are genotyped, all other SNP markers are imputed.

^bSNP marker positions according to NCBI Build 36.

^cMinor allele in controls (MA).

^dMA frequencies (MAF) in controls.

^eMAF in patients.

^f*P*-values of the single-marker association analysis.

^g*P*-values after permutation correction.

haplotype GTAT was significantly more frequent in individuals with schizophrenia than controls (7.0% versus 3.6%, *P* = 0.0037) (Table 2). Because the results suggested an extended haplotype architecture at this locus, we conducted a combined block 22–23 analysis. This revealed two 13-marker risk haplotypes—GTCGACTGGGTGT and GTCGACTGGGTAT—that

were both significant as well as more frequent in females with schizophrenia than female controls (32.5% versus 25.2%, *P* = 0.0027, and 6.6% versus 3.3%, *P* = 0.0048) (Table 2). The less frequent 13-marker risk haplotype differed at SNP rs17770256 only compared with the common risk haplotype (alleles G versus A, underlined above and below). This

Table 2. Haplotype association analysis in female cases and female controls within *DISC1* haplotype blocks 22 and 23

Haplotype block	Sample/sub-sample	Haplotype	% Patients ^a	% Controls ^a	<i>P</i> -value	Global <i>P</i> -value
Block 22 9-SNP-haplotype ^b	Female	GTCGACTGG	0.411	0.311	0.00009	0.0184
		ACCAACGAG	0.162	0.210	0.01860	
	Female EAO	GTCGACTGG	0.469	0.307	0.00008	
		ACCAACGAG	0.130	0.213	0.01586	
Block 23 4-SNP-haplotype ^c	Female	GTAT	0.070	0.036	0.00372	0.0448
	Female EAO	GTAT	0.098	0.035	0.00074	0.0155
Block 22–23 13-SNP-haplotype ^d	Female	GTCGACTGGGTGT	0.325	0.252	0.00279	0.0265
		GTCGACTGGGTAT	0.066	0.033	0.00483	
		ACCAACGAGGTGT	0.151	0.201	0.01428	
	Female EAO	GTCGACTGGGTGT	0.365	0.254	0.00464	
		GTCGACTGGGTAT	0.089	0.033	0.00207	
		ACCAACGAGGTGT	0.132	0.204	0.03770	
Block 22–23 12-SNP-haplotype ^e	Female	GTCGACTGGGT	0.388	0.284	0.00004	0.0367
		ACCAACGAGGT	0.149	0.200	0.01146	
	Female EAO	GTCGACTGGGT	0.444	0.285	0.00009	
		ACCAACGAGGT	0.129	0.204	0.02841	

Only haplotypes with significant differences between cases and controls are presented and only *P*-values <0.05 are given in bold.

^aEstimated haplotype frequencies in patients and controls. Estimations are based on each analyzed sample (all female patients and all female controls, EAO female patients and all female controls).

^bSNP-marker combination rs1015101–rs999710–rs11122355–rs9431711–rs17820909–rs11122362–rs4658890–rs4333837–rs11122366.

^cSNP-marker combination rs11585831–rs967433–rs17770256–rs821723.

^dSNP-marker combination rs1015101–rs999710–rs11122355–rs9431711–rs17820909–rs11122362–rs4658890–rs4333837–rs11122366–rs11585831–rs967433–rs17770256–rs821723.

^eSNP-marker combination rs1015101–rs999710–rs11122355–rs9431711–rs17820909–rs11122362–rs4658890–rs4333837–rs11122366–rs11585831–rs967433–rs821723.

haplotype most likely arose from the common risk haplotype after the mutation event had occurred. The combined 12-marker risk haplotype GTCGACTGGGT-G/A-T was more common in individuals with schizophrenia (38.8% versus 28.4% in controls) and was significantly associated with the disease ($P = 0.00004$) (Table 2).

Consistent with the single-marker results, the risk haplotypes were even more frequent in EAO females with schizophrenia (Table 2). In this homogenous sample, the most discriminative 12-marker risk haplotype had a frequency of 44.4% (versus 28.4% in controls) and was significantly associated with schizophrenia ($P = 0.00009$) (Table 2).

Interplay and functional *DISC* variants in the central European schizophrenia sample

Next, we tested for the locus-specific interaction observed in the schizophrenia study by Hennah *et al.* (7) and adopted their marker–marker–interplay approach (see Materials and Methods). At both of the previously implicated markers (7)—rs1538979 and rs821633—we stratified our cases and controls by grouping homo- and heterozygote carriers of the same alleles together (e.g. grouping of all individuals carrying the genotypes AA and AG as well as all individuals carrying the genotypes GG and AG at rs1538979). Within the allele-stratified samples, we assessed the association at the other reported interaction-marker. Because the procedure represents a confirmative test approach, no correction was applied in this analysis.

We observed the same rs1538979–rs821633 interaction that had previously been described in schizophrenia (7). The SNP rs1538979 (allele A) was significantly associated in males with schizophrenia (15.3% cases versus 10.3% in controls, $P = 0.016$, OR 1.57) by using rs821633 (allele G) as a conditional marker (Supplementary Material, Table S5). The association at rs1538979 was thus detectable only under the interaction model (Supplementary Material, Table S5). We also found that, on its own, allele G at rs821633 was a significant protective factor against schizophrenia (31.3% versus 35.9% in controls, $P = 0.038$, OR 0.83) (Supplementary Material, Table S2); notably, the same effect had been observed by Hennah *et al.* (7).

We then focused on those *DISC1* variants that might be of functional relevance; these SNPs cause an amino acid substitution and were associated with schizophrenia in some previous studies (4). We found no association for rs6675281 (Leu607Phe) or rs821616 (Ser704Cys) in our overall or gender-specific analysis of individuals with schizophrenia (Supplementary Material, Table S2), as well as in our phenotypic strata analysis (data not shown).

DISC meta-analysis across European schizophrenia samples and studies

The third part of our study was a *DISC* meta-analysis using nine schizophrenia samples from different European populations ($n = 10\,064$ maximum sample size/SNP, $n = 3694$ minimum sample size/SNP). All studies that used a

gene-based association approach (*DISC* marker set of more than five SNPs/study) and that were performed on independent case–control samples with a broad definition of European ancestry (Supplementary Material, Table S6) were included. In total, we tested 50 locus-specific SNPs for association using the weighted Z-score method and Comprehensive Meta Analysis v. 2 (see Materials and Methods).

In this mixed European sample, evidence for common schizophrenia risk variants was found at 10 *DISC* SNPs (P -values between 0.002 and 0.048) (Table 3). All studies contributed equally to the findings (heterogeneity test: P -values between 0.988 and 0.100) (Table 3) and the funnel-plot analysis did not indicate the presence of publication bias effects for the associated variants (data not shown).

Nevertheless, after correction by a factor of 23 was applied, only one marker, rs17817356, still remained significant ($P = 0.002$, $P = 0.046$ corrected, OR 1.27) (Table 3); this correction factor represents the number of haplotype blocks in which the 50 meta-analysis markers were located. Although the use of the haplotype structure observed in our sample might not be representative for all samples tested in the meta-analysis, a Bonferroni correction by a factor of 50 (number of tested markers) seemed to be overly conservative, given the strong inter-marker LD within each haplotype block. The significant SNP (rs17817356) is located within a *DISC1* intron 4–6 interval, where four additional markers showed a nominally significant association in the meta-analysis (rs1538979 to rs1322784) (Table 3). Interestingly, rs1538979—the marker that showed an interplay effect in our study as well as the one by Hennessey *et al.* (7)—is one of the associated markers in the identified intron 4–6 interval.

DISCUSSION

Notably, this analysis is one of the most comprehensive *DISC* studies in schizophrenia to date and comprises the application of three different, but complementary, approaches. Specifically, (i) in the first schizophrenia sample from a central European population, we analyzed 556 *DISC* markers ($n = 1621$). The LD mapping included a detailed phenotype–genotype examination that further increased sample homogeneity. In this population, a circumscribed *DISC1* intron 9 interval was associated with schizophrenia in females ($P = 0.00004$), and this risk effect was strongest in EAO female patients ($P = 0.00009$, OR 1.88); (ii) we then assessed the previously reported locus-specific *DISC* interaction (7) using the same central European sample. Our results confirmed the SNP interplay effect at rs1538979–rs821633 that significantly conferred risk of schizophrenia in males ($P = 0.016$, OR 1.57); (iii) finally, we performed a meta-analysis using 50 *DISC* markers and nine schizophrenia samples ($n = 10\,064$ individuals maximum, $n = 3694$ minimum) with the goal of detecting additional schizophrenia variants across European populations. We found evidence for a common risk interval within *DISC1* intron 4–6 ($P = 0.002$, OR 1.27).

The *DISC* findings described here point to a complex association profile in schizophrenia, including the presence of different risk loci and SNP interplay effects. In addition, our phenotype–genotype results highlight the value of

homogenous samples in mapping risk genes for complex disorders. Below, we review some of the relevant findings in this area, and discuss the implications of the current study.

Different schizophrenia risk intervals at the *DISC* locus

The *DISC1* intron 9 risk region implicated in schizophrenia risk for females in the central European sample has, to date, attracted only limited attention. Hodgkinson *et al.* (10) found an association at three SNPs within this locus in a European-American schizophrenia sample. Two of those three SNPs were analyzed in our study and showed a significant association (rs999710: $P = 0.0002$; rs9432024: $P = 0.0013$). In addition, Hennessey *et al.* (7) found an association within haplotype block 22 in two schizophrenia samples (from London and Edinburgh). Consistent with the results of the present study, this association was most prominent in females. However, the same alleles were implicated by the present study and the one by Hodgkinson *et al.* (10), whereas the findings of Hennessey *et al.* (7) differ on the allelic level. This may reflect the presence of a different haplotype structure or risk haplotypes in the UK/Scottish and central European populations.

In addition, the meta-analysis points to a common schizophrenia risk interval in *DISC1* intron 4–6. Within this region, one marker was significantly associated with disease risk, and four neighboring markers showed a nominally significant association. The clustering may reflect the robustness of our findings. In addition, one of the associated variants in this interval was rs1538979, which conferred risk of schizophrenia in the present study, as well as in a previous one (7), through a SNP–SNP interaction.

On the basis of our findings and those of independent association studies, it appears that both *DISC1* regions (within intron 9 and intron 4–6) are interesting candidates for schizophrenia follow-up studies. Furthermore, the extent of the identified risk intervals are circumscribed; the intron 9 interval spans between 27 and 47 kb (defined by markers of haplotype blocks 22 and 23 as well as 21 and 24), and the size of the intron 4–6 interval spans between 32 and 62 kb (inter-marker distances of the associated SNPs and first flanking markers without association). The limited extent of both regions should facilitate re-sequencing efforts, an important next step in determining the true underlying *DISC* risk variant(s) at both loci.

Locus-specific *DISC* interaction

The rs1538979–rs821633 interaction confirms the recently reported SNP interplay effects at the *DISC* locus (7). In that study as well as in the present one, the combination of the same alleles at both markers increased schizophrenia risk. Furthermore, we confirmed that rs821633-allele G by itself was a protective factor (7).

Nevertheless, the results obtained here are not a complete replication. Hennessey *et al.* (7) obtained their results in female individuals with schizophrenia, whereas we found ours in males with schizophrenia. The findings may point to differences in the underlying genetic structure of schizophrenia between both populations. Alternatively, additional variants

Table 3. DISC meta-analysis in nine schizophrenia case-control samples from European populations

SNP	Position ^a	Inter-bp	Study and Sample	Cases	Controls	OR	z-value	P-value	P-value Heterogeneity
rs4658879	229809198		Present Study	782	839	1.21	2.087	0.037	
			Wood <i>et al.</i> (11)	311	291	1.00	0.000	1.000	
			Hennah <i>et al.</i> (7), Edinburgh	328	315	1.06	0.428	0.668	
			Hennah <i>et al.</i> (7), Helsinki	343	342	1.05	0.353	0.724	
			Hennah <i>et al.</i> (7), Aberdeen	256	255	1.22	1.256	0.209	
			Hennah <i>et al.</i> (7), London	348	351	1.08	0.538	0.591	
			Meta-analysis	2368	2393	1.12	2.126	0.033	
rs1572899	229892113	82915	Present study	782	839	1.08	0.858	0.391	
			Hennah <i>et al.</i> (7), Edinburgh	328	315	1.17	1.117	0.264	
			Hennah <i>et al.</i> (7), Helsinki	343	342	1.00	0.022	0.982	
			Hennah <i>et al.</i> (7), Aberdeen	256	255	1.35	1.856	0.063	
			Hennah <i>et al.</i> (7), London	348	351	1.06	0.402	0.688	
			Hodgkinson <i>et al.</i> (10)	196	217	1.46	2.127	0.033	
			Meta-analysis	2253	2319	1.13	2.353	0.019	
rs2812385	229930625	38512	Present study	782	839	1.04	0.447	0.655	
			Wood <i>et al.</i> (11)	311	291	1.04	0.256	0.798	
			Hennah <i>et al.</i> (7), Edinburgh	328	315	1.14	0.889	0.374	
			Hennah <i>et al.</i> (7), Helsinki	343	342	1.17	1.141	0.254	
			Hennah <i>et al.</i> (7), Aberdeen	256	255	1.46	2.343	0.019	
			Hennah <i>et al.</i> (7), London	348	351	1.08	0.571	0.568	
			Meta-analysis	2368	2393	1.12	2.097	0.036	
rs1538979	229963491	32866	Present study	782	839	1.14	1.421	0.155	
			Sullivan <i>et al.</i> (13)	738	733	1.12	1.195	0.232	
			Hennah <i>et al.</i> (7), Edinburgh	328	315	1.03	0.229	0.819	
			Hennah <i>et al.</i> (7), Helsinki	343	342	1.37	2.269	0.023	
			Hennah <i>et al.</i> (7), Aberdeen	256	255	1.29	1.588	0.112	
			Hennah <i>et al.</i> (7), London	348	351	1.06	0.398	0.691	
			Meta-analysis	2795	2835	1.04	2.860	0.004	
rs16854957	229979516	16025	Present study	782	839	0.85	0.171	0.864	
			Wood <i>et al.</i> (11)	311	291	0.79	0.330	0.741	
			Sullivan <i>et al.</i> (13)	738	733	0.84	0.066	0.947	
			Hennah <i>et al.</i> (7), Edinburgh	328	315	0.98	2.075	0.038	
			Hennah <i>et al.</i> (7), Helsinki	343	342	0.84	0.707	0.480	
			Hennah <i>et al.</i> (7), Aberdeen	256	255	0.93	2.394	0.017	
			Hennah <i>et al.</i> (7), London	348	351	0.96	1.677	0.093	
Meta-analysis	3106	3126	1.22	2.368	0.017	0.247			
rs17817356	229986844	7328	Present study	782	839	0.86	0.254	0.800	
			Sullivan <i>et al.</i> (13)	738	733	0.93	1.137	0.256	
			Hennah <i>et al.</i> (7), Edinburgh	328	315	0.77	0.166	0.868	
			Hennah <i>et al.</i> (7), Helsinki	343	342	0.98	2.137	0.033	
			Hennah <i>et al.</i> (7), Aberdeen	256	255	0.82	3.157	0.002	
			Hennah <i>et al.</i> (7), London	348	351	0.94	1.534	0.125	
			Meta-analysis	2795	2835	1.27	3.007	0.002	
rs1322784	229995558	8714	Present study	782	839	0.92	1.012	0.311	
			Sanders <i>et al.</i> (12)	1870	2002	0.93	0.714	0.475	
			Hennah <i>et al.</i> (7), - Edinburgh	328	315	0.81	0.492	0.623	
			Hennah <i>et al.</i> (7), Helsinki	343	342	0.96	1.638	0.101	
			Hennah <i>et al.</i> (7), Aberdeen	256	255	0.80	0.576	0.565	
			Hennah <i>et al.</i> (7), London	348	351	0.91	1.260	0.208	
			Hodgkinson <i>et al.</i> (10)	196	217	0.97	1.800	0.072	
Meta-analysis	4123	4321	1.19	2.430	0.015	0.716			
rs860275	230102768	107210	Present study	782	839	1.00	2.004	0.045	
			Wood <i>et al.</i> (11)	311	291	0.79	0.331	0.741	
			Hennah <i>et al.</i> (7), Edinburgh	328	315	0.85	0.840	0.401	
			Hennah <i>et al.</i> (7), Helsinki	343	342	0.93	1.438	0.151	
			Hennah <i>et al.</i> (7), Aberdeen	256	255	0.93	1.519	0.129	
			Hennah <i>et al.</i> (7), London	348	351	0.84	0.674	0.501	
			Meta-analysis	2368	2393	1.29	2.896	0.004	
rs7541019	230117808	15040	Present study	782	839	0.93	1.208	0.227	
			Sullivan <i>et al.</i> (13)	738	733	0.90	0.837	0.403	
			Hennah <i>et al.</i> (7), Edinburgh	328	315	0.94	1.523	0.128	
			Hennah <i>et al.</i> (7), Helsinki	343	342	0.82	0.572	0.568	
			Hennah <i>et al.</i> (7), Aberdeen	256	255	0.86	1.004	0.315	
			Hennah <i>et al.</i> (7), London	348	351	0.81	0.383	0.702	
			Meta-analysis	2795	2835	1.22	2.227	0.025	
rs2038636	230121872	4064	Present study	782	839	0.95	1.361	0.174	
			Wood <i>et al.</i> (11)	311	291	0.87	0.997	0.319	

Continued

Table 3. Continued

SNP	Position ^a	Inter-bp	Study and Sample	Cases	Controls	OR	z-value	P-value	P-value Heterogeneity
rs821577	230133680	11808	Sanders <i>et al.</i> (12)	1870	2002	0.96	1.264	0.206	0.838
			Meta-analysis	2963	3132	1.20	2.023	0.043	
			Present study	782	839	0.92	1.066	0.286	0.988
			Sullivan <i>et al.</i> (13)	738	733	0.94	1.254	0.210	
			Hennah <i>et al.</i> (7), Edinburgh	328	315	0.80	0.373	0.709	
			Hennah <i>et al.</i> (7), Helsinki	343	342	0.91	1.303	0.193	
			Hennah <i>et al.</i> (7), Aberdeen	256	255	0.85	0.982	0.326	
			Hennah <i>et al.</i> (7), London	348	351	0.82	0.556	0.578	
			Meta-analysis	2795	2835	1.23	2.285	0.022	

In bold results with significant disease association across samples are presented (P -values association < 0.05 , P -values heterogeneity > 0.05) using the weighted Z-score method (Comprehensive Meta Analysis v. 2)

^aSNP marker positions according to NCBI Build 36.

(at other loci) may be involved in this two-marker interaction and they show sex differences between the populations. Finally, we cannot exclude the possibility that our interplay findings represent type-I errors, although they (*ad hoc*) confirm both of the previously reported *DISC* effects (rs1538979–rs821633 interplay and protective effect of rs821633). We therefore recommend that the potential epistasis between both variants be an a priori consideration in all future schizophrenia *DISC1* association studies.

Phenotype–genotype results

Our analysis points to a gender-specific *DISC* contribution in schizophrenia. This is supported by most other *DISC* studies that stratified their samples according to gender (7,14–16). The results are also in accordance with clinical findings, where age of onset, premorbid functioning, symptomatic characteristics and course of illness are well-established gender differences (17). Furthermore, the present phenotype–genotype analysis suggests that the identified *DISC* intron 9 interval contributes most strongly to EAO in females with schizophrenia. The finding is particularly consistent with the expectation that genetic effects should be more pronounced in patients with an earlier phenotypic expression. Unfortunately, we could not use a phenotypically more homogeneous sample within our meta-analysis, because the raw data necessary for a detailed stratification (e. g. for gender) were not available for most of the included studies (see Materials and Methods). Despite this limitation, the comparatively large size of this sample provided sufficient power to detect more common risk loci at the *DISC* locus that contribute to schizophrenia across European populations.

Conclusion

Our results concur with previous *DISC* findings and point to a complex genetic architecture underlying schizophrenia at this locus, including the presence of private mutations as observed in the initially reported ‘breakpoint family’. To address this complexity, we applied two different, but complementary approaches. We identified a circumscribed risk interval in *DISC1* intron 9 by using a sample that might better represent greater etiological homogeneity (central European population,

gender-stratification, EAO). In addition, we identified a risk interval in the *DISC1* intron 4–6 across European populations by performing a meta-analysis on a more heterogeneous but maximally sized sample. Both approaches are recommended when studying complex genetic disorders in general, and both led to the successful identification of two *DISC* risk intervals in the present study. On the basis of our results, future research to identify the underlying *DISC* risk variants at both loci can be conducted, with the ultimate goal of increasing our understanding of the etiology of schizophrenia.

MATERIALS AND METHODS

SNP marker selection

We genotyped 121 SNP markers, tagging all haplotypes at a frequency $> 1\%$ in the CEPH Utah (CEU) population using HapMap phase I+II data (HapMap release #20). Haplotype blocks were defined by the algorithm developed by Gabriel *et al.* (9) implemented in HaploView (v. 3.32) using the default settings. The selected SNPs covered 507 660 bp of the *DISC* locus (Supplementary Material, Table S2 and Fig. S1). The boundaries of the analyzed region ranged from 229 736 450 to 230 244 110 bp and the average inter-marker distance was 4195 bp.

Sample characteristics and diagnostic assessment

All 121 SNP markers were genotyped in a sample of 782 patients with schizophrenia (354 females, 428 males) and 839 controls (358 females, 481 males). All individuals were of German descent and were recruited in the local areas around the cities of Bonn and Mannheim (central western part of Germany). The genetic homogeneity of the sample was also determined using Structure v. 2.2 (18,19). (see Supplementary Material). The diagnostic assessment of patients was done using a best estimate approach and IPGS, a comprehensive inventory for phenotype characterization (20). This included a SCID-I Interview for Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) and the OPCRIT system, as well as a review of medical records (21,22). All control subjects were screened for psychiatric disorders (see Supplementary Material).

SNP marker genotyping and association statistics

Genotyping was performed using Golden Gate Assay (Illumina, San Diego, CA, USA). Genotype call rate was > 98% for all markers, and there were no replicate errors among the 2% of the samples genotyped in duplicate. Departure from Hardy–Weinberg equilibrium (HWE), single-marker and multi-marker haplotype association analyses were performed using PLINK software (<http://pngu.mgh.harvard.edu/~purcell/plink/index.shtml>) (23). SNPs showing significant deviation from HWE are listed in Supplementary Material, Table S7. To prevent type-I association errors, we also used PLINK to perform 10 000 permutations of each of the markers and samples studied [all, female, male, and using specific phenotypic characteristics (see below)]. However, PLINK only allows permutation results from single-marker association analyses. The results achieved by the multi-marker analyses were not permuted and are, thus, uncorrected. We also performed a power analysis of our samples using Genetic Power Calculator (<http://pngu.mgh.harvard.edu/~purcell/gpc>) (24). Under two different assumptions on the genotypic relative risk (GRR 1.25 and 1.5), the statistical power of the entire sample was between 0.53 and 0.96 [between 0.26 and 0.67 for the female sub-sample and between 0.32 and 0.78 for the male sub-sample (80% power, alpha 0.05, allelic test (df 1), risk allele frequency 0.10, disease prevalence 0.015)].

Haplotype definition and marker imputation

To obtain the most precise estimate of locus-specific genomic architecture, we used the genotypes of all 121 analyzed markers in our sample of 1621 individuals (cases and controls) and imputed genotypes of 435 additional SNPs using the CEU data released by the most updated phase of HapMap (HapMap release #22), as well as a Markov-chain approach as implemented by MACH (25). The haplotype block structure was defined according to Gabriel *et al.* (9) using all 556 markers and HaploView (v. 4.0 beta 14).

Detailed phenotype–genotype analysis

Given that some previous studies found evidence of a gender-specific *DISC* association in their schizophrenia samples (4) (Supplementary Material, Table S1), we further stratified our sample according to gender. In addition, we stratified our patient sample for specific phenotypic characteristics that might represent greater etiological homogeneity. Patients were stratified based on FAM in first- and second-degree relatives [FAM, $n = 131$ ($n = 67$ females, $n = 64$ males)], lifetime history of depressive symptoms [DEP, $n = 232$ ($n = 127$ females, $n = 105$ males)] and EAO [EAO < 21 years, $n = 194$ ($n = 81$ females, $n = 113$ males)]; for additional details on defining characteristics, see Supplementary Material. Supplementary Material, Table S8 provides a detailed overview of the composition of the stratified sample.

SNP marker interplay

For the locus-specific interaction, we used the marker–marker interplay approach reported by Hennah *et al.* (7). At the two SNPs most implicated in their study (7)—rs1538979 and

rs821633—we stratified our cases and controls by grouping homo- and heterozygote carriers of the same alleles together. Using the allele-stratified samples at the conditional marker, we tested for an association at the other reported interplay SNP. Phenotypic strata were not used for the interplay tests. The sample sizes after stratifying for allele carriers and phenotypic characteristics appeared to be too small for a powerful association.

Meta-analysis across samples and studies

For the meta-analysis, we included all studies that used a gene-based association approach (defined by a marker set of more than five SNPs) and that were performed on independent case–control samples with a broad definition of European ancestry. Markers were only selected, if they had been analyzed in at least three of the included studies and showed the same allele tendencies in cases and controls across all samples. In total, we tested 50 markers equally distributed across the *DISC* locus (average inter-marker distance of 8.502 bp). The sample sizes analyzed per SNP ranged between 3.694 and 10.064 individuals.

For the association statistics, we applied a weighted Z-score method using Comprehensive Meta Analysis v. 2 (<http://www.meta-analysis.com/pages/comparisons.html>). This program also allows the detection of possible publication bias effects by performing a funnel-plot analysis, as well as an estimate of between-group heterogeneity by performing a heterogeneity test. A meta-analysis based on allelic and/or genotypic data or using information on gender could not be applied, because the raw data necessary for this test statistic were not available for most of the included samples and studies. The *P*-values for each marker and sample association were downloaded from the Schizophrenia Research Forum database (<http://www.schizophreniaforum.org>) (10–12) or, if not available on the website, results were obtained by contacting the groups personally (7,13).

SUPPLEMENTARY MATERIAL

Supplementary Material is available at *HMG* online.

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Conflict of Interest statement. None declared.

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