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ORIGINAL ARTICLE

Association of *DISC1* with autism and Asperger syndrome

H Kilpinen[1], T Ylisaukko-oja[1,2], W Hennah[1], OM Palo[1], T Varilo[1,2], R Vanhala[3], T Nieminen-von Wendt[3,4], L von Wendt[3], T Paunio[1,5] and L Peltonen[1,2,6]

[1]*Department of Molecular Medicine, National Public Health Institute, Biomedicum, Helsinki, Finland;* [2]*Department of Medical Genetics, University of Helsinki, Biomedicum, Helsinki, Finland;* [3]*Unit of Child Neurology, Hospital for Children and Adolescents, Helsinki, Finland;* [4]*Department of Adolescent Psychiatry, Jorvi Hospital, Espoo, Finland;* [5]*Department of Psychiatry, Helsinki University Central Hospital, Helsinki, Finland and* [6]*Program in Medical and Population Genetics, The Broad Institute, MIT and Harvard University, Cambridge, MA, USA*

The *DISC1* gene at 1q42 has generated considerable interest in various psychiatric diseases, since a balanced translocation interrupting the gene was found to cosegregate with schizophrenia and related mental illnesses in a large Scottish pedigree. To date, linkage and association findings to this locus have been replicated in several study samples ascertained for psychotic disorders. However, the biological function of *DISC1* in neuronal development would suggest a potential role for this gene also in other, early onset neuropsychiatric disorders. Here we have addressed the allelic diversity of the *DISC1*, *DISC2* and *TRAX* genes, clustered in 1q42, in Finnish families ascertained for infantile autism (97 families, $n_{affected}=138$) and Asperger syndrome (29 families, $n_{affected}=143$). We established association between autism and a *DISC1* intragenic microsatellite (D1S2709; $P=0.004$). In addition, evidence for association to Asperger syndrome was observed with an intragenic single nucleotide polymorphism (SNP) of *DISC1* (rs1322784; $P=0.0058$), as well as with a three-SNP haplotype ($P=0.0013$) overlapping the HEP3 haplotype, that was previously observed to associate with schizophrenia in Finnish families. The strongest associations were obtained with broad diagnostic categories for both disorders and with affected males only, in agreement with the previous sex-dependent effects reported for *DISC1*. These results would further support the involvement of *DISC1* gene also in the etiopathogenesis of early onset neuropsychiatric disorders.
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Introduction

Autism spectrum disorders (ASD (MIM 209850)) affect at least 0.1–0.6% of children.[1–3] The most common disorders of this broad group, known also as pervasive developmental disorders (PDDs), are infantile autism and Asperger syndrome (AS). The etiology of both conditions has remained unknown, but they are generally considered multifactorial diseases with a strong genetic component involving several predisposing genes. Concordance rates observed in twin studies suggest distinct heritability for infantile autism, whereas for AS, no systematic twin studies have been performed. In infantile autism, concordance rates vary from 69–98% among monozygotic twins to 0–30% in dizygotic twins,[4–6] resulting in the high-heritability estimate of over 90%.[7] For AS, most descriptions of familial transmission have been reports of a few families or single cases, creating challenges in the estimation of the extent of genetic effects. A consistent feature for both infantile autism and AS is that males have a remarkably higher prevalence than females with a ratio of approximately 3:1.[1,8] As a distinction from AS, approximately 75–80% of children with infantile autism suffer from severe mental retardation and 25–30% from epilepsy.[9,10]

According to the International Classification of Diseases (ICD-10, World Health Organization), infantile autism is characterized by qualitative impairment or delayed development in reciprocal social interaction, verbal and nonverbal communication and behavioral skills before the age of three. As the milder form, AS shares the basic clinical features of infantile autism, but individuals with AS lack major cognitive deficiencies and have fairly normal language development and average basic verbal skills (for communication). Typical features for AS include difficulties in socialization, one-sided way of communication, dependence on routines and rituals,

Correspondence: Dr L Peltonen, Department of Molecular Medicine, National Public Health Institution, Biomedicum, Haartmaninkatu 8, Helsinki 00251, Finland.
E-mail: leena.peltonen@ktl.fi
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unusual patterns of interest and formal and pedantic speech.[11] AS is usually recognized after the age of three years.[12]

To date, linkage and association findings between psychotic disorders and the *DISC1* (Disrupted in Schizophrenia 1) gene at the 1q42 locus have been replicated in several independent studies across populations. The initial Scottish finding[13] was supported by the linkage of *DISC1* intragenic markers to schizophrenia in a population-based nationwide sample of Finnish families ascertained for schizophrenia.[14,15] Furthermore, a single nucleotide polymorphism (SNP) haplotype (HEP3) of *DISC1* demonstrated family-based association to schizophrenia when the Finnish population frequency was taken into account, and this same allelic haplotype was later shown to associate also with impaired visual working memory functions in schizophrenia, predominantly in males.[16,17] SNPs defining the HEP3 haplotype (rs751229 and rs3738401) and its vicinity have since been found to associate not only with schizophrenia[18–23] but also with bipolar disorder (BPD), schizoaffective disorder[20,21,23] and major depression,[24] as well as with endophenotypes defined using neurocognitive and neuronal imaging measurements.[18,19,25,26]

Besides *DISC1*, the 1q42 locus also contains *TRAX* (translin-associated factor X; also known as *TSNAX*) and *DISC2*, which is not known to encode a protein, but instead is thought to regulate *DISC1* expression through its transcript.[27–29] Analysis of the function of DISC1 binding partners has implicated DISC1, for example, in neurite outgrowth, neuronal migration, synaptogenesis, glutamatergic neurotransmission and cAMP signaling[30–36] and led to the hypothesis of *DISC1* being a critical molecule in neuronal development. The discovery of *DISC1* interacting genes has provided valuable insight to the possible molecular mechanisms leading to cognitive and affective dysfunctions of the human brain.

Due to (i) established biological functions of the DISC1 protein, (ii) the versatility of the neuropsychiatric conditions found to be related to *DISC1*, and (iii) shared neurocognitive defects between schizophrenia and ASDs such as impaired executive function and social functioning,[37,38] we wanted to study if the gene would also play a role in the etiology of infantile autism and AS. Bearing in mind the history of the isolated Finnish population with a previous established association of *DISC1* with schizophrenia, the probability of finding a common genetic variant for these disorders in these study samples might be higher compared to study samples with mixed backgrounds. Interestingly, we have observed some genealogical links between Finnish ASD and schizophrenia families (unpublished data). Thus, we hypothesize that *DISC1* might be involved in various neurobiological processes, which, if disturbed, could lead to several slightly differing and even overlapping phenotypes such as ASDs and schizophrenia.

Materials and methods

Study samples

The families in this study were recruited through Finnish university and central hospitals. All diagnoses were assessed according to detailed structured interviews based on the ICD-10 (World Health Organization 1993) and DSM-IV (American Psychiatric Association 1994) criteria for the disorders in question. Additional diagnostic information was routinely collected using instruments such as the Childhood Autism Rating Scale, Asperger syndrome screening questionnaire,[8,39,40] Asperger syndrome diagnostic interview[41] and the criteria proposed by Gillberg *et al*.[8,40] Thorough medical and clinical examinations were performed for all of the study subjects, including neurological examinations and psychological and neuropsychological evaluations. The ascertainment of diagnoses was carried out by multidisciplinary teams with extensive experience and common training, using the same set of diagnostic instruments, as described elsewhere.[42–44] These studies have been approved by relevant ethical committees and informed written consent was received from all the participating families.

The autism study sample consists 97 Finnish families with 138 affected individuals diagnosed with infantile autism, AS or developmental dysphasia. Only families with at least one child with autism were included in the study, while families with associated medical conditions such as fragile X syndrome or profound mental retardation were excluded. Individuals with AS ($n=8$) and developmental dysphasia ($n=12$) were included in this study sample because approximately one-third of the autistic probands had a first-degree relative with these conditions. Because of our original hypothesis of *DISC1* being involved in a wide range of neurological processes, we included all individuals affected either with infantile autism, AS or developmental dysphasia in our analyses instead of narrowing the diagnosis only to infantile autism. Altogether, 118 individuals with infantile autism, 8 with AS and 12 with developmental dysphasia were included. Interestingly, three individuals had an overlapping diagnosis of schizophrenia.

The AS sample, with no overlap with the autism sample, contains 29 large Finnish pedigrees, which contain only AS cases in multiple subsequent generations. Only individuals with normal cognitive development before the age of 3 were included in the study. The study sample is an extension from the 17 multiplex families reported originally by Ylisaukko-oja *et al*.[42] The addition of 12 new families, described by Rehnström *et al*.,[43] raised the total number of affected individuals to 143. Altogether 119 individuals fulfilled the ICD-10 criteria for AS. Additional 24 individuals had AS-like features but did not completely meet all of the criteria. As with the autism study sample, we included both of these diagnostic groups as a broad AS phenotype with a total of 143 affected individuals. Five of these had an

overlapping diagnosis of schizophrenia and one also of BPD. The overlapping diagnoses of schizophrenia and BPD in both the autism and AS samples were given according to patient records, based on the DSM-IV criteria. The whole-study samples were not systematically screened for these two disorders.

In addition, all markers were analyzed in a special extended pedigree from Central Finland consisting of 18 Finnish families, which have been genealogically traced back to the 17th century and found to originate from common ancestors. The pedigree was originally described by Auranen *et al.*,[45] after which six new families have been added and more detailed genealogical links established (Ylisaukko-oja and Kilpinen *et al.*, unpublished data). Altogether, the pedigree includes 33 affected individuals ($n_{\text{aff.males}} = 24$, $n_{\text{aff.females}} = 9$), of which 16 are diagnosed with infantile autism, 14 with AS, 1 with developmental dysphasia and 2 with PDD not otherwise specified. Of the families, 15 are included in the autism sample, 2 in the AS sample, 1 in both and 1 in neither. The advantages of using population isolates in genetic mapping are well recognized. Based on the tendency of the affected individuals to share ancestral haplotypes derived from a small number of founders, a lower degree of genetic heterogeneity can be expected.[46] The study samples are summarized in Figure 1.

To properly control for the diversity of background alleles in different regional study samples, independent regional controls ($n = 93$) were collected from the same geographical area where the families in the extended pedigree originate. Randomly selected trios ($n = 57$) representative of the Finnish population were used as a population-wide control sample (all Finland).

Laboratory methods and statistical analyses

DNA was extracted from ethylenediamine-tetraacetic acid-treated blood according to standard procedure.[47]

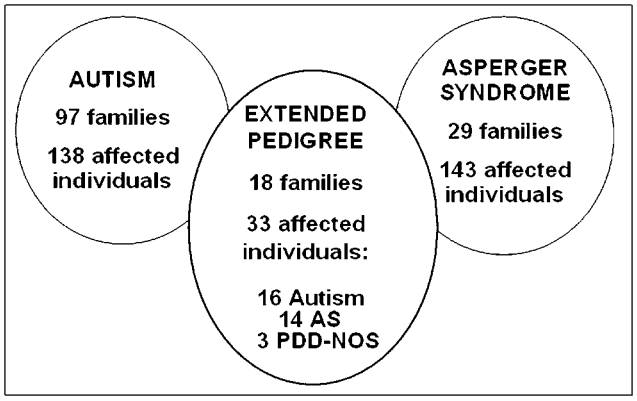

Figure 1 A description of the study samples used, showing the number of families and affected individuals in each category. In the extended pedigree, AS indicates Asperger syndrome and PDD-NOS indicates pervasive developmental disorder not otherwise specified.

Eleven SNP markers spanning *DISC1*, *DISC2* and *TRAX* (see Figure 2)[17,21] were genotyped with the homogenous MassEXTEND (hME) technology using the MassARRAY Platform (Sequenom Inc., San Diego, CA, USA), as specified by manufacturer's instructions. Also, two microsatellite markers from the same region, D1S251 and D1S2709 (Figure 2), were genotyped with the ABI 3730 DNA sequencer and analyzed with GeneMapper v.3.0 software (Applera Corporation, Norwalk, CT, USA).[14,15] Genotypes were checked for correct Mendelian transmission using PEDCHECK v.1.1[48] and monitored for Hardy–Weinberg equilibrium. All markers accepted for analysis displayed a minimum genotyping success rate of 92%, with the majority of markers having a success rate of over 98%. The borderline for the minor allele frequency of SNP markers was 5%.

Both single-marker and haplotype association analyses were performed using FBAT 1.5.5[49] and TRANSMIT 2.5.4;[50] these can test for transmission of a haplotype even when the parental genotypes are not complete and the phase is unknown. To account for any effects of linkage on the results, we used the empirical variance option of FBAT and performed 100 000 bootstrap replicates with TRANSMIT for all analyses. Both of these programs test for family-based association. Pseudomarker vs 0.9.7 beta software, a method ideal for the joint and separate analysis of association and linkage in family-based samples, was used to monitor single-marker association in the extended pedigree, since it is capable of handling different kinds of data and pedigree structures in the same analysis.[51] Pseudomarker was also used to monitor for two-point linkage in all study samples. Marker allele frequencies were estimated with DOWNFREQ 2.1 program from the genotypes of all the individuals in each study sample.[52] The degree of linkage disequilibrium (LD) between the SNPs and haplotype blocks was monitored with the Haploview v3.2 program.[53] In all analyses, individuals were treated only as affected or as unknown. Analyses were performed separately for the autism, and AS study samples and the Central Finland extended pedigree.

Results

We tested a total of 11 SNPs over the ~600 kb region of 1q42 with the *TRAX*, *DISC1* and *DISC2* genes. The LD patterns of the SNPs were monitored with the solid spine of LD option of Haploview ($D' > 0.8$). In the total study sample (autism and AS samples combined), four LD blocks were identified (Figure 3). Due to the previous sex-dependent association findings reported for *DISC1* and the overall higher prevalence of all ASDs in males, we performed statistical analyses also with affected males only (autism sample $n = 105$, AS sample $n = 85$), using the genotypes of females only for phase determination. An analysis with only affected females

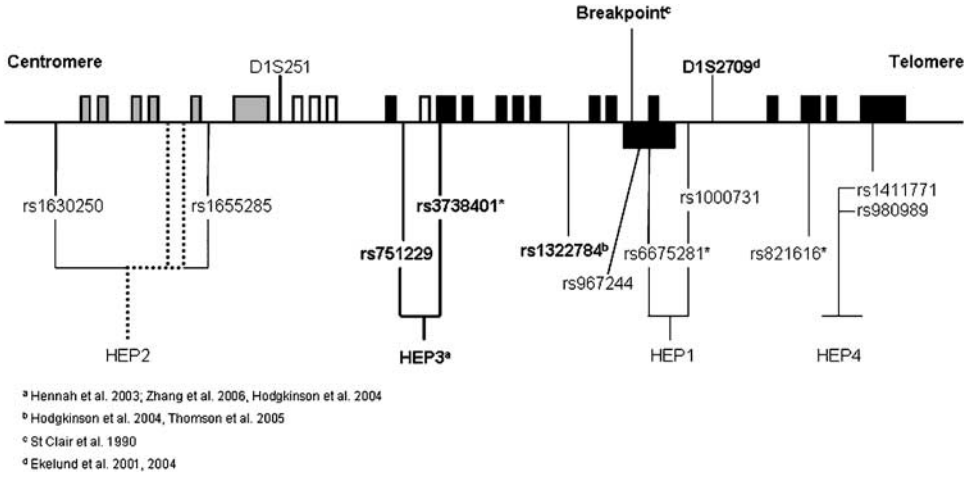

Figure 2 A diagram showing the exonic structure of the two *DISC* genes (black) and *TRAX* (gray). Intergenic exons are marked in white. The markers used in this study are shown in relation to the exons. The original location of the HEP2 haplotype is indicated with dotted lines, and the markers showing association in this study with bold. Non-synonymous single nucleotide polymorphisms (SNPs) are indicated with a star (*). Letters from a to d mark previous association findings (Figure modified from Hennah *et al*.[17]).

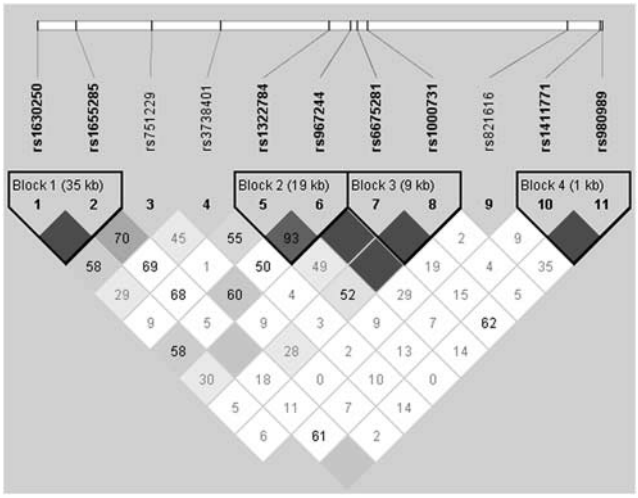

Figure 3 The linkage disequilibrium (LD) structure of the *TRAX/DISC* region in the total study sample according to Haploview (solid spine of LD, $D' > 0.8$; http://www.broad.mit.edu/mpg/haploview/). Dark gray indicates LOD > 2 and $D' = 1$, while lighter shades of gray represent lesser degree of LD. White indicates LOD < 2 and $D' < 1$.

was not considered due to the small number of affected females in the samples (autism sample $n = 33$, AS sample $n = 58$). No evidence of linkage to the *DISC1* locus was observed either in the combined study sample (autism + AS) or infantile autism or AS families separately (data not shown). In the AS families, however, some modest evidence for linkage emerged when only affected AS males were considered with both rs1000731 (Lod = 1.23) and rs821616 (Lod = 1.18) with a dominant mode of inheritance (dominant pseudomarker) (Table 1).

Association analysis

In the autism study sample, the only marker displaying family-based association was the intragenic multiallelic marker D1S2709 (TRANSMIT global $P = 0.022$, FBAT $P = 0.010$). When only affected males were considered, TRANSMIT yielded a global $P = 0.019$ and FBAT $P = 0.004$ for this marker. In the AS study sample, no evidence of single-marker association was seen. However, when only affected AS males were considered, the SNP rs1322784 displayed family-based association using both TRANSMIT (global $P = 0.0058$) and FBAT tests ($P = 0.0195$). When comparing allele frequencies for rs1322784, the major allele frequency was 0.83 in AS cases compared to 0.78 in the regional controls and 0.72 in the all Finland controls (Table 1). In the combined study sample, modest association was seen only with rs1411771 (FBAT $P = 0.042$; $P = 0.029$ males only) but this could not be detected with TRANSMIT.

Haplotype association analysis

To avoid problems with multiple testing, we restricted the haplotype association analyses to the four HEP haplotypes that showed association in the Finnish families ascertained for schizophrenia.[17] We tested the same haplotypes as in the original study, except in the case of HEP2 where rs1630250 and rs1655285 were used as surrogates for the haplotype information provided by the original haplotype consisting of rs1615344, rs1615409 and rs766288 (see Figure 2). We detected suggestive association with the HEP3 haplotype (rs751229 and rs3738401) in the AS study sample with TRANSMIT, but with HEP1, 2 and 4 no association evidence was observed. In the autism sample and in the combined sample, no evidence of association could be detected with any of the tested haplotypes (best *P*-values 0.135 and 0.107,

Table 1 Major allele frequencies and association analysis *P*-values for all the markers in *DISC1* genotyped in this study

Marker information			*Major allele frequencies*					*Association analysis* P-*values AUT sample*				*AS sample*			
Marker	*Major*	*Minor*	*AUT cases*	*AS cases*	*Extended pedigree cases*	*Regional controls*	*All Finland controls*	*FBAT all*	*Males only*	*TRANSMIT all*	*Males only*	*FBAT all*	*Males only*	*TRANSMIT all*	*Males only*
rs1630250	G	C	0.73	0.72	0.59	0.67	0.73	0.823	0.444	0.649	0.289	0.138	0.220	0.147	0.303
rs1655285	G	C	0.93	0.96	0.94	0.96	0.92	0.847	0.549	0.456	0.272	1.000	0.739	0.912	0.506
D1S251								0.897	0.609	0.849	0.691	0.205	0.196	0.268	0.107
rs751229	T	C	0.56	0.58	0.55	0.55	0.60	0.553	0.972	0.796	0.737	0.889	0.402	0.766	0.282
rs3738401	G	A	0.74	0.72	0.72	0.77	0.74	0.426	0.197	0.788	0.522	0.423	0.170	0.324	0.237
rs1322784	A	G	**0.83**	**0.83**	**0.98**	**0.78**	**0.72**	0.279	0.542	0.250	0.408	0.086	**0.0195**	0.160	**0.0058**
rs967244	A	G	0.82	0.81	0.90	0.83	0.83	0.453	0.705	0.405	0.821	0.346	0.718	0.295	0.415
rs6675281	C	T	0.89	0.89	0.95	0.88	0.88	0.724	0.945	0.867	0.986	0.175	0.564	0.226	0.455
rs1000731[a]	C	T	0.64	0.68	0.50	0.62	0.70	0.643	0.536	0.583	0.446	0.285	0.385	0.284	0.272
D1S2709								**0.010**	**0.004**	**0.022**	**0.019**	0.351	0.972	0.579	0.979
rs821616[b]	A	T	0.63	0.65	0.66	0.70	0.66	0.783	0.592	0.568	0.271	0.701	0.403	0.469	**0.038**
rs1411771	T	C	0.71	0.67	0.86	0.74	0.76	0.106	0.050	0.076	0.077	0.299	0.290	0.471	0.591
rs980989	G	T	0.79	0.73	0.89	0.78	0.77	0.267	0.210	0.208	0.282	0.541	0.403	0.609	0.415

Abbreviations: AS, Asperger; AUT, autism.
TRANSMIT *P*-values are 100 000 × bootstrap global *P*-values and FBAT *P*-values obtained with the empirical variance option to account for effects of linkage. The markers displaying association in this study and all *P*-values < 0.05 are marked in bold.
[a]Linkage in the AS syndrome study sample; Lod = 1.23 (males only).
[b]Linkage in the AS syndrome study sample; Lod = 1.18 (males only).

respectively). In the AS sample, TRANSMIT yielded a global *P*-value = 0.030 for HEP3 (best allele combination TA; 17.6 observed transmissions, 12.7 expected transmissions). The same alleles were associated also when only affected AS males were used (TRANSMIT global $P = 0.015$; 13.5 observed, 9.0 expected). HBAT did not show association with HEP3 SNPs.

Next, we combined the HEP3 SNPs with neighboring SNPs in both directions to further define the extent of the potentially associated haplotype. In the complete AS sample, none of the combinations (rs1655285 + HEP3/HEP3 + rs1322784) appeared significant. In the AS males, however, the combination of the HEP3 SNPs plus the additional rs1322784 in the telomeric direction (HEP3 + 1; Table 2) yielded a global *P*-value of 0.0013 (TRANSMIT; best allele combination TAA; 13.4 observed, 9.0 expected). The HBAT analysis displayed a $P = 0.024$ with only two of these three SNPs, rs3738401 and rs1322784.

Extended pedigree

Finally, we analyzed all markers in the extended pedigree with multiple-affected individuals originating from an internal isolate in Central Finland.[45] Modest linkage was observed only with marker rs1630250 (Lod = 1.01; dominant and recessive pseudomarker). Due to the small number of informative transmissions in the pedigree, association was monitored only with pseudomarker. We employed the regional control data to get more reliable allele frequency estimates. When comparing allele frequencies in the extended pedigree cases ($n_{\text{affected}} = 33$, $n_{\text{aff.males}} = 24$, $n_{\text{aff.females}} = 9$), we noticed that the allele frequency for the SNP rs1322784 was 0.98 compared with the frequency of the regional controls of 0.78 and the Finnish average frequency of 0.72 (Table 1). In fact, all except one of the affected individuals in the extended pedigree were AA homozygotes for rs1322784. Thus, using Fisher's exact test, this deviation between these cases and both regional and all Finland controls appeared highly significant ($P = 9.3 \times 10^{-5}$ and $P = 9.89 \times 10^{-7}$, respectively). This deviation could also be detected with pseudomarker, which yielded $P = 0.002$ for the best SNP (rs1322784; LD + Linkage, recessive model). The most significant association was observed when calculating LD under the assumption of linkage, with a *P*-value of 0.0007 (LD | Linkage; recessive model). With the two microsatellite markers, modest association with D1S2709 was detected by pseudomarker ($P = 0.0376$, LD + Linkage and $P = 0.0260$, LD | Linkage; recessive model).

Discussion

DISC1 is one of the most interesting genes in psychiatric genetics today. In this study we analyzed

Table 2 Haplotype association analysis *P*-values for the haplotypes tested in this study

Haplotype	*SNP ID*	*AUT sample*				*AS syndrome sample*			
		HBAT all	*Males only*	*TRANSMIT all*	*Males only*	*HBAT all*	*Males only*	*TRANSMIT all*	*Males only*
Initial analysis									
HEP2	rs1630250 rs1655285	0.912	0.519	0.492	0.242	0.244	0.463	0.173	0.143
HEP3	rs751229 rs3738401	0.707	0.592	0.954	0.704	0.288	0.115	**0.030**[a]	**0.015**[a]
HEP1	rs6675281 rs1000731	0.805	0.668	0.796	0.851	0.299	0.573	0.229	0.416
HEP4	rs1411771 rs980989	0.263	0.135	0.242	0.159	0.553	0.571	0.819	0.449
Further characterization									
HEP3 −1	rs1655285 rs751229 rs3738401	x	x	x	x	0.578	0.212	0.308	0.143
HEP3 + 1	rs751229 rs3738401 rs1322784	x	x	x	x	0.197	0.670	0.110	**0.0013**[b]

Abbreviations: AS, Asperger; AUT, autism; SNP, single nucleotide polymorphism.
The haplotypes are presented in the genomic order. TRANSMIT *P*-values are 100 000 × bootstrap global *P*-values and HBAT *P*-values obtained with the empirical variance option to account for effects of linkage. The haplotypes displaying association in this study and all *P*-values < 0.05 are marked in bold. HEP3 + 1 and HEP3−1 were not tested in the autism sample (x), since no evidence for HEP3 was seen in the initial analysis. For a comparison of associating haplotypes in previous *DISC1* studies, see Hennah *et al*.[65]
[a]Alleles TA.
[b]Alleles TAA.

16 *DISC1* markers in Finnish ASD families and established association between autism and an intragenic marker, D1S2709, as well as between AS and an intragenic SNP, rs1322784, located ~101 kb apart from D1S2709. Best allelic association was observed in AS males with a three-SNP haplotype overlapping the previously associated HEP3 haplotype. Although earlier genetic studies have demonstrated association of *DISC1* with schizophrenia-related psychotic disorders and traits, already the original Scottish translocation finding showed that the disruption of *DISC1* was not associated with schizophrenia alone. This well-known pedigree contained individuals with a spectrum of psychiatric diagnoses, such as recurrent major depression and BPD, but also milder phenotypes such as adolescent conduct disorder, anxiety and minor depression.[27] Furthermore, Sachs *et al*.[23] reported a frameshift mutation in *DISC1* in an American pedigree with eight individuals diagnosed with various forms of schizophrenia and major depression. Interestingly, the pedigree also contained two individuals with a possible ASD, two others with mental retardation and one with both of these disorders. This finding was later challenged by Green *et al*.,[54] who did not identify the mutation in any of their schizophrenia cases ($n = 655$), but instead in two of their controls ($n = 694$). Recent linkage and association findings concerning *DISC1* have been reported within schizoaffective, BPD and major depression study samples, in addition to schizophrenia. Traditionally, for example, schizophrenia and BPD have been considered to be separate entities, but it has been suggested that patients having multiple symptoms of both disorders are often given the hybrid diagnosis of schizoaffective disorder.[55] Considering the well-recognized difficulties in diagnostics of mental disorders, it seems reasonable to assume that *DISC1* might be involved in various neurobiological processes, which, if disturbed, could lead to several slightly differing and even overlapping phenotypes. The association of *DISC1* with deficits in learning and memory functions[16,18,19,25,26] would provide a suggestive clue for mechanisms through which *DISC1* affects cognitive functioning in such phenotypes. These traits are an integral part of schizophrenia, and evidence exists of their abnormal function in infantile autism and AS as well.[56,57] This would be consistent with the neurodevelopmental hypothesis associated with *DISC1* functioning.

Even though the diagnoses of ASDs and schizophrenia exclude each other, according to the official diagnostic criteria (ICD-10 and DSM-1V) especially childhood-onset schizophrenia greatly resembles ASDs. Infantile autism has remained more or less a separate entity, but the overlap of AS and schizophrenia spectrum diagnoses is repeatedly reported, in particular with AS and schizotypal-,[58] schizoid- and paranoid personality disorders.[59] PDDs in adulthood often fulfil criteria for schizotypal personality disorder[60] and there is evidence that children diagnosed with schizophrenia often have an early history indicating an ASD.[61] Especially persons with AS seem to have an increased risk of BPD, depression and schizophrenia spectrum disorders,[62,63] which further indicates possible shared etiological factors. In this study, three individuals diagnosed with infantile autism and five individuals with AS had a previous clinical diagnosis of schizophrenia. One of these individuals was diagnosed with both schizophrenia and BPD. Interestingly, in our earlier genome-wide linkage studies, we have identified overlapping loci for ASDs and schizophrenia on 1q21–23.[42,44,64] Also, the previous *DISC1* association to schizophrenia in the Finnish family sample[17] was obtained with a broad diagnostic category involving schizoaffective disorder, other schizophrenia spectrum diagnoses and bipolar- or major depressive disorders. Therefore, it seems encouraging to establish association to *DISC1* in the Finnish ASD study samples, especially with some of the ASD families having genealogical links to Finnish schizophrenia families. Our suggestive findings, with most of the association evidence emerging from affected males, are in line with previous association findings to this locus, and would further emphasize the wide range of neurological effects of *DISC1*. Based on the striking prevalence difference of ASDs in males and females, it is reasonable to assume that at least some of the predisposing genetic factors are sex specific.

The association signal for the location including the HEP3 SNPs originates from a 160 kb region delimited by markers rs751229 and rs1322784. Weak linkage was observed at the 3′ end of the gene. Similar association results have been reported by Zhang *et al*.,[22] who found association to schizophrenia with a haplotype including the HEP3 SNPs and a third SNP located further along *DISC1* in the area preceding rs1322784 in this study (Figure 2). Hodgkinson *et al*.[20] have also reported association to schizophrenia with rs1322784, as well as association to schizoaffective disorder with the HEP3 haplotype. Thomson *et al*.[21] could not see association precisely with HEP3, but instead found association to BPD and schizophrenia with haplotypes including rs1322784, as well as with haplotypes including the HEP3 SNPs. A recent study in Finland found overtransmission of the same HEP3 haplotype as Hennah *et al*.[17] to males affected with psychotic disorder as well as a haplotype in the 3′ end of *DISC1* associated with bipolar spectrum disorder (Palo *et al*., unpublished data). Associations to the 3′ end have been reported also in other studies.[17,18,21,23,25] Thus, with most findings localizing in two distinct regions of *DISC1*, the actual risk allele(s) remains unidentified (for a review on *DISC1* findings see Hennah *et al*.[65]). Instead of a single narrow diagnosis, our association signal seems to emerge from a broad diagnostic category of ASDs, affected males in particular. It should be noted that most affected individuals in both autism and AS study samples are males.

The observation that all except one of the affected individuals in the extended pedigree from an internal

isolate are AA homozygotes for the best SNP of this study (rs1322784) is highly interesting, and might indicate enrichment of a specific risk allele of *DISC1* in this unique isolate sample with a restricted number of founders. According to the allele frequencies of the rest of the SNPs in the extended pedigree (Table 1) and corresponding frequencies in the HapMap (CEU), no such homozygosity as with rs1322784 can be seen in the region (lowest minor allele frequencies 0.05 and 0.11, respectively). Also, in our recent 317k SNP scan using Finnish DNA samples, no traces of higher homozygosity within the *DISC1* region were observed (Rehnström *et al.*, unpublished data). Because of the small size of our extended pedigree, the number of informative transmissions remains small, due to which association was monitored primarily with pseudomarker, which is able to combine family and case–control data in the same analysis.

Considering the general difficulties to replicate linkage and association findings within the field of complex genetics we realize that these results must be interpreted with caution. Since our sample sizes are relatively small, these results require replication and further studies before they can be regarded as confirmed associations. However, we have made an effort to carefully harmonize the diagnostic criteria across all families and firmly establish the diagnosis, not only for infantile autism but also for AS. The exceptional structure and size of our AS pedigrees leads to the question whether or not this kind of complexity will bring about problems with the analysis programs used, resulting in an increased type I or type II error rate. However, the fact that our peak AS association colocalizes with the same HEP3 region of *DISC1* that has been replicated several times to date makes the finding highly interesting, especially with the associating alleles being the same that showed association both in Finnish schizophrenia and BPD study samples. Also, D1S2709, that shows association in the autism study sample, is the best marker in the Finnish schizophrenia linkage study on chromosome 1q.[14]

There has already been suggestions of the existence of a common '*DISC1* pathway', involving several *DISC*-interacting genes such as *NDE1* and *PDE4B*.[35,36,66] Such a pathway would appear to contribute to common cellular signaling mechanisms affecting brain development and function,[67] thus providing a biological link between major mental illnesses. Interestingly, *DISC1* can also be connected to ASDs via one of the genes encoding a *DISC1* interacting protein, MAP1A.[34] MAP1A physically interacts with DLG4 (alias PSD95),[68] that is known to interact with neuroligin genes.[69–71] Neuroligins function as neuronal cell adhesion molecules essential in synaptogenesis,[71] and are known to be mutated in rare cases of autism or AS.[72,73]

As a conclusion, finding association to *DISC1* in all of our ASD study samples further supports the role of *DISC1* in a broad range of neurological processes capable of causing multiple psychiatric conditions when disrupted. This leads to the question whether also the other genes in the *DISC1* pathway could be involved in the etiology of these conditions.

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