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Genetic and Functional Analyses Identify *DISC1* as a Novel Callosal Agenesis Candidate Gene

Nathan Osbun[1], **Jiang Li**[1], **Mary C. O'Driscoll**[2], **Zoe Strominger**[1], **Mari Wakahiro**[1], **Eric Rider**[1], **Polina Bukshpun**[1], **Elena Boland**[2], **Cailyn H. Spurrell**[3], **Wendy Schackwitz**[3], **Len A. Pennacchio**[3], **William B. Dobyns**[4], **Graeme C.M. Black**[2,*], and **Elliott H. Sherr**[1,**]

[1]Department of Neurology, University of California San Francisco, San Francisco, California

[2]Genetic Medicine, University of Manchester, Manchester, UK

[3]U.S. Department of Energy Joint Genome Institute, Walnut Creek, California

[4]Division of Genetic Medicine, University of Washington, Seattle, Washington

Abstract

Agenesis of the corpus callosum (AgCC) is a congenital brain malformation that occurs in approximately 1:1,000–1:6,000 births. Several syndromes associated with AgCC have been traced to single gene mutations; however, the majority of AgCC causes remain unidentified. We investigated a mother and two children who all shared complete AgCC and a chromosomal deletion at 1q42. We fine mapped this deletion and show that it includes Disrupted-in-Schizophrenia 1 (*DISC1*), a gene implicated in schizophrenia and other psychiatric disorders. Furthermore, we report a de novo chromosomal deletion at 1q42.13 to q44, which includes *DISC1*, in another individual with AgCC. We resequenced *DISC1* in a cohort of 144 well-characterized AgCC individuals and identified 20 sequence changes, of which 4 are rare potentially pathogenic variants. Two of these variants were undetected in 768 control chromosomes. One of these is a splice site mutation at the 5' boundary of exon 11 that dramatically reduces full-length mRNA expression of *DISC1*, but not of shorter forms. We investigated the developmental expression of mouse *DISC1* and find that it is highly expressed in the embryonic corpus callosum at a critical time for callosal formation. Taken together our results suggest a significant role for *DISC1* in corpus callosum development.

Keywords

agenesis of the corpus callosum; schizophrenia; genetics; *DISC1*

[*]Correspondence to: Graeme C.M. Black, DPhil, FRCOphth, Genetic Medicine, Manchester Academic Health Science Centre, University of Manchester, Central Manchester University Hospitals NHS Foundation Trust, St. Mary's Hospital, Oxford Road, Manchester M13 9WL, UK. graeme.black@manchester.ac.uk. [**]Correspondence to: Elliott H. Sherr, M.D., Ph.D., Department of Neurology, University of California, San Francisco, 505 Parnassus Ave, Box 0114, San Francisco, CA 94143-0114. sherre@neuropeds.ucsf.edu.

Nathan Osbun and Jiang Li contributed equally to this work.

INTRODUCTION

Agenesis of the corpus callosum (AgCC), a congenital malformation resulting from a failure to develop the brain's largest cerebral commissure, occurs in approximately 1:1,000–1:6,000 individuals [Guillem et al., 2003; Wang et al., 2004; Glass et al., 2008]. While identifiable causes of AgCC are only known for an estimated 25% of cases [Paul et al., 2007], the etiology of AgCC is likely to contain a large as yet unidentified genetic component. Even the known genetic causes are variable, reflecting the complex and multigenic nature of callosal development. Patients with AgCC present with a wide variety of cognitive deficits that often fall within the autism spectrum [Badaruddin et al., 2007; Paul et al., 2007]. Volume based morphometry (VBM) analyses of the brain in autism consistently show a smaller corpus callosum [Boger-Megiddo et al., 2006; Just et al., 2007; Frazier and Hardan, 2009; Keary et al., 2009; Hardan et al., 2009], and recent studies of the most common copy number variant in autism, 16p11.2, show AgCC in a subset of these patients [Weiss et al., 2008; Bedoyan et al., 2010; Rosenfeld et al., 2010]. There may also be behavioral symptom overlap between AgCC and other neurodevelopmental disorders. Individuals with AgCC, similar to those with schizophrenia or autism, tend to exhibit concrete thinking, impaired social skills, lack of introspection, and poor social judgment [Paul et al., 2007]. Abnormal interhemispheric transfer has been proposed as an explanation for some symptoms within schizophrenia [David, 1994], and several manuscripts report identification of patients with AgCC in cohorts of schizophrenia screened for anatomic brain changes [David et al., 1993; Motomura et al., 2002; Chinnasamy et al., 2006; Hallak et al., 2007]. Moreover, we have shown that AgCC patients have a reduction in the size and in the fractional anisotropy (FA) of the cingulum bundle, as compared to matched controls [Nakata et al., 2009a]. Impairment of the cingulum bundle has also been implicated in schizophrenia (with a similarly reduced size and FA), suggesting a possible mechanistic overlap between a disorder grouped by its most visible anatomic change(AgCC) and one defined by its constellation of clinical symptoms (schizophrenia) [Kubicki et al., 2005; Nestor et al., 2008].

Millar et al.[2000] originally reported a large Scottish family with a balanced translocation with one chromosomal breakpoint at 1q42.1, which disrupts a gene eventually termed Disrupted-in-Schizophrenia 1 (*DISC1*). The majority of the family members with this translocation, and none without the translocation, presented with schizophrenia or related psychiatric disorders including schizoaffective disorder and bipolar disorder, although brain imaging for this family has not been reported. Since this report, additional studies have strengthened the argument that *DISC1* plays an important role in schizophrenia, and recent work has suggested that *DISC1* mutations disrupt neurite outgrowth and normal cerebral cortex development [Ozeki et al., 2003; Kamiya et al., 2005]. Furthermore, *DISC1* has been implicated as a candidate gene for autism [Kilpinen et al., 2008; Williams et al., 2009; Crepel et al., 2010]. The phenotypic similarities between patients with AgCC and those with autism reinforce the need to investigate *DISC1* as a potential candidate gene for callosal development as well.

Puthuran et al. [2005] described a mother and two children who all carried an interstitial 1q42 deletion and had AgCC. In this present study, we have performed further molecular analysis on the affected mother of the family using a genome-wide microarray and FISH to

define the boundaries and architecture of the deletion in more detail. In addition to this family, we report on another individual with AgCC and a de novo interstitial deletion that includes *DISC1*. We also sequenced all 13 exons of the full-length *DISC1* gene in a cohort of 144 individuals with MRI characterized AgCC and identified four potentially deleterious mutations, two of which were not found in a large control population (n = 768 chromosomes). Finally, we are the first to show that *DISC1* is highly expressed in the developing corpus callosum in embryonic mice. Taken together, these data suggest an important functional role for *DISC1* in callosal development.

MATERIALS AND METHODS

FISH and Microarray Analysis

Protocols from the BlueGnome Cytochip reference manual 0.9 were used throughout to label and purify patient DNA and reference DNA (female pooled, Promega, Madison, WI) (Invitrogen BioPrime labelling kit followed by GE Healthcare Autoseg G50 columns) and hybridized to version v1.1 CytoChip. Perkin Elmer Proscanarray captured images were processed using Blue Fuse v3.4. Clinical microarray analysis in the individual identified in Figure 1D was performed at Emory Genetics Laboratory on the EmArray 60K platform.

DISC1 Sequencing and Variant Genotyping

DNA was prepared from whole blood samples using the Puregene DNA extraction method according to standard protocols. Primers for *DISC1* exon sequencing were chosen using Primer3 software (sequences available upon request). Rare variant alleles were screened in controls using the Custom Taqman SNP Genotyping Assay (Applied Biosystems, Carlsbad, CA). The genotyping PCRs were run on a Dual GeneAmp PCR System 9700 and allelic discrimination was called with SDS 2.0 software on an ABI 7900 Analyzer. SNPs rs3738401 (R264Q), rs6675281 (L607F), and rs821616 (S704C) were genotyped in controls using the Sequenom iPLEX genotyping assay on the MassARRAY system. *P*-values for allele frequencies were determined using a two-tailed Fisher's exact test. Amino acid sequence alignments were created using the HomoloGene program (http://www.ncbi.nlm.nih.gov/homologene). DNA sequence alignments were created using the ClustalW2 program (http://www.ebi.ac.uk/Tools/clustalw2/index.html).

Real-Time PCR

Total RNA was isolated using the PAXgene Blood RNA Kit (Qiagen, Valencia, CA), and 1 μg total RNA was used for first-strand cDNA synthesis using the SuperScript First-Strand Synthesis System (Invitrogen, Carlsbad, CA). Real-time reverse transcriptase PCR was performed by iCycler (Bio-Rad, Hercules, CA) in a SYBR green I mix. PCR conditions were 95°C for 15 sec, 57°C for 30 sec and 72° C for 30 sec. The forward and reverse primer sequences used for *DISC1* exon 10 were AGCACCAGGAGACTGCCTATGAAA and AGT CAGCTT CCC ACA CTT TCC CAA; for exon 11 of *DISC1* Lv isoform the primers were TTG GGA AAGTGTGGG AAG CTG ACT and AGC AGC TCC CTC TAA GTC ATC CAT; for exon 11 of *DISC1* L isoform the primers were TTG GGA AAG TGT GGG AAG CTG ACT and CTC TTT CTG TTC ACC TCC AGC ACA G; for GAPDH the primers were TGC ACC ACC AAC TGC TTA GC and GGC ATG GAC TGT GGT CAT GAG. PCR

specificity was examined on a 2% agarose gel using 10 ml from each reaction and each sample was run in triplicate. Statistical analysis was performed using the $2^{-\Delta\Delta CT}$ method, as described by a previous protocol [Livak and Schmittgen, 2001].

Immunohistochemistry

Whole brains were fixed in 4% formalin solution and embedded in paraffin, and sections were cut at 5 mm. After dewaxing and hydration, endogenous peroxidase was quenched with 0.3% hydrogen peroxide in methanol for 20 min at room temperature. Rabbit polyclonal antibodies specific for *DISC1* (c-terminus) (Zymed, Carlsbad, CA) and for the neuronal marker PGP9.5 (Abcam, Cambridge, MA) were used at 1:200 dilution and expression was detected with the ABC kit (Vector Laboratories, Burlingame, CA) and 3,3'-diaminobenzidine (DAB) (Vector Laboratories). All slides were counterstained with cresyl violet.

RESULTS

Clinical Reports

Proposita 1650-0—This patient is now a 3-year-old female who was born at term but was small for her gestational age throughout the pregnancy. She is noted to have multiple cardiac abnormalities, including an atrial septal defect, two ventricular septal defects, and Wolff–Parkinson–White syndrome. Additionally, the patient was born with microcephaly and bilateral dislocated hips, and has severe gastro-esophageal reflux as well as grade IV vesico-ureteral reflux. EEG at 4 months of age appeared normal. MRI showed a thin and short corpus callosum with absent rostrum, minimal genu, and absent splenium.

Propositus 1148-0—This patient is now a 9-year-old male, born at term via cesarean indicated by hydrocephalus. A ventricular peritoneal shunt was placed shortly after birth. MRI revealed partial AgCC with possible polymicrogyria in the perisylvian regions. The patient also was noted to have right eye esotropia with nystagmus, and global hypotonia.

Propositus 1132-0—This patient is now an 8-year-old male with craniofacial dysmorphisms (including hypertelorism, frontal bossing, and simplified low set helices), global developmental delay, and is non-verbal. He was noted to have infantile spasms and more recently has been evaluated for possible absence seizures. He has severe chronic constipation requiring frequent enemas, kyphoscoliosis, and short stature. Imaging studies show partial AgCC with only the superior genu and the anterior body of the corpus callosum present, inferior placement of the posterior pituitary, bilateral perisylvian polymicrogyria, and a sphenoid encephalocele.

Propositus 1294-0—This patient is now a 17-year-old male born to a 28-year-old mother. He was noted to have testicular hydrocele at birth. He has ataxia and developed partial-complex and absence seizures at age 9. His full-scale IQ is 73 on the WISC-III. MRI studies revealed isolated AgCC with no other remarkable findings.

Propositus 1145-0—This patient is now a 9-year-old male with no prenatal complications, although he had respiratory distress requiring resuscitation after delivery. He demonstrated left arm paralysis and moderate developmental delays with walking at 20 months, crawling at 10–11 months, and speaking after 1 year. Chromosome analysis including subtelomeric FISH revealed no deletions, duplications, or translocations. MRI showed isolated partial AgCC.

Proposita 1058-0—This patient is now an 18-year-old female with severe language delays, a non-verbal IQ of 44 and behavior on the autism spectrum. She was found to have a bicuspid aortic valve and secondary mitral valve regurgitation. MRI reveals partial AgCC, with only a residual component of the posterior body. She also does not have probst bundles.

Refinement of 1q42 Deletion in Family With Callosal Agenesis and Identification of 1q42.13 to q44 Deletion in Individual With AgCC

The clinical details of three members of the same family (mother and two children) with AgCC (Fig. 1A – C) and chromosome 1q42 deletion have been described in a previous report [Puthuran et al. [2005]]. Using standard cytogenetic analysis with FISH the maternal karyotype was as follows: 46,XX,del(1)(q42.1-q42.3).ish del(1)(q42.13-q42.2)(RP11–449N9+, RP11–520H14–, RP11–353A5–, RP11–284L19–,RP11–285B4+). Follow-up BAC microarray analysis of her DNA confirmed the deletion and refined its size and boundaries to a 5.8 Mb region spanning BAC clones RP11-375H24 to RP11-87P4 (Figs. 2A and 3). Metaphase spreads counterstained with DAPI illustrate the hybridization of probes RP11-520H14, RP11-353A5, and RP11-284L19 (Fig. 2C–E). Detailed information about the clones used in this analysis (Fig. 2B) can be obtained from the Ensembl database. In addition to this family, clinical microarray and confirmatory G-banding analyses in a separate individual with partial AgCC (proposita 1650-0) (Figs. 1D and 3) identified a 13.7 Mb deletion at 1q42.13 to q44 (between 226.5 and 240.2 Mb). G-banding analysis of both parents indicated that the deletion occurred de novo.

Detection of *DISC1* Nucleotide Changes in AgCC Population

The two-generation family with AgCC and 1q42 deletion, the prevalence of schizophrenia among AgCC individuals, and in vitro evidence linking *DISC1* to neurite outgrowth highlighted *DISC1* as an important AgCC candidate gene. To address this hypothesis, we sequenced all 13 exons and intron–exon boundaries of the long form of the *DISC1* gene in a cohort of 144 MRI-verified AgCC individuals [Hetts et al., 2006]. A total of 20 nucleotide changes were observed in the sequencing, 10 of which were previously unreported in dbSNP (Table I). Three of these nucleotide changes result in non-conservative amino acid substitutions (P287L in propositus 1148-0, T453M [rs28930675] in propositus 1132-0 and 1294-0, and P540Q in propositus 1145-0), and are predicted to impair *DISC1* protein function using the PolyPhen and SIFT protein folding prediction programs. The wild type amino acid at these three positions appears to be highly conserved in mammals (Table II). Additionally, we detected a novel change at the 5' intron–exon boundary of exon 11 (IVS10 –2; A–G nucleotide change) in proposita 1058-0 that is predicted to disrupt mRNA splicing, as it occurs at the invariant second position in the splice acceptor site (see below). In mammals that express exon 11 of *DISC1*, this IVS10 –2 A nucleotide at the splice site is

highly conserved (Table III). In all cases, we confirmed the detection of these nucleotide changes by resequencing fresh DNA samples. Parents were also genotyped to determine whether the mutations were inherited or occurred de novo; all four changes were inherited, with no obvious gender predilection. To determine the population frequency of these genetic changes, we then screened a Caucasian control population. P287L and rs28930675 were detected at low frequencies (1/764 and 3/694 chromosomes, respectively). However, P540Q and IVS10 –2 were absent in all genotyped controls (0/756 and 0/706, respectively).

DISC1 variants R264Q, L607F, and S704C have been associated with a higher risk for developing schizophrenia [Zhang et al., 2006; Qu et al., 2007; Song et al., 2008; Nakata et al., 2009b]. We compared the allele frequencies at these genetic loci between AgCC patients and controls to inquire whether these variants also segregate with the AgCC phenotype, While the allele frequencies differed somewhat between the two groups, the associations did not reach a threshold of statistical significance.

Mutation at *DISC1* Exon 11 Splice Site and Reduction of mRNA Levels

As IVS10 – 2 is located two bases upstream of the start of exon 11, we hypothesized that the mutant G allele at this locus may disrupt expression of the long forms of *DISC1* mRNA (L and Lv, which differ by a length of 66 base pairs in exon 11). To address the functional significance of this change in vitro, we conducted realtime PCR to measure the steady state levels of the three main *DISCI* mRNA splice products in total RNA freshly isolated from whole blood of a matched healthy control and of proposita 1058-0 with the IVS10 – 2 mutation. This analysis demonstrated a dramatic reduction of exon 11 mRNA levels (Fig. 4B,C) in proposita 1058-0 compared to the control, with no significant difference in the overall mRNA levels of exon 10 (Fig. 4D). We propose that the reduction in exon 11 mRNA expression in the proposita corresponds to a reduction in both *DISCI* L and *DISCI* Lv mRNA expression, whereas the unaffected expression of exon 10 signifies normal levels of short *DISCI* isoforms. This differential expression between total mRNA abundance and the abundance of specific isoforms serves as an internal control for this measurement.

DISC1 Expression in Mouse Developing Corpus Callosum

To address whether and when DISC1 is expressed in the developing corpus callosum, we performed immunohistochemical staining with a DISC1 polyclonal antibody in E14–E17 mice. A high level of *DISC1* expression was observed throughout the corpus callosum during E14–E17 (Fig. 5A – D). In addition to being expressed in commissural axons, *DISC1* is also found in the cerebral cortex and in the dorsal hippocampal commissure and the fornices, regions that have been previously reported to show *DISC1* expression [Schurov et al., 2004]. While the hippocampus and cerebral cortex have been previously shown to express *DISC1*, our findings are the first to show that *DISC1* is highly expressed in the developing corpus callosum.

DISCUSSION

In this present study, we provide multiple lines of evidence to support a role for *DISC1* in callosal development. This includes refinement of the 1q42 deletion that contains *DISC1* in

a family with AgCC [Puthuran et al. [2005]] as well as identification of a different interstitial deletion including *DISC1* in an unrelated individual with AgCC. Our findings also encompass identification of multiple rare, likely pathogenic *DISC1* variants in a cohort of 144 well-characterized ACC patients, including a splice site mutation that we demonstrated diminishes the long forms of *DISC1* mRNA. We also provide evidence that *DISC1* is highly expressed in the developing corpus callosum, a previously unreported finding.

Others have also shown deletions in or near the chromosomal region of 1q42 in patients with readily apparent callosal abnormalities (Fig. 3) [Gentile et al., 2003; Kato et al., 2007; Filges et al., 2010]. However, Rice et al. [2006] report a patient with an interstitial deletion in this region that includes *DISC1* but with normal callosal development. Furthermore, patients with callosal abnormalities and deletions just proximal [Filges et al., 2010] or distal [Gentile et al., 2003] to *DISC1* have been reported in the literature as well. It is also possible that genes deleted in this region other than *DISC1* are responsible for the callosal malformations in these patients. For example, CDC42 binding protein kinase A (CDC42BPA), a gene included in both deleted regions in the family with AgCC originally reported by Puthuran et al. and proposita 1650-0 in this study, is expressed in the developing CNS. CDC42 has been implicated in establishing neuronal polarity, and CDC42-null mice show defects in developing axonal tracts [Garvalov et al., 2007]; however, little information exists for CDC42BPA itself. Exocyst complex component 8 (EXOC8) is also included within these deleted intervals, and the exocyst complex has been shown to promote neurite outgrowth [Vega and Hsu, 2001]. Nevertheless, we hypothesize that AgCC is likely a polygenic phenotype, and thus we would anticipate that deletion of *DISC1* could quite likely have variable penetrance for the acallosal phenotype. Additionally, a deletion adjacent to *DISC1* (as in the Filges et al. and Gentile et al. manuscripts) may alter *DISC1* transcription through a secondary effect on the chromatin structure of the *DISC1* gene.

Recently, Shen et al. [2008] created a transgenic mouse, that in addition to expressing one allele of the endogenous *DISC1*, expressed a truncated *DISC1* gene encoding only the first eight exons to model the *DISC1* translocation originally identified in the large Scottish pedigree. These mice exhibited reduced neuronal proliferation as well as reduced neurite outgrowth, consistent with earlier data [Ozeki et al., 2003]. Morphological analysis also showed partial AgCC in the transgenic mice, with thinning at the rostral portion of the corpus callosum and failure to cross the midline caudally. These findings provide potential corroboration for the findings in our AgCC patient cohort. The IVS10 – 2 mutation found in one AgCC individual in our study also nearly mimics the *DISC1* translocation in the original Scottish family as it selectively diminishes expression of the long forms of *DISC1*. There are currently no reports of brain imaging in this family, but we hypothesize that callosal anomalies are likely to be identified in this and similar individuals. Another genetic locus that has been associated with schizophrenia, duplication of 16p11.2, has also been reported to have callosal anomalies [McCarthy et al., 2009; Rosenfeld et al., 2010].

This connection between schizophrenia and callosal anomalies may be more widespread. Diffusion tensor imaging has shown reduced interhemispheric connectivity (IHC) involving callosal fibers in schizophrenia [Kubicki et al., 2008]. Moreover, a recent meta-analysis of

28 studies that used magnetic resonance imaging to analyze brain morphology of schizophrenic individuals showed that over all, corpus callosum area was reduced when compared to healthy controls [Arnone et al., 2008]. Moreover, callosal agenesis has been reported in many case studies [David et al., 1993; Motomura et al., 2002; Chinnasamy et al., 2006; Hallak et al., 2007] and in one study callosal agenesis was found in two patients of 140 schizophrenics systematically imaged for observed structural changes. The callosal phenotype was the only notable brain finding [Swayze et al., 1990]. Another series of 52 cases of schizophrenia found AgCC in one individual [Scott et al, 1993]. Analogously, a population study of callosal agenesis in the United Kingdom found that, of the 56 adults with AgCC identified, 35% had psychiatric histories of which 8% had schizophrenia or bipolar psychosis [Taylor and David, 1998]. These observations suggest that callosal malformations may be an important underlying biological mechanism for schizophrenia.

Resequencing candidate genes in a large cohort of patients and controls provides an ideal method for identifying variants contributing to a disease phenotype, and recent analysis has suggested that the overwhelming majority of rare missense or splice site mutations are in fact detrimental and likely contribute to the phenotype of such diseases [Kryukov et al., 2007]. Indeed, rare variants have been shown to be pathogenic in more common diseases such as cardiovascular disease and autism [Barnby et al., 2005; Durand et al., 2007; Romeo et al., 2007]. Rare *DISC1* variants have also been shown to correlate with disease. Certain ENU-induced *DISC1* missense mutations are associated with phenotypes that attempt to model psychiatric symptoms in mice. Animals with Q31L *DISC1* mutation perform poorly on the forced swim test, a paradigm that has been used to demonstrate depressive behavior in rodents. These mice improved their performance after the administration of the antidepressant bupropion. Mice with L100P mutations have deficits in prepulse inhibition as well as latent inhibition, models used to demonstrate information-processing deficits in patients with schizophrenia. The deficits in these mice partially improved after antipsychotic treatment with haloperidol or clozapine [Clapcote et al., 2007].

A recent study reported resequencing *DISC1* in a cohort of 288 schizophrenia patients and found seven non-synonymous variants that were not found in 288 controls. Direct sequencing of the controls revealed only one novel variant, suggesting that rare *DISC1* variants occur at a lower frequency in phenotypically normal controls than in schizophrenic patients. Five of the variants (G14A, R37W, S90L, R418H, and T603I) were absent in 10,000 screened control alleles, and authors estimated a 2% schizophrenia attributable-risk for these rare variants [Song et al., 2008]. This frequency of rare *DISC1* alleles is comparable to the rate found in our AgCC patients, in which 2 variants (P540Q and IVS10 – 2) were present in the 144 AgCC individuals but were absent in all 764 genotyped control chromosomes.

Neuronal migration during development is regulated in part by microtubule dynamics [Smith et al, 2000; Hatten, 2002; Tischfield et al., 2010]. In support of this, patients with TUBB3 mutations and mice null for the microtubule-associated protein 1B (MAP1B) display AgCC [Meixner et al, 2000; Tischfield et al., 2010]. Since *DISC1* is a component of the dynein protein motor complex that participates in microtubule organization and dynamics [Kamiya et al., 2005] and has been shown to bind another microtubule associated protein, MAP1A

[Morris et al., 2003], it is reasonable to hypothesize that a mutant dynein motor complex could affect microtubule dynamics in the axon and prevent axons from traversing the midline, causing callosal agenesis.

In conclusion, researchers have long proposed a link between corpus callosum morphology and function and schizophrenia. Our data implicating *DISC1* mutations in callosal development also supports the hypothesis that abnormal callosal formation correlates with schizophrenia, and may provide insight into the correlation between aberrant cerebral connectivity and neurodevelopmental disorders more generally.

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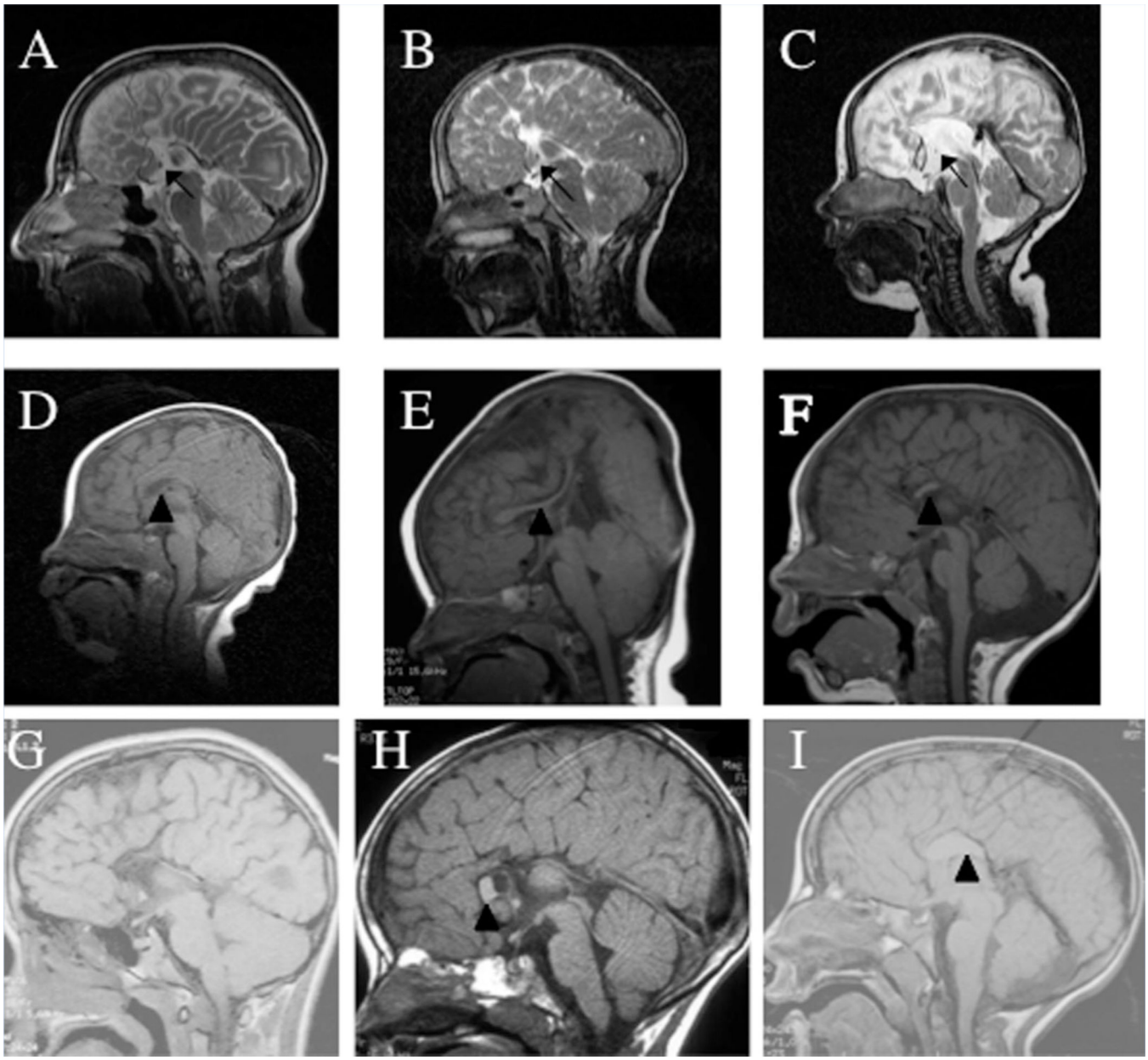

FIG. 1.

Patients with corpus callosum abnormalities and genetic aberrations of *DISC1.* Midline sagittal MRI of mother (A) and two sons (B,C) with 1q42 deletion demonstrate complete absence of the corpus callosum. The anterior commissure (arrows) is present in these three family members. D: A residual corpus callosum (arrowhead) is present in proposita 1650-0 with 1q42.13 to q44 deletion. E–I: Corpus callosum abnormalities are also present in individuals with single nucleotide changes affecting *DISC1.* E: Propositus 1148-0 with a thin and morphologically distorted corpus callosum (arrowhead). F:Propositus 1132-0 with only the superior genu and anterior body of the corpus callosum present (arrowhead). G: Propositus 1294-0 with isolated AgCC. H: Propositus 1145-0 with an absent corpus

callosum except for the anterior aspect of the genu (arrowhead). I:Proposita 1058-0 with only a residual component of the posterior body of the corpus callosum present (arrowhead).

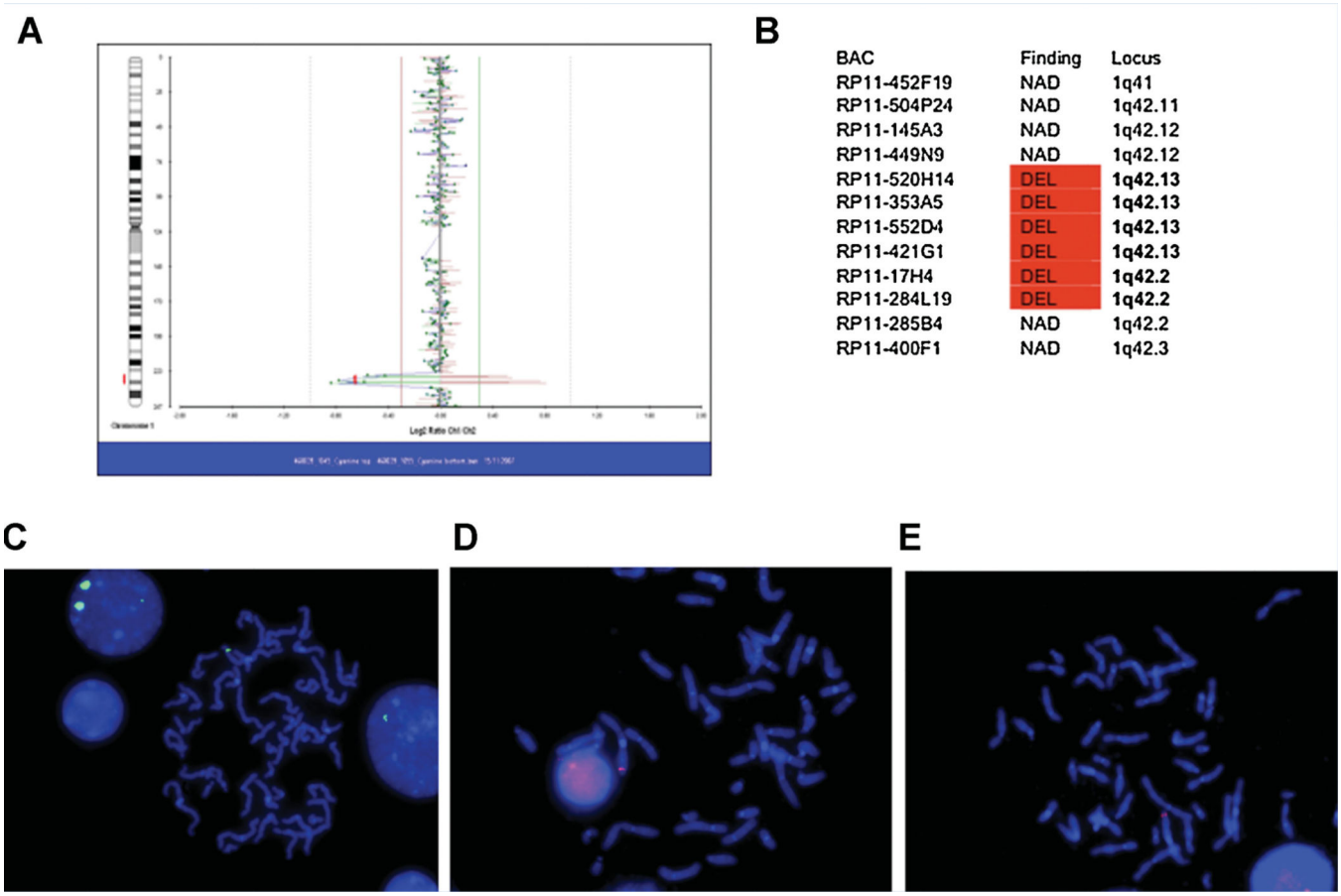

FIG. 2.

Refinement of 1q42 deletion in family with agenesis of the corpus callosum. Follow-up BAC clone microarray analysis of the mother of the family with AgCC and 1q42 deletion showed a deletion spanning six clones over approximately 5.8 Mb—arr cgh 1q42.13 → 1q42.2 (RP11-375H24 → RP11-87P4)x1 which refines the earlier karyotype results (A). The table in (B) gives the BAC clones used in microarray analysis. Detailed information about the clones can be obtained from the Ensembl database. Metaphase spreads counterstained with DAPI illustrate the hybridization of probes RP11-520H14 (C), RP11-353A5 (D), and RP11-284L19 (E).

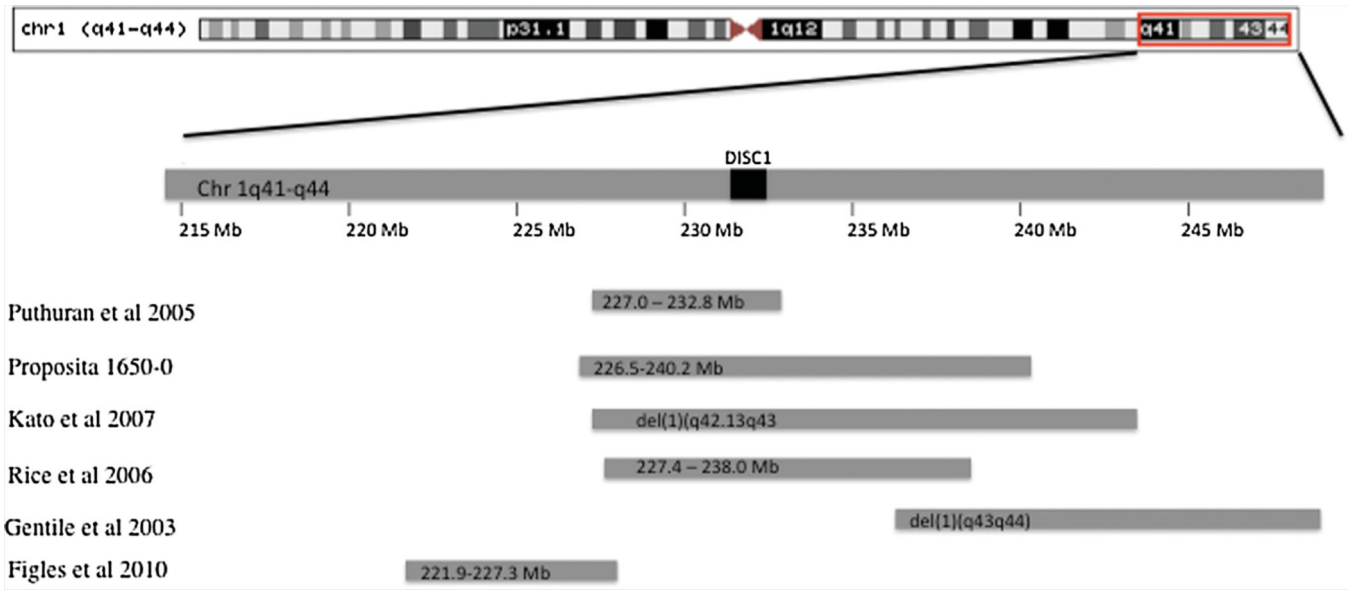

FIG. 3.

Genomic map of deletions in chromosomal region 1q42. Deletions in the chromosome region 1q42 including and adjacent to *DISC1* are found in patients who demonstrate callosal abnormalities. The deletion in the family with AgCC reported by Puthuran et al. spans a 5.8 Mb region between 227.0 and 232.8 Mb on chromosome 1q. Proposita 1650-0 with partial AgCC has a 13.7 Mb deletion between 226.5 and 240.2 Mb. Cytogenetic analysis by Kato et al. and Gentile et al. locate deletions in patients with partial AgCC at 1q42.13-q43 and 1q43-q44, respectively. The deletion described by Rice et al. is found in a patient with a normal corpus callosum and is located between 227.4 and 238.0 Mb. The deletion in a patient with AgCC reported by Filges et al. is located between 221.9 and 227.3 Mb.

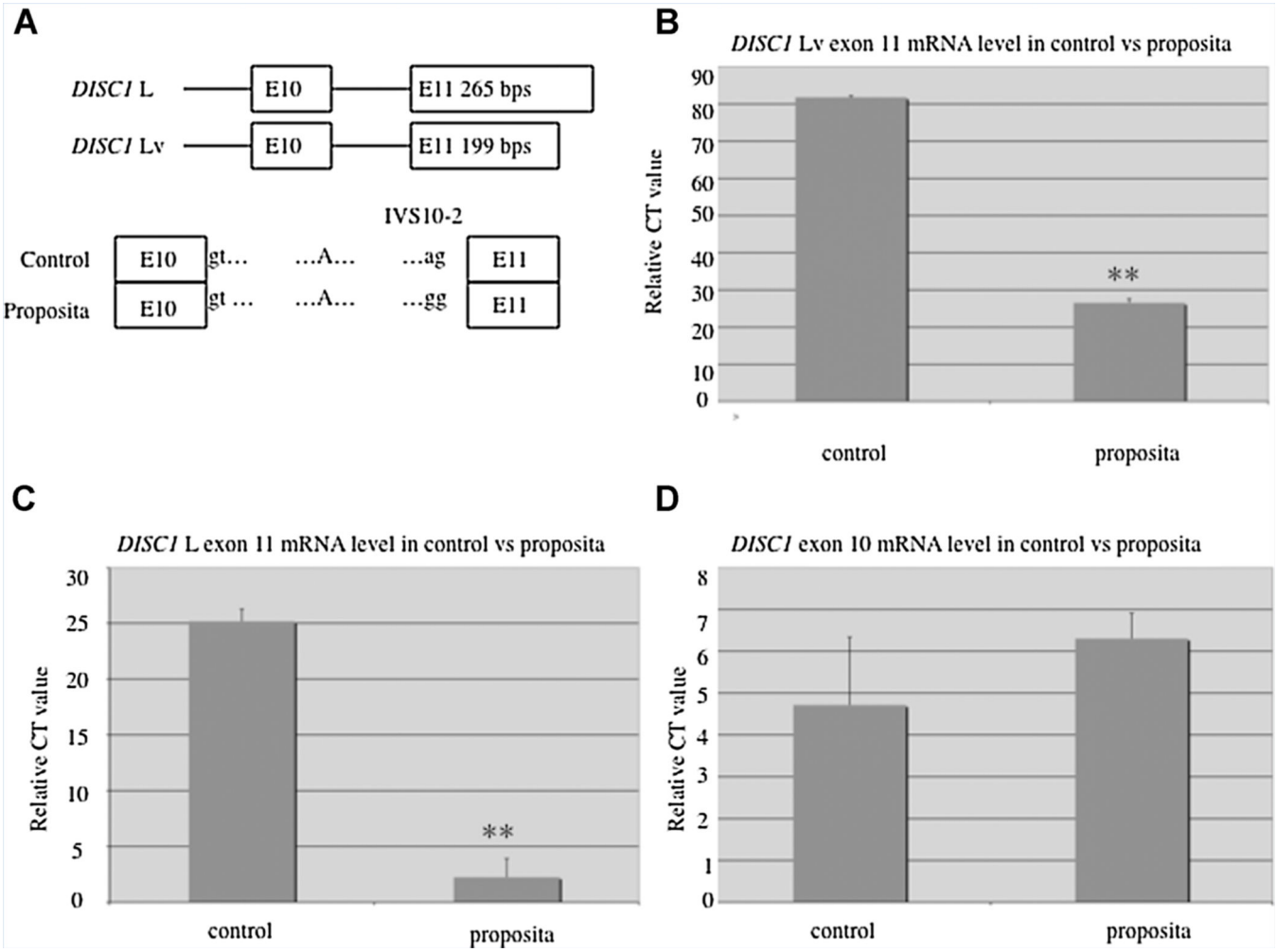

FIG. 4.

Splice site mutation resulting in reduced expression of *DISC1* exon 11 mRNA. A: Top, The two *DISC1* long isoforms only differ for the length of exon 11: 265 bp for *DISC1* L and 199 bp for *DISC1* Lv. Bottom, Sequence analysis revealed a splice site mutation found in proposita 1058-0 where one nucleotide at the acceptor site changed from A to G in intron 10 (SNP IVS10 2). B–D: Quantitative measurement of *DISC1* mRNA levels from whole blood samples in control and proposita. Compared with the control, both *DISC1* Lv and *DISC1* L exon 11 mRNA are significantly decreased in the proposita [mean + SD, $P < 0.01$] (B,C). However, exon 10 expression was similar in the proposita and control (D).

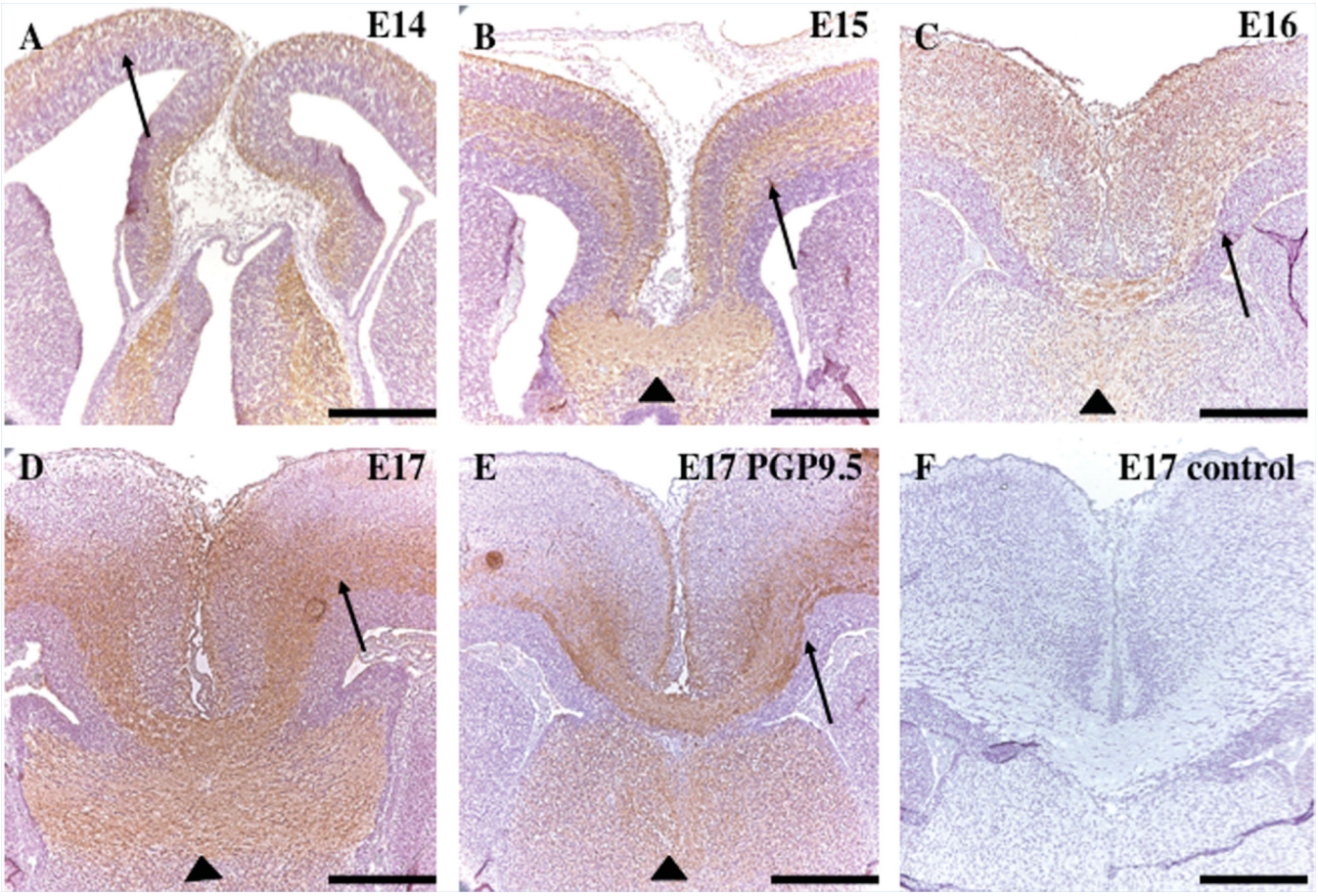

FIG. 5.

Developmental expression of *DISC1* in mouse corpus callosum. Immunohistochemistry demonstrates expression of *DISC1* in the cerebral cortex as early as E14 (A, arrow). During corpus callosum development from E16–17 (B–D), strong expression of *DISC1* is found in commissural neurons (arrows), the dorsal hippocampus commissure, and the fornices (arrowheads). Similar expression is also found for neuronal marker PGP9.5 (E, arrow and arrowhead). Panel F shows the negative control with secondary anti-rabbit antibody only. Scale bar, 50 μm.

Table I

Summary of *DISC1* Nucleotide Changes in AgCC Patients and Controls

AA change	Rs#	Location	PolyPhen prediction	SIFT prediction	Patient genotype frequency	Control genotype frequency	Minor allele frequency	*P*value
V71L		Exon 2			GG = 139			
					GT = 1			
					TT = 0			
A83V		Exon 2			CC = 139			
					CT = 1			
					TT = 0			
R264Q	rs3738401	Exon 2			GG = 55	GG = 161	Patients = 0.384	0.23
					AG = 60	AG = 126	Controls = 0.337	
					AA = 23	AA = 51		
P287L		Exon 2	Probably damaging	Tolerated	CC = 137	CC = 381		
					CT = 1	CT = 1		
					TT = 0	TT = 0		
IVS4–36		Intron 4			TT = 139			
					CT = 2			
					CC = 0			
T453M	rs28930675	Exon 5	Possibly damaging	Damaging [low confidence]	CC = 139	CC = 344		
					CT = 2	CT = 3		
					TT = 0	TT = 0		
L465L	rs3738402	Exon 5			CC = 132			
					CT = 9			
					TT = 0			
I4691	rs2492367	Exon 6			CC = 115			
					CT = 20			
					TT = 5			
P540Q		Exon 6	Probably damaging	Damaging (low confidence)	CC = 138	CC = 378		
					AC = 1	AC = 0		
					AA = 0	AA = 0		

AA change	Rs#	Location	PolyPhen prediction	SIFT prediction	Patient genotype frequency	Control genotype frequency	Minor allele frequency	Pvalue
IVS6 + 31	rs16854940	Intron 6			CC = 128			
					CT = 8			
					TT = 0			
IVS6 - 89	rs41271515	Intron 6			GG = 138			
					AG = 1			
					AA = 0			
IVS7 + 6		Intron 7			TT = 104			
					CT = 32			
					CC = 4			
IVS8 + 60		Intron 8			GG = 128			
					AG = 2			
					AA = 0			
L607F	rs6675281	Exon 9			CC = 109	CC = 250	Patients = 0.124	0.36
					CT = 29	CT = 88	Controls = 0.148	
					TT = 3	TT = 7		
L621L	rs12133766	Exon 9			GG = 126			
					AG = 15			
					AA = 0			
IVS10 + 95		Intron 10			GG = 72			
					AG = 53			
					AA = 13			
IVS10–2		Intron 10			AA = 140	AA = 353		
					AG = 1	AG = 0		
					GG = 0	GG = 0		
S704C	rs821616	Exon 11			AA = 85	AA = 185	Patients = 0.225	0.15
					AT = 50	AT = 133	Controls = 0.271	
					TT = 7	TT = 27		
L792L		Exon 12			GG = 140			
					AG = 1			
					AA = 0			

AA change	Rs#	Location	PolyPhen prediction	SIFT prediction	Patient genotype frequency	Control genotype frequency	Minor allele frequency	*P*value
IVS12 – 64	rs17773715	Intron 12			GG = 92			
					AG = 43			
					AA = 4			

Resequencing of all 13 exons in 144 individuals with AgCC detected several SNPs and previously unreported single nucleotide variants. The rare variants indicated in bold result in an amino acid substitution with significantly different biochemical properties from the wild type amino acid at that position. The IVS10 – 2 mutation was detected in a splice site two bases upstream of exon 11. These rare variants as well as other selected SNPs were then genotype-screened in a control population. While P287L and rs28930675 [T453M] were detected at low frequencies, P540Q and IVS10 – 2 were found only in the AgCC cohort. The variation in minor allele frequencies of rs3738401 (R264Q), rs6675281 (L607F), and rs821616 (S704C) between patients and controls did not reach statistical significance.

Table II

Alignment of *DISCI* Amino Acid Sequences in Mammals

Species	Amino Acid Sequence
Homo sapiens	270 ATRVSADLAQAAR–NSSR**P**ER 289
Pan troglodytes	270 ATRVSADLAQAAR–NSSR**P**ER 289
Canis lupus familiaris	304 TIPSLADSAQTTG–GSHR**P**EC 323
Bos taurus	----------
Mus musculus	271 AAPGLADLAQVTRSSSRQ**S**EC 290
Rattus norvegicus	266 AAPGLVDLAQGTR-SNRQ**P**EC 285
Homo sapiens	440 LEPTAQDSLHV–SI**T**RRDWLL 459
Pan troglodytes	440 LEPTAQDSLHV–SI**T**RRDWLL 459
Canis lupus familiaris	469 WEPTAQDTLRV–SI**T**RRDWLL 488
Bos taurus	65 LEAAAQDSLRV–SI**T**RRDWLL 84
Mus musculus	440 ---TAQDSLPA–SI**T**RRDWLI 456
Rattus norvegicus	432 ---TAQDSLPGLAV**T**RRDWLM 449
Homo sapiens	530 AGQIPFHAEP**P**ETIRSLQER 549
Pan troglodytes	530 AGQIPFHAEP**P**ETIRSLQER 549
Canis lupus familiaris	559 ANQIPICAEP**P**ETIRSLQER 578
Bos taurus	153 AEQIPLHAEP**P**ETIRSLQER 172
Mus musculus	527 ANQAPFQVEP**P**ETLRSLRER 546
Rattus norvegicus	520 ARWAPFRVEP**P**ETLRSLRER 539

Protein sequence alignment using HomoloGene reveals that amino acids at positions 287, 453, and 540 (bold) in humans are highly conserved in several other mammal species.

Table III

DNA Sequence Alignment of the 5° Splice Site of Exon 11 of DISC1 in Mammals

Species	DNA sequence
Homo sapiens	ctc**a**gCTGCAAGTGTCCACTGCTTG
Pan troglodytes	ctg**a**cCTGCAAGTGTCCACTGCTCG
Pongo pygmaeus	ctc**a**gCTGCAAGTGTCCACTGCTTG
Macaca mulatta	ctc**a**gCTGCAAGTGTCCACTGCTTG
Equusferus caballus	ctt**a**gCTGCAAGTGTCCGCTGCTTG
Canis lupus familiaris	---**a**gCTGCAAGTGTCCACTGCTTG
Cavia porcellus	ctt**a**gCTGCAAGTGTCCGCTGCTTG

The “A nucleotide” (bold) at the 5′ splice site of exon 11 if *DISC1* is highly conserved in primates and other mammals.