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Mutations in DCC cause isolated Agenesis of the Corpus Callosum with incomplete penetrance

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ABSTRACT

Brain malformations involving the corpus callosum are common in children with developmental disabilities. We report that *DCC* mutations cause isolated agenesis of the corpus callosum (ACC), without intellectual disability, in four families and five sporadic individuals. *DCC* mutations result in variable dominant phenotypes with reduced penetrance including mirror movements and ACC associated with a favorable developmental prognosis. Possible phenotype modifiers include the type and location of mutation and sex of the individual.

MAIN TEXT

The corpus callosum (CC) is the main cerebral commissure in placental mammals with a key role in communication between the brain hemispheres¹. Formation of the CC is a complex process involving ligands such as those in the Netrin, Ephrin, Semaphorin and Slit families and their receptors². Agenesis of the corpus callosum (ACC) is the complete or partial absence of the CC. This frequent brain malformation affects ~1/4,000 newborns and 3-5% of children with intellectual disability (ID)^{3,4} and is a common cause of late pregnancy termination⁵. Mutations in many genes cause syndromes with ID and ACC, whereas the genetics of isolated ACC remain poorly understood^{3,6,7}. The Netrin receptor Dcc plays a critical role in CC development in mice by guiding callosal axons at the midline⁸. While mutations in *DCC* have been associated with congenital mirror movements (MM) in humans⁹, they have not been described in individuals with ACC.

We investigated four multigenerational families with individuals presenting with ACC, MM or both phenotypes segregating as autosomal dominant traits (Fig. 1a). Neuroimaging and clinical studies confirmed that complete or partial ACC was isolated in most cases (Fig. 1b, Fig. S1) and

associated with a range of intellectual abilities (normal-borderline impaired); additionally, specific cognitive impairments, including language delay or visuospatial deficits, were documented (Table S1). Diffusion MRI tractography using probabilistic constrained spherical deconvolution identified reduced crossing of descending corticospinal tract projections at the pyramidal decussation in all affected individuals in families 2 and 4 with either ACC and MM (n=5) or MM only (n=2) (Fig. 1c, Fig. S2). The tractography results for other commissural fibers, including the decussation of the superior cerebellar peduncles, anterior commissure, posterior commissure and optic chiasm, were comparable between the affected individuals and controls.

Linkage analysis and exome sequencing of three affected individuals in family 1 identified two shared candidate variants in the 16 linkage regions (Fig. S3, Table S2) including a truncating mutation in *DCC* (NM_005215.3:c.925delA, p.(Thr309ProfsTer26)). For family 2, 48 candidate variants were identified in 28 linkage regions (Fig. S4, Table S3), including a missense variant (c.2378T>G, p.(Val793Gly)) in *DCC*. The previously-reported nonsense mutation (c.823C>T, p.(Arg275Ter)) in *DCC* segregated with MM in five individuals of family 3¹⁰. Further investigation revealed two additional female mutation carriers with ACC and a male carrier with MM who had a thin rostrum. Direct screening in family 4 identified a heterozygous *DCC* missense variant (c.2414G>A, p.(Gly805Glu)). All four *DCC* mutations were absent from public databases, including 1000 Genomes and ExAC, and segregated with ACC and/or MM in all available individuals tested (Fig. 1a). In addition, we sequenced *DCC* in 70 unrelated individuals with ACC including 46 with normal cognitive development. Five individuals, all with isolated complete ACC, had at least one heterozygous missense variant altering a conserved amino acid of *DCC* (Fig. 1d, Fig. S1 and S5, Table 1). Analysis of all available imaging in mutation-positive individuals with complete ACC also showed absence of the hippocampal commissure and

cingulate gyri, and dysmorphic lateral ventricles (usually colpocephaly) as would be expected (detailed in Online methods). Apart from this, no consistent additional brain malformations were seen.

We analyzed the phenotype of individuals with *DCC* mutations reported in the literature and in this study (Table S4) to assess the penetrance of MM and ACC. Of the 88 individuals with *DCC* mutations identified to date, 50 had MM; among the 39 who had brain imaging, 19 exhibited ACC. Excluding the index individuals from the analysis, the penetrance of MM was estimated to be 42% and the penetrance of ACC to be 26% (Table S5). Overall, males (n=31) exhibited MM more frequently than females (n=19, male:female ratio=1.8, $p=0.0027$, Fisher's Exact test; Table S5) while, in individuals with truncating *DCC* variants, ACC was more often present in females (n=7) than males (n=1, male:female ratio=0.2). Sex differences in CC anatomy have been associated with testosterone levels during prenatal brain development¹¹⁻¹³; therefore, we tested the effect of androgens on *DCC* expression. Independent analysis by RNAseq and RT-qPCR demonstrated a significant dose-dependent increase in *DCC* expression in human neural stem cells treated with 10 nM or 100 nM testosterone (Fig. S6). Since variants introducing a premature stop codon generally result in haploinsufficiency due to nonsense mediated decay of the mutant mRNA, it is possible that ACC may occur when the amount of *DCC* mRNA/protein falls below a threshold level during CC development, which would occur more frequently in females. However, given the incomplete penetrance observed in both sexes, the phenotypic outcome must also be influenced by additional genetic, epigenetic and/or environmental factors. Interestingly, families 1 and 3, in which a majority of females display ACC, are both of North African background, supporting the hypothesis of genetic modifiers.

Contrary to truncating variants, missense mutant proteins are usually present in the cell and can interfere with the function of the wildtype protein, potentially resulting in differing phenotypes compared to haploinsufficiency for the same protein. Binding of Netrin-1 to DCC results in intracellular homodimerization or heterodimerization with UNC5, another axon guidance receptor, and is critical for both the chemoattractive and chemorepulsive properties of the signaling complexes^{15,16}. The Netrin-1 binding region involves the 4th, 5th and 6th fibronectin type III-like domains of DCC^{14,15}, therefore amino acid substitutions in this binding region may compromise DCC function. Five of the eight *DCC* missense variants identified in individuals with ACC are located in the Netrin-1 binding region (Fig. 1d), which represents a considerable enrichment compared to missense variants located in this domain in ExAC (5/74, 6.7% *versus* 519/~60000, 0.86%; $p=5 \times 10^{-4}$ (all rare variants) or 284/~60000, 0.47%; $p=3 \times 10^{-5}$ (rare variants predicted to be damaging by SIFT), Fisher's exact test (Table S6)). Given the reduced penetrance and mild phenotype of *DCC*-related ACC, it is possible that some individuals described in ExAC have pathogenic *DCC* mutations and undiagnosed ACC.

Modelling of *DCC* missense variants revealed that the amino acid substitutions in families 2 and 4, both located within the *DCC*/Netrin-1 binding interface, are predicted to be most disruptive. The p.(Val793Gly) substitution abolishes a hydrophobic interaction with Thr147 of Netrin-1 while p.(Gly805Glu) introduces a highly unfavorable charged moiety within a hydrophobic pocket, disrupting interaction with Leu113 of Netrin-1 (Fig. S7-8). The predicted effects of the three substitutions within the Netrin-1 binding region but outside the binding interface (Fig. S9-12) are consistent with *in vitro* studies demonstrating that even conservative mutations to residues in this binding region can disrupt *DCC* dimerization, Netrin-1 binding and axon guidance¹⁴.

In addition to the effect of sex hormones and the type and location of *DCC* mutations, developmental differences between the CC and corticospinal tract may also contribute to the variable ACC/MM phenotypes. Callosal and sub-cerebrally projecting pyramidal neurons of the cortex are specified at early stages of development and the molecular identity of each population directly affects its axonal connectivity¹⁶. While corticospinal axons utilise DCC/Netrin-1 signalling to reach the midline, callosal axons use DCC/Netrin-1 chemoattraction to attenuate ROBO1/SLIT-2-mediated chemorepulsion to approach and cross the midline¹⁷. Therefore, a *DCC* mutation may differentially affect commissural versus subcerebral axon trajectories, leading to ACC, MM or both. MM were consistently associated with reduced crossing of descending corticospinal tract projections at the pyramidal decussation in this study as well as in individuals with *RAD51*-related MM¹⁸, suggesting that *DCC*-mediated MM are primarily the result of corticospinal tract decussation abnormalities.

In conclusion, our results provide compelling evidence that *DCC* mutations cause isolated ACC in humans, in addition to the previously-reported MM phenotype. The factors determining the phenotypic variability are complex and likely include the hormonal context during development, the type and location of *DCC* mutation, and the genetic background of the individual. Although the full spectrum of phenotypes associated with *DCC* mutations remains to be fully characterized, individuals described in this study have an intellectual quotient within the normal/borderline range. Heterozygous mutations in *DCC* therefore appear to result in isolated ACC with a mild phenotype and favorable cognitive outcomes, contrasting with the unfavourable developmental outcomes associated with syndromic ACC. Given the high frequency of *DCC* mutations detected in our cohorts, this observation has prenatal diagnostic and parental counselling implications for fetuses with ACC as the condition currently has unclear prognostication. Our data suggest that

214 the prenatal detection of isolated ACC related to a pathogenic *DCC* mutation indicates a lower
215 risk of an abnormal neurodevelopmental outcome.

216 **URLs.** 1000 Genomes Project, <http://www.1000genomes.org/>; Exome Variant Server,
217 <http://exac.broadinstitute.org/>; SIFT, <http://sift.jcvi.org/>; PolyPhen-2,
218 <http://genetics.bwh.harvard.edu/pph2/>; dbSNP, <https://www.ncbi.nlm.nih.gov/SNP/>; PyMOL,
219 <https://www.pymol.org/>.

220

221 **Data Availability.**

222 Families included in this study have not consented to have Next Generation Sequence data
223 publicly released. Variants identified in this study have been deposited into ClinVar for
224 immediate release (SUB2184411) and accession numbers are pending final processing.

225

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Ashley Marsh - formulation of theory and prediction, contributions to experimental conception and design, acquisition, analysis and/or interpretation of data and drafting the article and revising it critically for important intellectual content

Delphine Héron - contributions to experimental conception and design, interpretation of data and revising the article critically for important intellectual content

Timothy Edwards - formulation of theory and prediction, contributions to experimental conception and design, acquisition, analysis and/or interpretation of data and drafting the article and revising it critically for important intellectual content

Charles Galea - formulation of theory and prediction and acquisition, analysis and/or interpretation of data

Angélique Quartier- acquisition, analysis and interpretation of data

Caroline Nava - contributions to experimental conception and design, acquisition, analysis and interpretation of data, and revising the article critically for important intellectual content

Agnès Rastetter - acquisition, analysis and interpretation of data

Marie-Laure Moutard - acquisition, analysis and interpretation of data and revising the article critically for important intellectual content

264 Vicki Anderson - acquisition, analysis and/or interpretation of data and drafting the article or
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266 Pierre Bitoun - acquisition, analysis and interpretation of data

267 Jens Bunt - acquisition, analysis and/or interpretation of data

268 Anne Faudet - acquisition, analysis and/or interpretation of data

269 Catherine Garel - acquisition, analysis and/or interpretation of data

270 Greta Gillies - acquisition, analysis and interpretation of data

271 Ilan Gobius - acquisition, analysis and interpretation of data

272 Justine Guegan - analysis of data

273 Solveig Heide - acquisition, analysis and/or interpretation of data

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276 Vesna Lukic - acquisition, analysis and/or interpretation of data

277 Simone Mandelstam- acquisition, analysis and/or interpretation of data and drafting the article or
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279 George McGillivray - acquisition, analysis and/or interpretation of data and drafting the article or
280 revising it critically for important intellectual content

281 Alissandra McIlroy - acquisition, analysis and/or interpretation of data

282 Aurélie Meneret - acquisition, analysis and/or interpretation of data

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292 Myriam Srouf - acquisition, analysis and/or interpretation of data
293 Sarah Stephenson - contributions to experimental conception and design
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322 and design, acquisition, analysis and/or interpretation of data and drafting the article and revising
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327 Christel Depienne - formulation of theory and prediction, contributions to experimental
328 conception and design, acquisition, analysis and/or interpretation of data and drafting the article
329 and revising it critically for important intellectual content

330

331 **COMPETING FINANCIAL INTERESTS**

332 The authors declare no competing financial interests.

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Figure legend

Figure 1 *DCC* mutations cause isolated ACC and/or MM associated with significantly reduced crossing of descending corticospinal tract projections at the pyramidal decussation.

a. All available family samples were analysed; m=mutation; black dot=mutation carrier; blue=partial ACC; black=complete ACC and grey=MM. * indicates neuroimaging data for mutation carrier or individual with MM.

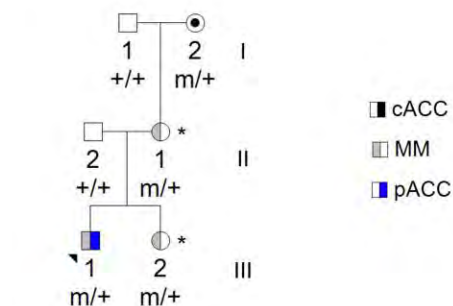
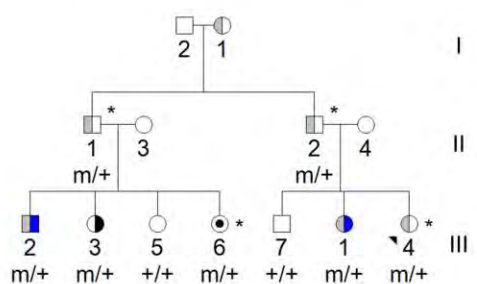
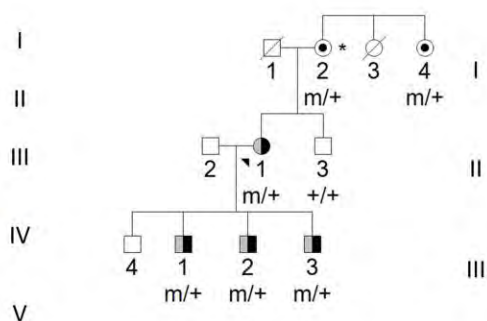
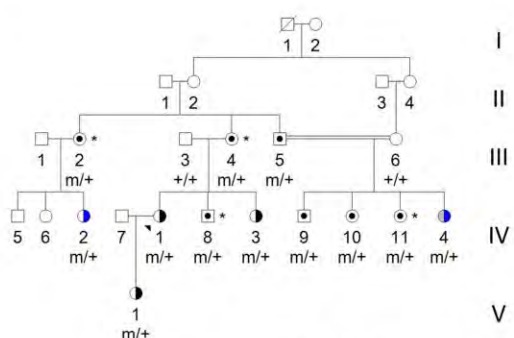
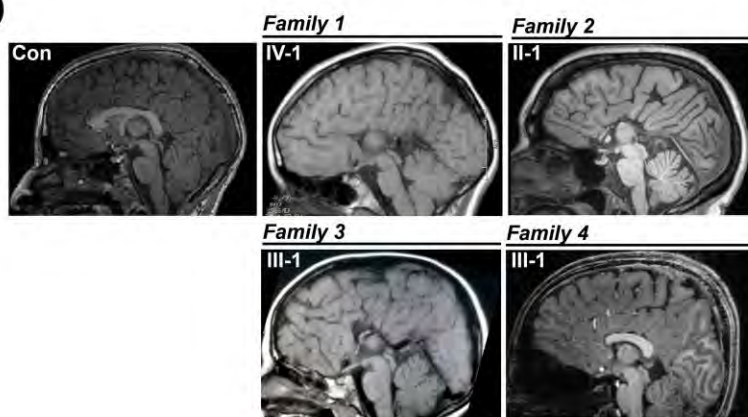
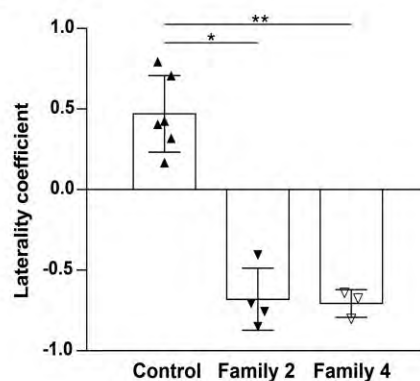
b. Midsagittal MRI of control and family proband/representative individual (1-2=complete ACC; 3=near complete ACC with thin rostrum and genu remaining and 4=partial ACC with absence of the rostrum and genu).

c. Group-wise comparison of laterality coefficient in both families (family 2, n=4; family 4, n=3) were compared to controls (n=6). For each individual, a laterality coefficient for the corticospinal tract was calculated as the ratio of the difference between the numbers of crossed and uncrossed streamlines to the total number of streamlines. Right and left coefficients were averaged to find the laterality coefficient of each individual. Greater positive values indicate more crossed and negative values more uncrossed streamlines (mean +/- S.D, * p=0.0238 ; ** p=0.0095; two-tailed Mann-Whitney U-test).

d. Protein domain structure depicting the location of the *DCC* truncation (red square and triangle) and missense mutations (colored dots). The Netrin-1 binding region is indicated, IgC2, immunoglobulin-like type C2 domain; FN3, fibronectin type III-like domain; TM, transmembrane domain; P1-3, conserved motifs.

375 **Table 1:** Summary of DCC mutations identified in individuals with ACC (+/-MM) in this study. cACC, complete isolated agenesis of
376 the corpus callosum; pACC, partial isolated agenesis of the corpus callosum; MM, mirror movements; IgC2, immunoglobulin-like type
377 C2 domain; FN3, fibronectin type III-like domain; ExAC, Exome Aggregation Consortium; dbSNP, dbSNP reference SNP
378 identification number. Reference sequences used are NM_005215.3 and NP_005206.2.

Family number	Phenotype	cDNA	Protein	Protein domain	SIFT	PolyPhen-2	ExAC	dbSNP
1	cACC or pACC ± MM	c.925delA	p.(Thr309ProfsTer26)	IgC2-3	-	-	No	-
2	cACC & MM	c.2378T>G	p.(Val793Gly)	FN3-4	Deleterious	Probably damaging	No	-
3	cACC or MM ± pACC	c.823C>T	p.(Arg275Ter)	IgC2-3	-	-	No	-
4	pACC &/or MM	c.2414G>A	p.(Gly805Glu)	FN3-4	Deleterious	Probably damaging	No	-
5	cACC	c.1790G>C	p.(Arg597Pro)	FN3-2	Deleterious	Probably damaging	No	-
6	cACC	c.2227A>T	p.(Met743Leu)	FN3-4	Deleterious	Benign	No	rs199651452
7	cACC	c.2260G>A	p.(Val754Met)	FN3-4	Deleterious	Possibly damaging	Yes (x19)	-
8	cACC	c.2677G>A	p.(Ala893Thr)	FN3-5	Deleterious	Benign	No	-
9	cACC	c.3649A>G; c.3748G>A	p.(Met1217Val); p.(Ala1250Thr)	Cytoplasmic	Tolerated; Tolerated	Benign; Probably damaging	No; Yes (x2)	-

a**b****c****d**