

## Mutations in DCC cause isolated agenesis of the corpus callosum with incomplete penetrance

Ashley P. L. Marsh, Delphine Héron, Timothy J. Edwards, Angélique Quartier, Charles Galea, Caroline Nava, Agnès Rastetter, Marie-Laure Moutard, Vicki Anderson, Pierre Bitoun, et al.

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

3  
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93

94 **ABSTRACT**

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

101

102 **MAIN TEXT**

103 The corpus callosum (CC) is the main cerebral commissure in placental mammals with a key role  
104 in communication between the brain hemispheres<sup>1</sup>. Formation of the CC is a complex process  
105 involving ligands such as those in the Netrin, Ephrin, Semaphorin and Slit families and their  
106 receptors<sup>2</sup>. Agenesis of the corpus callosum (ACC) is the complete or partial absence of the CC.  
107 This frequent brain malformation affects ~1/4,000 newborns and 3-5% of children with  
108 intellectual disability (ID)<sup>3,4</sup> and is a common cause of late pregnancy termination<sup>5</sup>. Mutations in  
109 many genes cause syndromes with ID and ACC, whereas the genetics of isolated ACC remain  
110 poorly understood<sup>3,6,7</sup>. The Netrin receptor *Dcc* plays a critical role in CC development in mice  
111 by guiding callosal axons at the midline<sup>8</sup>. While mutations in *DCC* have been associated with  
112 congenital mirror movements (MM) in humans<sup>9</sup>, they have not been described in individuals with  
113 ACC.

114

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

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

126  
127 Linkage analysis and exome sequencing of three affected individuals in family 1 identified two  
128 shared candidate variants in the 16 linkage regions (Fig. S3, Table S2) including a truncating  
129 mutation in *DCC* (NM\_005215.3:c.925delA, p.(Thr309ProfsTer26)). For family 2, 48 candidate  
130 variants were identified in 28 linkage regions (Fig. S4, Table S3), including a missense variant  
131 (c.2378T>G, p.(Val793Gly)) in *DCC*. The previously-reported nonsense mutation (c.823C>T,  
132 p.(Arg275Ter)) in *DCC* segregated with MM in five individuals of family 3<sup>10</sup>. Further  
133 investigation revealed two additional female mutation carriers with ACC and a male carrier with  
134 MM who had a thin rostrum. Direct screening in family 4 identified a heterozygous *DCC*  
135 missense variant (c.2414G>A, p.(Gly805Glu)). All four *DCC* mutations were absent from public  
136 databases, including 1000 Genomes and ExAC, and segregated with ACC and/or MM in all  
137 available individuals tested (Fig. 1a). In addition, we sequenced *DCC* in 70 unrelated individuals  
138 with ACC including 46 with normal cognitive development. Five individuals, all with isolated  
139 complete ACC, had at least one heterozygous missense variant altering a conserved amino acid of  
140 *DCC* (Fig. 1d, Fig. S1 and S5, Table 1). Analysis of all available imaging in mutation-positive  
141 individuals with complete ACC also showed absence of the hippocampal commissure and

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

145  
146 We analyzed the phenotype of individuals with *DCC* mutations reported in the literature and in  
147 this study (Table S4) to assess the penetrance of MM and ACC. Of the 88 individuals with *DCC*  
148 mutations identified to date, 50 had MM; among the 39 who had brain imaging, 19 exhibited  
149 ACC. Excluding the index individuals from the analysis, the penetrance of MM was estimated to  
150 be 42% and the penetrance of ACC to be 26% (Table S5). Overall, males (n=31) exhibited MM  
151 more frequently than females (n=19, male:female ratio=1.8, p=0.0027, Fisher's Exact test; Table  
152 S5) while, in individuals with truncating *DCC* variants, ACC was more often present in females  
153 (n=7) than males (n=1, male:female ratio=0.2). Sex differences in CC anatomy have been  
154 associated with testosterone levels during prenatal brain development<sup>11-13</sup>; therefore, we tested  
155 the effect of androgens on *DCC* expression. Independent analysis by RNAseq and RT-qPCR  
156 demonstrated a significant dose-dependent increase in *DCC* expression in human neural stem  
157 cells treated with 10 nM or 100 nM testosterone (Fig. S6). Since variants introducing a premature  
158 stop codon generally result in haploinsufficiency due to nonsense mediated decay of the mutant  
159 mRNA, it is possible that ACC may occur when the amount of *DCC* mRNA/protein falls below a  
160 threshold level during CC development, which would occur more frequently in females.  
161 However, given the incomplete penetrance observed in both sexes, the phenotypic outcome must  
162 also be influenced by additional genetic, epigenetic and/or environmental factors. Interestingly,  
163 families 1 and 3, in which a majority of females display ACC, are both of North African  
164 background, supporting the hypothesis of genetic modifiers.

165



166 Contrary to truncating variants, missense mutant proteins are usually present in the cell and can  
167 interfere with the function of the wildtype protein, potentially resulting in differing phenotypes  
168 compared to haploinsufficiency for the same protein. Binding of Netrin-1 to DCC results in  
169 intracellular homodimerization or heterodimerization with UNC5, another axon guidance  
170 receptor, and is critical for both the chemoattractive and chemorepulsive properties of the  
171 signaling complexes<sup>15,16</sup>. The Netrin-1 binding region involves the 4th, 5th and 6th fibronectin  
172 type III-like domains of DCC<sup>14,15</sup>, therefore amino acid substitutions in this binding region may  
173 compromise DCC function. Five of the eight *DCC* missense variants identified in individuals  
174 with ACC are located in the Netrin-1 binding region (Fig. 1d), which represents a considerable  
175 enrichment compared to missense variants located in this domain in ExAC (5/74, 6.7% *versus*  
176 519/~60000, 0.86%;  $p=5 \times 10^{-4}$  (all rare variants) or 284/~60000, 0.47%;  $p=3 \times 10^{-5}$  (rare variants  
177 predicted to be damaging by SIFT), Fisher's exact test (Table S6)). Given the reduced penetrance  
178 and mild phenotype of *DCC*-related ACC, it is possible that some individuals described in ExAC  
179 have pathogenic *DCC* mutations and undiagnosed ACC.

180  
181 Modelling of *DCC* missense variants revealed that the amino acid substitutions in families 2 and  
182 4, both located within the *DCC*/Netrin-1 binding interface, are predicted to be most disruptive.  
183 The p.(Val793Gly) substitution abolishes a hydrophobic interaction with Thr147 of Netrin-1  
184 while p.(Gly805Glu) introduces a highly unfavorable charged moiety within a hydrophobic  
185 pocket, disrupting interaction with Leu113 of Netrin-1 (Fig. S7-8). The predicted effects of the  
186 three substitutions within the Netrin-1 binding region but outside the binding interface (Fig. S9-  
187 12) are consistent with *in vitro* studies demonstrating that even conservative mutations to residues  
188 in this binding region can disrupt *DCC* dimerization, Netrin-1 binding and axon guidance<sup>14</sup>.

189

190 In addition to the effect of sex hormones and the type and location of *DCC* mutations,  
191 developmental differences between the CC and corticospinal tract may also contribute to the  
192 variable ACC/MM phenotypes. Callosal and sub-cerebrally projecting pyramidal neurons of the  
193 cortex are specified at early stages of development and the molecular identity of each population  
194 directly affects its axonal connectivity<sup>16</sup>. While corticospinal axons utilise *DCC*/*Netrin-1*  
195 signalling to reach the midline, callosal axons use *DCC*/*Netrin-1* chemoattraction to attenuate  
196 *ROBO1*/*SLIT-2*-mediated chemorepulsion to approach and cross the midline<sup>17</sup>. Therefore, a  
197 *DCC* mutation may differentially affect commissural versus subcerebral axon trajectories, leading  
198 to ACC, MM or both. MM were consistently associated with reduced crossing of descending  
199 corticospinal tract projections at the pyramidal decussation in this study as well as in individuals  
200 with *RAD51*-related MM<sup>18</sup>, suggesting that *DCC*-mediated MM are primarily the result of  
201 corticospinal tract decussation abnormalities.

202  
203 In conclusion, our results provide compelling evidence that *DCC* mutations cause isolated ACC  
204 in humans, in addition to the previously-reported MM phenotype. The factors determining the  
205 phenotypic variability are complex and likely include the hormonal context during development,  
206 the type and location of *DCC* mutation, and the genetic background of the individual. Although  
207 the full spectrum of phenotypes associated with *DCC* mutations remains to be fully characterized,  
208 individuals described in this study have an intellectual quotient within the normal/borderline  
209 range. Heterozygous mutations in *DCC* therefore appear to result in isolated ACC with a mild  
210 phenotype and favorable cognitive outcomes, contrasting with the unfavourable developmental  
211 outcomes associated with syndromic ACC. Given the high frequency of *DCC* mutations detected  
212 in our cohorts, this observation has prenatal diagnostic and parental counselling implications for  
213 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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255 and revising it critically for important intellectual content

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298 Alexis Brice - acquisition, analysis and/or interpretation of data and revising the article critically

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303 important intellectual content

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324 Paul Lockhart - formulation of theory and prediction, contributions to experimental conception  
325 and design, acquisition, analysis and/or interpretation of data and drafting the article and revising  
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### 331 **COMPETING FINANCIAL INTERESTS**

332 The authors declare no competing financial interests.



333 **REFERENCES**

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355 **Figure legend**

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

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

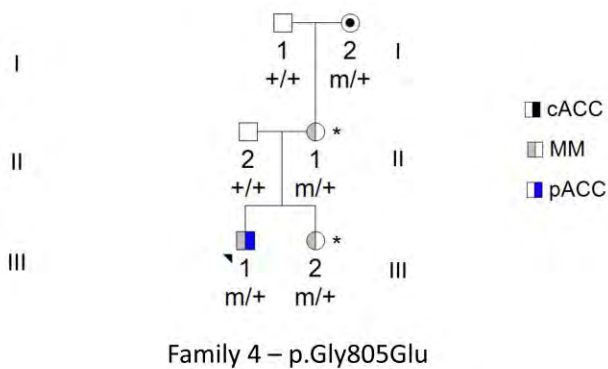
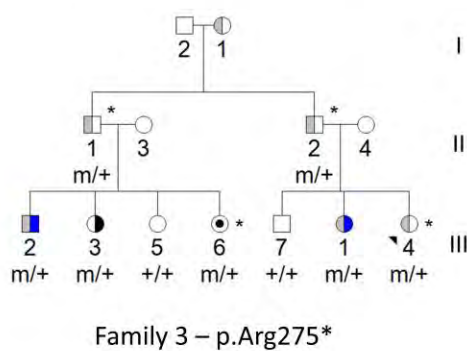
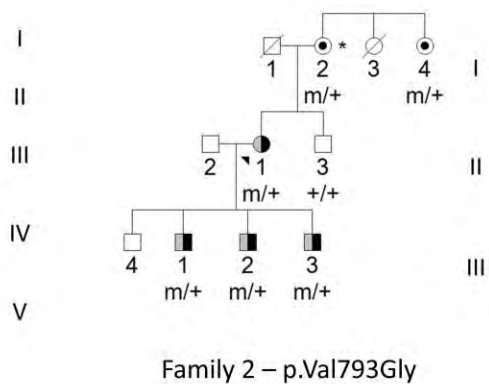
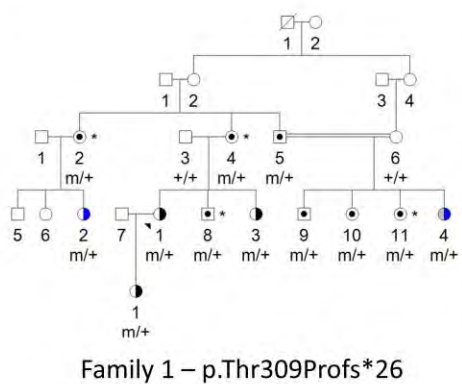
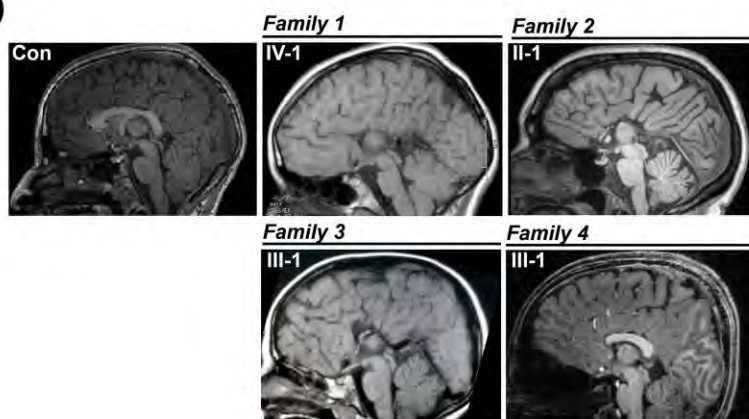
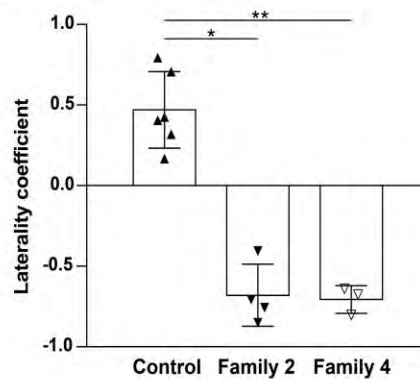
361 b. Midsagittal MRI of control and family proband/representative individual (1-2=complete ACC;  
362 3=near complete ACC with thin rostrum and genu remaining and 4=partial ACC with absence of  
363 the rostrum and genu).

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

371 d. Protein domain structure depicting the location of the *DCC* truncation (red square and triangle)  
372 and missense mutations (colored dots). The Netrin-1 binding region is indicated, IgC2,  
373 immunoglobulin-like type C2 domain; FN3, fibronectin type III-like domain; TM,  
374 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**