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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 hemispheres¹. 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 receptors². 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)^{3,4} and is a common cause of late pregnancy termination⁵. Mutations in
109 many genes cause syndromes with ID and ACC, whereas the genetics of isolated ACC remain
110 poorly understood^{3,6,7}. The Netrin receptor *Dcc* plays a critical role in CC development in mice
111 by guiding callosal axons at the midline⁸. While mutations in *DCC* have been associated with
112 congenital mirror movements (MM) in humans⁹, 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 3¹⁰. 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 development¹¹⁻¹³; 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^{15,16}. The Netrin-1 binding region involves the 4th, 5th and 6th fibronectin
172 type III-like domains of DCC^{14,15}, 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 guidance¹⁴.

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 connectivity¹⁶. 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 midline¹⁷. 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 MM¹⁸, 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.

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248 Ashley Marsh - formulation of theory and prediction, contributions to experimental conception
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251 Delphine Héron - contributions to experimental conception and design, interpretation of data and
252 revising the article critically for important intellectual content

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

256 Charles Galea - formulation of theory and prediction and acquisition, analysis and/or
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258 Angélique Quartier- acquisition, analysis and interpretation of data

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261 Agnès Rastetter - acquisition, analysis and interpretation of data

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

264 Vicki Anderson - acquisition, analysis and/or interpretation of data and drafting the article or
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270 Greta Gillies - acquisition, analysis and interpretation of data

271 Ilan Gobius - acquisition, analysis and 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

294 Rick Tankard - acquisition, analysis and/or interpretation of data

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297 Amanda Wood - acquisition, analysis and/or interpretation of data

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301 for important intellectual content

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320 content

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324 Paul Lockhart - formulation of theory and prediction, contributions to experimental conception
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327 Christel Depienne - formulation of theory and prediction, contributions to experimental
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330

331 **COMPETING FINANCIAL INTERESTS**

332 The authors declare no competing financial interests.

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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.

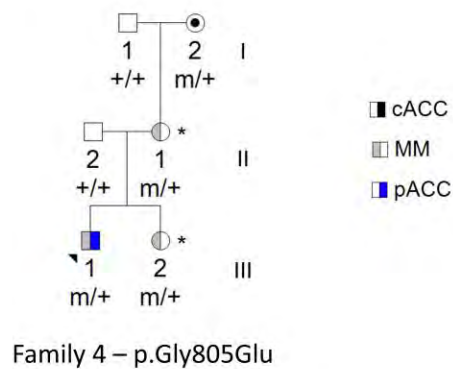
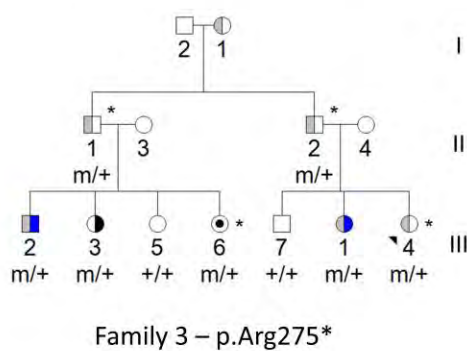
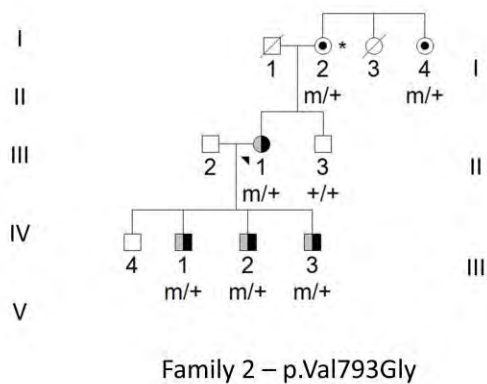
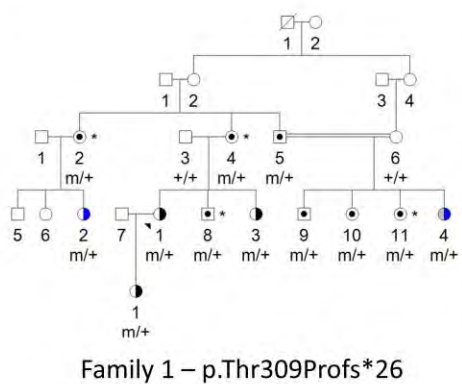
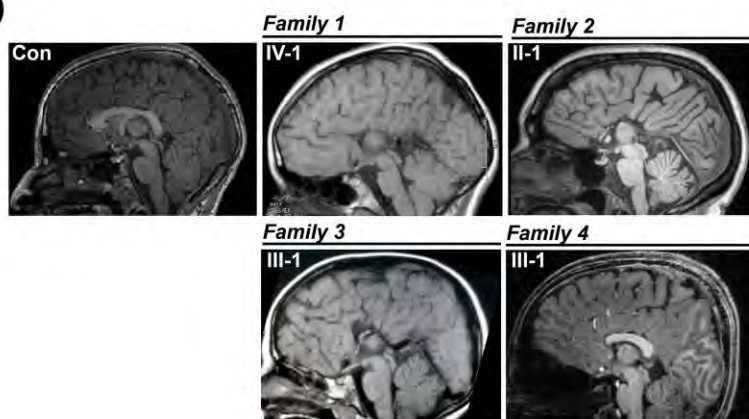
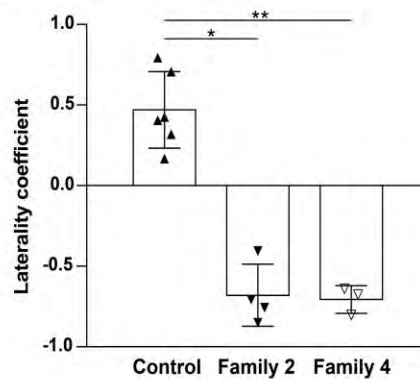
361 b. Midsagittal MRI of control and family proband/representative individual (1-2=complete ACC;
362 3=near complete ACC with thin rostrum and genu remaining and 4=partial ACC with absence of
363 the rostrum and genu).

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**