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

2    **penetrance**

3

4    Ashley PL Marsh[1,2] †, Delphine Heron[3,4,5] †, Timothy J Edwards[6,7] †, Angélique Quartier[8],

5    Charles Galea[9], Caroline Nava[3,10], Agnès Rastetter[10], Marie-Laure Moutard[11,12,13], Vicki

6    Anderson[14], Pierre Bitoun[15], Jens Bunt[6], Anne Faudet[3], Catherine Garel[16], Greta Gillies[1], Ilan

7    Gobius[6], Justine Guegan[17], Solveig Heide[3,4], Boris Keren[3,10], Fabien Lesne[3], Vesna Lukic[18],

8    Simone A Mandelstam[2,19,20], George Mcgillivray[21], Alissandra McIlroy[14], Aurélie Méneret[10,22] ,

9    Cyril Mignot[3,4,5], Laura R Morcom[6], Sylvie Odent[23,24], Annalisa Paolino[6,] Kate Pope[1], Florence

10   Riant[25], Gail A Robinson[26], Megan Spencer-Smith[14,27], Myriam Srour[28,29], Sarah EM

11   Stephenson[1,2], Rick Tankard[30,31], Oriane Trouillard[10], Quentin Welniarz[10,32], Amanda Wood[14.33],

12   Alexis Brice,[3,10] Guy Rouleau[29,34], Tania Attié-Bitach[35], Martin B Delatycki[1,2,36] Jean-Louis

13   Mandel[8,37], David J Amor[1,2], Emmanuel Roze[10,22], Amélie Piton[8,37], Melanie Bahlo[30,31], Thierry

14   Billette de Villemeur[5,11,12,38], Elliott H Sherr[39], Richard J Leventer[2,40,41], Linda J Richards[6,42]§*,

15   Paul J Lockhart[1,2] §*, Christel Depienne[3,8,10,37]§*

16

17   [1] Bruce Lefroy Centre for Genetic Health Research, Murdoch Childrens Research Institute, Royal

18   Children's Hospital, Parkville, Victoria 3052, Australia.

19   [2] Department of Paediatrics, University of Melbourne, Parkville, Victoria 3052, Australia.

20   [3] AP-HP, Hôpital de la Pitié-Salpêtrière, Département de Génétique, F-75013, Paris, France.

21   [4] Groupe de Recherche Clinique (GRC) "déficience intellectuelle et autisme" UPMC, 75013

22   Paris, France.

23   [5] Centre de Référence "déficiences intellectuelles de causes rares", 75013 Paris, France.

24   [6] The University of Queensland, Queensland Brain Institute, St Lucia, Brisbane, 4072, Australia.

25   [7] The University of Queensland, School of Medicine, Herston, Brisbane, 4006, Australia.

26   [8] Département de Médicine translationnelle et Neurogénétique, IGBMC, CNRS UMR

27   7104/INSERM U964/Université de Strasbourg, 67400 Illkirch, France.

28   [9] Drug Delivery, Disposition and Dynamics (D4), Monash Institute of Pharmaceutical Sciences,

29   Monash University, Parkville, Victoria, 3052 Australia.

30   [10] INSERM, U 1127, CNRS UMR 7225, Sorbonne Universités, UPMC Univ Paris 06 UMR S

31   1127, Institut du Cerveau et de la Moelle épinière, ICM, F-75013, Paris, France

32   [11] AP-HP, Hôpital Trousseau, service de neuropédiatrie, 75012 Paris, France.

33   [12] UPMC, GRC ConCer-LD, Sorbonne université, Paris France.

34   [13] Centre de référence "Neurogénétique", 75013 Paris France.

35   [14] Developmental Imaging and Child Neuropsychology Research groups, Murdoch Childrens

36   Research Institute, Parkville, Victoria 3052, Australia.

37   [15] Génétique Médicale, CHU Paris Nord, Hôpital Jean Verdier, 93140 Bondy, France.

38   [16] AP-HP, GHUEP, Hôpital Armand-Trousseau, Service de Radiologie, 75012 Paris, France.

39   [17] iCONICS facility, ICM, 75013, Paris, France.

40   [18] Bioinformatics Division, The Walter and Eliza Hall Institute of Medical Research, 1G Royal

41   Parade, Parkville, Victoria 3052, Australia.

42   [19] The Florey Institute of Neuroscience and Mental Health, Melbourne, Victoria, Australia.

43   [20] Department of Radiology, University of Melbourne, Royal Children's Hospital, Parkville,

44   Victoria 3052, Australia.

45   [21] Victorian Clinical Genetics Services, Murdoch Childrens Research Institute, Parkville, Victoria

46   3052, Australia.

47   [22] AP-HP, Hôpital de la Pitié-Salpêtrière, Département de Neurologie, F-75013, Paris, France.

48  [23] Service de Génétique Clinique, Centre de référence CLAD-Ouest, CHU Rennes, 35000

49  Rennes, France.

50  [24] UMR 6290 CNRS, IGDR Institut de Génétique et développement de Rennes, Université de

51  Rennes1, Rennes France.

52  [25] AP-HP, Groupe Hospitalier Saint Louis, Lariboisière, Fernand Widal, Laboratoire de

53  Génétique, 75010 Paris, France.

54  [26] Neuropsychology Research Unit, School of Psychology, The University of Queensland,

55  Brisbane QLD 4072, Australia.

56  [27] School of Psychological Sciences and Monash Institute of Cognitive and Clinical

57  Neurosciences, Monash University, Clayton Campus, Clayton Victoria 3800, Australia.

58  [28] Department of Pediatrics, Montreal Children's Hospital, McGill University, Montréal, Quebec,

59  H4A 3J1, Canada.

60  [29] Department of Neurology and Neurosurgery, McGill University Health Center, Montreal,

61  Quebec, H3A 2B4 Canada.

62  [30] Population Health and Immunity Division, The Walter and Eliza Hall Institute of Medical

63  Research, Parkville, Victoria, 3052, Australia.

64  [31] Department of Medical Biology, The University of Melbourne, Parkville, Victoria, 3052,

65  Australia.

66  [32] Institut de Biologie Paris Seine, Neuroscience Paris Seine, Sorbonne Universités, UPMC Univ

67  Paris 06, INSERM, CNRS, F-75005, Paris, France

68  [33] School of Life and Health Sciences, Aston University, Birmingham, B4 7ET, United-Kingdom.

69  [34] Montreal Neurological Institute and Hospital, McGill University, Montréal, Quebec H3A 2B4,

70  Canada.

71  [35] INSERM U1163, Laboratory of Embryology and Genetics of Congenital Malformations, Paris

72  Descartes University, Sorbonne Paris Cité and Imagine Institute, 75015 Paris, France;

73  Département de Génétique, Hôpital Necker - Enfants Malades, Assistance Publique - Hôpitaux

74  de Paris, 75015 Paris, France.

75  [36] Victorian Clinical Genetics Services, Parkville, Victoria, 3052, Australia.

76  [37] Laboratoires de génétique, Institut de génétique médicale d'Alsace, Hôpitaux Universitaires de

77  Strasbourg, 67 000 Strasbourg, France.

78  [38] INSERM U1141, 75019 Paris, France.

79  [39] Department of Neurology, UCSF Benioff Children's Hospital, San Francisco, California, USA.

80  [40] Neuroscience Research Group, Murdoch Childrens Research Institute, Parkville, Victoria 3052,

81  Australia.

82  [41] Department of Neurology, University of Melbourne, Royal Children's Hospital, Parkville,

83  Victoria 3052, Australia.

84  [42] The University of Queensland, School of Biomedical Sciences, St Lucia, Brisbane, 4072,

85  Australia.

86

87  † These authors contributed equally to this work.

88  § Equal last and senior authors

89  * Corresponding authors. E-mail: depiennc@igbmc.fr (CD), paul.lockhart@mcri.edu.au (PJL),

90  richards@uq.edu.au (LJR)

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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[1]. 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[2]. 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 termination[5]. 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 midline[8]. While mutations in *DCC* have been associated with

112 congenital mirror movements (MM) in humans[9], 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[10]. 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[11-13]; 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%; $p=5\times10^{-4}$ (all rare variants) or 284/~60000, 0.47%; $p=3\times10^{-5}$ (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[14].

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[16]. 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[17]. 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[18], 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

246

247    **AUTHOR CONTRIBUTIONS**

248    Ashley Marsh - formulation of theory and prediction, contributions to experimental conception

249    and design, acquisition, analysis and/or interpretation of data and drafting the article and revising

250    it critically for important intellectual content

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

257    interpretation of data

258    Angélique Quartier- acquisition, analysis and interpretation of data

259    Caroline Nava - contributions to experimental conception and design, acquisition, analysis and

260    interpretation of data, and revising the article critically for important intellectual content

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

265    revising it critically for important intellectual content

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

274    Boris Keren - acquisition, analysis and/or interpretation of data

275    Fabien Lesne - acquisition, analysis and/or interpretation of data

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

278    revising it critically for important intellectual content

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

283    Cyril Mignot - acquisition, analysis and/or interpretation of data, and revising the article critically

284    for important intellectual content

285    Laura Morcom - acquisition, analysis and/or interpretation of data

286    Sylvie Odent - acquisition, analysis and/or interpretation of data

287    Annalisa Paolino - acquisition, analysis and/or interpretation of data

288    Kate Pope - acquisition, analysis and/or interpretation of data

289    Florence Riant - acquisition, analysis and/or interpretation of data

290    Gail Robinson - acquisition, analysis and/or interpretation of data

291    Megan Spencer-Smith - acquisition, analysis and/or interpretation of data

292    Myriam Srour - 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

295    Oriane Trouillard - acquisition, analysis and/or interpretation of data

296    Quentin Welniarz - acquisition, analysis and/or interpretation of data

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

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

299    for important intellectual content

300    Guy Rouleau - acquisition, analysis and/or interpretation of data and revising the article critically

301    for important intellectual content

302    Tania Attié-Bitach- contributions to experimental design, and revising the article critically for

303    important intellectual content

304    Martin Delatycki - drafting the article or revising it critically for important intellectual content

305    Jean Louis Mandel - contributions to experimental conception and design, interpretation of data

306    and revising the article critically for important intellectual content

307    David Amor - drafting the article or revising it critically for important intellectual content

308    Emmanuel Roze - acquisition, analysis and/or interpretation of data and revising the article

309    critically for important intellectual content

310    Amélie Piton - contributions to experimental conception and design, interpretation of data and

311    revising the article critically for important intellectual content

312 Melanie Bahlo - acquisition, analysis and/or interpretation of data and drafting the article or

313 revising it critically for important intellectual content

314 Thierry Billette de Villemeur - acquisition, analysis and/or interpretation of data and revising the

315 article critically for important intellectual content

316 Elliott Sherr - formulation of theory and prediction, acquisition, analysis and/or interpretation of

317 data and drafting the article and revising it critically for important intellectual content

318 Richard Leventer - formulation of theory and prediction, acquisition, analysis and/or

319 interpretation of data and drafting the article and revising it critically for important intellectual

320 content

321 Linda Richards - formulation of theory and prediction, contributions to experimental conception

322 and design, acquisition, analysis and/or interpretation of data and drafting the article and revising

323 it critically for important intellectual content

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

326 it critically for important intellectual content

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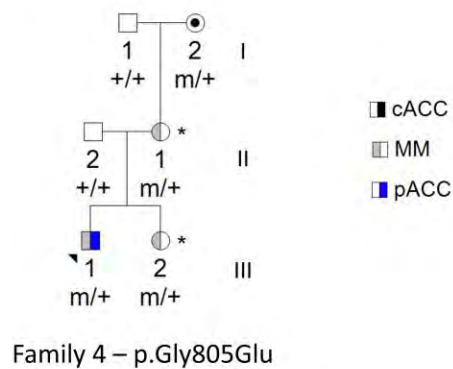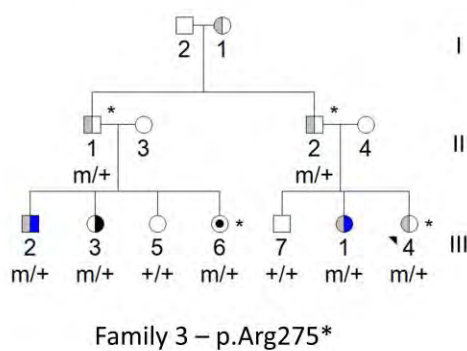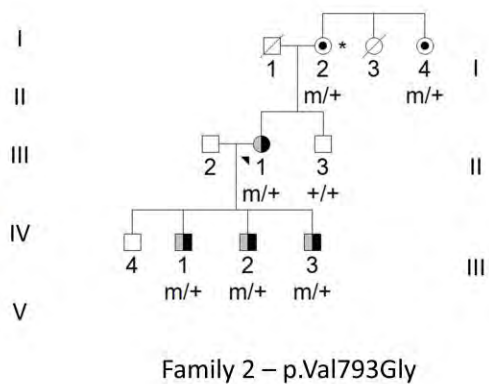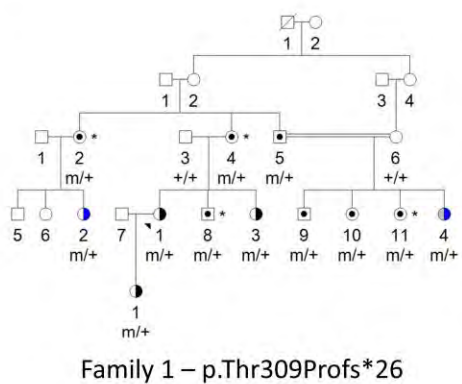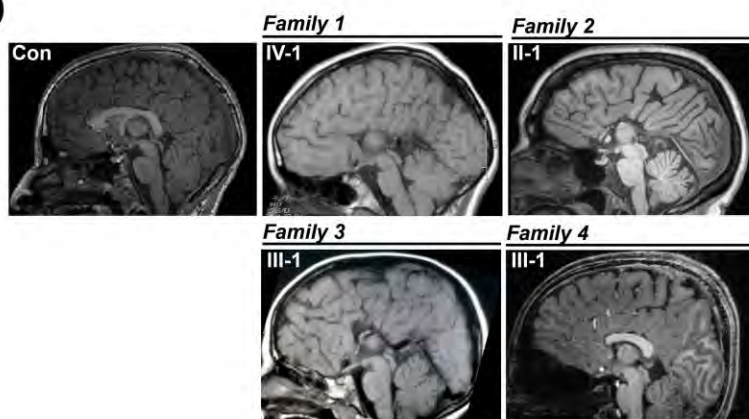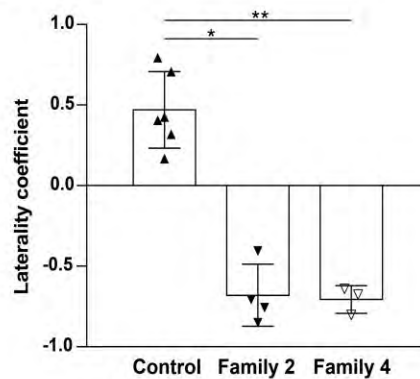
b. Midsagittal MRI of control and family proband/representative individual (1-2=complete ACC; 3=near complete ACC with thin rostrum and genu remaining and 4=partial ACC with absence of the rostrum and genu).

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) | - |

0

**a**

Family 1 – p.Thr309Profs*26

Family 2 – p.Val793Gly

Family 3 – p.Arg275*

Family 4 – p.Gly805Glu

cACC
MM
pACC

**b**

Con

Family 1
IV-1

Family 2
II-1

Family 3
III-1

Family 4
III-1

**c**

Laterality coefficient

Control   Family 2   Family 4

**d**

Netrin-1

1
N

IgC2  IgC2  IgC2  IgC2  FN3  FN3  FN3  FN3  FN3  FN3  TM  P1  P2  P3  1447
C

R275X
T309Profs*26
R597P
M743L
V754M
V793G
G805E
A893T
M1217V:A1250T

Extracellular Domain

Cytoplasmic Domain