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

Ashley PL Marsh[1,2] †, Delphine Heron[3,4,5] †, Timothy J Edwards[6,7] †, Angélique Quartier[8], Charles Galea[9], Caroline Nava[3,10], Agnès Rastetter[10], Marie-Laure Moutard[11,12,13], Vicki Anderson[14], Pierre Bitoun[15], Jens Bunt[6], Anne Faudet[3], Catherine Garel[16], Greta Gillies[1], Ilan Gobius[6], Justine Guegan[17], Solveig Heide[3,4], Boris Keren[3,10], Fabien Lesne[3], Vesna Lukic[18], Simone A Mandelstam[2,19,20], George Mcgillivray[21], Alissandra McIlroy[14], Aurélie Méneret[10,22] , Cyril Mignot[3,4,5], Laura R Morcom[6], Sylvie Odent[23,24], Annalisa Paolino[6,] Kate Pope[1], Florence Riant[25], Gail A Robinson[26], Megan Spencer-Smith[14,27], Myriam Srour[28,29], Sarah EM Stephenson[1,2], Rick Tankard[30,31], Oriane Trouillard[10], Quentin Welniarz[10,32], Amanda Wood[14.33], Alexis Brice,[3,10] Guy Rouleau[29,34], Tania Attié-Bitach[35], Martin B Delatycki[1,2,36] Jean-Louis Mandel[8,37], David J Amor[1,2], Emmanuel Roze[10,22], Amélie Piton[8,37], Melanie Bahlo[30,31], Thierry Billette de Villemeur[5,11,12,38], Elliott H Sherr[39], Richard J Leventer[2,40,41], Linda J Richards[6,42]§*, Paul J Lockhart[1,2] §*, Christel Depienne[3,8,10,37]§*

[1] Bruce Lefroy Centre for Genetic Health Research, Murdoch Childrens Research Institute, Royal Children's Hospital, Parkville, Victoria 3052, Australia.

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

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

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

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

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

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

[8] Département de Médicine translationnelle et Neurogénétique, IGBMC, CNRS UMR 7104/INSERM U964/Université de Strasbourg, 67400 Illkirch, France.

[9] Drug Delivery, Disposition and Dynamics (D4), Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, Victoria, 3052 Australia.

[10] INSERM, U 1127, CNRS UMR 7225, Sorbonne Universités, UPMC Univ Paris 06 UMR S 1127, Institut du Cerveau et de la Moelle épinière, ICM, F-75013, Paris, France

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

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

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

[14] Developmental Imaging and Child Neuropsychology Research groups, Murdoch Childrens Research Institute, Parkville, Victoria 3052, Australia.

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

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

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

[18] Bioinformatics Division, The Walter and Eliza Hall Institute of Medical Research, 1G Royal Parade, Parkville, Victoria 3052, Australia.

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

[20] Department of Radiology, University of Melbourne, Royal Children's Hospital, Parkville, Victoria 3052, Australia.

[21] Victorian Clinical Genetics Services, Murdoch Childrens Research Institute, Parkville, Victoria 3052, Australia.

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

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

[24] UMR 6290 CNRS, IGDR Institut de Génétique et développement de Rennes, Université de Rennes1, Rennes France.

[25] AP-HP, Groupe Hospitalier Saint Louis, Lariboisière, Fernand Widal, Laboratoire de Génétique, 75010 Paris, France.

[26] Neuropsychology Research Unit, School of Psychology, The University of Queensland, Brisbane QLD 4072, Australia.

[27] School of Psychological Sciences and Monash Institute of Cognitive and Clinical Neurosciences, Monash University, Clayton Campus, Clayton Victoria 3800, Australia.

[28] Department of Pediatrics, Montreal Children's Hospital, McGill University, Montréal, Quebec, H4A 3J1, Canada.

[29] Department of Neurology and Neurosurgery, McGill University Health Center, Montreal, Quebec, H3A 2B4 Canada.

[30] Population Health and Immunity Division, The Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, 3052, Australia.

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

[32] Institut de Biologie Paris Seine, Neuroscience Paris Seine, Sorbonne Universités, UPMC Univ Paris 06, INSERM, CNRS, F-75005, Paris, France

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

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

[35] INSERM U1163, Laboratory of Embryology and Genetics of Congenital Malformations, Paris Descartes University, Sorbonne Paris Cité and Imagine Institute, 75015 Paris, France; Département de Génétique, Hôpital Necker - Enfants Malades, Assistance Publique - Hôpitaux de Paris, 75015 Paris, France.

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

[37] Laboratoires de génétique, Institut de génétique médicale d'Alsace, Hôpitaux Universitaires de Strasbourg, 67 000 Strasbourg, France.

[38] INSERM U1141, 75019 Paris, France.

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

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

[41] Department of Neurology, University of Melbourne, Royal Children's Hospital, Parkville, Victoria 3052, Australia.

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

† These authors contributed equally to this work.

§ Equal last and senior authors

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

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 hemispheres[1]. Formation of the CC is a complex process involving ligands such as those in the Netrin, Ephrin, Semaphorin and Slit families and their receptors[2]. 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 termination[5]. 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 midline[8]. While mutations in *DCC* have been associated with congenital mirror movements (MM) in humans[9], 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 3[10]. 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 development[11-13]; 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%; $p=5x10^{-4}$ (all rare variants) or 284/~60000, 0.47%; $p=3x10^{-5}$ (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 guidance[14].

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 connectivity[16]. 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 midline[17]. 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 MM[18], 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.

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

Data Availability.

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

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AUTHOR CONTRIBUTIONS

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

Vicki Anderson - acquisition, analysis and/or interpretation of data and drafting the article or revising it critically for important intellectual content

Pierre Bitoun - acquisition, analysis and interpretation of data

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

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

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

Greta Gillies - acquisition, analysis and interpretation of data

Ilan Gobius - acquisition, analysis and interpretation of data

Justine Guegan - analysis of data

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

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

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

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

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

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

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

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

Cyril Mignot - acquisition, analysis and/or interpretation of data, and revising the article critically for important intellectual content

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

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

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

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

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

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

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

Myriam Srour - acquisition, analysis and/or interpretation of data

Sarah Stephenson - contributions to experimental conception and design

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

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

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

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

Alexis Brice - acquisition, analysis and/or interpretation of data and revising the article critically for important intellectual content

Guy Rouleau - acquisition, analysis and/or interpretation of data and revising the article critically for important intellectual content

Tania Attié-Bitach- contributions to experimental design, and revising the article critically for important intellectual content

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

Jean Louis Mandel - contributions to experimental conception and design, interpretation of data and revising the article critically for important intellectual content

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

Emmanuel Roze - acquisition, analysis and/or interpretation of data and revising the article critically for important intellectual content

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

Melanie Bahlo - acquisition, analysis and/or interpretation of data and drafting the article or revising it critically for important intellectual content

Thierry Billette de Villemeur - acquisition, analysis and/or interpretation of data and revising the article critically for important intellectual content

Elliott Sherr - formulation of theory and prediction, acquisition, analysis and/or interpretation of data and drafting the article and revising it critically for important intellectual content

Richard Leventer - formulation of theory and prediction, acquisition, analysis and/or interpretation of data and drafting the article and revising it critically for important intellectual content

Linda Richards - 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

Paul Lockhart - 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

Christel Depienne - 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

COMPETING FINANCIAL INTERESTS

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.

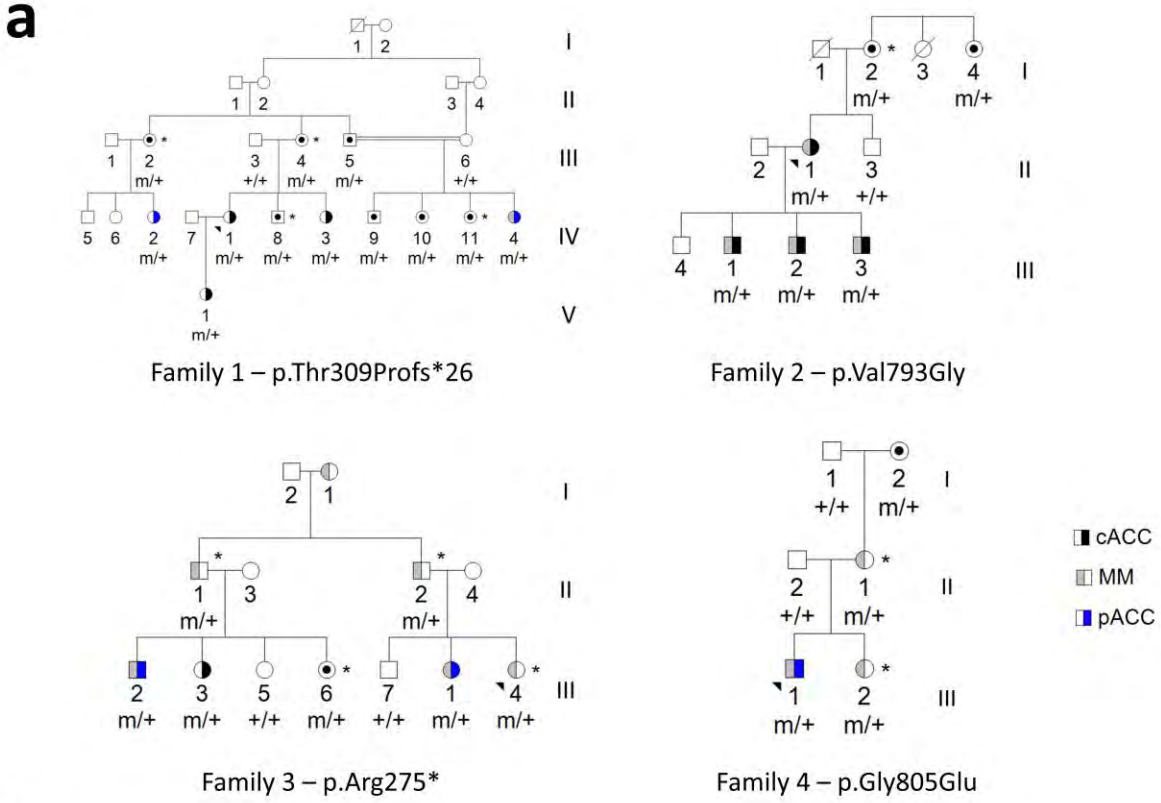
b. Midsagittal MRI of control and family proband/representative individual (1-2=complete ACC; 3=near complete ACC with thin rostrum and genu remaining and 4=partial ACC with absence of the rostrum and genu).

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.

Table 1: Summary of DCC mutations identified in individuals with ACC (+/-MM) in this study. cACC, complete isolated agenesis of the corpus callosum; pACC, partial isolated agenesis of the corpus callosum; MM, mirror movements; IgC2, immunoglobulin-like type C2 domain; FN3, fibronectin type III-like domain; ExAC, Exome Aggregation Consortium; dbSNP, dbSNP reference SNP 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
I
II
III
IV
V
Family 1 – p.Thr309Profs*26
Family 2 – p.Val793Gly
Family 3 – p.Arg275*
Family 4 – p.Gly805Glu
cACC
MM
pACC
b
Family 1
Family 2
Family 3
Family 4
Con
IV-1
II-1
III-1
III-1
c
Laterality coefficient
1.0
0.5
0.0
-0.5
-1.0
**
*
Control
Family 2
Family 4
d
Netrin-1
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
IgC2
FN3
P1
P2
P3
1447
N
C
R275X
T309Profs*26
R597P
M743L
V754M
V793G
G805E
A893T
TM
M1217V:A1250T
Extracellular Domain
Cytoplasmic Domain