Received: 13 August 2017 | Revised: 8 October 2017 | Accepted: 11 October 2017

DOI: 10.1002/humu.23361

MUTATION UPDATE

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DCC mutation update: Congenital mirror movements, isolated agenesis of the corpus callosum, and developmental split brain syndrome

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Funding information
Contract Grant Sponsors: National Health and Medical Research Council (NHMRC) Australia (GNT1059666, GNT1126153, GNT1032364); Campbell Edwards Trust; Victorian Government's Operational Infrastructure Support Program; Australian Government NHMRC IRIISS; Boston Children's Hospital; National Institutes of Health IDDRC (1U54 HD090255); Australian Postgraduate Award; University of Queensland Research Scholarship; Thierry and Annick Desmarest Foundation.

Abstract

The deleted in colorectal cancer (*DCC*) gene encodes the netrin-1 (NTN1) receptor DCC, a transmembrane protein required for the guidance of commissural axons. Germline *DCC* mutations disrupt the development of predominantly commissural tracts in the central nervous system (CNS) and cause a spectrum of neurological disorders. Monoallelic, missense, and predicted loss-of-function *DCC* mutations cause congenital mirror movements, isolated agenesis of the corpus callosum (ACC), or both. Biallelic, predicted loss-of-function *DCC* mutations cause developmental split brain syndrome (DSBS). Although the underlying molecular mechanisms leading to disease remain poorly understood, they are thought to stem from reduced or perturbed NTN1 signaling. Here, we review the 26 reported *DCC* mutations associated with abnormal CNS development in humans, including 14 missense and 12 predicted loss-of-function mutations, and discuss their associated clinical characteristics and diagnostic features. We provide an update on the observed genotype–phenotype relationships of congenital mirror movements, isolated ACC and DSBS, and correlate this to our current understanding of the biological function of DCC in the development of the CNS. All mutations and their associated phenotypes were deposited into a locus-specific LOVD (https://databases.lovd.nl/shared/genes/DCC).

KEYWORDS
ACC, agenesis of the corpus callosum, axon guidance, DCC, developmental split brain syndrome, horizontal gaze palsy with progressive scoliosis, mirror movements, mutation, Netrin-1, NTN1

1 | BACKGROUND

Deleted in colorectal cancer (*DCC*) (MIM# 120470) encodes the DCC netrin-1 (NTN1) receptor protein, an evolutionarily conserved, single-pass transmembrane glycoprotein belonging to the immunoglobulin (Ig) superfamily of cell adhesion molecules (Fearon et al., 1990; Keino-Masu et al., 1996). *DCC* is located at 18q21.2 and spans chr18:52,340,172–53,535,903 (GRCh38/hg38) (Hedrick et al., 1994). The canonical *DCC* transcript consists of 29 exons that encode a 159 kDa (1,447 amino acid) protein. DCC is a putative dependence receptor originally thought to function as a tumor suppressor gene, although this role has not been proven in vivo and remains controversial (Bin et al., 2015; Fearon et al., 1990; Llambi, Causeret, Bloch-Gallego, & Mehlen, 2001; Williams et al., 2006). More recently, the role of DCC in the development of the central nervous system (CNS) was established (Fazeli et al., 1997; Finger et al., 2002; Jamuar et al., 2017; Keino-Masu et al., 1996; Marsh, et al., 2017; Srour et al., 2010).

DCC is expressed by commissural axons and binds NTN1, a secreted protein encoded by the *NTN1* gene (MIM# 601614), which functions both locally and diffusely as a bifunctional guidance cue (Blasiak, Kilinc, & Lee, 2017; Keino-Masu et al., 1996; Kennedy, Serafini, de la Torre, & Tessier-Lavigne, 1994). Both *DCC* and *NTN1* are expressed throughout the developing mouse and human brain, with distinct but complementary spatial and temporal expression patterns (Harter et al., 2010; Jamuar et al., 2017; Ren et al., 2006; Shu, Valentino, Seaman, Cooper, & Richards, 2000). DCC is required for the transduction of NTN1-induced attractive and long-range repulsive signaling in the coordinated outgrowth and guidance of commissural axons that cross the anatomical midline of the body (Chan et al., 1996; Hong et al., 1999; Keino-Masu et al., 1996).

The development of commissural tracts is dependent on neurons forming synaptic connections with their target cells located on the opposite side of the CNS. To achieve this, neurons extend commissural axons tipped with a specialized, motile sensing device called a growth cone (Tessier-Lavigne & Goodman, 1996). Growth cones express an array of axon guidance receptors such as DCC. The binding of a chemotactic (diffusible) or haptotactic (substrate-bound) guidance cue to an axon guidance receptor generates a permissive or non-permissive axonal outgrowth signal, or an attractive or repulsive directional response (Raper & Mason, 2010; Tessier-Lavigne & Goodman, 1996). The signals transduced by axon guidance receptors converge to modulate the assembly of growth cone filamentous actin and cytoskeletal microtubules required for directing axon outgrowth and guidance toward or away from a guiding cell population (Kahn & Baas, 2016; Luo, 2002).

Monoallelic and biallelic germline *DCC* mutations disrupt commissural axon guidance (Jamuar et al., 2017; Marsh, et al., 2017; Srour et al., 2010; Welniarz et al., 2017b). These disruptions impair the normal development and function of tracts such as the corticospinal tract (CST) and corpus callosum (CC). Monoallelic *DCC* mutations cause congenital mirror movements (MMs; MIM# 157600) in association with abnormal midline crossing of the CST, isolated agenesis of the

CC (iACC; MIM# 217990), or both (Marsh et al., 2017; Srour et al., 2010). Alternatively, biallelic *DCC* mutations leading to predicted loss-of-function (LoF) cause developmental split brain syndrome (DSBS; MIM# 617542), a more complex syndrome associated with agenesis of the corpus callosum (ACC) as well as widespread failure of commissural tracts throughout the rest of the CNS, with or without MMs (Jamuar et al., 2017).

2 | VARIANT DATABASE

We created a new repository for all reported disease-associated *DCC* sequence variants using the Leiden Open Variation Database (LOVD 3.0: https://databases.lovd.nl/shared/genes/DCC) (Fokkema et al., 2011). The International Research Consortium for the Corpus Callosum and Cerebral Connectivity (IRC^5, https://www.irc5.org) encourages users to register and submit data, including clinical and neuroimaging phenotypic data.

3 | VARIANT NOMENCLATURE

The nomenclature for DNA and protein sequence variants adheres to the guidelines of the Human Genome Variation Society (den Dunnen & Antonarakis, 2000). Variant descriptions were based on the following Genbank reference sequences: NG_013341.2, NM_005215.3, and NP_005206.2. We utilized the Mutalyzer program (https://mutalyzer.nl/) to validate variant descriptions. At the time of publication, the *DCC* Locus Reference Genomic sequence (LRG_1107) was under curation and pending approval (MacArthur et al., 2014).

4 | VARIANTS

To date, 26 unique *DCC* mutations have been associated with MMs, iACC, and DSBS in 66 individuals from 25 unrelated families (Figure 1). There are 29 reported unaffected heterozygous mutation carriers; 11 confirmed carriers from seven unrelated families assessed for MMs and brain abnormalities using magnetic resonance imaging (MRI) or computed tomography (CT), and 18 carriers from nine unrelated families that appear clinically unaffected but have not been assessed for MMs and brain abnormalities. There are no reported unaffected individuals with biallelic, predicted LoF *DCC* mutations.

Monoallelic or biallelic missense (14 out of 24; 58%) and monoallelic, predicted LoF (10 out of 24; 42%) *DCC* mutations may cause MMs, iACC, or both (Table 1). These include one case each where two heterozygous missense mutations were inherited in cis p.(Met1217Val;p.Ala1250Thr) or in trans p.(Gly470Asp);(p.(Gly803Arg) and a biallelic p.(Gln691Lys) mutation observed in conjunction with an interhemispheric cyst and modest learning difficulties (Jamuar et al., 2017; Marsh et al., 2017; Méneret et al., 2014a). Alternatively, biallelic, predicted LoF (two out of two; 100%) *DCC* mutations cause DSBS. Germline transmission of the mutant allele, except for a p.(Gly803Arg) mutation that arose de novo, was confirmed in all pedigrees where parental genotypes were available (Méneret et al., 2014a). *DCC*-MMs and *DCC*-iACC are both associated with reduced penetrance: MMs ≈42% penetrance and iACC ≈26% penetrance (Marsh et al., 2017). In contrast, DSBS appears to be fully penetrant. Monoallelic *DCC* mutations are also associated with variable expressivity and affected individuals within one family may present with MMs, iACC, or both (Marsh et al., 2017). All DCC residues altered by a missense mutation are conserved throughout vertebrate evolution, although it should be noted that Dcc has been lost in passeriformes and galliformes during bird evolution, and are either novel (eight out of 14; 57%) or found at a minor allele frequency of less than 0.5% (six out of 14; 43%) in the Exome Aggregation Consortium (ExAC) (Supp. Table S1) (Friocourt et al., 2017). Predicted LoF *DCC* mutations include nine frameshift mutations, two truncating mutations and an intragenic deletion encompassing exons 4 and 5 (Table 1).

5 | STRUCTURE AND FUNCTION

DCC is composed of four structurally distinct, evolutionarily conserved regions: four extracellular Ig-like domains, six extracellular fibronectin

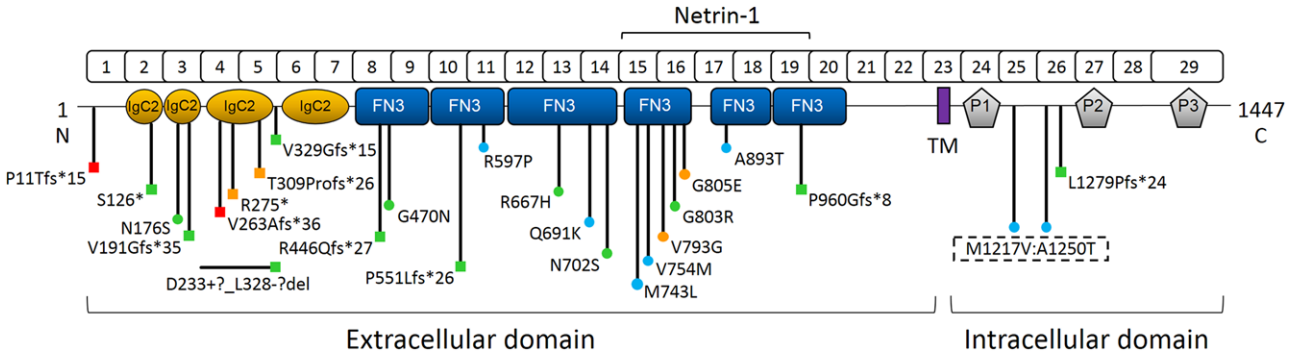

FIGURE 1 Linear *DCC* gene schematic with protein domain structure depicting the location of all reported mutations. Square, predicted loss-of-function; circle, missense mutation; red, DSBS; green, MMs; blue, iACC; orange, MMs and iACC. The NTN1 binding region is indicated. IgC2, immunoglobulin-like type C2 domain; FN3, fibronectin type III-like domain; TM, transmembrane domain; P1–3, conserved P motifs. The NP_005206.2 reference sequence is used. Image modified from Marsh et al. (2017)

TABLE 1 Overview of all reported *DCC* mutations linked to MMs, iACC, and DSBS

Family	Phenotype	No. of affected	Sex of affected	Variant identification	Inheritance (transmission)	Allele	Exon/intron	cDNA	Protein	Protein domain	ExAC	dbSNP	Reference
1	DSBSΔ	2	2M	Hom. + SEQ	Germline (both)*	Bi.	1	c.31_91 +7622del	p.(Pro11 Thrfs*15)	–	–	–	Jamuar et al. (2017)
2	MMs	2	2M	SEQ	Germline (paternal)	Mono.	2	c.377C>A	p.(Ser126*)	IgC2-1	–	–	Méneret et al. (2014a)
3	MMs†	1	1M	SEQ	Germline (maternal)	Mono.	3	c.527A>G	p.(Asn176Ser)	IgC2-2	5/121228	rs138724679	Méneret et al. (2014a)
4	MMs†	4	4M	SEQ	Germline (either)*	Mono.	3	c.571dupG	p.(Val191Glyfs*35)	IgC2-2	–	–	Sharafaddinzadeh et al. (2008) Srour et al. (2010)
5	MMs	1	1F	SEQ	Unknown (unknown)	Mono.	3i_5i	c.(697+1_698-1)_ (985+1_986-1) del	p.(Asp233+?_ Leu328-?del)	IgC2-3	–	–	Borgheresi et al. (2010) Méneret et al. (2014a)
6	DSBSΔ	1	1F	SEQ	Germline (both)	Bi.	4	c.788_794del	p.(Val263Alafs*36)	IgC2-3	–	–	Jamuar et al. (2017)
7	cACC or MMs ± pACC†	7	3M:4F	SEQ	Germline (either)*	Mono.	4	c.823C>T	p.(Arg275*)	IgC2-3	–	–	Méneret et al. (2014a) Marsh et al. (2017) Welniarz et al. (2017b)
8	MM ± pACC	3	1M:2F	SEQ	Germline (either)	Mono.	4	c.823C>T	p.(Arg275*)	IgC2-3	–	–	Méneret et al. (2014a) Meneret et al. (2015) Welniarz et al. (2017b)
9	cACC or pACC ± MMs†	5	5F	Linkage + ES + SEQ	Germline (either)	Mono.	5	c.925delA	p.(Thr309 Profs*26)	IgC2-3	–	–	Marsh et al. (2017)
10	MMs†	11	9M:2F	Linkage + SEQ	Germline (either)	Mono.	6i	c.1140+1G>A	p.(Val329 Glyfs*15)	IgC2-3-IgC2-4 linker	–	–	Srour et al. (2009) Srour et al. (2010)
11	MMs†	1	1M	SEQ	Germline (maternal)	Mono.	8	c.1336_1337 insAGCC	p.(Arg446 Glnfs*27)	FN3-1	–	–	Méneret et al. (2014a)
12	MMs†	1	1F	SEQ	Germline (maternal); de novo (in trans)	Bi.	8;16	c.1409G>A; c.2407G>A	p.(Gly470Asp); p.(Gly803Arg)	FN3-1;FN3-4	385/121006;-	rs141813053/-	Méneret et al. (2014a)
13	MMs	4	3M:1F	Linkage + ES + SEQ	Germline (maternal)*	Mono.	10	c.1652delC	p.(Pro551 Leufs*26)	FN3-2	–	–	Franz et al. (2015)

(Continues)

TABLE 1 (Continued)

Family	Phenotype	No. of affected	Sex of affected	Variant identification	Inheritance (transmission)	Allele	Exon/ intron	cDNA	Protein	Protein domain	ExAC	dbSNP	Reference
14	cACC†	1	1F	SEQ	Germline (paternal)	Mono.	11	c.1790G>C	p.(Arg597Pro)	FN3-2	–	–	Marsh et al. (2017)
15	MMs†	1	1M	SEQ	Germline (unknown)	Mono.	13	c.2000G>A	p.(Arg667His)	FN3-3	194/121316	rs200099519	Méneret et al. (2014a)
16	cACC	1	1M	SEQ	Unknown (unknown)	Bi.	14	c.2071C>A	p.(Gln691Lys)	FN3-3	–	–	Griebel et al. (1995) Jamuar et al. (2017)
17	MMs	1	1F	SEQ	Unknown (unknown)	Mono.	14	c.2105A>G	p.(Asn702Ser)	FN3-3	372/121354	rs35691189	Djarmati-Westenberger et al. (2011) Méneret et al. (2014a)
18	cACC†	1	1M	SEQ	Germline (paternal)	Mono.	15	c.2227A>T	p.(Met743Leu)	FN3-4	–	rs199651452	Marsh et al. (2017)
19	cACC†	2	1M:1F	SEQ	Germline (paternal)	Mono.	15	c.2260G>A	p.(Val754Met)	FN3-4	19/121284	–	Marsh et al. (2017)
20	cACC and MMs†	4	3M:1F	Linkage + ES + SEQ	Germline (maternal)*	Mono.	16	c.2378T>G	p.(Val793Gly)	FN3-4	–	–	Marsh et al. (2017)
21	pACC and/or MMs†	3	1M:2F	SEQ	Germline (maternal)	Mono.	16	c.2414G>A	p.(Gly805Glu)	FN3-4	–	–	Marsh et al. (2017)
22	cACC	1	1F	SEQ	Unknown (unknown)	Mono.	17	c.2677G > A	p.(Ala893Thr)	FN3-5	–	–	Marsh et al. (2017)
23	MMs	3	3F	SEQ	Germline (maternal)*	Mono.	19	c.2873 _2877dup	p.(Pro960 Glyfs*8)	FN3-6	–	–	Méneret et al. (2014a)
24	cACC†	1	1F	SEQ	Germline (in cis) (maternal)*	Mono.	25;26	c.3649A>G; c.3748G>A	p.(Met1217Val); p.(Ala1250Thr)	P1-P2 linker	-;2/121388	–	Marsh et al. (2017)
25	MMs	4	2M:2F	SEQ	Germline (either)*	Mono.	26	c.3836_3837del	p.(Leu1279 Profs*24)	P1-P2 linker	–	–	Cincotta et al. (2002) Depienne et al. (2012)

DSBS, developmental split brain syndrome; cACC, complete isolated agenesis of the corpus callosum; pACC, partial isolated agenesis of the corpus callosum; MMs, mirror movements; Δ, unaffected carrier; †, incomplete penetrance; ES, exome sequencing; SEQ, direct sequencing of *DCC*; Hom.; homozygosity mapping; *, inferred transmission of mutant allele; Bi., biallelic; Mono., monoallelic; IgC2, immunoglobulin-like type C2 domain; FN3, fibronectin type III-like domain; ExAC, Exome Aggregation Consortium; dbSNP, dbSNP reference SNP identification number. The NM_005215.3 and NP_005206.2 reference sequences are used.

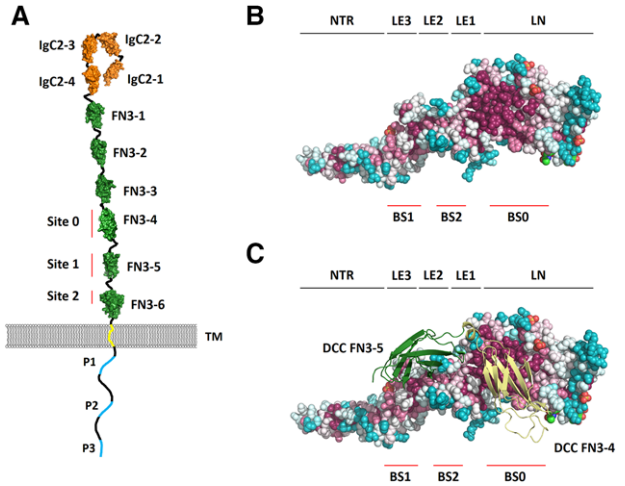

FIGURE 2 **A**: Schematic drawing of DCC with protein domain structure depicting the location of the NTN1 binding sites (BS) 0, 1, and 2. **B**: Structure of NTN1 protein depicting the location of its DCC binding sites BS0, BS1, and BS2. **C**: NTN1 residues (colored spheres) that bind the 4th and 5th FN3 domains of DCC (yellow and green ribbons, respectively) are conserved throughout evolution. Conserved residues are colored red, non-conserved residues are colored blue. IgC2, immunoglobulin-like type C2 domain; FN3, fibronectin type III-like domain; P1–3, conserved P motifs; NTR, netrin-like domain; LE, laminin-type epidermal growth factor-like domain; LN, laminin-like domain

type III-like (FN3) domains, a transmembrane domain, and an intracellular domain with three conserved P motifs (Supp. Figure S1) (Kolodziej et al., 1996). Each region of DCC appears to have a unique functional role required for the transduction of NTN1 signaling.

The distal N-terminal region of DCC contains four Ig-like domains and three inter-domain linkers that together comprise 371 amino acids (residues 46–417). These domains fold into a horseshoe-like configuration that appears necessary for DCC to transduce NTN1-signaling (Chen et al., 2013). The proximal N-terminal region of DCC contains six FN3 domains and five inter-domain linkers that together comprise 612 amino acids (residues 429–1041). Regions within the 4th, 5th, and 6th FN3 domains bind NTN1 (Bennett et al., 2002; Finci et al., 2014; Geisbrecht, Dowd, Barfield, Longo, & Leahy, 2003; Xu, et al., 2014). This extracellular binding brings two DCC proteins into close proximity, enabling their intracellular domains to homodimerize and initiate the recruitment of multi-protein complexes required to transduce NTN1 signaling (Mille et al., 2009; Stein, Zou, Poo, & Tessier-Lavigne, 2001).

The DCC ligand, NTN1, is an extracellular protein belonging to the laminin superfamily and is composed of three structurally distinct regions: a laminin-like domain (LN), three laminin-type epidermal growth factor-like domains (LE) and a netrin-like domain (NTR) (Serafini et al., 1994). NTN1 has three distinct DCC-binding sites (binding site 1, 2, and 0; BS1, BS2, and BS0) (Figure 2). BS1 is located on the third LE of NTN1 and interacts exclusively with the 5th FN3 domain of DCC. This site functions as a DCC-specific binding site and is stabilized by several hydrophobic interactions supported by a surrounding network of hydrogen bonds (Figure 3A) (Finci et al., 2014). BS2 is located on the first and second LE domains of NTN1 and interacts with a distinct region of the 5th FN3 domain as well as the N-terminal region of the 6th FN3 domain of DCC. This site functions as a generic binding site that can interact with receptors other than DCC (Finci et al., 2014). BS2 is predominantly stabilized by a group of sulfate and chloride anions that neutralize positively charged patches on NTN1 and DCC residues located at the binding interface (Figure 3B) (Finci et al., 2014). The adaptable nature of BS2 allows binding to the 1st and 2nd Ig-like domains of members of the unc-5 netrin receptor (UNC5; MIM# 603610) family of repulsive axon guidance proteins, which appear to have a higher affinity for BS2 relative to DCC (Finci et al., 2014; Geisbrecht et al., 2003; Hong et al., 1999; Leonardo et al., 1997). As a result, UNC5 are able to outcompete DCC at BS2 and switch an attractive growth cone response (mediated via NTN1-induced DCC homodimerization) to a repulsive response (mediated via NTN1-induced DCC and UNC5 heterodimerization) (Finci et al., 2014; Grandin et al., 2016; Hong et al., 1999). In contrast to BS1 and BS2, BS0 is located on the LN of NTN1 and interacts with the 4th FN3 domain of DCC (Xu et al., 2014). This NTN1 protein is different to the one simultaneously engaged at BS1 and BS2, but binds the same DCC protein occupied at BS1 (Figure 3C). The binding of DCC by a second NTN1 protein at BS0 is thought to generate a signaling cluster important for the transduction of a directional response toward or away from a guiding cell population (Finci, Zhang, Meijers, & Wang, 2015).

Taken together, these studies indicate that NTN1 forms two distinct binding clusters through BS0, BS1, and BS2 and that these clusters underpin its bifunctionality as an attractive and repulsive guidance cue. The first cluster involves the 4th and 5th FN3 domains of one DCC protein at BS0 and BS1, while the second cluster involves the 5th and 6th FN3 domains of a second DCC protein or the 1st and 2nd Ig-like domains of an UNC5 family member protein at BS2. However, it should be noted that the actual in vivo binding structure may differ as these studies utilized a recombinant NTN1 protein lacking its NTR domain and different truncated DCC fragments, both lacking the NTN1 binding region in its entirety (Finci et al., 2014; Xu et al., 2014).

The importance of the 4th, 5th, and 6th FN3 domains are highlighted by the significant enrichment of *DCC* missense mutations linked to MMs and/or iACC within these NTN1 binding regions compared with missense variants located in these domains in the ExAC (Figure 4A and B) (Marsh et al., 2017). This enrichment is most significant for monoallelic, missense mutations linked to iACC (five out of nine; 56%) and suggests that missense mutations may cause disease through different, perhaps more complex mechanisms compared with those mutations leading to predicted LoF and haploinsufficiency. This may be due to reduced or perturbed NTN1-induced axon repulsion and/or attraction caused by dysfunction of the mutant DCC protein in both a mutant-mutant homodimer and wild-type–mutant homodimer complex. Additional studies are required to determine whether these missense alterations cause complete or partial LoF or perhaps gain-of-function as antimorphic, hypermorphic, or neomorphic mutations. For instance, it remains to be determined whether mutant DCC proteins are stably expressed and trafficked to the plasma membrane or aberrantly sequestered in the cytoplasm like mutant NTN1 proteins

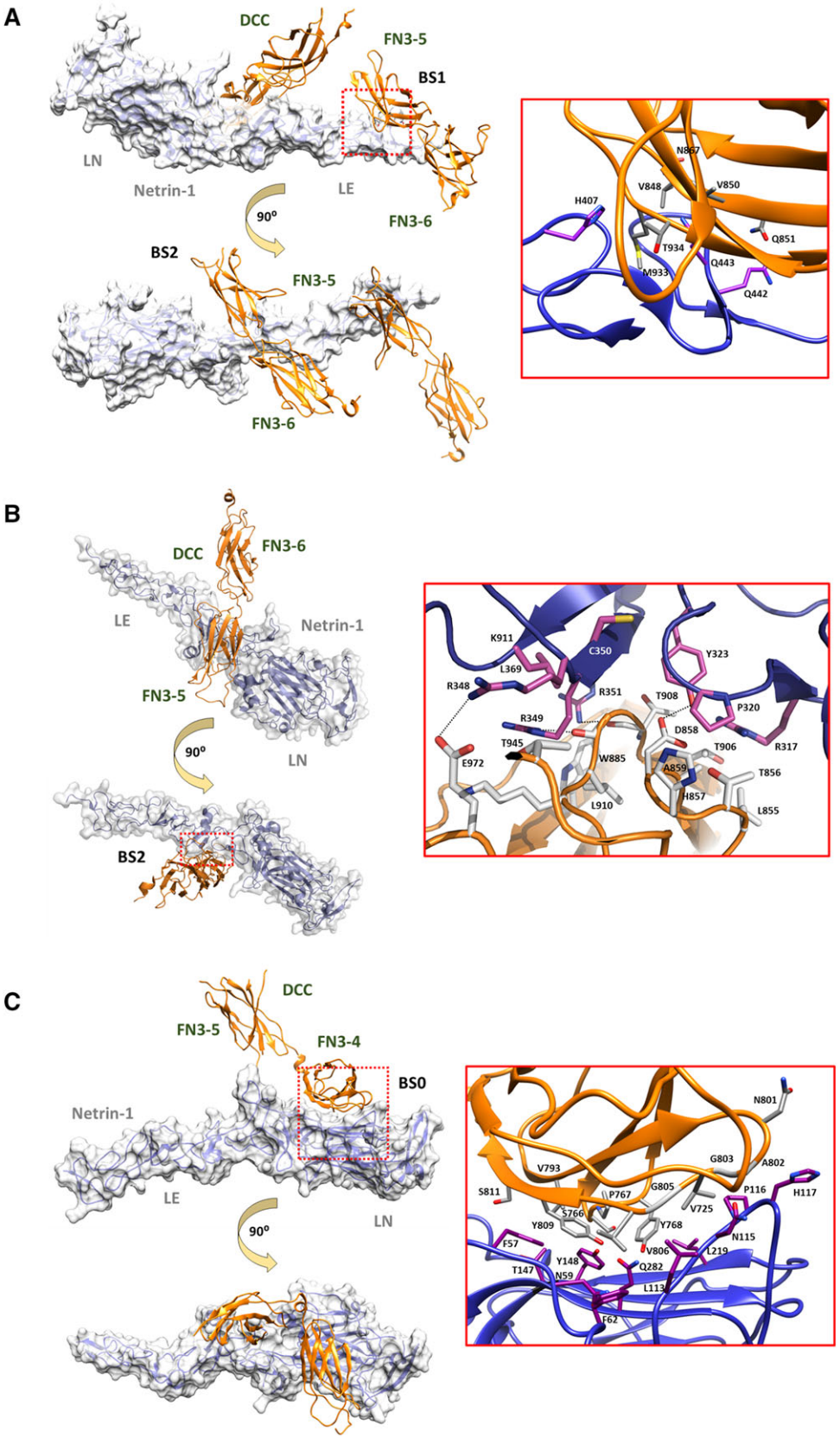

FIGURE 3 **A**: Binding site 1 (BS1) and BS2 of the NTN1/DCC complex (PDB ID: 4URT). **B**: BS2 of the NTN1/DCC complex (PDB ID: 4URT). **C**: BS0 of the NTN1/DCC complex (PDB ID: 4PLO). Structure of NTN1 (transparent molecular surface) bound to DCC FN3 domains (orange ribbons). The expansion of the red dotted box region shows the NTN1 (magenta sticks) and DCC (gray or white sticks) involved in binding at BS1, BS2, or BS0. Hydrogen bonds are represented by dotted black lines. FN3, fibronectin type III-like domain; PDB ID, Protein Data Bank identification

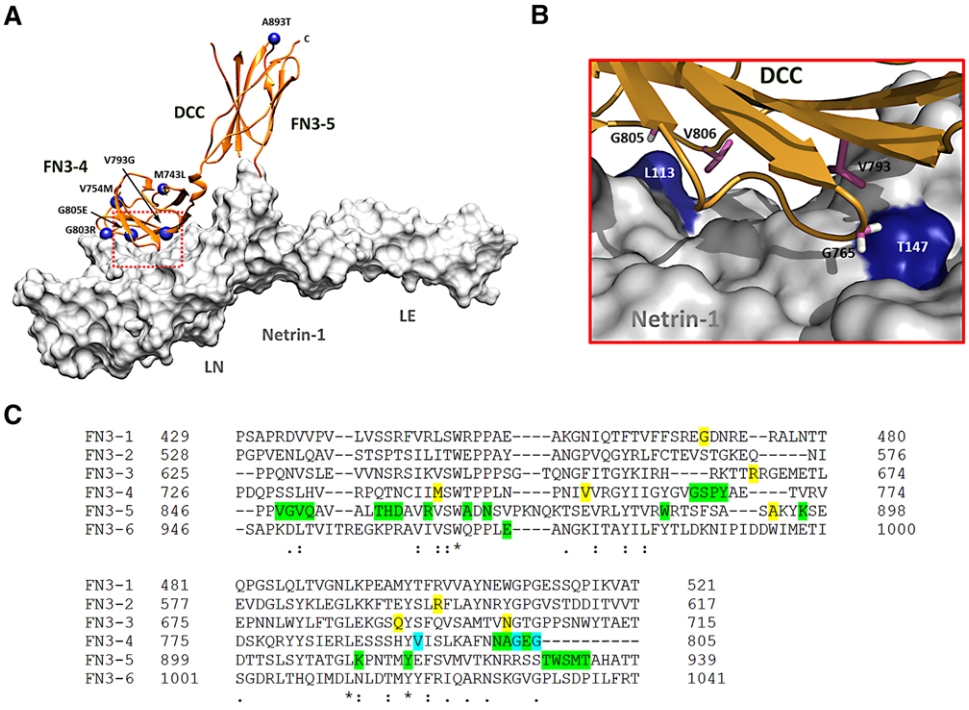

FIGURE 4 The fibronectin type III-like (FN3) domains are required for NTN1 binding and DCC function. **A**: Enrichment of *DCC* missense mutations linked to MMs and/or iACC within the NTN1 binding region. Structure of NTN1/DCC complex. DCC is depicted as an orange ribbon and NTN1 as a white solvent accessible surface. *DCC* missense mutations located within the FN3-4 and FN3-5 domains are represented as blue spheres (PDB ID: 4PLO). **B**: Expansion of the binding interface (red dotted box in A) with NTN1 residues colored blue and critical DCC residues represented as purple sticks. Mutation of V793 and G805 to Gly and Glu, respectively, is associated with both MMs and iACC. **C**: Sequence alignment of the DCC FN3 domains. Residues highlighted in green are predicted to be directly involved in NTN1 binding by Finci et al. 2014 and/or Xu et al. (2014). Residues in yellow are missense mutations associated with MMs and/or iACC. Residues in blue are missense mutations associated with MMs and/or iACC that are also predicted to be directly involved in NTN1 binding. The NP_005206.2 reference sequence is used. PDB ID, Protein Data Bank identification. Image modified from Marsh et al. (2017)

(Méneret et al., 2017). However, absence of the DSBS phenotype in an individual with a biallelic p.(Gln691Lys) missense mutation suggests that these mutant proteins may retain some residual function (Jamuar et al., 2017).

The DCC transmembrane domain comprises 24 amino acids (residues 1,098–1,122). To bind NTN1, DCC must correctly partition its transmembrane domain within the cell plasma membrane. The palmitoylation and localization of the transmembrane domain to lipid rafts (cholesterol- and sphingolipid-enriched membrane subdomains of the plasma membrane) has been demonstrated to be necessary for DCC to transduce NTN1-induced axon guidance and outgrowth (Guirland, Suzuki, Kojima, Lu, & Zheng, 2004; Herincs et al., 2005).

The intracellular, C-terminal region of DCC contains 324 amino acids (residues 1,123–1,447) and is required to transduce NTN1 signals into appropriate growth cone responses. Within the C-terminal region there are three conserved P motifs referred to as P1 (encoded by residues 1,150–1,167), P2 (encoded by residues 1,330–1,365), and P3 (encoded by residues 1,424–1,447) (Kolodziej et al., 1996). These P motifs are involved in numerous protein–protein interactions and phosphorylation cascades required for the intracellular transduction of NTN1 signaling (Fothergill et al., 2014; Hong et al., 1999; Li et al., 2004; Ren et al., 2004; Stein & Tessier-Lavigne, 2001). For example, the NTN1-induced, intracellular homodimerization of the DCC P3 motifs initiates downstream signaling events that generate an attractive growth cone response (Li et al., 2002; Li et al., 2004; Liu et al., 2004; Meriane et al., 2004; Ren et al., 2004; Ren et al., 2008; Stein et al., 2001). Alternatively, the NTN1-induced, intracellular heterodimerization of the DCC P1 motif and the DCC binding (DB) motif of UNC5 family members initiates downstream signaling events that generate a long-range, repulsive growth cone response (Hong et al., 1999; Keleman & Dickson, 2001; Li, Aurandt, Jürgensen, Rao, & Guan, 2006; Norris, Sundararajan, Morgan, Roberts, & Lundquist, 2014).

6 | GENOTYPE–PHENOTYPE CORRELATION

Monoallelic frameshift and nonsense *DCC* mutations are predicted to result in haploinsufficiency via nonsense mediated mRNA decay or rapid turnover of the truncated protein, resulting in reduced NTN1 binding (Srour et al., 2010). This is in contrast to *DCC* missense mutations that likely generate a full-length protein within the cell (Finci et al., 2014; Meriane et al., 2004). DCC proteins carrying a missense mutation may be non-functional, hypomorphic, or may have an altered function that imparts a dominant effect through perturbed NTN1 binding, receptor multimerization and/or downstream signaling (Finci et al., 2014; Meriane et al., 2004). Monoallelic, truncating *DCC* mutations

predicted to result in haploinsufficiency are frequently associated with MMs (eight out of 10; 80%) or MMs with iACC (two out of 10; 20%). *DCC* missense mutations are less frequently associated with MMs (five out of 14; 36%). Instead, the majority of these mutations are associated with iACC (seven out of 14; 50%) or MMs with iACC (two out of 14; 14%). This disparity may be due to developmental differences between the CC and the subcerebrally projecting neurons that comprise the CST. The neuronal populations that comprise each of these tracts are molecularly distinct and uniquely employ DCC and NTN1 signaling to reach their contralateral targets (Fothergill et al., 2014; Molyneaux, Arlotta, Menezes, & Macklis, 2007). Therefore, a missense or predicated LoF *DCC* mutation may differentially affect commissural versus subcerebral axon trajectories, thus leading to iACC, MMs or both (Marsh et al., 2017). Although these observations do support the hypothesis that distinct disease mechanisms contribute to *DCC*-MMs or *DCC*-iACC, additional functional investigations are required to confirm this correlation. These investigations could utilize in vivo techniques such as in utero electroporation or conditional gene knockout to study the effect loss or dysfunction of Dcc has on the outgrowth and guidance of axons extending from specific populations of projection neurons during development.

Monoallelic, missense mutations located within the NTN1 binding sites of DCC appear to be strongly associated with iACC (Marsh et al., 2017). Indeed, the majority of *DCC* mutations located within the 4th and 5th FN3 domains are associated with iACC (five out of six; 83%). Interestingly, the p.(Val793Gly) and p.(Gly805Glu) missense mutations located directly within the NTN1 binding interface are associated with a more severe phenotype that features both iACC and MMs (Marsh et al., 2017). Previous studies have shown a differential disruption to NTN1-induced axon attraction and repulsion dependent on the location of the mutated DCC residue within the NTN1 binding interface (Figure 4C) (Finci et al., 2014). Consequently, *DCC* mutations located within the NTN1 binding interface may cause more severe disruptions to commissural axon outgrowth and guidance that result in a phenotype that features both iACC and MMs. Overall, it appears that the majority of missense iACC mutations localize to the FN3 domains (seven out of nine; 78%), highlighting the importance of these domains to DCC function and callosal development.

The observed structure of pedigrees with monoallelic, predicted LoF *DCC* mutations has suggested a sex-bias in MMs and iACC phenotype expression (Marsh et al., 2017; Sharafaddinzadeh, Bavarsad, Yousefkhah, & Aleali, 2008; Srour et al., 2009). Indeed, within these pedigrees a significant proportion of males displayed MMs, while iACC was almost exclusively detected in females (Marsh et al., 2017). Sex differences in callosal morphology have previously been linked with testosterone levels during prenatal brain development (Chura et al., 2010; Moffat, Hampson, Wickett, Vernon, & Lee, 1997). Consistent with these observations, RNAseq and RT-qPCR analyses have detected a significant dose-dependent increase in *DCC* expression in testosterone-treated neural stem cells derived from human embryonic stem cells (Marsh et al., 2017). These findings suggest that iACC may occur when the expression of *DCC* falls below a threshold level during CC development, as would occur more commonly in females. However, given the incomplete penetrance observed in both sexes, the phenotype must also be influenced by additional genetic, epigenetic, and/or environment factors (Marsh et al., 2017). Furthermore, the sex-specific nature of this phenotype imbalance remains to be verified as reciprocal analyses utilizing estrogens have not been reported. Additional in vivo investigations are required to evaluate the potential impact the hormonal context during brain development has on *DCC* expression and whether it influences the expressivity of the MMs and iACC phenotype.

7 | BIOLOGICAL SIGNIFICANCE

Commissural axons form connections between the left and right sides of the brain that are required for the transfer and integration of information generated by sensory, motor, and associative neurons. These connections are defined anatomically as either commissures or decussations. While commissures cross the midline to form predominantly homotopic connections, decussations descend or ascend along the neuraxis before crossing the midline to form connections with different neuronal populations. For instance, axons within the CC arch over the lateral ventricles in a segregated, orderly manner to form predominantly homotopic connections with their target cells in the contralateral cerebral hemisphere (Zhou et al., 2013). These connections function by mediating higher-order brain processes and facilitating the integration of sensory and motor information between the two cerebral hemispheres (Paul et al., 2007). Within the CST, axons cross the midline at the medulla to form the pyramidal decussation between the brainstem and the spinal cord (Nathan, Smith, & Deacon, 1990). The connections formed by axons within the crossed CST facilitate the voluntary and unilateral movement of the contralateral distal limbs (Welniarz, Dusart, & Roze, 2017a). Several additional tracts within the brainstem are disrupted in DSBS (Jamuar et al., 2017). These appear to include the decussation of the superior cerebellar peduncles (also referred to as the commissure of Wernekinck) that connects the cerebellum to the midbrain, and the commissural tract linking the lateral abducens and medial occulomotor nerves, required for conjugate horizontal eye movement (Jamuar et al., 2017; Jen, 2008).

The function of each commissural tract is underpinned by the transcriptionally distinct neuronal subtypes that comprise it (Harada, Sato, & Nakamura, 2016; Molyneaux et al., 2007). For instance, the CC is formed from pioneer axons extending from neurons in the cingulate cortex and follower axons extending from callosal projection neurons located within layers II/III and Va of the cerebral cortex (Fame, MacDonald, & Macklis, 2011). In comparison, the CST is comprised of an array of axons extending from subcerebrally projecting neurons situated within layer Vb of the cortex (Dum & Strick, 1991; Harwell et al., 2012). Notably, the tracts associated with the brainstem originate from diverse nuclei. For example, the transverse pontocerebellar projections arise from pontine nuclei while cranial nerves originate from nuclei located throughout the midbrain, pons and medulla. To reach their contralateral targets, these neuronal subtypes extend axons that express a unique repertoire of receptors, including DCC, on their growth cones (Dickson & Zou, 2010; Evans and Bashaw, 2010). The tightly regulated, spatiotemporal expression of axon guidance

receptors and their ligands control the development of tracts such as the CC and CST (Chedotal & Richards, 2010). As highlighted below, our understanding of the development of these tracts and the associated role of *DCC* and *NTN1* signaling in humans has been predominantly informed by the study of these biological processes in mice.

The formation of the CC is complex and dependent on the execution of several developmental steps. Briefly, these include midline patterning and remodeling of the interhemispheric fissure by astroglia to create a permissible substrate for commissural axons to cross the midline (Gobius et al., 2016; Hayhurst, Gore, Tessier-Lavigne, & McConnell, 2008; Okada, et al., 2008; Silver, Lorenz, Wahlsten, & Coughlin, 1982); secretion of guidance cues by populations of glia and neurons situated around the midline (Shu & Richards, 2001; Shu, Li, Keller, & Richards, 2003; Unni et al., 2012); pioneering of the callosal tract by axons extending from neurons in the cingulate cortex (Rash & Richards, 2001); and fasciculation of callosal axons with pioneer axons as they grow toward and across the midline (Koester & O'Leary, 1994; Rash & Richards, 2001). Dcc and Ntn1 are required for the normal development of the CC and their loss or dysfunction in mice is associated with complete ACC (Fazeli et al., 1997; Finger et al., 2002; Serafini et al., 1996). While both pre-crossing pioneer and callosal axons express *Dcc*, only the former axonal population appears to be attracted toward the midline by Ntn1 (Fothergill et al., 2014; Shu et al., 2000). Instead, it has been shown that pre-crossing callosal axons utilize Dcc and Ntn1 signaling to attenuate the repulsive signaling of Robo1 and Slit2 (another axon guidance receptor-ligand pair) to approach and cross the midline (Fothergill et al., 2014; Stein & Tessier-Lavigne, 2001). The expression of *Dcc* in post-crossing callosal axons is subsequently downregulated, thereby restoring the repulsive effect of Robo1 and Slit2 signaling to direct axons away from the midline and toward their targets in the contralateral cerebral hemisphere (Fothergill et al., 2014; Shu et al., 2000).

The subcerebrally projecting neurons that comprise the CST mainly originate from the primary motor and premotor areas of the cortex and follow a stereotyped route to innervate their targets in the spinal cord (Dum & Strick, 1991). During development, axons from these projection neurons converge and descend through the internal capsule and cerebral peduncles of the midbrain before entering the ventral brainstem where they form the medullary pyramids. Most CST projections then cross the midline in the caudal region of the medulla to form the pyramidal decussation, before projecting inferiorly in the spinal cord to synapse with lower motor neurons or interneurons in the ventral spinal cord to facilitate voluntary movement of the limbs (Welniarz et al., 2017a). Dcc is required for the normal development of the CST in mice and Dcc dysfunction is associated with a failure of the CST to cross the midline at the level of the pyramidal decussation (Finger et al., 2002; Welniarz et al., 2017b). However, Dcc does not appear to be expressed in the brainstem CST of mice as it is significantly downregulated in the distal portion of the axon that grows beyond the internal capsule (Finger et al., 2002; Shu et al., 2000). Interestingly, the role of Dcc in the development of the CST at the midline was reported to be non-cell autonomous as conditional knockout of *Dcc* in the cortex (and therefore the CST) caused ACC but not a failure of the CST to cross the midline (Welniarz et al., 2017b).

In the brainstem and spinal cord, Dcc is present on commissural axons, which are guided circumferentially from the dorsal roof plate toward the ventral floor plate (where midline crossing occurs) in response to Ntn1 (Dominici et al., 2017; Holley, 1982; Tessier-Lavigne, Placzek, Lumsden, Dodd, & Jessell, 1988; Varadarajan, et al., 2017; Yee, Simon, Tessier-Lavigne, & O'Leary, 1999). The attractive response of these commissural axons to Ntn1 is regulated by Dcc in partnership with Robo3, another axon guidance receptor required for normal development of the hindbrain and spinal cord (Chen, Gore, Long, Ma, & Tessier-Lavigne, 2008; Marillat et al., 2004; Sabatier et al., 2004; Zelina et al., 2014). Pioneering studies utilizing embryonic chick spinal cord demonstrated that this axonal navigation is reliant on a ventral–dorsal gradient of Ntn1 diffused from the floor plate (Kennedy et al., 1994). Subsequent studies in mice also detected a similar graded distribution of Ntn1 extending from the floor plate, supporting the observation that Ntn1 functions as a long-range diffusible chemoattractant (Kennedy, Wang, Marshall, & Tessier-Lavigne, 2006; Serafini et al., 1996). However, recent reports have revised this model by demonstrating that Ntn1 produced by neural progenitors in the ventral ventricular zone of hindbrain and spinal cord neuroepithelium are essential for commissural axon extension toward the ventral midline in embryonic mice (Dominici et al., 2017; Varadarajan, et al., 2017; Yamauchi et al., 2017). These reports propose that, in mice, commissural axons are directed via Dcc-dependent haptotaxis toward the ventral midline by Ntn1 produced and deposited at the pial surface of the hindbrain and spinal cord, not via a diffusible gradient of Ntn1 emanating from the floor plate (Dominici et al., 2017; Varadarajan & Butler, 2017; Varadarajan et al., 2017; Yamauchi et al., 2017). Therefore, regardless of how Ntn1 is presented to axons, it is clear that it functions to direct Dcc-expressing commissural axons toward the ventral midline of the hindbrain and spinal cord during development.

8 | CLINICAL RELEVANCE

MMs are involuntary movements on one side of the body that mirror voluntary movements made on the opposite side (Cincotta & Ziemann, 2008). *DCC*-MMs commonly present in infancy or early childhood and persist stably into adulthood (Depienne et al., 2011; Franz et al., 2015; Marsh et al., 2017; Méneret et al., 2014a; Srour et al., 2009; Srour et al., 2010). *DCC*-MMs manifest in the fingers and hands but may also be present in the forearms, toes, and feet in a subset of affected individuals (Supp. Table S2) (Marsh et al., 2017; Méneret et al., 2014a; Srour et al., 2010). With effort, some affected individuals can partly suppress these involuntary movements (Srour et al., 2009). Individuals with *DCC*-MMs exhibit a range of functional disabilities besides difficulties in fine bimanual activities. These include fatigue, spontaneous muscle contractions, and pain in the upper limbs during extended manual activities such as writing, as well as general clumsiness and compensatory maneuvers to inhibit involuntary movements (Depienne et al., 2011; Marsh et al., 2017; Méneret et al., 2014a; Meneret, Trouillard, Brochard, & Roze, 2015). As a result, *DCC*-MMs may preclude affected individuals from professions and social activities that demand sustained or complex bimanual coordination. Individuals with *DCC*-MMs

do not appear to exhibit any additional clinical manifestations and have a normal developmental outcome (Depienne et al., 2011; Franz et al., 2015; Marsh et al., 2017; Méneret et al., 2014a; Meneret, et al., 2015; Sharafaddinzadeh et al., 2008; Srour et al., 2009; Srour et al., 2010; Welniarz et al., 2017b). However, only a subset of individuals diagnosed with *DCC*-MMs are reported to have undergone brain imaging (12 out of 42; 29%) and formal neuropsychological evaluation (two out 42; 5%) and therefore the phenotypic spectrum of *DCC*-MMs remains to be defined (Marsh et al., 2017).

DCC-MMs are observed in association with midline axon guidance defects, evidenced by decreased crossing of descending corticospinal motor projections at the pyramidal decussation (Marsh et al., 2017; Welniarz et al., 2017b). This reduction of crossed projections occurs in conjunction with a relative, reciprocal increase of uncrossed projections (Marsh et al., 2017; Welniarz et al., 2017b). Concordantly, unilateral transcortical stimulation of the primary hand motor area elicits both normal, contralateral and abnormal, ipsilateral motor evoked potentials in individuals with *DCC*-MMs (Cincotta et al., 1994; Srour et al., 2010; Welniarz et al., 2017b). As a result, *DCC*-MMs are thought to originate from the bilateral transmission of motor commands through normally crossed and abnormally uncrossed, fast-conducting CST projections in the spinal cord (Srour et al., 2010; Welniarz et al., 2017b; Welniarz, Dusart, Gallea, & Roze, 2015).

Individuals with monoallelic, missense *DCC* mutations may also present with iACC, with or without MMs (Marsh et al., 2017). ACC describes the partial or complete absence of the CC and is characterized by the failure of callosal axons to cross the midline. Apart from the abnormalities typically expected to be associated with ACC, such as the absence of the hippocampal commissure and cingulate gyrus and dysmorphic lateral ventricles or colpocephaly, individuals with iACC do not present with additional brain abnormalities (Cesaretti et al., 2016; Kuker, Mayrhofer, Mader, Nagele, & Krageloh-Mann, 2003). Individuals with *DCC*-iACC may present with complete iACC (14 out of 19; 74%) or partial iACC (five out of 19; 26%) (Marsh et al., 2017). Approximately half of these individuals also present with MMs (nine out of 19; 47%), which suggests that *DCC*-iACC may frequently present as part of a global disorder of midline crossing (Marsh et al., 2017; Parrish, Roessmann, & Levinsohn, 1979; Paul et al., 2007). Other callosal abnormalities such as hypoplasia (a uniformly thin CC) and/or dysplasia (abnormal CC shape) have not been observed in individuals with *DCC*-iACC (Marsh et al., 2017). Individuals with *DCC*-iACC show no consistent additional gross brain abnormalities in the posterior commissure, ventricular system, cerebral cortex, white matter, hippocampi, brainstem, basal ganglia, cerebral and cerebellar peduncles, cerebellar vermis and hemispheres, optic chiasm, and pituitary gland (Figure 5) (Marsh et al., 2017). However, the anterior commissure of some *DCC*-iACC individuals may be enlarged (Marsh et al., 2017). Enlargement of the anterior commissure is also observed in a minority of individuals with ACC and has been suggested to represent a compensatory mechanism to maintain connectivity between the cerebral hemispheres (Barr and Corballis, 2002; Hetts, Sherr, Chao, Gobuty, & Barkovich, 2006).

The clinical manifestations of *DCC*-iACC vary, but they are generally associated with a favorable development outcome with no intellectual disability (Marsh et al., 2017) (Supp. Table S2). Still, individuals with *DCC*-iACC do commonly exhibit typical neurobehavioral consequences associated with ACC, such as impairments in emotional and social functioning in addition to attention, language, visuospatial, and literacy and numeracy deficits (Marsh et al., 2017; Paul et al., 2007; Sotiriadis & Makrydimas, 2012).

Biallelic, *DCC* mutations leading to predicted LoF are associated with DSBS, a complex syndrome associated with a broad disorganization of white-matter tracts throughout the CNS (Jamuar et al., 2017). The features of DSBS include absence of all commissures (including the CC, anterior, and posterior), brainstem defects (including hypoplasia of the pons and midbrain), horizontal gaze palsy and progressive scoliosis with a variable age of onset (Figure 5).

Several features of DSBS overlap with HGPPS1 (MIM# 607313), a congenital syndrome caused by biallelic mutations in the axon guidance receptor *ROBO3* (MIM# 608630) (Chan et al., 2006; Jen et al., 2004; Sicotte et al., 2006). Robo3 is a functional, intracellular binding partner of Dcc, expressed by commissural axons in the brainstem and spinal cord (Marillat et al., 2004; Sabatier et al., 2004; Zelina et al., 2014). The overlapping feature of horizontal gaze palsy in DSBS and HGPPS1 affected individuals appears to originate from hindbrain midline axon guidance defects in tracts that control conjugate horizontal eye movement (Chan et al., 2006; Jamuar et al., 2017; Jen et al., 2004; Renier et al., 2010; Sicotte et al., 2006). The pathogenesis of progressive scoliosis in both these disorders is unknown, but may stem from defective spinal commissural interneurons or abnormal development of extrapyramidal projections (Jamuar et al., 2017; Jen et al., 2004; Rabe Bernhardt et al., 2012). Diffusion MRI of one DSBS individual with a biallelic p.(Val263Alafs*36) mutation revealed several tract defects, including absence of the decussation of the superior cerebellar peduncles and transverse pontocerebellar projections (Jamuar et al., 2017). These commissural defects are also observed in individuals with HGPPS1 (Sicotte et al., 2006). Individuals with DSBS may also present with MMs, which are associated with the reduced midline crossing of descending CST projections at the pyramidal decussation (Jamuar et al., 2017). This is in contrast to individuals with HGPPS1 who do not display MMs, a feature attributed to an uncrossed CST leading to reversed lateralization of motor control (Jen et al., 2004). It is currently unknown why some individuals with DSBS and complete loss of DCC do not present with MMs. However, like HGPPS1, this may be due to a complete, rather than partial, failure of the descending CST to cross the midline. The other major clinical manifestations of DSBS include intellectual disability, global developmental delay, and hypotonia (Supp. Table S2) (Jamuar et al., 2017). Individuals with DSBS have a poor developmental outcome compared to individuals with *DCC*-iACC, likely attributed to additional brain abnormalities affecting the formation of other commissural tracts (Jamuar et al., 2017; Marsh et al., 2017).

9 | DIAGNOSTIC STRATEGIES

The clinical assessment and diagnosis of MMs is commonly based on the Woods and Teuber severity scale developed in 1978 (Woods & Teuber, 1978). The assessment typically consists of three different

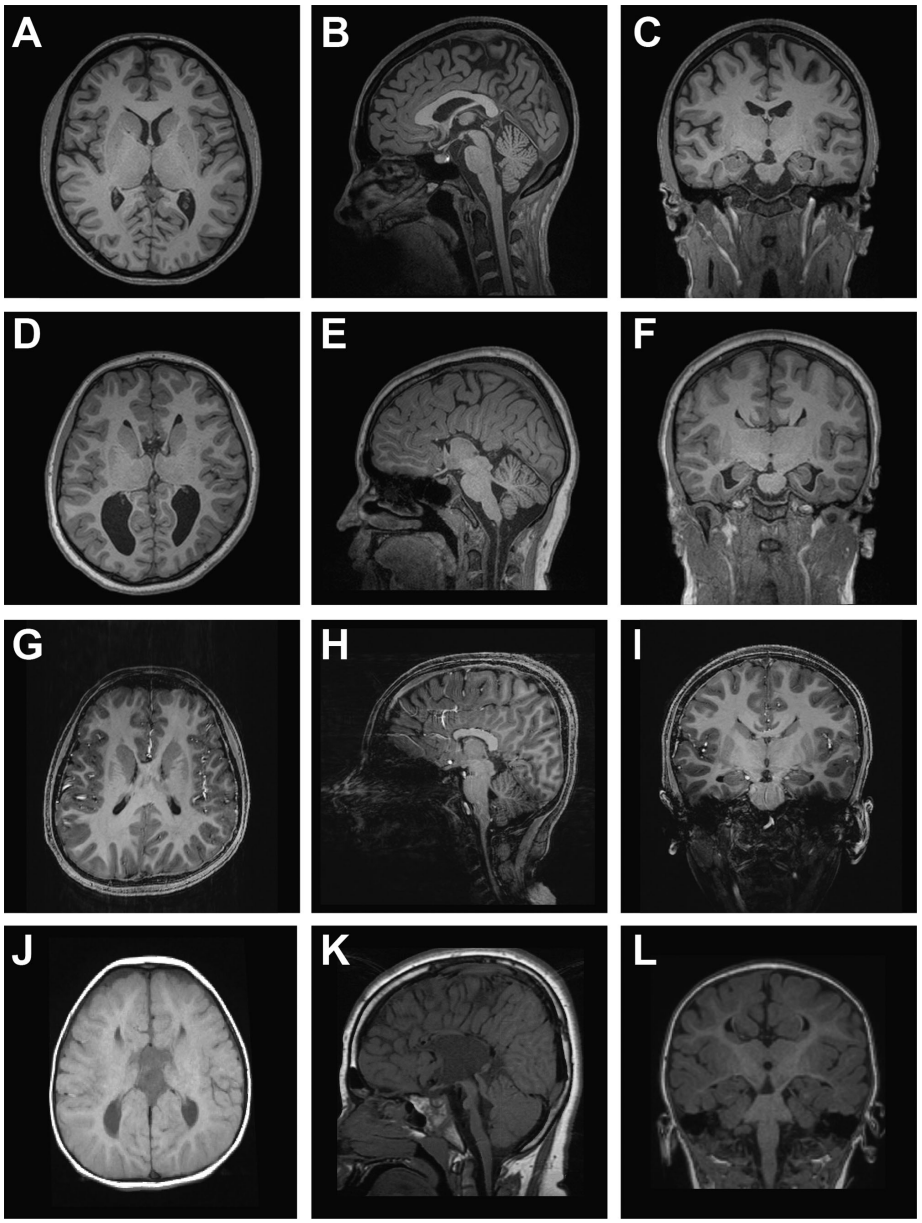

FIGURE 5 Axial MRI of control (**A**) and individuals with complete *DCC*-iACC (**D**), partial *DCC*-iACC (**G**) and DSBS (**J**). Midsagittal MRI of control (**B**) and individuals with complete *DCC*-iACC (**E**), partial *DCC*-iACC with absence of the rostrum and genu (**H**) and DSBS (**K**). Coronal MRI of control (**C**) and individuals with complete *DCC*-iACC (**F**), partial *DCC*-iACC (**I**), and DSBS (**L**). iACC, isolated agenesis of the corpus callosum; DSBS, developmental split brain syndrome. Images A–I adapted from Marsh et al. (2017). Images J–L adapted from Jamuar et al. (2017)

hand motor tasks. The level of MMs visible in the mirror hand is scored on a scale between 1—barely discernible repetitive movement and 4—movement equal to that expected for the intended hand (Woods & Teuber, 1978). The majority of individuals with *DCC*-MMs score between 2 (slight but unsustained or stronger, but briefer, repetitive movement) and 3 (strong and sustained repetitive movement). Recently, accelerometer gloves were utilized to quantitatively evaluate MMs in a multiplex MMs family with a monoallelic, p.(Pro551Leufs*26) *DCC* mutation (Franz et al., 2015). These gloves detect subtle movement, in contrast to electromyography that measures the electrical activity produced by skeletal muscle (Franz et al., 2015). Interestingly, the accelerometer gloves led to the diagnosis of subclinical MMs in two additional family members initially diagnosed as unaffected carriers following standardized neurological assessment. The current penetrance of *DCC*-MMs is approximately 42% (Marsh et al., 2017). In light of these findings, it appears likely that a proportion of clinically unaffected mutation carriers have subclinical MMs and that the true prevalence of *DCC*-MMs may be higher. It remains to be determined whether subclinical *DCC*-MMs are associated with a similar, or perhaps less severe, failure of the CST to cross the midline.

Brain abnormalities such as ACC can be readily identified utilizing ultrasonography, CT, and MRI. MRI is considered the gold standard for the diagnosis of ACC because of its superior sensitivity and ability to differentiate iACC and complex ACC (ACC with additional brain abnormalities) (Rapp et al., 2002; Tercanli & Prufer, 2016; Wright, Sibley, & Baker, 2010). The distinction of the latter is important in a

prenatal setting as individuals with iACC generally have a favorable developmental outcome compared with those with complex ACC (D'Antonio et al., 2016; des Portes et al., 2017; Edwards, Sherr, Barkovich, & Richards, 2014; Sotiriadis & Makrydimas, 2012). Likewise, individuals with *DCC*-iACC have a favorable developmental outcome while individuals with DSBS have a poor developmental outcome. The current penetrance of *DCC*-iACC is approximately 26% (Marsh et al., 2017). However, brain imaging studies have not been completed for the majority of affected individuals originating from *DCC*-MMs-only families (29 out of 36; 81%). Given its mild clinical phenotype and concomitance with *DCC*-MMs, it is probable that further brain imaging studies will identify *DCC*-iACC in a proportion of these mutation-positive individuals. Like *DCC*-MMs, the true prevalence of *DCC*-iACC is likely to be higher than current estimates.

Genetic screening for monoallelic *DCC* mutations is recommended for individuals presenting with MMs, iACC or both, in the absence of intellectual disability. Alternatively, screening for biallelic *DCC* mutations with predicted LoF is recommended for individuals presenting with characteristic features of DSBS, including horizontal gaze palsy, ACC, brainstem defects, and progressive scoliosis. Direct sequencing of coding exons and flanking intronic regions using germline DNA is advised when screening for *DCC* mutations. Given the mild clinical phenotype and high rates of incomplete penetrance associated with *DCC*-MMs and *DCC*-iACC, cascade testing should be considered for all extended family members at risk of inheriting a mutant allele. Differential genetic diagnoses for *DCC*-MMs include *NTN1* and *RAD51* (MIM# 179617) (Depienne et al., 2012; Méneret et al., 2014a; Méneret et al., 2017); for *DCC*-iACC include *CDK5RAP2* (MIM# 608201, although only one family has been described to date (Jouan et al., 2016)); and for DSBS include *ROBO3* (note: individuals with HGPPS1 do not suffer from intellectual disability and have normal forebrain commissures, consistent with the predominant role of *ROBO3* in the developing hindbrain and spinal cord) (Chan et al., 2006; Jen et al., 2004; Sicotte et al., 2006). However, the low diagnostic yield of genetic screening studies indicates that additional MMs and iACC disease genes remain to be identified and therefore mutations in *DCC*, *NTN1*, *RAD51*, and *CDK5RAP2* will not always be identified in individuals with these phenotypes (Franz et al., 2015; Jamuar et al., 2017; Marsh et al., 2017; Méneret et al., 2014a; Méneret et al., 2017; Méneret, et al., 2014b).

10 | VARIANTS OF UNKNOWN SIGNIFICANCE

The classification and clinical interpretation of a *DCC* sequence variant is challenged by the variable expressivity of monoallelic *DCC* mutations and the incomplete penetrance associated with *DCC*-MMs and *DCC*-iACC. It may also be complicated by phenotypic heterogeneity and the identification of other potential pathogenic variants in an individual. In this context and in the absence of sufficient evidence to classify a *DCC* variant as pathogenic or not, it is recommended to report such a genetic alteration as a variant of unknown significance (VUS).

To illustrate these issues, we describe individual N-0083-01 with two novel, monoallelic VUS predicted to be damaging by most in silico tools: a maternally inherited NM_005215.3:c.916G > A:p.(Gly306Arg) *DCC* alteration and a de novo NM_001270399.1:c.1246G > A:p.(Gly416Ser) *TUBA1A* (MIM# 602529) alteration (Supp. Table S3) (Jamuar et al., 2017). The individual has complete ACC with a small pons and small inferior aspect of the midbrain, but no definite cortical malformation as would be expected to result from a pathogenic *TUBA1A* mutation (Keays et al., 2007). However, the individual does not exhibit the mild clinical manifestations associated with *DCC*-iACC. Instead, she has global developmental delay and severe cognitive impairments with significant emotional and behavioral problems in addition to spastic diplegia, strabismus and cortical visual impairment (Supp. Table S4). As exemplified by this case, additional disease modeling and physiologically relevant functional assays are required to better inform the classification and clinical interpretation of *DCC* sequence variants, particularly for those causing missense alterations.

11 | FUTURE PROSPECTS

Mutations in *DCC* disrupt the development of predominantly commissural tracts in the CNS and cause a spectrum of neurological disorders ranging from MMs and iACC with a normal or favorable developmental outcome, to DSBS with a poor developmental outcome. These findings are consistent with previously reported animal models and support the notion that DCC, in partnership with NTN1, functions as a master regulator of commissural axon guidance at the midline (Fazeli et al., 1997; Finger et al., 2002; Varadarajan et al., 2017; Welniarz et al., 2017b). Future investigations into the underlying molecular mechanisms leading to these disorders will strengthen current genotype–phenotype correlations. However, insightful correlations will be dependent on the multidisciplinary phenotyping of affected individuals, employing standardized neurological assessment, formal neuropsychological evaluation, and brain imaging studies. Utilization of advanced neuroimaging modalities, such as diffusion MRI-based tractography and functional MRI, will also aid our understanding of how the brains of these affected individuals is wired in the context of DCC dysfunction or LoF during development. We anticipate the *DCC* locus-specific LOVD will function as a central data repository that will assist researchers to establish these important genotype-phenotype correlations.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the participation of the patients and their families in these studies and the support of the Australian Disorders of the Corpus Callosum (AusDoCC) organization, as well as the generous financial support of the Lefroy and Handbury families. The authors also acknowledge and thank Ivo Fokkema and Johan T. den Dunnen from the Leiden University Medical Center for their assistance in establishing the DCC LOVD.

DISCLOSURE STATEMENT

A.M. received travel funding from Zambon Company and AbbVie Inc. E.R. has received research support from Merz-Pharma, Orkyn,

Aguettant, IP Santé, Ultragenix, IPSEN, Association Française pour l'Hémiplégie Alternante, AMADYS; served on scientific advisory boards for Orkyn, Ultragenix, Retrophin, and Merz Pharma; received speech honoraria from Orkyn, Aguettant, Merz Pharma, and Ultragenix; and received travel funding from the Dystonia Coalition, the Dystonia Medical Research Foundation, the Movement Disorders Society, and the European Academy of Neurology. S.S.J. is co-founder of Global Gene Corporation. T.W.Y. is co-founder of and part-time consultant to Claritas Genomics.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Marsh APL, Edwards TJ, Galea C, et al. *DCC* mutation update: Congenital mirror movements, isolated agenesis of the corpus callosum and developmental split brain syndrome. *Human Mutation*. 2018;39:23–39. https://doi.org/10.1002/humu.23361

APPENDIX

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