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De novo variants predicting haploinsufficiency for *DIP2C* are associated with expressive speech delay

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Abstract

The disconnected (disco)-interacting protein 2 (DIP2) gene was first identified in *D. melanogaster* and contains a DNA methyltransferase-associated protein 1 (DMAP1) binding domain, Acyl-CoA synthetase domain and AMP-binding sites. DIP2 regulates axonal bifurcation of the mushroom body neurons in *D. melanogaster* and is required for axonal regeneration in the neurons of *C. elegans*. The DIP2 homologues in vertebrates, Disco-interacting protein 2 homolog A (*DIP2A*), Disco-interacting protein 2 homolog B (*DIP2B*), and Disco-interacting protein 2 homolog C (*DIP2C*), are highly conserved and expressed widely in the central nervous system. Although there is evidence that *DIP2C* plays a role in cognition, reports of pathogenic variants in these genes are rare and their significance is uncertain. We present 23 individuals with heterozygous *DIP2C* variants, all manifesting developmental delays that primarily affect expressive language and speech articulation. Eight patients had de novo variants predicting loss-of-function in the *DIP2C* gene, two patients had de novo missense variants, three had paternally inherited loss of function variants and six had maternally inherited loss-of-function variants, while inheritance was unknown for four variants. Four patients had cardiac defects (hypertrophic cardiomyopathy, atrial septal defects, and bicuspid aortic valve). Minor facial anomalies were inconsistent but included a high anterior hairline with a long forehead, broad nasal tip, and ear anomalies. Brainspan analysis showed elevated *DIP2C* expression in the human neocortex at 10–24 weeks after conception. With the cases presented herein, we provide phenotypic and genotypic data supporting the association between loss-of-function variants in *DIP2C* with a neurocognitive phenotype.

KEYWORDS
developmental delay, DIP2, DIP2C, intellectual disability, speech articulation, speech delay

1 | INTRODUCTION

The current yield of exome sequencing (ES) in neurocognitive disorders implies there are still new genes associated with disease to identify, including genes in which pathogenic or likely pathogenic variants are accompanied by reduced penetrance (Kaplanis et al., 2020). DIP2 was identified in *D. melanogaster* and contains a DNA methyltransferase-associated protein 1 (DMAP1) binding domain, acyl-CoA synthetase (AMP-forming; Caic) domain, and AMP-binding sites (Xing et al., 2020). DIP2 regulates axonal bifurcation of mushroom body neurons in *D. melanogaster* and is required for axonal regeneration in the mature neurons of *C. elegans* (Nitta et al., 2017; Noblett et al., 2019). The disconnected (disco)-interacting protein 2 (DIP2) homologues in vertebrates, disco-interacting protein 2 homolog A (*DIP2A*; OMIM 607711), disco-interacting protein 2 homolog B (*DIP2B*; OMIM 611379), and disco-interacting protein 2 homolog C (*DIP2C*; OMIM 611380), are highly conserved and broadly expressed in the central nervous system (Oo et al., 2020; Xing et al., 2020; Zhang et al., 2015).

In humans, deletions of *DIP2C* have been observed in association with deletions of *ZMYND11* (OMIM 608668) in chromosome 10p15.3 microdeletion syndrome, which is characterized by developmental delays affecting motor function and speech, behavioral disturbances, dysmorphic features, brain anomalies, seizures, low birth weight and short stature (Chen et al., 2019; DeScipio et al., 2008; Eggert et al., 2016; Fernández et al., 2016; Poluha et al., 2017; Ravnan et al., 2006; Tumiene et al., 2017; Vargiami et al., 2014). The critical gene for 10p15.3 microdeletion syndrome was previously identified as *ZMYND11* after heterozygous, loss-of-function variants in this gene were found in 6 out of 4716 individuals with developmental delays or autism (Coe et al., 2014). Haploinsufficiency for *ZMYND11* has also been associated with speech delay and intellectual disability, epilepsy, aggressive behavior, social difficulties, wide-spaced eyes, ptosis, and a wide mouth (Cobben et al., 2014; Coe et al., 2014). No deleterious variants in the *DIP2C* gene were found in the same large patient cohort, while truncating mutations in *DIP2C* were demonstrated in two of 2193 controls (Coe et al., 2014), leading the authors to conclude that *ZMYND11* was the gene responsible for the neurocognitive findings. However, mosaicism for a 67.6 kb deletion that impacted at least two exons of *DIP2C* has also been reported in two patients diagnosed with hemiplegic cerebral palsy, one of whom manifested attention deficit hyperactivity disorder (ADHD) inattentive subtype and

TABLE 1 Sequence variants in *DIP2C*.

Patient (Table S1)	Nucleotide alteration	Amino acid alteration	Inheritance	Mutation taster	CADD[a] score	GnomAD[b]	Prediction	ACMG criteria[c]
DIP2C	NM_014974.2/ NM_014974.3							
23	c.467dup	*p.(Ser157Glnfs*53)*	Paternal	DC[d]; 1.0	NA	–	NMD[e]; splice[f]	PVS1; PM2; PP3
1	c.820C>T	*p.(Arg274*)*	de novo	DC; 1.0	37	–	NMD; splice	PVS1; PS2; PM2; PP3
12	c.898dup	p.(Ala300Glyfs*84)	de novo	DC; 1.0	NA	–	NMD; splice	PVS1; PS2; PM2
10	c.4028del	p.(Leu1343Argfs*14)	Maternal	DC; 1.0	NA	–	NMD; splice	PVS1; PM2
5	c.1441C>T	p.(Arg481*)	de novo	DC; 1.0	39	–	NMD	PVS1; PS2; PM2; PP3
2	c.2130_2131dup	p.(Ala711Glufs*7)	de novo	DC[f]; 1.0	NA	–	NMD; splice	PVS1; PM2
6 and 11	c.2208_2209dup	p.(Ala737Valfs*15)	Maternal and de novo	DC; 1.0	NA	–	NMD	PVS1: PM2
4	c.3699 C>G	p.(Tyr1233*)	de novo	DC; 0.999	36	–	NMD; splice	PVS1; PS2; PM2; PP3
7, 8 and 9	c.3757C>T	p.(Arg1253*)	Maternal (2) and unknown	DC; 0.999	40	–	NMD; splice	PVS1; PM2; PP3
3	c.4615C>T	p.(Arg1539*)	Paternal	DC; 0.999	44	–	NMD	PVS1; PM2; PP3
Splice								
14	c.4419-1G>A	–	de novo	–	NA	–	Splice	PVS1; PS2; PM2
15	c.1598-2A>G	–	Paternal	–	NA	–	Splice	PVS1; PM2
17 and 18	c.4045-2A>G	–	Unknown	–	NA	–	Splice	PVS1; PM2
20–22	c.4045-5A>G	–	Maternal (2) and unknown	–	NA	–	Splice	PVS1; PM2
19	c.1385-2A>G	–	de novo	–	NA	–	Splice	PVS1; PS2; PM2
Missense								
13	c.217C>G	p.(Arg73Gly)	de novo	DC; 0.931	21.5	–	Aa[g] change	PS2; PM2; PP3
16	c.956C>T	p.(Ser319Leu)	de novo	DC; 0.999	23.6	–	Aa change	PS2; PM2; PP3
Previous reports								
Yuen et al. (2017)	c.637_638insGT:	p.(Tyr213Cysfs*39)	Inherited	DC; 1.0	NA	–	NMD	PVS1; PM2
Maddirevula et al. (2018)	c.3283C>T	p.(Arg1095Trp)	Biparental	DC; 0.999	32	8 het./249,844	Missense; splice	PM2; PP3
Yang et al. (2022)	c.1057 + 2T>G	–	de novo	–	NA		Splice	PVS1

[a]Combined annotation dependent depletion score.
[b]gnomAD browser.
[c]Joint Consensus Recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (Richards et al., 2015).
[d]Disease causing.
[e]Predicts nonsense mediated decay.
[f]Predicts altered splicing.
[g]Amino acid.

was noted to be a 'slow learner' (Zarrei et al., 2018). In another study using genome sequencing (GS) to identify candidate genes in 5205 patients with autism, a de novo frameshift variant was identified in *DIP2C* in one patient (Yuen et al., 2017). In addition, a pathogenic variant in *DIP2C* was published in a child who presented with speech apraxia without developmental delays or intellectual disability (Kaspi et al., 2023). The clinical description included asthma and migraines in addition to autism, but further details were not provided. Thus, although there is some evidence that *DIP2C* plays a role in cognition, reports of pathogenic variants in this gene are rare (Yang et al., 2022). We describe the clinical findings in 21 patients with heterozygous variants predicting loss of function in *DIP2C* and two patients with heterozygous missense variants in *DIP2C*, to provide further data on the clinical findings associated with variants in this gene.

2 | MATERIALS AND METHODS

2.1 | Case reports

All families signed written consent for publication of clinical details. Patients were ascertained through GeneMatcher (Sobreira et al., 2015). For all patients, clinical findings and *DIP2C* variant information were recorded (Table S1). Patient 14 has previously been reported (Kaspi et al., 2023) and was ascertained due to childhood apraxia of speech; we have provided additional clinical details for this child.

2.2 | Exome and genome sequencing

Brief descriptions of the methods used for sequencing are provided in Table S1 when available. The pathogenicity of each variant was examined using MutationTaster (Schwarz et al., 2010) and combined annotation dependent depletion (CADD) scores (Kircher et al., 2014).

2.3 | BrainSpan

The expression of *DIP2C* from 8 post-conception weeks (pcw) to 40 years in the human neocortex and non-neocortex was examined using the BrainSpan Atlas of the developing human brain according to prior methods (Dias et al., 2022).

3 | RESULTS

3.1 | Exome and genome sequencing

All the *DIP2C* variants were heterozygous and all but two *DIP2C* variants were predicted to result in loss of function (Table 1). The gene has a pLI score of 1.0 in gnomAD (Karczewski et al., 2020). In ClinVar (Landrum et al., 2018), there were no reported pathogenic variants involving *DIP2C* alone, although p.(Arg1539*) was noted as a variant of uncertain significance (VUS). Two missense variants in *DIP2C* were recorded in ClinVar and one, p.(Arg88Trp), was associated with developmental delays and obesity. Several patients had inherited variants that imply variable expressivity (Table S1). For example, a stop variant in *DIP2C*, p.(Arg1539*), was paternally inherited from a father who reportedly had marked speech delay in childhood, and a brother and sister both had p.(Arg1253*) in *DIP2C* that was inherited from their mother who had speech delay, learning difficulties and poor articulation as an adult, although she was without intellectual disability, able to read and write and was independent with an occupation.

We have included two patients with de novo missense variants, p.(Arg73Gly) and p.(Ser319Leu), in *DIP2C* in this report (Table 1). We considered that they warranted inclusion, given the de novo nature of the variants, their absence from gnomAD, and the clinical similarity of these children to the other patients with loss of function variants in *DIP2C*.

TABLE 2 Summary of phenotypic findings in patients with *DIP2C* variants from this report.

Clinical finding	Frequency	Frequency (%)
Failure to thrive	3/22	13.6
Feeding difficulties	5/21	23.8
Developmental delays	23/23	100
Delayed sitting	9/14	64.3
Delayed walking	16/21	76.2
Delayed first word	12/19	63.2
Delayed speech	15/16	93.8
Speech difficulties	20/20	100
Behavioral differences	18/22	81.8
Autism	6/22	27.3
Seizures	4/23	17.4
Regression/plateau in skills	6/23	26.1
Hypotonia	5/23	21.7
Sleeping difficulties	4/21	19.0

3.2 | Case reports

The clinical features of 23 patients with heterozygous variants predicting loss of function or amino acid substitutions in *DIP2C* are summarized in Table 2 and provided in Table S1. Eleven female and 12 male patients were examined between 13 months and 41 years of age. Ten of the patients had a de novo *DIP2C* variant, three had paternally inherited variants, and six patients, including two pairs of siblings, had a maternally inherited variant (Table 1). Most babies experienced a normal neonatal period with few pregnancy complications; however, when known, 3/13 (23.1%) patients had head circumferences below the 3rd percentile at birth. Feeding difficulties were present in 5/21 babies (23.8%), with two children requiring

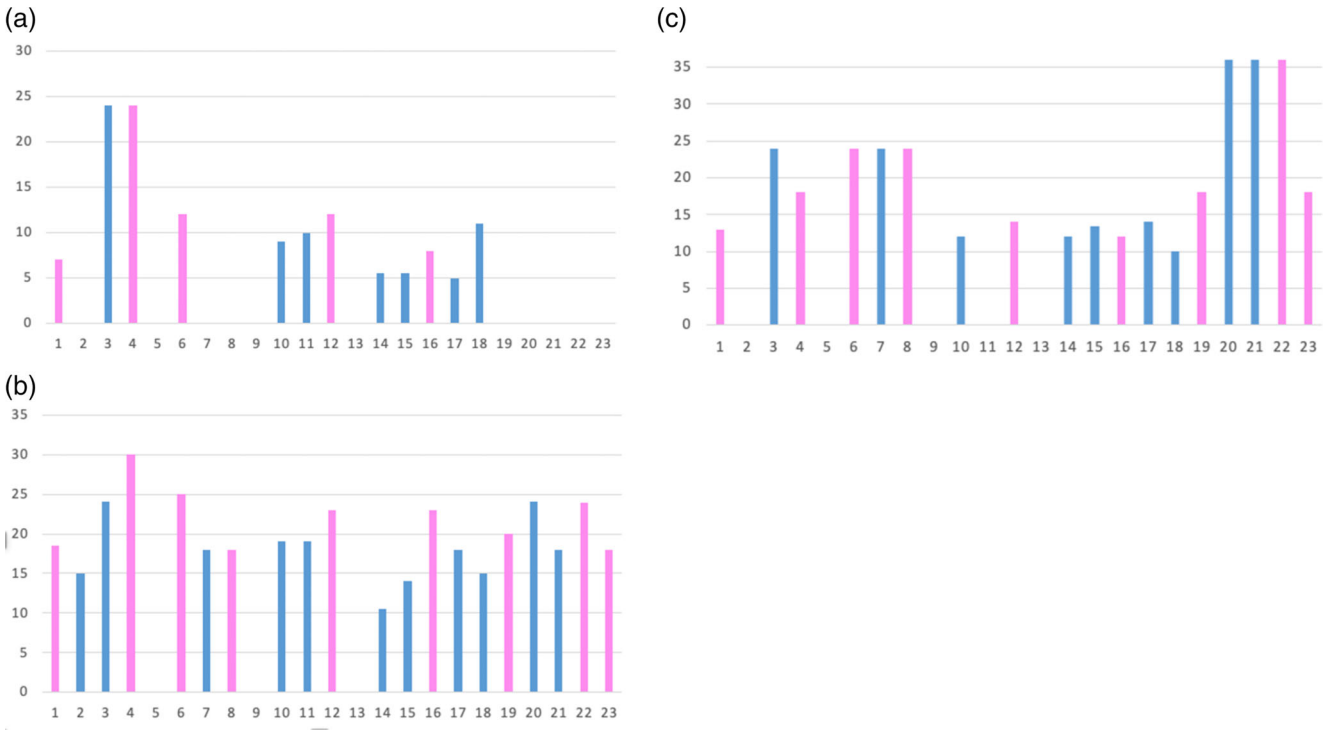

FIGURE 1 Column graphs showing age at independent sitting, walking and first words in patients with *DIP2C* variants. Patients are represented by solid columns numbered 1–23 on the *X* axis with girls represented by pink columns and boys represented by blue columns. The Y axis is labeled in months. (a) Ages of independent sitting in 23 patients with *DIP2C* variants. (b) Ages of independent walking in 23 patients with *DIP2C* variants. (c) Ages of first words in 23 patients with *DIP2C* variants. Columns have been left blank when no numerical data is available. Measurements provided as a range have been plotted as the mean of the range.

nasogastric (NG) and/or percutaneous endoscopic gastrostomy (PEG) feeding. Failure to thrive was present in 3/22 children (13.6%).

Developmental delays were observed in all 23 patients. Motor milestones were delayed in 16 patients, who walked independently at or after the age of 10–15 months (Figure 1). The ages of sitting ranged from 4 to 7 months up to 2 years and the ages of independent ambulation ranged from 12 months to 2.5 years. Age at first word ranged from 10 months to 3 years, and 11 patients spoke their first word after 14 months (Figure 1). Eight patients had poor articulation. Formal intelligence quotient (IQ) testing was conducted in four patients and full-scale IQs were measured at 61, 66, 83 and 94, respectively, as evidence that development can fall into the typical range. Autism was diagnosed in 6/22 (27.3%) patients and 6/23 (26.1%) patients regressed or plateaued in skill development. Hypotonia was observed in 5/23 (21.7%) patients.

Behavioral or personality attributes were reported for 18/22 children (81.8%). Eight had hyperactivity and attention disorders and two had anxiety, with one child demonstrating self-harm while anxious, irrational thoughts, perseveration, and insensitivity to heat. Mood disorders and depression were found in two patients, but three were described as sociable, happy, and loving. Four patients had sleeping difficulties, with altered day and night rhythms and frequent nocturnal awakening.

Organ malformations were rare; however, abnormal cardiac findings were the most common (5/19; 26.3%). One patient was diagnosed with hypertrophic cardiomyopathy, and two others had a bicuspid aortic valve. Two patients had an atrial septal defect (ASD) and one patient, who also had first-degree atrioventricular block, required surgical repair of the septal defect. An enlarged aorta was noted in one patient in the context of a three generation, maternal family history of the same condition. One patient had mild dilatation of the renal pelvis. Twelve children had magnetic resonance imaging (MRI) or computerized tomography (CT) of the brain (43.5%). One child who had a thin corpus callosum developed generalized, myoclonic, and absence seizures from 6 months of age that were successfully treated with medication. A 17-month-old female infant with a de novo variant predicted to affect *DIP2C* splicing (NM_014974.3: c.1057 + 2T>G) and cause an 80 basepair deletion in exon 8 was reported to have focal to bilateral tonic–clonic seizures and focal motor seizures that were controlled with sodium valproate (Yang et al., 2022). Complete or partial deletions involving *DIP2C* have also been associated with focal seizures in two other patients, but few details are available (Yang et al., 2022). Another patient had a thin corpus callosum without seizures and a third had a small/absent pituitary gland. Mild optic anomalies included strabismus in five patients, refractive errors in four patients, and one patient had astigmatism. Hearing loss or a failed hearing screen that did not resolve was

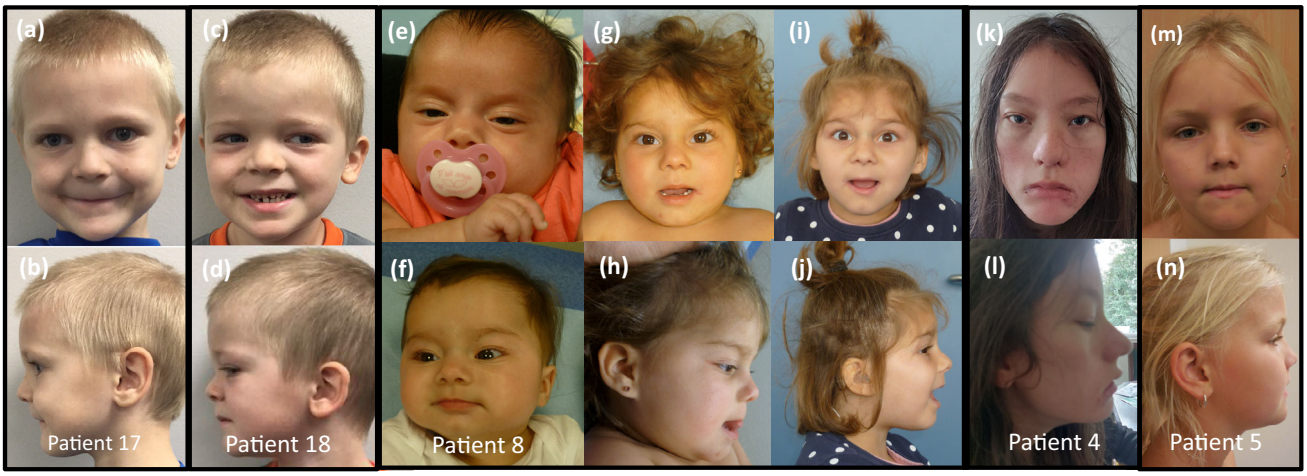

FIGURE 2 Photograph of patients with heterozygous *DIP2C* variants. (Patient ages at the time of photographs may differ from age recorded in Table S1.) (a, b) Frontal and profile views of a 7-year-10-month-old male (patient 17); pictures are bracketed together. (c, d) Frontal and profile views of a 6-year-4-month-old male (patient 18); pictures are bracketed together. (e, f) Frontal view of a 6-month-old female (patient 8) and frontal view of the same child at 9 months of age. (g, h) Frontal and profile views of a 2-year-old female, the same child as in figures e and f. (i, j) Frontal and profile views of a 5-year-old female, the same child as in figures e–h. Figures e–i are bracketed together. (k, l) Frontal and profile views of a 15-year-old female (patient 4); pictures are bracketed together. (m, n) Frontal and profile views of a 10-year-old female (patient 5); pictures are bracketed together.

detected in four children (19.0%) and 5/21 (23.8%) patients had recurrent episodes of otitis media.

Facial anomalies (Figures 2 and 3) were subtle and did not lead to a clearly recognizable gestalt, although 5/22 patients (22.7%) had triangular facies and six patients were reported to have large, high, and/or broad foreheads. A high anterior hairline was present in 10/22 patients (45.5%). Additional anomalies of the eyes, nose, ears, and mouths were present, but did not appear distinctive. Widely spaced eyes were noted in 3/22 (13.6%) and 8/23 (34.8%) had small, upslanting, or almond-shaped palpebral fissures. Three out of 21 patients (14.3%) had a broad nasal bridge. Several patients had anomalous nasal tips that were variably described as bulbous in seven patients, broad in one, full and everted in another, and small in a single patient. Ear anomalies were common (7/23; 30.4%) but were not specific or consistent. Three out of 23 (13.0%) children had a high-arched palates and 3/22 (13.6%) had a thin upper lip. Minor digital anomalies were also not distinctive. Three out of 23 (13.0%) of patients had 5th finger clinodactyly, three had slender fingers, and two had brachydactyly of the fingers. Additional findings included broad halluces, a wide sandal gap, hallux valgus, genu valgum, minimal two to three toe syndactyly, fifth toe clinodactyly, and long toes.

3.3 | BrainSpan

Spatiotemporal bioinformatic analyses of *DIP2C* expression in the human brain across developmental stages showed high *DIP2C* expression in the neocortex prior to birth (Figure 4a). Boxplots of gene expression (RPKM) of various other regions in the brain, including the hippocampus, amygdala, the ventral forebrain, the cerebral cortex and the mediodorsal nucleus of thalamus, demonstrated higher prenatal expression of *DIP2C* compared to postnatal expression (Figure 4b).

4 | DISCUSSION

We present 23 patients with *DIP2C* variants, all with developmental delays affecting expressive language and speech articulation and most with mild developmental delays and intellectual disability. Additional phenotypic findings were non-specific and there was no recognizable facial gestalt, but recurrent anomalies did include a high anterior hairline, prominent forehead, and a broad nasal tip. Structural cardiac anomalies without a family history were noted in four patients and two patients had a bicuspid aortic valve and one patient had hypertrophic cardiomyopathy.

The clinical findings in this patient group support a role for this gene in speech development and cognition. The results are consistent with a recent study that found a significant association between several single nucleotide polymorphisms (SNPs) in *DIP2C* (rs3740304, rs2288681, rs7088729, rs4242757, rs10795060, and rs10904083) and autism susceptibility in the Han population (Li et al., 2022). As further evidence for the importance of *DIP2C* in expressive language, a high confidence variant was found in a group of children ascertained due to childhood onset of apraxia involving speech (Kaspi et al., 2023). Several of the patients in this paper had inherited *DIP2C* variants with variable penetrance, and the clinical manifestations associated with this gene are likely to prove variable, with incomplete penetrance for some individuals that suggest a role for genetic modifiers or environmental influences. Several patients had parents or family members with mild delays or intellectual disability who did not have a *DIP2C* variant, thus supporting a multifactorial etiology of the

FIGURE 3 Circular figure showing relative frequencies of facial and digital anomalies associated with heterozygous variants in *DIP2C*.

cognitive differences. In addition, several patients had copy number variants (such as patients 2, 17 and 18) or variants in other genes (e.g., patients 19 and 23) that could influence the penetrance of the *DIP2C* phenotype. Our findings are in contrast to those described in a patient with a homozygous missense variant in *DIP2C*, c.3283C>T:p.(Arg1095Trp) (NM_014974.2), who had a skeletal dysplasia comprising bilateral short femora, a short humerus, reduced length of the metacarpal bones, bilateral metatarsus adductus and shortening of the first metatarsals (Larsson et al., 2017; Maddirevula et al., 2018). None of the patients in this report had biallelic variants or similar skeletal findings.

The mechanism for the developmental differences associated with *DIP2C* variants is unclear. Brainspan analysis showed elevated *DIP2C* expression in the human neocortex at 10–24 weeks after conception, with higher expression in prenatal developmental stages compared to postnatal stages. *Dip2c* is expressed in the brain of postnatal mice and targeted homozygous deletions of the murine gene, including a two base pair deletion (Dip2c$^{\Delta 2bp}$) in exon 4, have been generated (Oo et al., 2020). RNA-Seq comparing gene expression in the brain of 10, day old male Dip2c$^{\Delta 2bp}$ mice compared to wildtype controls revealed 838 differentially expressed genes (DEGs) with a fold change greater than two that were enriched for roles in neurocognitive function, including 'memory', 'neuropeptide signaling pathway' and 'response to amphetamines' (Oo et al., 2020). The genes encoding glutamate receptors involved in neuronal excitation, *Grid2ip* and *Grin2a*, were upregulated, whereas *Grin2c* and *Grm4* were

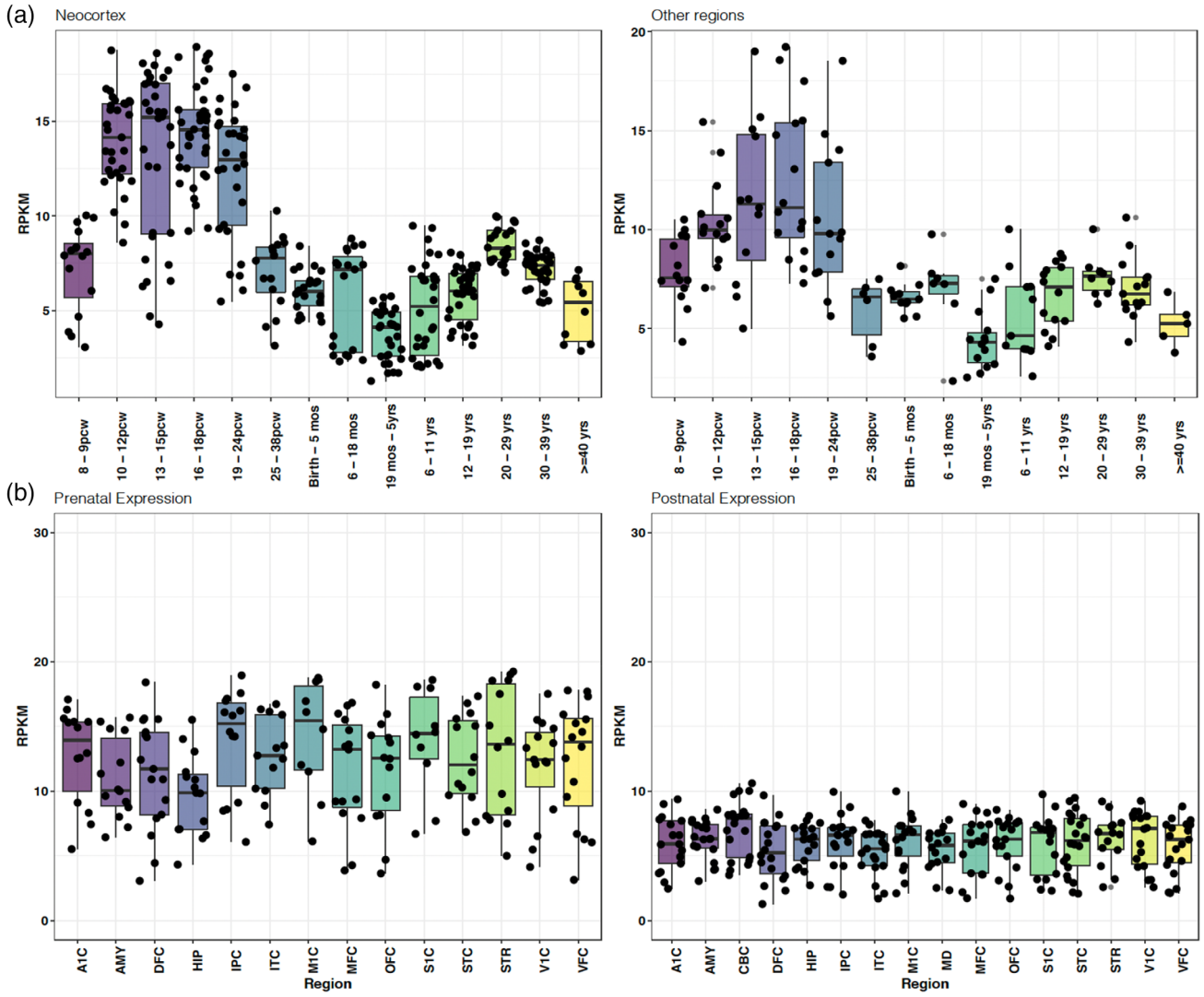

FIGURE 4 (a) Spatiotemporal bioinformatic analyses of *DIP2C* expression in the human brain across developmental stages. *DIP2C* expression across pre- and postnatal stages shows higher expression in early stages of brain development. (a) Boxplots of gene expression (reads per kilobase per million, RPKM) of the neocortex shows that *DIP2C* is highly expressed during early stages of development. Right. Boxplots of gene expression (RPKM) of various other regions in the brain pooled including the hippocampus, amygdala, the ventral forebrain, the cerebral cortex and the Mediodorsal nucleus of thalamus. Stages correspond to the following post-conception weeks (pcw): 8–9 pcw, 10–12 pcw, 13–15 pcw, 16–18 pcw, 19–24 pcw, 25–38 pcw, birth to 5 months, 6–18 months, 19 months to 5 years, 6–11 years, 12–19 years, 20–29 years, 30–39 years, 40–49 years. (b) Spatiotemporal bioinformatic analyses of *DIP2C* expression across human brain regions for prenatal and postnatal time periods. Regions of the brain correspond to the following: A1C, primary auditory cortex; AMY, amygdala; CBC, cerebellar cortex; DFC, dorsolateral prefrontal cortex; HIP, hippocampus; IPC, posterior inferior parietal cortex; ITC, inferior temporal cortex; M1C, primary motor cortex; MD, mediodorsal nucleus of the thalamus; MFC, medial prefrontal cortex; OFC, orbital prefrontal cortex; S1C, primary somatosensory cortex; STC, superior temporal gyrus; STR, striatum; V1C, occipital cortex; VFC, ventrolateral prefrontal cortex.

downregulated (Oo et al., 2020). Genes encoding GABA receptors were also dysregulated, with upregulation of *Gabbr2*, *Gabra5*, *Gabre* and *Gabrq* and downregulation of *Gabra6* and *Gabrr2* (Oo et al., 2020). Loss of *Dip2c* did not result in detectable alterations in murine brain morphology. The authors concluded that *Dip2c* may play an important role in brain development and function (Oo et al., 2020). In colorectal cancer cells, knockout of *DIP2C* resulted in cell enlargement and abnormal growth, with differential regulation of genes involved in epithelial-mesenchymal transition and cell death (Larsson et al., 2017).

5 | CONCLUSION

We present 23 patients with heterozygous *DIP2C* variants who had mild developmental delays predominantly affecting expressive speech and articulation. Additional phenotypic findings in some patients with *DIP2C* variants included facial anomalies with a high anterior hairline, prominent forehead, broad nasal tip, and a low incidence of structural cardiac anomalies. Eight of the *DIP2C* variants were de novo loss of function variants, suggesting haploinsufficiency as a mechanism, and

several variants were inherited with variable expressivity. Although heterozygous deletions of *DIP2C* have most frequently been noted with deletions of *ZMYND11* in 10p15.3 deletion syndrome (DeScipio et al., 2012), the findings in the group of patients reported herein suggest that variants predicting haploinsufficiency for *DIP2C* may also be an independent risk factor for delays in expressive language and speech articulation.

AUTHOR CONTRIBUTIONS

Thoa Ha: Conceptualization, Data curation, Writing – review and editing. **Angela Morgan**: Data curation, Writing – review and editing. **Meghan N. Bartos**: Data curation, Writing – review and editing. **Katelyn Beatty**: Formal analysis, Writing – original draft. **Benjamin Cogné**: Data curation, Writing – review and editing. **Dominique Braun**: Data curation, Writing – review and editing. **Céline B. Gerber**: Data curation, Writing – review and editing. **Harald Gaspar**: Data curation, Writing – review and editing. **Anna M. Kopps**: Data curation, Writing – review and editing. **Claudine Rieubland**: Data curation, Writing – review and editing. **Anna C. E. Hurst**: Data curation, Writing – review and editing. **David J. Amor**: Data curation, Writing – review and editing. **Mathilde Nizon**: Data curation, Writing – review and editing. **Laurent Pasquier**: Data curation, Writing – review and editing. **Rolph Pfundt**: Data curation, Writing – review and editing. **André Reis**: Data curation, Writing – review and editing. **Victoria Mok Siu**: Data curation, Writing – review and editing. **Marine Tessarech**: Data curation, Writing – review and editing. **Michelle L. Thompson**: Data curation, Writing – review and editing. **Marie Vincent**: Data curation, Writing – review and editing. **Bert B. A. deVries**: Data curation, Writing – review and editing. **Matthew B. Walsh**: Data curation, Writing – review and editing. **Stephanie Burns Wechsler**: Data curation, Writing – review and editing. **Christiane Zweier**: Data curation, Writing – review and editing. **Rhonda E. Schnur**: Data curation, Writing – review and editing. **Maria J. Guillen Sacoto**: Data curation, Writing – review and editing. **Henri Margot**: Data curation, Writing – review and editing. **Barbara Masotto**: Data curation, Writing – review and editing. **Maria Irene Valenzuela Palafoll**: Data curation, Writing – review and editing. **Urwah Nawaz**: Data curation, Writing – review and editing, Methodology, Formal analysis. **Irina Voineagu**: Data curation, Writing – review and editing, Methodology, Formal analysis. **Anne Slavotinek**: Data curation, Writing – original draft, Writing – review and editing, Methodology, Formal analysis.

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DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

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REFERENCES

Chen, C. P., Ko, T. M., Wang, L. K., Chern, S. R., Wu, P. S., Chen, S. W., Wu, F. T., Chen, L. F., & Wang, W. (2019). Inv dup del(10p): Prenatal diagnosis and molecular cytogenetic characterization. *Taiwanese Journal of Obstetrics & Gynecology*, *58*(5), 698–703. https://doi.org/10.1016/j.tjog.2019.07.021 PMID: 31542096.

Cobben, J. M., Weiss, M. M., van Dijk, F. S., De Reuver, R., de Kruiff, C., Pondaag, W., Hennekam, R. C., & Yntema, H. G. (2014). A de novo mutation in ZMYND11, a candidate gene for 10p15.3 deletion syndrome, is associated with syndromic intellectual disability. *European Journal of Medical Genetics*, *57*(11–12), 636–638. https://doi.org/10.1016/j.ejmg.2014.09.002

Coe, B. P., Witherspoon, K., Rosenfeld, J. A., van Bon, B. W., Vulto-van Silfhout, A. T., Bosco, P., Friend, K. L., Baker, C., Buono, S., Vissers, L. E., Schuurs-Hoeijmakers, J. H., Hoischen, A., Pfundt, R., Krumm, N., Carvill, G. L., Li, D., Amaral, D., Brown, N., Lockhart, P. J., … Eichler, E. E. (2014). Refining analyses of copy number variation identifies specific genes associated with developmental delay. *Nature Genetics*, *46*(10), 1063–1071. https://doi.org/10.1038/ng.3092

DeScipio, C., Conlin, L., Rosenfeld, J., Tepperberg, J., Pasion, R., Patel, A., McDonald, M. T., Aradhya, S., Ho, D., Goldstein, J., McGuire, M., Mulchandani, S., Medne, L., Rupps, R., Serrano, A. H., Thorland, E. C., Tsai, A. C.-H., Hilhorst-Hofstee, Y., Ruivenkamp, C. A. L., … Krantz, I. D. (2012). Subtelomeric deletion of chromosome 10p15.3: Clinical findings and molecular cytogenetic characterization. *American Journal of Medical Genetics. Part A*, *158A*, 2152–2161.

DeScipio, C., Spinner, N. B., Kaur, M., Yaeger, D., Conlin, L. K., Ambrosini, A., Hu, S., Shan, S., Krantz, I. D., & Riethman, H. (2008). Fine-mapping subtelomeric deletions and duplications by comparative genomic hybridization in 42 individuals. *American Journal of Medical Genetics. Part A*, *146A*(6), 730–739.

Dias, K. R., Carlston, C. M., Blok, L. E. R., De Hayr, L., Nawaz, U., Evans, C. A., Bayrak-Toydemir, P., Htun, S., Zhu, Y., Ma, A., Lynch, S. A., Moorwood, C., Stals, K., Ellard, S., Bainbridge, M. N., Friedman, J., Pappas, J. G., Rabin, R., Nowak, C. B., … Roscioli, T. (2022). De novo ZMYND8 variants result in an autosomal dominant neurodevelopmental disorder with cardiac malformations. *Genetics in Medicine*, *24*(9), 1952–1966. https://doi.org/10.1016/j.gim.2022.06.001

Eggert, M., Müller, S., Heinrich, U., & Mehraein, Y. (2016). A new familial case of microdeletion syndrome 10p15.3. *European Journal of Medical Genetics*, *59*(4), 179–182. https://doi.org/10.1016/j.ejmg.2016.02.008

Fernández, R. M., Sánchez, J., García-Díaz, L., Peláez-Nora, Y., González-Meneses, A., Antiñolo, G., & Borrego, S. (2016). Interstitial 10p deletion derived from a maternal ins(16;10)(q22;p13p15.2): Report of the first familial case of 10p monosomy affecting to two familial members of different generations. *American Journal of Medical Genetics. Part A*, *170A*(5), 1268–1273. https://doi.org/10.1002/ajmg.a.37559

Kaplanis, J., Samocha, K. E., Wiel, L., Zhang, Z., Arvai, K. J., Eberhardt, R. Y., Gallone, G., Lelieveld, S. H., Martin, H. C., McRae, J. F., Short, P. J.,

Torene, R. I., de Boer, E., Danecek, P., Gardner, E. J., Huang, N., Lord, J., Martincorena, I., Pfundt, R., … Retterer, K. (2020). Evidence for 28 genetic disorders discovered by combining healthcare and research data. *Nature*, *586*(7831), 757–762.

Karczewski, K. J., Francioli, L. C., Tiao, G., Cummings, B. B., Alföldi, J., Wang, Q., Collins, R. L., Laricchia, K. M., Ganna, A., Birnbaum, D. P., Gauthier, L. D., Brand, H., Solomonson, M., Watts, N. A., Rhodes, D., Singer-Berk, M., England, E. M., Seaby, E. G., Kosmicki, J. A., … MacArthur, D. G. (2020). The mutational constraint spectrum quantified from variation in 141,456 humans. *Nature*, *581*(7809), 434–443. https://doi.org/10.1038/s41586-020-2308-7

Kaspi, A., Hildebrand, M. S., Jackson, V. E., Braden, R., van Reyk, O., Howell, T., Debono, S., Lauretta, M., Morison, L., Coleman, M. J., Webster, R., Coman, D., Goel, H., Wallis, M., Dabscheck, G., Downie, L., Baker, E. K., Parry-Fielder, B., Ballard, K., … Morgan, A. T. (2023). Genetic aetiologies for childhood speech disorder: Novel pathways co-expressed during brain development. *Molecular Psychiatry*, *28*(4), 1647–1663. https://doi.org/10.1038/s41380-022-01764-8

Kircher, M., Witten, D. M., Jain, P., O'Roak, B. J., Cooper, G. M., & Shendure, J. (2014). A general framework for estimating the relative pathogenicity of human genetic variants. *Nature Genetics*, *46*(3), 310–315. https://doi.org/10.1038/ng.2892

Landrum, M. J., Lee, J. M., Benson, M., Brown, G. R., Chao, C., Chitipiralla, S., Gu, B., Hart, J., Hoffman, D., Jang, W., Karapetyan, K., Katz, K., Liu, C., Maddipatla, Z., Malheiro, A., McDaniel, K., Ovetsky, M., Riley, G., Zhou, G., … Maglott, D. R. (2018). ClinVar: Improving access to variant interpretations and supporting evidence. *Nucleic Acids Research*, *46*, D1062–D1067.

Larsson, C., Ali, M. A., Pandzic, T., Lindroth, A. M., He, L., & Sjöblom, T. (2017). Loss of DIP2C in RKO cells stimulates changes in DNA methylation and epithelial-mesenchymal transition. *BMC Cancer*, *17*(1), 487. https://doi.org/10.1186/s12885-017-3472-5

Li, Y., Sun, C., Guo, Y., Qiu, S., Li, Y., Liu, Y., Zhong, W., Wang, H., Cheng, Y., & Liu, Y. (2022). DIP2C polymorphisms are implicated in susceptibility and clinical phenotypes of autism spectrum disorder. *Psychiatry Research*, *316*, 114792. https://doi.org/10.1016/j.psychres.2022.114792

Maddirevula, S., Alsahli, S., Alhabeeb, L., Patel, N., Alzahrani, F., Shamseldin, H. E., Anazi, S., Ewida, N., Alsaif, H. S., Mohamed, J. Y., Alazami, A. M., Ibrahim, N., Abdulwahab, F., Hashem, M., Abouelhoda, M., Monies, D., Al Tassan, N., Alshammari, M., Alsagheir, A., … Alkuraya, F. S. (2018). Expanding the phenome and variome of skeletal dysplasia. *Genetics in Medicine*, *20*(12), 1609–1616. https://doi.org/10.1038/gim.2018.50

Nitta, Y., Yamazaki, D., Sugie, A., Hiroi, M., & Tabata, T. (2017). DISCO interacting protein 2 regulates axonal bifurcation and guidance of drosophila mushroom body neurons. *Developmental Biology*, *421*(2), 233–244.

Noblett, N., Wu, Z., Ding, Z. H., Park, S., Roenspies, T., Flibotte, S., Chisholm, A. D., Jin, Y., & Colavita, A. (2019). DIP-2 suppresses ectopic neurite sprouting and axonal regeneration in mature neurons. *The Journal of Cell Biology*, *218*(1), 125–133. https://doi.org/10.1083/jcb.201804207

Oo, Z. M., Adlat, S., Sah, R. K., Myint, M. Z. Z., Hayel, F., Chen, Y., Htoo, H., Bah, F. B., Bahadar, N., Chan, M. K., Zhang, L., Feng, X., & Zheng, Y. (2020). Brain transcriptome study through CRISPR/Cas9 mediated mouse Dip2c gene knock-out. *Gene*, *758*, 144975. https://doi.org/10.1016/j.gene.2020.144975

Poluha, A., Bernaciak, J., Jaszczuk, I., Kędzior, M., & Nowakowska, B. A. (2017). Molecular and clinical characterization of new patient with 1,08 Mb deletion in 10p15.3 region. *Molecular Cytogenetics*, *10*, 34.

Ravnan, J. B., Tepperberg, J. H., Papenhausen, P., Lamb, A. N., Hedrick, J., Eash, D., Ledbetter, D. H., & Martin, C. L. (2006). Subtelomere FISH analysis of 11 688 cases: An evaluation of the frequency and pattern of subtelomere rearrangements in individuals with developmental disabilities. *Journal of Medical Genetics*, *43*(6), 478–489.

Richards, S., Aziz, N., Bale, S., Bick, D., Das, S., Gastier-Foster, J., Grody, W. W., Hegde, M., Lyon, E., Spector, E., Voelkerding, K., Rehm, H. L., & ACMG Laboratory Quality Assurance Committee. (2015). Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genetics in Medicine*, *17*(5), 405–424. https://doi.org/10.1038/gim.2015.30

Schwarz, J. M., Rödelsperger, C., Schuelke, M., & Seelow, D. (2010). MutationTaster evaluates disease-causing potential of sequence alterations. *Nature Methods*, *7*, 575–576.

Sobreira, N., Schiettecatte, F., Valle, D., & Hamosh, A. (2015). GeneMatcher: A matching tool for connecting investigators with an interest in the same gene. *Human Mutation*, *36*(10), 928–930. https://doi.org/10.1002/humu.22844

Tumiene, B., Čiuladaitė, Ž., Preikšaitienė, E., Mameniškienė, R., Utkus, A., & Kučinskas, V. (2017). Phenotype comparison confirms ZMYND11 as a critical gene for 10p15.3 microdeletion syndrome. *Journal of Applied Genetics*, *58*(4), 467–474.

Vargiami, E., Ververi, A., Kyriazi, M., Papathanasiou, E., Gioula, G., Gerou, S., Al-Mutawa, H., Kambouris, M., & Zafeiriou, D. I. (2014). Severe clinical presentation in monozygotic twins with 10p15.3 microdeletion syndrome. *American Journal of Medical Genetics. Part A*, *164A*(3), 764–768.

Xing, Z. K., Zhang, L. Q., Zhang, Y., Sun, X., Sun, X. L., Yu, H. L., Zheng, Y. W., He, Z. X., & Zhu, X. J. (2020). DIP2B interacts with α-tubulin to regulate axon outgrowth. *Front Cell Neurosci*, (14), 29. https://doi.org/10.3389/fncel.2020.00029

Yang, L., Zhao, S., Ma, N., Liu, L., Li, D., Li, X., Wang, Z., Song, X., Wang, Y., & Wang, D. (2022). Novel DIP2C gene splicing variant in an individual with focal infantile epilepsy. *American Journal of Medical Genetics. Part A*, *188*(1), 210–215.

Yuen, R. K. C., Merico, D., Bookman, M., Howe, J. L., Thiruvahindrapuram, B., Patel, R. V., Whitney, J., Deflaux, N., Bingham, J., Wang, Z., Pellecchia, G., Buchanan, J. A., Walker, S., Marshall, C. R., Uddin, M., Zarrei, M., Deneault, E., D'Abate, L., Chan, A. J., … Scherer, S. W. (2017). Whole genome sequencing resource identifies 18 new candidate genes for autism spectrum disorder. *Nature Neuroscience*, *20*(4), 602–611.

Zarrei, M., Fehlings, D. L., Mawjee, K., Switzer, L., Thiruvahindrapuram, B., Walker, S., Merico, D., Casallo, G., Uddin, M., MacDonald, J. R., Gazzellone, M. J., Higginbotham, E. J., Campbell, C., de Veber, G., Frid, P., Gorter, J. W., Hunt, C., Kawamura, A., Kim, M., … Scherer, S. W. (2018). De novo and rare inherited copy-number variations in the hemiplegic form of cerebral palsy. *Genetics in Medicine*, *20*(2), 172–180.

Zhang, L., Mabwi, H. A., Palange, N. J., Jia, R., Ma, J., Bah, F. B., Sah, R. K., Li, D., Wang, D., Bah, F. B., Togo, J., Jin, H., Ban, L., Feng, X., & Zheng, Y. (2015). Expression patterns and potential biological roles of Dip2a. *PLoS One*, *10*(11), e0143284.

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