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ORIGINAL ARTICLE

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Extending the phenotypes associated with *TRIO* gene variants in a cohort of 25 patients and review of the literature

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Abstract

The *TRIO* gene encodes a rho guanine exchange factor, the function of which is to exchange GDP to GTP, and hence to activate Rho GTPases, and has been described to impact neurodevelopment. Specific genotype-to-phenotype correlations have been established previously describing striking differentiating features seen in variants located in specific domains of the *TRIO* gene that are associated with opposite effects on RAC1 activity. Currently, 32 cases with a *TRIO* gene alteration have been published in the medical literature. Here, we report an additional 25, previously unreported individuals who possess heterozygous *TRIO* variants and we review the literature. In addition, functional studies were performed on the c.4394A > G (N1465S) and c.6244-2A > G *TRIO* variants to provide evidence for their pathogenicity. Variants reported by the current study include missense variants, truncating nonsense variants, and an intragenic deletion. Clinical features were previously described and included developmental delay, learning difficulties, microcephaly, macrocephaly, seizures, behavioral issues (aggression, stereotypies), skeletal problems including short, tapering fingers and scoliosis, dental problems (overcrowding/delayed eruption), and variable facial features. Here, we report clinical features that have not been described previously, including specific structural brain malformations such as abnormalities of the corpus callosum and ventriculomegaly, additional psychological and dental issues along with a more recognizable facial gestalt linked to the specific domains of the *TRIO* gene and the effect of the variant upon the function of the encoded protein. This current study further strengthens the genotype-to-phenotype correlation that was previously established and extends the range of phenotypes to include structural brain abnormalities, additional skeletal, dental, and psychiatric issues.

KEYWORDS
GEFD, macrocephaly, microcephaly, phenotype, spectrin, *TRIO* gene

1 | INTRODUCTION

Guanine exchange factors (GEFs), such as TRIO, are known to activate Rho guanosine triphosphatases (Rho GTPases) by facilitating the guanosine diphosphate (GDP)-guanosine triphosphate (GTP) exchange. Rho GTPases play a central part in neurodevelopment as they have a central regulatory role in actin cytoskeleton dynamics (Govek et al., 2005; Paskus et al., 2020; Tolias et al., 2011). TRIO is highly expressed in the developing brain (Ba et al., 2016; McPherson et al., 2005; Paskus et al., 2020; Portales-Casamar et al., 2006) and it is well recognized to have a major impact on several important processes including cell migration, axonal outgrowth and dendritic arborization and synaptogenesis (Ba et al., 2013; Briancon-Marjollet et al., 2008; Herring & Nicoll, 2016; Iyer et al., 2012; Schmidt & Debant, 2014). TRIO is highly conserved across the species and comprises two GEF domains and accessory motifs such as the spectrin repeats (Debant et al., 1996). The first GEF domain, GEFD1, modulates RAC1 and RHOG while the second GEF domain, GEFD2 regulates RHOA (Bellanger et al., 1998; Blangy et al., 2000).

Previous studies have described *TRIO* variants in individuals with neurodevelopmental disorders and associated features included macrocephaly, microcephaly, behavior problems, seizures, gastrointestinal problems, skeletal features, dental problems, hyperactivity, aggression, autism spectrum disorder, and hand anomalies among others (Ba et al., 2016; Barbosa et al., 2020; Pengelly et al., 2016; Sadybekov et al., 2017).

TRIO gene missense variants have been shown to cluster in two main mutational hotspots, namely the spectrin and GEFD1 domains, which are linked to two distinct phenotypes. Pathogenic and likely pathogenic variants within the spectrin domain have been associated with more severe developmental issues and macrocephaly, via TRIO-induced RAC1 hyperactivity and a gain-of-function mechanism, whereas variants in the GEFD1 domain were shown to lead to a milder phenotype with less pronounced neurodevelopmental delay and microcephaly, with demonstrable reduced activity of the TRIO protein and a loss-of-function mechanism (Barbosa et al., 2020).

Here, we describe detailed phenotype information of an additional 25 patients and review previously published cases. The current

study extends the spectrum of phenotypes with additional skeletal features, dental anomalies, and structural brain malformations. In addition, psychiatric issues including bipolar disorders and anxiety are described.

This is the largest cohort of patients with a *TRIO* variant to date, with a potential to extend and refine the phenotypic associations with mutations in this gene.

2 | PATIENTS AND METHODS

Patients and families wishing to participate in the current project contacted our team via a patient support group called Team TRIO (www.teamtrio.org) between July 2021 and August 2022. We also included cases referred to us by clinicians directly or via existing collaboration with other research teams and patient databases including the DECIPHER, Matchmaker, and the Human Disease Genes Database. Clinicians and participating families were asked to complete a clinical data collection form and provide photographs of the affected individual. Consent for publication was obtained from all patients and their legal guardians conforming to the standards of the Declaration of Helsinki.

Phenotype and genotype data of individuals with a reported pathogenic or likely pathogenic *TRIO* gene variant have been analyzed and photographs (face, hands, and feet) have been collated to delineate facial and limb features. The only exception were Patients 14 and 15, whose variants [c.4394A > G, p.(Asn1465Ser)] were classed as variants of uncertain significance (VUS), and Patient 23 with the splice site variant c.6244-2A > G; therefore, we endeavored to perform functional studies detailed below to provide evidence for their pathogenicity.

3 | FUNCTIONAL STUDY ON TRIO VARIANT N1465S

HEK293T and N1E-115 neuroblastoma cells were cultured and transfected as described in the study by Barbosa et al. (2020). Immunoblot analysis for quantification of Phospho-PAK levels was also performed as described in the study Barbosa et al. (2020), as was the quantification of neurites in N1E-115 cells. Primary antibodies used for immunoblotting: phospho-Ser144 PAK antibody (rabbit; Cell Signaling, #2606S, 1/1000), PAK1 antibody (mouse; Santa Cruz sc-166887, 1/1000), GFP antibody (rabbit; Torrey Pines Biolabs, #TP401, 1/3000).

Statistical analyses of phospho-PAK levels were made by non-parametric one-way ANOVA, Kruskal–Wallis test, and Dunns' post-test. Asterisks indicate datasets significantly different from WT (***$p < 0.001$). Statistical analyses of neurite outgrowth were made by one-way ANOVA, Dunnett's test (***$p < 0.001$) (Figure 1).

4 | FUNCTIONAL STUDY ON SPLICE SITE VARIANT C.6244-2A > G

Primers were designed to span exon 43 (ACACGAGAGAAGGTTGCACA) and exon 45 (ATGCACATGACTTCCACAGC) of TRIO

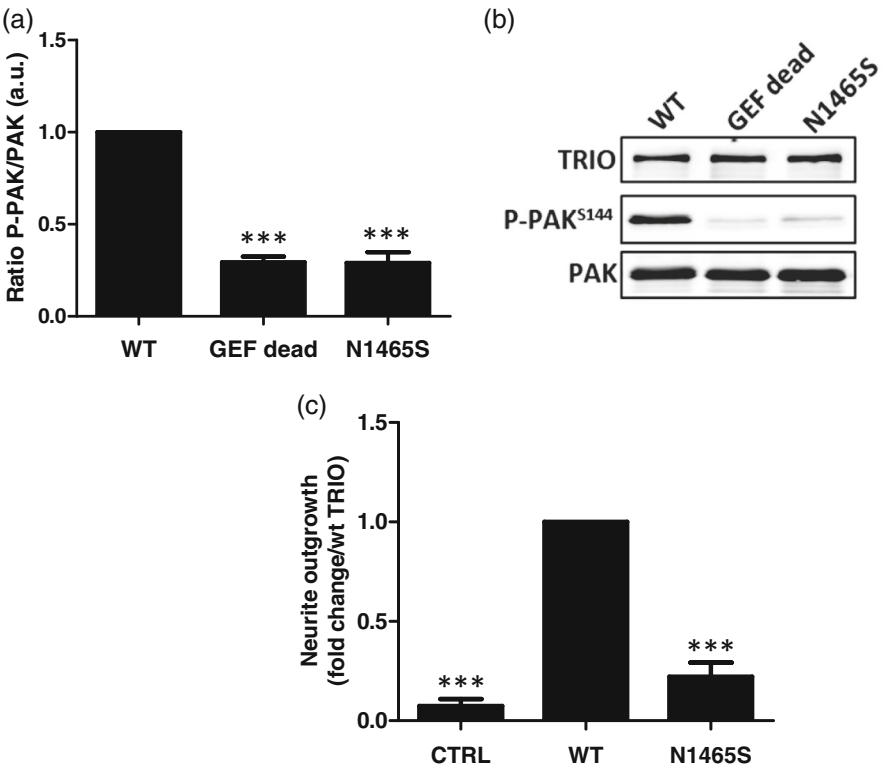

FIGURE 1 TRIO-N1465S variant leads to decreased neurite outgrowth and impaired RAC1 activation.
(a) Quantification of the ratio of phospho-PAK1 levels over total PAK1 expression, measured from HEK293T cell lysates transfected with the indicated GFP-TRIO constructs. GEF-dead is a TRIO form mutated in its GEF domain and unable to activate RAC1. Data are presented as the mean ± SEM of at least four independent experiments. (b) Representative immunoblot of HEK293T cell lysates transfected with the indicated GFP-TRIO constructs and detected with an anti-GFP antibody. PAK1 phosphorylation and total levels are detected with PAK1 antibodies, recognizing phosphorylated PAK on Ser144 or total PAK, respectively.
(c) Quantification of neurite outgrowth induced by WT or TRIO-N1465S variant in N1E-115 cells. Data are presented as *n*-fold change over WT TRIO, which was arbitrarily set to 1. Data are presented as the mean ± SEM of at least four independent experiments.

TABLE 1 Clinical and anthropometric details of nine children with a reported likely pathogenic or pathogenic variant in the spectrin domain of the TRIO gene.

Patient number	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9
Gender	Male	Female	Male	Female	Female	Female	Female	Female	Female
Age	6 years 4 months	10 years 2 months	5 years 11 months	2 years 11 months	14 years 10 months	3 years 1 month	20 years	11 years	8 years 7 months
Variant classification on report	Pathogenic	Pathogenic	Pathogenic	Pathogenic	Pathogenic	Pathogenic			Likely pathogenic
Inheritance	De novo	De novo	De novo	de novo	De novo	De novo	Unknown	De novo	De novo
Genotype	c.3211C > G p.Leu1071Val	c.3229G > C p.Ala1077Pro	c.3232C > T p.Arg1078Trp	c.3232C > T p.Arg1078Trp	c.3232C > T p.Arg1078Trp	c.3232 C > T p.Arg1078Trp	c.3371 T > C p.Leu1124Ser	c.3421G > A p.Val1141Met	c.3475G > A p.Glu1159Lys
Protein domain	Spectrin	Spectrin	Spectrin	Spectrin	Spectrin	Spectrin	Spectrin	Spectrin	Spectrin
Development	Severe delay	Severe delay	Severe delay	Severe delay	Moderate delay	Severe	Moderate delay	Severe delay	Moderate delay
First smile	NK	NK	5 months	2 months	6 weeks	4 weeks		Motor and language disorder	12 weeks
Sitting unsupported	20 months	20 months	2 years 6 months	24 months	NK	21 months			14 months
Walking unaided	Not walking	Not walking	Not walking	NK	2 years	Not yet			20 months
First words	Nonverbal	Nonverbal	Nonverbal	35 months	1 year, slow progress thereafter	Not yet			15 months Behind peers with schoolwork
Growth									
Height (cm)	88.3 (−4.61 SD)	150 (+1.59 SD)			165 (+0.51 SD)	87 cm (−1.92 SD)			120 (−1.85 SD)
Weight (kg)	11.5 (−4.48 SD)	46.3 (+1.43 SD)	16 (−2.05 SD)	9.5 (−3.76 SD)	71.6 (+1.47 SD)	11 kg (−2.29 SD)			34 (+1.06 SD)
OFC (cm)	52.6 (−0.07 SD)	58 (+3.28 SD)	55 (+0.7 SD)	52.5 (+2.69 SD)	59.8 (+3.482 SD)	Macrocephaly			54.5 (+1.12 SD)
Neuro/behavioral									
Stereotypies	−	−	+	+	−	−	NK	NK	+rocking, snapping
Aggression	−	−	+	-	+	−	NK	NK	fingers
Poor attention	−	−	+	+	+	+	NK	NK	+
OCD traits	−	−	+	-	+	−	NK	NK	+ADHD
Seizures	-	+ nocturnal seizures	+absence seizures	-	+	−	−	NK	+hand washing
Other	Hypotonia	-	-	hydrocephalus	ASD, ADHD, new onset tremor, toe walking, distal dystonic tone	−	Mild myoclonus dystonia	Hyperactivity ASD	−Autism

(Continues)

TABLE 1 (Continued)

Gastrointestinal									
Infantile feeding difficulties	+	−	+	+	−	+	NK	NK	+
Tube feeding	+	−	−	−	−	−	NK	NK	+ng tube till 3 years
Constipation	−	−	+	+constipation	+	−	NK	NK	+ NG
Other	−	−	Intestinal malrotation requiring surgery Eosinophilic oesophagitis	−	−	−	NK	NK	+Increased appetite
Skeletal									
Scoliosis	+	−	Kyphosis	−	−	−	NK	NK	−
Short tapering fingers	−	−	−	+	−	−	NK	NK	+
2/3 toe syndactyly	−	−	−	−	−	−	NK	NK	−
Other	−	−	−	−	High arched feet	−	NK	NK	+short stature
Cardiac									
Structural cardiac	Bicuspid aortic valve	−	−	−	−	−	NK	NK	−
Other	Aortic regurgitation, prominence of aortic root	−	−	−	−	−	NK	NK	Echo at 5 years-N
Dysmorphism	+	+	+Synophyris Inverted upper lip Low-set ears	+ prominent forehead, inverted upper lip	+High forehead, high frontal hairline, long nose	+	NK	NK	+high forhead, short paplebral fissures, hooded eyelids, LSE, overfolded helix of right ear
Dental									
Dental overcrowding	−	−	−	−	+	−	NK	NK	+
Delayed dental eruption	−	−	−	−	+	−	NK	NK	−
Other	−	−	−	−	−	No second molars	NK	NK	−

TABLE 1 (Continued)

Genitourinary															
Absent vagina	–		–		–		–		–		–		–		
Hypospadias	–		–		–		–		–		N/A				
Other	–		–	Neuropathic bladder	–		–		–		UTIs, leakage, dry during nights				
Growth retardation	+		–	+	+		–		+		+				
Other findings			–	IVH in neonatal period, mild hydrocephalus	Arnold-Chiari malformation		Chronic urticaria, autoimmune hypothyroidism		Thin CC, delayed myelin maturation		Inverted nipples				
							left supernumerary nipple, 2CALs								
Additional findings on genetic testing	COL1A1 c. [3424-1G > A] pathogenic variant, has OI						Paternally inherited 16p13.3 microdeletion, paternally inherited DYNC1H1 VUS				Pathogenic variant in intron 16 of FOXP1 c.1428+ 1G > C				

Abbreviations: ADHD, attention deficit hyperactivity disorder; ASD, autism spectrum disease; CAL, café-au-lait patch; CC, corpus callosum; IVH, intraventricular hemorrhage; LSE, low-set ears; N/A, not applicable; OI, osteogenesis imperfecta; UTI, urinary tract infection; VUS, variant of unknown significance.

transcript NM_007118.4. cDNA was synthesized using High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, USA). PCR was performed using GoTaq G2 DNA polymerase kit (Promega, USA). Gel was prepared at 3% agarose in Nancy-520 DNA gel stain (Sigma, USA). Gel picture was documented using Invitrogen iBirght Imager (Thermo Fisher Scientific). RT-PCR products were purified by GeneJET PCR Purification Kit (Thermo Fisher Scientific) and bidirectional Sanger sequencing was carried out by Source Bioscience. PCR experiments were repeated at least twice for reproducibility.

5 | RESULTS

A total number of 25 individuals with a pathogenic or likely pathogenic *TRIO* variant (reference sequence: NM_007118.4) have been recruited into the current cohort, 9 of which had a variant in the spectrin domain (Individuals 1–9, Table 1), 7 had a variant in or around the GEFD1 and GEFD2 domains (Individuals 10–16, Table 2), 8 had truncating variants and 1 participant had an in-frame intragenic deletion (Individuals 17–25, Table 3). Of the 25 individuals, 13 were females and 12 males with the age range being 22 months to 46 years.

Patients 14 and 15 carried a variant that was classified as variant of uncertain significance (VUS) (p.Asn1465Ser). Of note, these individuals were unrelated. We therefore performed functional studies on this variant to provide evidence for pathogenicity. Since this mutation is located in the RAC-activating GEFD1 domain of TRIO, we monitored the effect of the N1465S variant on the phosphorylation levels of PAK1, a target of RAC1, which serves as a read-out for RAC1 activation, as described previously (Barbosa et al., 2020). As shown in Figure 1a,b, the N1465S variant impaired the ability of TRIO to activate RAC1, as phosphoPAK levels were significantly reduced, similarly to the GEF dead control. To confirm this effect, we used the N1E-115 neuroblastoma cell line, in which TRIO expression leads to enhanced neurite outgrowth, in a RAC1-dependent manner. As shown in Figure 1c, the N1465S variant was impaired in inducing neurite outgrowth. These two data allowed us to reclassify the p.Asn1465Ser VUS into the likely pathogenic category.

In addition, the pathogenicity of the variant c.6244-2A > G identified in Patient 23 was confirmed by splicing analysis. This has shown aberrant alternative acceptor site activation causing a 26 base pair loss and subsequent frame shift confirming pathogenicity. This result was confirmed by Sanger sequencing (see Figure S1).

5.1 | Phenotypic characteristics of patients with a missense variant in the spectrin domain

All nine cases who possessed missense variants in the spectrin domain had moderate-to-severe delay in their development, with a

TABLE 2 Clinical and anthropometric details of 13 individuals with a likely pathogenic or pathogenic variant in or around the GEFD1 and GEFD2 domains of the TRIO gene.

Patient number	Patient 10	Patient 11	Patient 12	Patient 13	Patient 14	Patient 15	Patient 16
Gender	Female	Male	Male	Male	Female	Male	Female
Age	3.5 years		6 years 8 months	7 years	4 years	1 years 10 months	8 years 1 month
Variant classification on report	Likely pathogenic	Pathogenic	Pathogenic	Likely pathogenic	VUS but functional data provide evidence for pathogenicity	VUS but functional data provide evidence for pathogenicity	Pathogenic
Inheritance	De novo	De novo	De novo	De novo	Unknown	Unknown	De novo
Genotype	c.4112 A > G p. (His1371Arg)	c.4283 G > A p.Arg1428Gln	c.4283 G > A p.Arg1428Gln	c.4342G > A p.Gly1448Arg	c.4394A > G p.(Asn1465Ser)	c.4394A > G p.(Asn1465Ser)	c.6239 T > C p.Phe2080Ser
Protein domains	GEFD1	GEFD1	GEFD1	GEFD1	GEFD1	GEFD1	GEFD2
Development	Moderate	Global delay	Moderate delay	Moderate delay		Mild delay	Moderate delay
First smile	3–6 months		NK			1.5–2 months	NK
Sitting unsupported	3.5 years		10 months			8 months	NK
Walking unaided	Not walking yet		2 years			NK	9–10 months
First words	No words yet		1 year			17 months	Language delay
Growth		Microcephaly		Macrocephaly	Microcephaly		
Height (cm)	NK		121 (+0.25 SD)			87.5 (−0.09 SD)	145.9 (+2.82 SD)
Weight (kg)	NK		19.5 (−0.99 SD)			20.4 (+4.52 SD)	54.4 (+2.92 SD)
OFC (cm)	NK		46 (−4.0 SD)			43,7 (−3.34 SD)	53.4 (−0.29 SD)
Neurological							
Stereotypies	+		−			−	+fiddling w fins
Aggression	+		+			−	+ tantrums
Poor attention	−		+			+	+ reduced attention
OCD traits	−		+			−	+eating routines
Seizures	+ myoclonic seizures		−			−	Clumsiness
Other		Autism	−			Hyperactivity	
Gastrointestinal							
Infantile feeding difficulties	+		−			+	−
Tube feeding	+ has G button		−			−	−
Constipation	+		+			+	−
Other	−		−			−	+Colicky as baby

TABLE 2 (Continued)

Skeletal							
Scoliosis	−		−	NK		+mild	+
Short tapering fingers	+		+	NK		−	+
2/3 toe syndactyly	−		+	NK		+	-
Other	+Clubbing of feet		−	Pectus excavatum		−	+Pes planus, hypermobility
Cardiac							
Structural cardiac	−		−			−	No echo done
Other	−		−			−	
Dysmorphism	+	+	+ large ears, epicanthic folds, thin upper lip, broad nasal bridge, high arched palate		+	+Prominent eyes, micrognathia Broad nasal bridge, tubular nose, bulbous nasal tip	+Long, tubular nose, bulbous nasal tip, epicanthic folds, sparse lat eyebrows, fleshy ear helices
Dental							
Dental overcrowding	+		−			−	−
Delayed dental eruption	+significant delay		−			−	−
Other	−		+Early dental eruption			−	−
Genitourinary							
Absent vagina	−	−	−	−	−	−	−
Hypospadias	−	−	−	−	−	−	−
Other	−	−	−	−	−	−	Not examined
Growth retardation	+	NK	NK	+	NK	+	−
Other findings	MRI hypoplastic CC, dysmorphic lateral ventricle, dysmorphic basal ganglia	hypotonia		Insensitivity to pain Sacral dimple Syrinx on spine incontinent			Breast bud, inverted nipples MRI: gliotic scarring in right MCA (possible ischemic insult), duplication of the inferior branch of right MCA
Additional findings on genetic testing		SHANK3 VUS c.748 G > T, p.Gly250Cys; SHANK3 VUS c.768G > T, p.Gln256His			ZSWIM6 VUS C.1165a > C p.(Asn389His)		Maternally inherited heterozygous POLG variant c.2209G > C p.(Gly737Arg) unlikely to be significant

Abbreviations: ADHD, attention deficit hyperactivity disorder; ASD, autism spectrum disease; CC, corpus callosum; G button, gastrostomy button; ID, intellectual disability; MCA, middle cerebral artery; MRI, magnetic resonance imaging; NG tube, nasogastric tube; NK, not known; PEG, percutaneous endoscopic gastrostomy; TOF, tetralogy of fallot; VUS, variant of uncertain significance.

TABLE 3 Clinical and anthropometric details of eight individuals with a truncating variant and one individual with an intragenic deletion in the TRIO gene.

Patient number	Patient 17	Patient 18	Patient 19	Patient 20	Patient 21	Patient 22	Patient 23	Patient 24	Patient 25
Gender	Male	Male	Male	Female	Male	Male	Female	Male	Female
Age	10 years 3 months	7 years 6 months	16 years	4 years 11 months	21 years	11 years 8 months	6 years 4 months	46 years 5 months	15.5 years
Variant classification on report	Pathogenic	Likely pathogenic	Likely pathogenic; patient also reported by Kolbjer et al. (2021)	Likely pathogenic	Pathogenic	Likely pathogenic	Likely pathogenic	Likely pathogenic	Pathogenic
Inheritance	De novo	De novo	De novo	De novo	Inherited-NK	Unknown	De novo	Maternally inherited	De novo
Genotype	c.2926del p.Gln976Argfs*9	c.3727 del p.(Ser1244Leufs*23)	c.3949-122_4312-240del	c.4231C > T: R1411* p.Arg1411Ter	if maternally or paternally inherited c.5203 + 1dup	c.5419del p.Arg1807Alafs*33	c.6244-2A > G p?	c.6554_6557del p.(Glu2185Glyfs35)	c.6995del p.(Ser2332Thrfs*81)
Protein domains	Spectrin		GEFD1	GEFD1			GEFD2	GEFD2	
Development	Mild delay	Mild delay	Severe delay	Moderate delay	Mild–moderate	Mild delay	Moderate delay	Mild delay	Moderate delay
First smile	NK	NK	NK	8 months	NK		Delayed	Normal	Normal
Sitting unsupported	12 months	NK	Never	15 months	6 months	Tail end of normal range or just past	12 months	Normal	Normal
Walking unaided	18 months	18 months	Never	22 months	14 months		21 months	Normal	18 months
First words	Delayed	NK	No speech	4 years	5–6 years		20 months	Normal	After 2 years
Growth		At 7 years 1 month							
Height (cm)	142.5 (+0.4 SD)	110 (−2.29 SD)	148 (−4 SD)	94 (−3.39 SD)	NK	152.4 cm (+0.72 SD)	118 (+0.18 SD)	175 cm (−0.26 SD)	154 cm (−1.44 SD)
Weight (kg)	27.3 (−1.14 SD)	16 (−3.23 SD)	39 (−3.5 SD)	12 (−3.06 SD)	NK		17.7 (−1.28 SD)	114 kg (+2.4 SD)	48 kg (−0.9 SD)
OFC (cm)	50 (−2.8 SD)	48 (−3.5 SD)	38 cm at 12 months (−6.27 SD)	46 (−4.7 SD)	53 as adult (−2.4 SD)	38.5 kg (−0.05 SD) microcephaly	45 (−5.88SD)	54 cm (−1.9 SD)	55 cm (−0.15 SD)
Neurological									
Stereotypies	−	−	−	−	−	+	−	−	+
Aggression	−	−	−	−	−	++	−	+	+
Poor attention	+Mild attention problem	−	−	+poor attention	+	+	+	+	+
OCD traits	−	−	−	−	+	+	−	−	+
Seizures	−	−	+intractable seizures	−	−	−	−	+	+refractory seizures
Other	−	+Mood swings, hyperactivity, paroxysmal burst of laughter	−	+Hypotonia, brisk reflexes, ASD	+migraines, anxiety	Autism, bipolar disorder		Progressive leukoencephalopathy	Absences with eyelid and forehead myoclonus Self-mutilating behaviors (not aggressive toward others) since very young

TABLE 3 (Continued)

Gastrointestinal									
Infantile feeding difficulties	−	+	+	+ poor suck, choking	+	+	+	−	+
Tube feeding	−	−	+ PEG, jejunostomy	−		−		−	−
Constipation	+	−	−	+	+PEG/NG	−	−	−	−
Other	−	−	−	−	−	−	−	−	-
Skeletal									
Scoliosis	−	−	−	−	−	−	−	−	−
Short tapering fingers	−	−	−	+short tapering fingers	−	−	−	+	−
2/3 toe syndactyly	−	−	−	−	−	−	−	−	−
Other	−	−	−	+short fifth toe, short nails	−	−	−	−	+Abnormal gait with external rotation at hips and hindfoot valgus necessitating some insoles
Cardiac									
Structural cardiac	−	−	−	+Atrial septal defect	+	−	−	−	−
Other	−	−	−	−	TOF	−	−	+? arrhythmia	−
Dysmorphism	Abnormality of the crus of the helix Synophrys Highly arched eyebrow Sparse lateral eyebrow Long nose Thick lower lip vermilion Short lingual frenulum Thick upper lip vermilion Exaggerated cupid's bow	+ fullness of upper eyelid, broad nasal tip, smooth philtrum, wide mouth, thin upper lip vermilion, large ear lobe	+	+Midface hypoplasia, facial asymmetry, prominent cheeks, short and bulbous nose, round face, brachydactyly	+prominent nose, tipped back large ears, USPF, thin upper lip	+	−	−	−

(Continues)

TABLE 3 (Continued)

Dental									
Dental	–		–	–	–	+Possibly	–	–	–
Overcrowding	–		+	–	–	+	–	–	–
Delayed dental eruption	+Macrodontia of permanent		Large incisors	+small teeth, caries	–	–	–	–	–
Other	maxillary central incisor, abnormality of dental enamel								
Genitourinary									
Absent vagina	–	NA	NA	–	Not examined	–	–	–	–
Hypospadias	–	–	–	–	No abnormality reported	–	–	–	NA
Other	–	–	–	–		–	–	–	–
Growth retardation	–		+	+	–	+	+	–	+
Other findings	Nasolacrimal duct obstruction, unilateral cleft lip repaired in utero	MRI brain: ventriculomegaly myopia	Lissencephaly	Hypothyroidism-was on thyroxine, profound anemia at 2 weeks, had Transfusion	sleep paralysis/ REM sleep disorder from teens with hallucinations, on certaline, unilateral dystonic posturing of foot and arm, bilateral acessory nipples	Autism, bipolar disorder Very bad behavior	Global cerebral atrophy in brain MRI, secondary agenesis of corpus callosum	Progressive leukoencephalopathy	Visual problems (intermittent) suggestive of bilateral macular pathway dysfunction with pale optic disks
Additional findings on genetic testing				arr 6q13q14.1 (72,983,442-77,064,061) x3 arr 18p11.32p11.21 (136,226-15,054,986)x1 VUS arr 19p13.3 (685,215-1,334,735)x3					

Abbreviations: ADHD, attention deficit hyperactivity disorder; ASD, autism spectrum disease; MCA, middle cerebral artery; MRI, magnetic resonance imaging; NA, not applicable; NG, nasogastric; NK, not known; OCD, obsessive–compulsive disorder; PEG, percutaneous endoscopic gastrostomy; TOF, tetralogy of fallot; USPF, upslanting palpebral fissures.

recognizable facial gestalt including macrocephaly (Table 1 and Figure 2).

5.1.1 | Neurodevelopmental and neurological phenotype

All nine patients have significant developmental delay ranging from moderate to severe with four of the nine (44.4%) cases not walking at the time of their assessment and four individuals being nonverbal (three individuals were over 5 years of age at the time of their assessment and one patient was 3 years old). Four out of the nine (44.4%) cases developed seizures and these included nocturnal seizures, absence seizures, and myoclonus dystonia.

5.1.2 | Behavioral phenotype

Behavior issues were clearly observed, with three of the nine (33.3%) cases having stereotypies, three of the nine (33.3%) aggression, three of the nine (33.3%) obsessive–compulsive (OCD) traits, and five of the nine (55.5%) cases with a missense variant had poor attention. Of note, two cases had attention deficit hyperactivity disorder (ADHD), one had hyperactivity, and two had autism.

5.1.3 | Gastrointestinal, skeletal, dental, and other features

Infantile feeding difficulties were observed in five of the nine (55.5%) cases and two patients required tube feeding. Four of the nine (44.4%) cases had constipation, and intestinal malrotation, increased appetite and eosinophilic esophagitis was described in a single case. Of note, five of the nine cases had growth retardation. Skeletal features also occurred in single cases and included kyphosis, scoliosis, and high arched feet. Short, tapering fingers were observed in two cases and long toes in a single case. Dental overcrowding was reported in two cases, while delayed dental eruption and missing teeth were described in one case. One participant had cardiac problems with bicuspid aortic valve, prominence of the aortic root and mild aortic regurgitation. None of the cases had genitourinary problems apart from possible neuropathic bladder in one case. An intriguing finding was the presence of brain malformations in Patients 3, 4 and 6, including Arnold-Chiari malformation, thin corpus callosum, and delayed myelin maturation (Table 1).

5.1.4 | Facial characteristics

Characteristic facial features were recognized in the seven cases where this information was available and included high forehead,

FIGURE 2 Facial and limb features of individuals with likely pathogenic or pathogenic variant in the spectrin domain of the *TRIO* gene. Common features are high forehead, long nose with broad nasal tip, synophrys, and low-set ears.

frontal bossing, receding hairline, frontal baldness, low-set ears, broad nasal tip, thin upper lip, and synophrys (Figure 2).

5.2 | Phenotypic characteristics of patients with a missense variant in the GEFD1 and GEFD2 domains

5.2.1 | Neurodevelopmental and neurological phenotype

All the seven individuals with a likely pathogenic or pathogenic variant in the GEFD1 (six cases) or the GEFD2 domain (one case) had mild-to-moderate developmental delay with microcephaly, except for one case (Patient 13) who was described to have macrocephaly. Only Patient 10 was reported not to have any words at 3.5 years of age. Patient 10 had myoclonic seizures and Patient 11 had hypotonia (Table 2).

5.2.2 | Behavioral/psychiatric phenotype

Information regarding behavioral issues was provided in four out of the seven participants and two of the four (50%) had stereotypies, three of the four (75%) exhibited aggressive behavior, three of the four (75%) were described as having poor attention and two of the four (50%) had OCD traits. One of the seven cases had autism (Patient 11) and one of seven (Patient 15) had hyperactivity (Table 2).

5.2.3 | Gastrointestinal, skeletal, dental, and other features

Two of the four (50%) individuals were reported to have infantile feeding difficulties with one of the two cases requiring tube feeding. In addition, three of the four (75%) cases had constipation and three of the four (75%) cases had growth retardation.

Skeletal features were observed in this group of patients with two of the four (50%) having scoliosis, three of the four (75%) cases having short tapering fingers, and two of the four (50%) 2–3 toe syndactyly. Pectus excavatum, pes planus, and hypermobility were observed in single cases. Dental overcrowding was seen in one of the four cases (Patient 10) and the same individual (Patient 10) had delayed dental eruption (Table 2).

5.2.4 | Brain malformations

Structural brain abnormalities were described in two cases and included hypoplasia or agenesis of corpus callosum in one participant (Patient 10). Patient 10 also had dysmorphic lateral ventricles and dysmorphic basal ganglia. In addition, one patient (Patient 16) had gliotic scarring in the right middle cerebral artery (MCA) and duplication of the inferior branch of the right MCA and a further individual (Patient 13) had syrinx of the spine (Table 2).

5.2.5 | Facial characteristics

Photographs of five of the seven cases were made available, which revealed a recognizable facial gestalt including microcephaly with hypertelorism, upslanting palpebral fissures, synophrys; long tubular nose with a bulbous nasal tip and thin upper lip (Figure 3).

5.3 | Phenotypic characteristics of patients with truncating variants and the intragenic deletion in the TRIO gene

5.3.1 | Neurodevelopmental and neurological phenotype

Nine cases with a truncating variant (TV) or a deletion in the *TRIO* gene were identified in the current study. Of note, Patient 19 has already been included in a study by Kolbjer et al (Kolbjer et al., 2021). Seven of the nine cases had mild-to-moderate developmental delay and one individual (Patient 19) with the intragenic deletion had severe delay with all but one (Patient 25) participants having microcephaly. Interestingly, the early development of two patients (Patients 22 and 24) with truncating variants was within the normal range. Two cases (Patients 19 and 25) with a TV had seizures and another participant (Patient 20) had hypotonia.

The Patient 19 with in-frame intragenic deletion had severe delay and never walked or talked. He was also described as having intractable seizures (Table 3).

5.3.2 | Behavioral/psychiatric phenotype

Of the eight individuals with a truncating variant, two (25%) had stereotypies, three (37%%) had aggression, seven (87%) had attention deficit, and three (37%) had OCD traits. In addition, two of the eight (25%) had autism and one (12%) had hyperactivity. One patient (Patient 22) had a diagnosis of bipolar disorder and another individual (Patient 18) had mood swings and paroxysmal bursts of laughter. Patient 25 presented with self-mutilating behaviors since a very young age. Of note, one patient (Patient 21) had anxiety and sleep paralysis/REM sleep disorder associated with hallucinations from teenage years requiring sertraline medication (Table 3).

5.3.3 | Gastrointestinal, skeletal, dental, and other features

Infantile feeding problems were present in seven of the nine (78%) cases including the patient with the intragenic deletion with only the

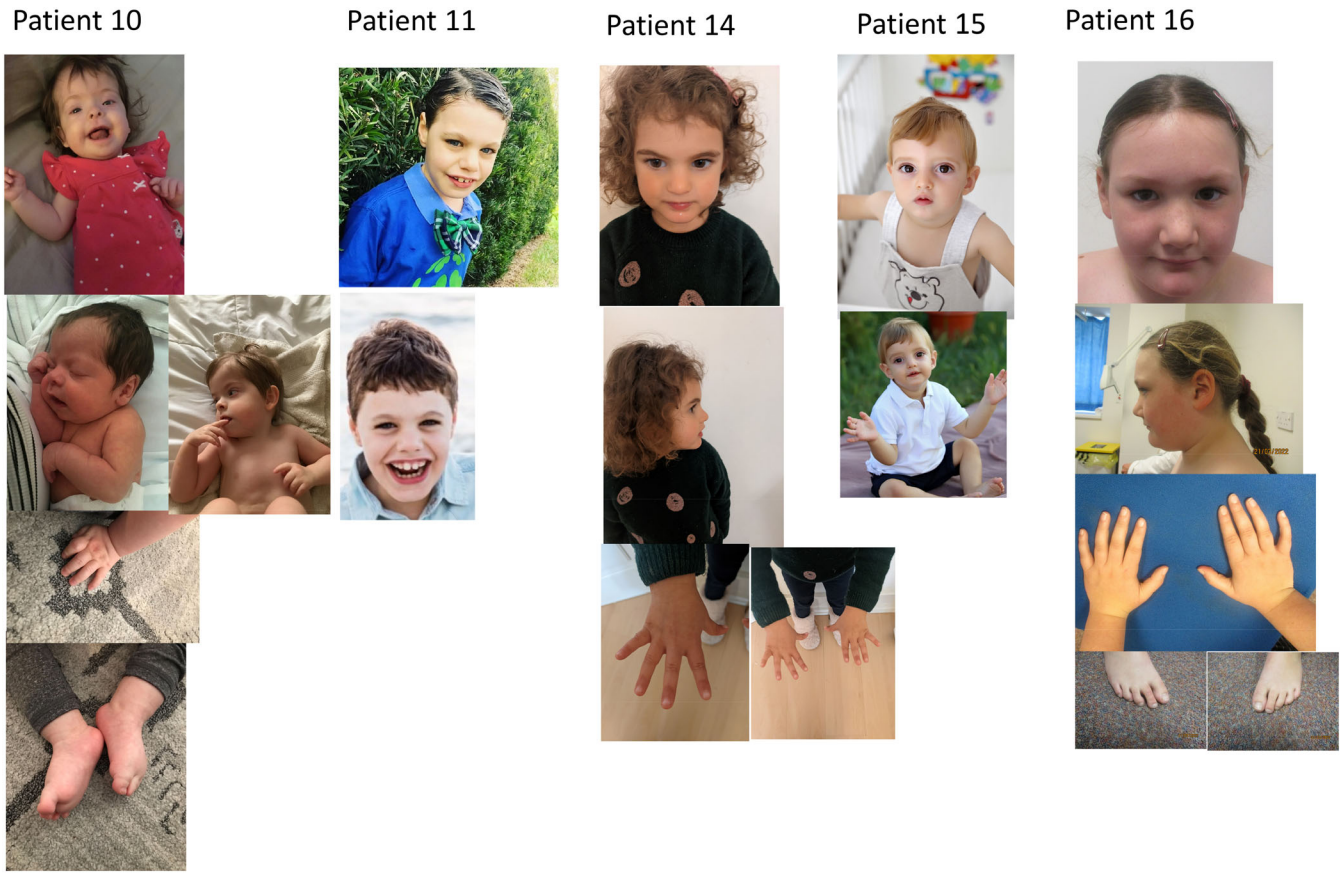

FIGURE 3 Facial and limb phenotypes of individuals with a missense variant in the GEFD1 or GEFD2 domain.

latter and another patient requiring tube feeding. Constipation was recorded in two of the nine (22%) cases and five out of the nine (55%) cases were described as having growth retardation. Skeletal features did not appear to be common in this group of patients, with short, tapering fingers described in two cases with TV; one of these individuals (Patient 20) also had a short fifth toe and short nails. Dental crowding was described in one individual (Patient 22) and delayed dental eruption was seen in two cases (Patients 19 and 22). Of note, large incisors were detected in two of the nine cases (Patients 17 and 19) and abnormality of the dental enamel (Patient 17) and small teeth (Patient 20) in single cases. In terms of cardiac features, one patient (Patient 20) with a TV had atrial septal defect, Patient 21 had tetralogy of Fallot and another case had suspected arrhythmia (Patient 24). One patient (Patient 20) had hypothyroidism requiring thyroxine and developed profound anemia necessitating blood transfusion (Table 3).

5.3.4 | Brain abnormalities

Structural brain malformation was seen in four cases (Patients 18, 19, 23, and 24) and these included ventriculomegaly, lissencephaly, global cerebral atrophy, secondary agenesis of corpus callosum, and progressive leucoencephalopathy on the MRI brain scan (Table 3).

5.3.5 | Facial characteristics

Photographs were provided for six of the nine cases and clinical features appeared to resemble those seen in individuals with missense variants in the GEFD1/2 domain. These included microcephaly, upslanting palpebral fissures, long, tubular nose with a bulbous nasal tip and thin upper lip (Figure 4).

6 | DISCUSSION

The *TRIO* gene encodes a protein that exchanges GDP to GTP on Rho GTPases and has a role in the remodeling of the cytoskeleton, hence impacting on cell migration and growth (Schmidt & Debant, 2014).

Pathogenic variants in the *TRIO* gene have been linked to neurodevelopmental problems including developmental delay, learning difficulties, behavior issues such as ADHD and autism. Ba et al. (Ba et al., 2016) described four individuals, one with an intragenic de novo deletion of *TRIO* and three with truncation variants. All cases were described to have mild intellectual disability along with behavior problems, comprising aggressive behavior, hyperactivity and autistic features. Pengelly et al. (2016) reported three members (proband, father, and paternal uncle) of the same family with the same frameshift

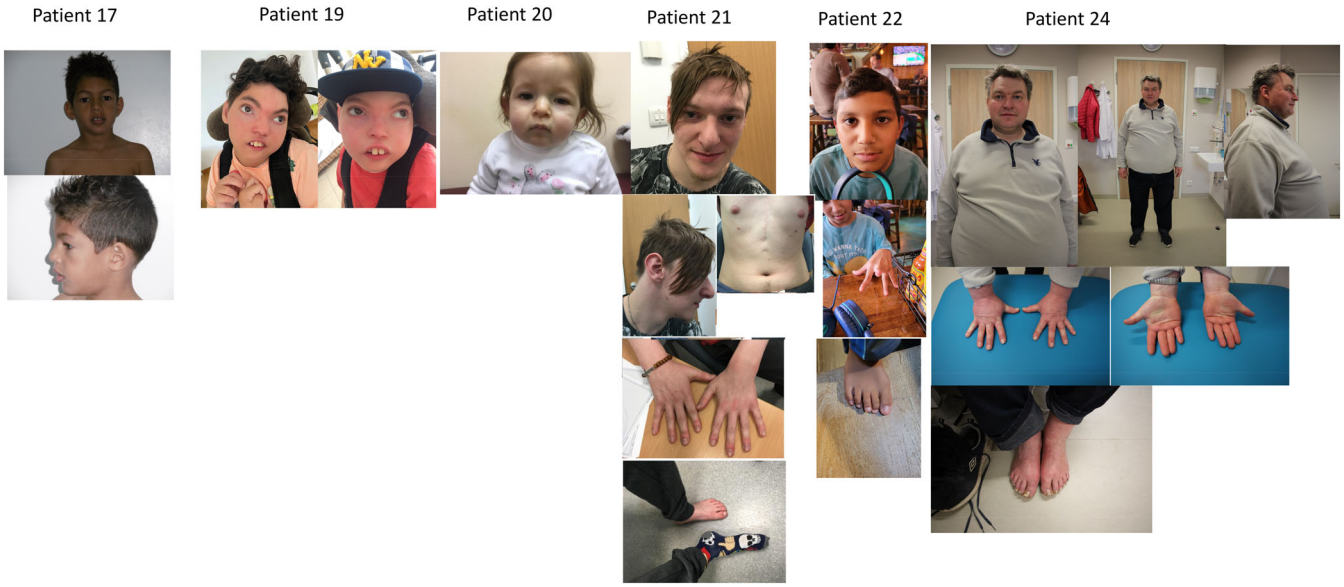

FIGURE 4 Facial and limb features of individuals with a truncating variant. Patient 19 possesses an in-frame intragenic deletion.

variant exhibiting similar facial features, mild learning difficulties, microcephaly, skeletal and dental features, as well as an additional three cases with missense variants (one in the spectrin and two in the GEFD1 domains). The two cases with missense variants in the GEFD1 domain had microcephaly and the third patient with the spectrin variant had macrocephaly. More recently, Barbosa et al. (Barbosa et al., 2020) reported a cohort of 24 individuals (which included cases reported by Pengelly et al.) with pathogenic nonsense and missense variants and reported a distinct phenotype and genotype effect between sequence changes in different domains. All individuals presented in that study had a degree of neurodevelopmental delay and behavioral issues, but those with missense variants in the spectrin domain were found to have severe intellectual disability and macrocephaly, while those with missense variants in the GEFD1 domain had a milder phenotype with microcephaly. Truncating variants were scattered across the gene and were associated with variable degree of neurodevelopmental problems. Kloth et al. (Kloth et al., 2021) also reported two cases with variants in the spectrin domain demonstrating macrocephaly, characteristic facial features, and moderate-to-severe developmental delay. One of the cases had stereoptypies and seizures and both cases presented with growth problems. Skeletal abnormalities and structural brain malformations were observed in one of the cases. In addition, Schultz-Rogers et al. (Schultz-Rogers et al., 2020) reported two cases with truncating variants who had developmental delay, one of them had macrocephaly and the other had microcephaly. Interestingly, Patient 2 presented with cutis aplasia. One of the patients had stereotypies, aggression, poor attention span, and seizures.

Here, we provide detailed description of clinical features of an additional 25 individuals, nine of whom have a missense variant in the spectrin domain, six cases with a missense variant in the GEFD1 domain, one case with a missense variant in the GEFD2 domain, eight patients with truncating variants, and one individual with an in-frame intragenic deletion (Figure 5).

Comparing the 25 cases presented here with those reported previously (see Table 4), there appears to be a recognizable split between clinical features of individuals with a missense variant in the spectrin domain and those with a missense variant in the GEFD1 or GEFD2 domains, truncating variants or deletions.

6.1 | Missense variants in the spectrin domain

All nine patients presented here were delayed in the acquisition of their developmental milestones ranging from moderate to severe, which is in keeping with findings from previous studies. Short stature was described in three cases here and growth retardation in five of the nine cases, in addition to one case that was published by Pengelly et al. (Pengelly et al., 2016). All cases, where measurements of the head circumference were available, had absolute—or in a few cases relative—macrocephaly, which is identical to *TRIO* cases in other case series with this finding. Neurobehavioral phenotypes were previously described in the study by Pengelly et al. (2016) paper and included stereotypies, aggression, and poor attention span. Barbosa et al. (Barbosa et al., 2020) also described an overall incidence of 36% for aggression, 27% for stereotypies, 45% OCD traits, 31% autistic traits, and 70% had poor attention in the *TRIO* cohort. In our spectrin cohort, the rate of stereotypies was 33.3% (3/9), aggression was 33.3% (3/9), poor attention was 55.5% (5/9), and OCD traits were 43% (3/7). In addition, hyperactivity, autism, and ADHD were described as in previous papers. Seizures occurred in four of the nine cases (44.4%), which is similar to those seen in the Barbosa paper (33.3%). Infantile feeding difficulties were delineated in the spectrin case of the Pengelly paper and found in 55.5% (5/9) in the cases here and constipation was seen

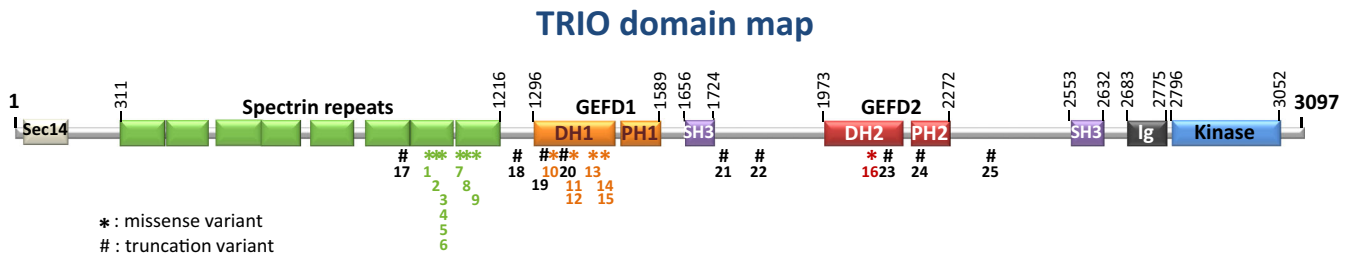

FIGURE 5 TRIO domains map showing the position of variants described in this study.

in 44.4% (4/9) of this patient cohort. Facial features were described in previous papers and add to the evidence for a recognizable facial gestalt comprising high forehead, frontal bossing, receding hairline, frontal baldness, low-set ears, broad nasal tip, thin upper lip, and synophrys. The presence of brain malfomations has been alluded to in the Barbosa et al., 2020 study and described here in detail, including Arnold-Chiari malformation, thin corpus callosum, and delayed myelin maturation.

6.2 | Missense variants in GEFD1 and GEFD2, intragenic deletion and truncating variants present with a similar phenotype

Of the seven cases with GEFD1/2 variants, five had microcephaly (or relative microcephaly) and all had mild-to-moderate developmental delay, which was consistent with findings from other studies although one patient (Patient 13) was reported to have macrocephaly with no actual measurement of the head circumference. Interestingly, patients with TVs and the deletion also had microcephaly with no reported cases with macrocephaly. All of the TV cases had mild-to=moderate delay versus the one deletion case who had severe learning issues. In contrast, there were two TV cases in the Barbosa et al. paper who had severe delays. None of the cases with GEFD1 or GEFD2 missense variants had short stature which was consistent across the case series in previous studies; however, 3 of 13 (23%) of all reported TV and deletion cases reported so far had short stature.

Neurobehavioral phenotypes were described in the current cohort (including patients with GEFD1/2 variants, TVs, and the intragenic deletion) with stereotypies (4/13, 33.3%), aggression (6/13, 46%), poor attention (10/13, 77%), OCD traits (5/13, 38%), and seizures (4/13, 31%) being described. These features were also delineated in the Barbosa study, although only an overall figure was presented for the *TRIO* cohort there. Of note, neurobehavioral phenotypes appeared to be slightly less common in the TV group (including the intragenic deletion) with the exception of poor attention that was seen in seven of the nine (78%) cases (Table 4). Infantile feeding difficulties (9/13, 61%), tube feeding (3/13, 23%), and constipation (5/13, 38%) were observed in the GEFD1/2 and the TV cohort as well, with similar ratios to previous reports (Ba et al., 2016; Pengelly et al., 2016). Facial features were similar in all of these cases and included microcephaly with hypertelorism, upslanting palpebral fissures, synophrys, long tubular nose with a bulbous nasal tip and thin upper lip.

The current study highlights possible psychiatric complications such as anxiety, sleep paralysis/REM sleep disorder associated with hallucinations, bipolar disorder and mood swings with paroxysmal bursts of laughter that are all reported in single cases.

In addition, we report brain abnormalities, namely hypoplastic or agenesis of corpus callosum, dysmorphic lateral ventricles, dysmorphic basal ganglia, global cerebral atrophy, lissencephaly, ventriculomegaly, progressive leukoencephalopathy, gliotic scarring in the right middle cerebral artery (MCA), and duplication of the inferior branch of the right MCA.

Patient 19 has an intragenic deletion of *TRIO* and 8 patients had truncating variants suggesting that haploinsufficiency of the *TRIO* gene through a loss of function mechanism is the likely molecular cause of the phenotypes observed in these cases. However, we do not know whether the frameshift variants result in truncated products of different sizes or nonsense-mediated decay of the aberrant transcripts. Similarities of clinical features observed in cases with variants in the GEFD1/GEFD2 and TVs are evident and could be explained by the effect of the GEFD1 variants on RAC1 leading to reduced activity of the TRIO protein and a loss-of-function mechanism as shown by Barbosa et al. (2020).

The current study further supports the finding that missense variants in the spectrin domain cause more significant developmental delay with prominent neurological and behavior features including seizures, stereotypies, autism, OCD, and hyperactivity, while frameshift and GEFD1/2 variants cause a milder phenotype with mild delay but also with significant neurological and behavior involvement. Difference in the phenotype was not observed in gastrointestinal, dental, and skeletal phenotypes.

In view of these findings, we recommend neurodevelopmental, dental, and spine surveillance for individuals with *TRIO* gene variants, with brain MRI performed at their initial assessment. In addition, gastrointestinal, psychiatric and behavioral assessments are recommended.

Further studies are required to determine the possibility of long-term complications related to *TRIO* and to uncover the significance of the structural brain malformations and the psychiatric issues.

In summary, this case series brings the number of reported *TRIO* cases to 57 patients with either a variant in spectrin and a likely gain-

TABLE 4 Summary of Clinical Phenotypes reported in this study and previously published cases.

This study, $n = 25$					Barbosa et al. (2020) and Pengelly et al. (2016); $n = 24$			Schultz-Rogers et al. (2020); $N = 2$	Ba et al. (2016); $n = 4$		Kloth et al. (2021); $N = 2$	Total, $n = 57$		
	Spectrin	GEFD1/2	TV	Deletion	Spectrin	GEFD1	TV	TV	TV	Deletions	Spect.	Spectrin	GEFD1/2	TV/DELS
Clinical features	MAX = 9	MAX = 7	MAX = 8	MAX = 1	MAX = 9	MAX = 7	MAX = 8	MAX = 2	MAX = 3	MAX = 1	MAX = 2	MAX = 20	MAX = 14	MAX = 23
Microcephaly	0/9	5/7	7/8	1/1	0/9	5/7	5/8	1/2	3/3	0/1	0/2	0/20	10/14	17/23
Macrocephaly	7/9	1/7	0/8	0/1	9/9	0/7	0/8	1/2	0/3	0/1	2/2	18/20	1/14	1/23
Short stature	3/9	0/7	2/8	1/1	NK	NK	NK	NK	0/3	0/1	2/2	5/11	0/7	3/13
Moderate-to-severe development delay	9/9	0/7	0/8	1/1	9/9	0/7	2/7	1/2	0/3	0/1	2/2	20/20	0/14	4/22
Mild-to-moderate development delay	0/9	7/7	8/8	0/1	0/9	7/7	5/7	1/2	3/3	1/1	0/2	0/20	14/14	18/22
Neurobehavior														
Stereotypies	3/9	2/4	2/8	0/1	1/1	1/2	0/1	1/1	NK	NK	1/2	5/12	3/6	3/11
Aggression	3/9	3/4	3/8	0/1	1/1	1/2	0/1	1/1	NK	NK	NK	4/10	4/6	4/11
Poor attention	5/9	3/4	7/8	0/1	0/1	1/2	1/1	1/1	NK	NK	NK	5/10	4/6	9/11
OCD traits	3/7	2/4	3/8	0/1	NK	NK	NK	NK	NK	NK	NK	3/9	2/4	3/9
Seizures	4/9	1/4	2/8	1/1	3/9	NK	2/5	1/1	NK	NK	1/2	8/20	1/4	6/15
Other	Autism 2/9; ADHD 2/9; HA1/9	Autism 1/5; HA 1/4	HA 1/8, autism 2/8	Intractable seizures	NK	NK	NK	Autism 1/1	Autism 1/3; ADHD 2/3					
GI														
Inf feeding probl.	5/9	2/4	6/8	1/1	1/1	NK	NK	NK	2/3	1/1	1/2	7/12	2/4	10/13
Tube feeding	2/9	1/4	1/8	1/1	1/1	NK	NK	NK	NK	NK	1/2	4/12	1/4	2/9
Constipation	4/9	3/4	2/8	0/1	NK	NK	NK	NK	1/3	0/1	NK	4/9	3/4	3/13
Growth ret.	5/9	3/4	4/8	1/1	1/1	NK	NK	NK	NK	NK	2/2	8/12	3/4	5/9
Dysmorphism														
High forehead, prominent ears	7/7	0/7	0/8	0/1	0/1	0/2	0/3	2/2	2/3	1/1	2/2	9/10	0/9	5/18
Upslanting palp. fissures, tubular nose, bulbous nasal tip	0/7	6/6	5/8	1/1	0/1	2/2	3/3	0/2	0/3	0/1	0/2	0/10	8/8	9/18

TABLE 4 (Continued)

	This study, $n = 25$				Barbosa et al. (2020) and Pengelly et al. (2016); $n = 24$			Schultz-Rogers et al. (2020); $N = 2$	Ba et al. (2016); $n = 4$		Kloth et al. (2021); $N = 2$	Total, $n = 57$		
Clinical features	Spectrin MAX = 9	GEFD1/2 MAX = 7	TV MAX = 8	Deletion MAX = 1	Spectrin MAX = 9	GEFD1 MAX = 7	TV MAX = 8	TV MAX = 2	TV MAX = 3	Deletions MAX = 1	Spect. MAX = 2	Spectrin MAX = 20	GEFD1/2 MAX = 14	TV/DELS MAX = 23
Dental														
Dental crowding	2/9	1/4	1/8	0/1	NK	1/2	NK	NK	3/3	0/1	NK	2/9	2/6	4/13
Delayed eruption	1/9	1/4	1/8	1/1	NK	NK	2/3	NK	NK	NK	NK	1/9	1/4	4/12
Brain abnormality	3/9	2/4	3/5	1/1	NK	NK	NK	NK	NK	NK	1/1	4/10	2/4	4/6
Skeletal														
Scoliosis/kyphosis	2/9	2/4	0/8	0/1	1/1	NK	2/3	NK	2/3	0/1	1/2	4/12	2/4	4/16
Short tap. fingers	2/9	3/4	2/8	0/1	NK	1/2	3/3	NK	NK	NK	1/2	3/11	4/6	5/12
2/3 toe syndactyly	0/9	2/4	0/8	0/1	NK	2/2	1/3	NK	NK	NK	0/2	0/11	4/6	1/12

Abbreviations: ADHD, attention deficit hyperactivity disorder; DELS, deletions; HA, hyperactivity; OCD, obsessive compulsive disorder; TV, truncating variant.

of-function mechanism or a variant in the GEFD1/2 domain, truncating variant or a deletion likely leading to a loss-of-function of the TRIO protein, resulting in distinguishable clinical features specific to the effect of the variant on the gene function. This current cohort further supports the contention raised by previous publications that missense variants in the spectrin domain result in a distinct phenotype with recognizable features (macrocephaly, frontal bossing, etc.) while truncating variants, deletions and missense variants in the GEFD1 and 2 domains are more likely to lead to a loss of protein function and characteristic features that include microcephaly, long, tubular nose, bulbous nasal tip, and thin upper lip among other features.

AUTHOR CONTRIBUTIONS

Conceptualization: Diana Baralle, Anne Debant, Susanne Schmidt, Gabriella Gazdagh; Data curation: Gabriella Gazdagh, David Hunt, Anna Maria Cueto Gonzalez, Monserrat Pons Rodriguez, Ayeshah Chaudhry, Marcos Madruga, Fleur Vansenne, Deborah Shears, Ayeshah Chaudhry, Eva-Lena Stattin, Britt-Marie Anderlid, Slavica Trajkova, Elena Sukarova Angelovska, Catherine McWilliam, Philip R. Wyatt, Mary O'Driscoll, Giles Atton, Anke K. Bergman, Pia Zacher, Leena D. Mewasingh, Antonio Gonzalez-Meneses López, Olga Alonso-Luengo, Pauline Boiroux, Htoo A. Wai, Ottilie Rohde; Formal Analysis of the clinical data: Gabriella Gazdagh, Analysis of functional data: Anne Debant, Susanne Schmidt, Htoo A. Wai, Ottilie Rohde, Diana Baralle; Investigation and interpretation of data: Gabriella Gazdagh, Htoo A. Wai, Ottilie Rohde, Diana Baralle, Susanne Schmidt, Anne Debant; Methodology: Gabriella Gazdagh, Anne Debant, Susanne Schmidt, Diana Baralle; Supervision: Diana Baralle, Anne Debant, Susanne Schmidt; Visualization: Gabriella Gazdagh, Susanne Schmidt, Anne Debant, Htoo A. Wai, Ottilie Rohde; Writing – original draft: Gabriella Gazdagh; Writing – review & editing: David Hunt, Anna Maria Cueto Gonzalez, Monserrat Pons Rodriguez, Ayeshah Chaudhry, Marcos Madruga, Fleur Vansenne, Deborah Shears, Ayeshah Chaudhry, Eva-Lena Stattin, Britt-Marie Anderlid, Slavica Trajkova, Elena Sukarova Angelovska, Catherine McWilliam, Philip R. Wyatt, Mary O'Driscoll, Giles Atton, Anke K. Bergman, Pia Zacher, Leena D. Mewasingh, Antonio Gonzalez-Meneses López, Olga Alonso-Luengo, Htoo A. Wai, Ottilie Rohde, Pauline Boiroux, Anne Debant, Susanne Schmidt, Diana Baralle.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request. These include the data collection forms and details of the functional studies.

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SUPPORTING INFORMATION

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