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RESEARCH ARTICLE

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The clinical and molecular spectrum of *QRICH1* associated neurodevelopmental disorder

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Abstract

De novo variants in *QRICH1* (Glutamine-rich protein 1) has recently been reported in 11 individuals with intellectual disability (ID). The function of QRICH1 is largely unknown but it is likely to play a key role in the unfolded response of endoplasmic reticulum stress through transcriptional control of proteostasis. In this study, we present 27 additional individuals and delineate the clinical and molecular spectrum of the individuals (n = 38) with *QRICH1* variants. The main clinical features were mild to moderate developmental delay/ID (71%), nonspecific facial dysmorphism (92%) and hypotonia (39%). Additional findings included poor weight gain (29%), short

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stature (29%), autism spectrum disorder (29%), seizures (24%) and scoliosis (18%). Minor structural brain abnormalities were reported in 52% of the individuals with brain imaging. Truncating or splice variants were found in 28 individuals and 10 had missense variants. Four variants were inherited from mildly affected parents. This study confirms that heterozygous *QRICH1* variants cause a neurodevelopmental disorder including short stature and expands the phenotypic spectrum to include poor weight gain, scoliosis, hypotonia, minor structural brain anomalies, and seizures. Inherited variants from mildly affected parents are reported for the first time, suggesting variable expressivity.

KEYWORDS

hypotonia, intellectual disability, QRICH1, short stature, variable expressivity, variant

1 | INTRODUCTION

Recent advances in sequencing technologies have enabled identification of multiple genes associated with rare intellectual disability (ID) disorders, including *QRICH1* (Glutamine-rich protein 1; MIM# 617387) which has 11 isoforms differing only at the 5′-end. All the isoforms encode the same 776 amino-acid-protein, which has two conserved domains: CARD domain (caspase activation and recruitment domain) at the N-terminal end and DUF3504 (domain of unknown function) at the C-terminal end, and a glutamine (Q) rich region in between (Figure 1). The DUF3504-domain is functionally uncharacterized, whereas CARD is a protein–protein interaction domain, and it is found in several proteins involved in apoptosis, inflammation and immune responses (Bouchier-Hayes & Martin, 2002). The function of QRICH1 is largely unknown, but a recent study suggests that it plays a key role in the unfolded response of endoplasmic reticulum (ER) stress through transcriptional control of proteostasis (You et al., 2021). *QRICH1* is conserved in chimpanzee, dog, cow, mouse, rat, chicken, zebrafish and frog (https://www.ncbi.nlm.nih.gov/gene/54870); and in human it is ubiquitously expressed in all investigated tissues with the highest expression in cerebellum and testis (https://www.gtexportal.org/home/).

Ten de novo loss-of-function (LoF) and one missense *QRICH1* variants have previously been described in individuals with developmental delay and variable clinical findings such as short stature with chondrodysplasia punctata, mild microcephaly, social behaviour and language deficits and elevated creatine phosphokinase (CK), (MIM# 617982) (Baruch et al., 2021; Cope et al., 2020; Föhrenbach et al., 2021; Lui et al., 2019; Ververi et al., 2018). Furthermore, *QRICH1* was suggested as a candidate gene for developmental delay (Deciphering Developmental Disorders S, 2017), and *QRICH1* variants have rarely been identified in large cohorts of individuals with autism spectrum disorder (ASD) (De Rubeis et al., 2014; Feliciano et al., 2019) and in one family with Gilles de la Tourette Syndrome (Wang et al., 2018).

Identification and phenotyping additional individuals with variants in *QRICH1* is important in defining the clinical spectrum associated with variants in this gene and identifying diagnostic clues that may assist clinicians in the evaluation of individuals with developmental delay. Here, we review the molecular and detailed clinical information of the 11 previously described individuals and report 27 additional individuals, further refining the clinical phenotypes associated with *QRICH1* variants.

2 | MATERIALS AND METHODS

2.1 | Editorial policies and ethical considerations

The genetic and clinical data were obtained as part of regular clinical care for most of the participants; IRB approvals have been obtained by the local clinicians/geneticists for those individuals who were investigated through research projects. Informed written consent for genetic testing and publication of the clinical information including clinical pictures was obtained from the parents or the legal guardians of each individual by the local clinicians/geneticists according to the Declaration of Helsinki.

2.2 | Cohort

In this series 27 new individuals with *QRICH1* variants were added to 11 previously reported individuals (Tables 1 and 2) (Baruch et al., 2021; Cope et al., 2020; Föhrenbach et al., 2021; Lui et al., 2019; Ververi et al., 2018). One of the individuals (Individual 19)

was reported as part of a larger study with limited clinical information and the full phenotype is presented in the current series (Cope et al., 2020). The 27 unpublished individuals were ascertained through GeneMatcher (https://genematcher.org/statistics, accessed December 31st, 2020) (Sobreira et al., 2015) or through ERN-ITHACA network (https://ern-ithaca.eu/), and phenotyping was carried out by local clinicians (Table 1 and Table S1). The frequency of a feature was calculated by taking into account all the individuals investigated for the given feature (either present or absent) and when data was not available these individuals were not included (Table 3).

3 | METHODS

QRICH1 variants were identified in the probands using massively parallel sequencing (next generation sequencing) based technologies (exome/genome sequencing with or without employing virtual gene panels) in either clinical diagnostic or research settings, and parental testing for the identified variant was performed in most cases. Pathogenicity of the identified *QRICH1* variants was established according to American College of Medical Genetics and Genomics/Association for Molecular Pathology (ACMG/AMP) criteria (Richards et al., 2015). The Genome Aggregation Database (gnomAD v.2.1.1; https://gnomad.broadinstitute.org/) was used to check the presence of variants in control populations. NMDEscPredictor tool (https://nmdprediction.shinyapps.io/nmdescpredictor/) was employed to predict whether the truncating variants escaped nonsense-mediated decay (NMD). SpliceAI (https://github.com/Illumina/SpliceAI), a deep learning-based splice variant prediction tool was used to annotate the variants for their predicted effect on splicing. All the variants are described using the NM_017730.3 (GRCh37/hg19) transcript of *QRICH1* according to Human Genome Variation Society recommendations (https://varnomen.hgvs.org/) and confirmed by Mutalyzer (https://mutalyzer.nl/).

To explore genotype-phenotype relationships, the frequency of specific clinical features in individuals with missense variants was compared to those with LoF variants using Fisher's exact test, and two-tailed *p* values were calculated (https://www.graphpad.com).

To model the 3D structure of QRICH1 and to identify its possible interactions with DNA "homology modelling" and "sequence-structure relationship" methods were employed. For homology modelling multiple sequence alignments including structures present in the Protein Data Bank database (https://www.rcsb.org/) and the SwissModel server (https://swissmodel.expasy.org/) were employed as previously described (Marcos-Alcalde et al., 2017). The sequence-structure relationship search was performed using the Phyre2 server (http://www.sbg.bio.ic.ac.uk/phyre2), based the construction of Hidden Markov models (HMMs) using multiple alignments and subsequent search using these HMMs in an internal database of solved structures (Kelley et al., 2015).

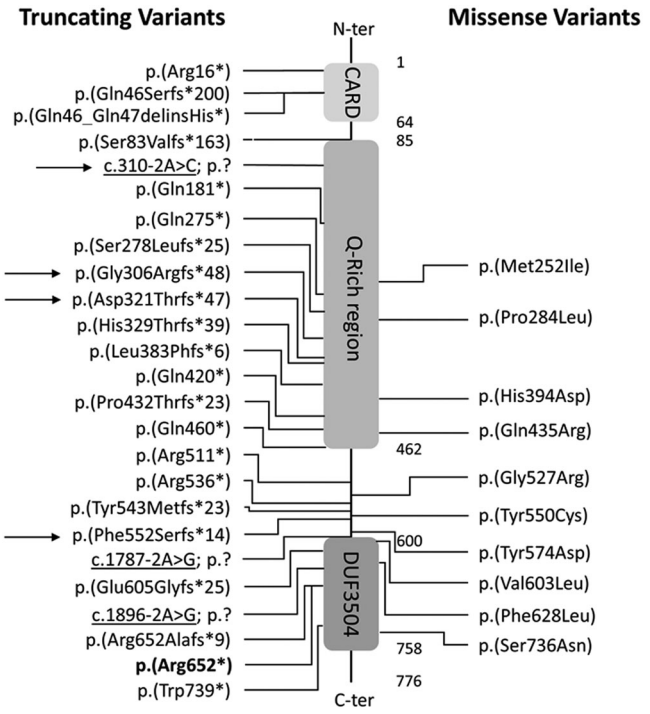

FIGURE 1 *QRICH1* domains and variants. CARD, caspase activation and recruitment domain (AA 1–64); Q-rich region, the region with several glutamine residues (AA 85–462), DUF, domain of unknown function (AA 600–758); N, N-terminal; C, C-terminal. The recurrent p.(Arg652*) variant identified in four unrelated individuals is shown in bold, inherited variants are marked with an arrow and the three splice variants are underlined

4 | RESULTS

4.1 | Phenotypic spectrum

The clinical features and molecular information of 38 probands with *QRICH1* variants are presented in Tables 1 and 2, Figure 1, and detailed information can be found in Table S1. The most common clinical features associated with *QRICH1* variants are presented in Table 3 and discussed below; and a chart showing systemic involvement in individuals with *QRICH1* variants are presented in Figure S1. Photographs of consenting individuals are included in Figure 2.

No sex difference was found in reported individuals (21 males; 17 females). The most common indications for genetic testing were developmental delay, epilepsy, and facial dysmorphism or a combination of these features. The age of first clinical evaluation ranged from 21 months to 26 years. Seven of 38 reported individuals (18%) were born preterm (<37 weeks gestation). Pregnancy-related complications likely contributing to prematurity, such as multiple gestation, maternal comorbidities, and placental problems were reported in six of these seven individuals. The few fetal abnormalities reported included intrauterine growth restriction in four individuals, increased nuchal translucency in one individual and a transient fetal pericardial effusion in one individual. Birth weights ranged from −2.87 to +1.27

TABLE 1 Phenotypic features in individuals with *QRICH1* variants

Individual number (reference)	Age and gender at evaluation	Variant type and inheritance	ID or GDD	Autism	Motor delay	Language delay	Facial dysmorphism	Short stature (height < −2SD)	Low weight (weight < −2SD)	Feeding difficulties/ GORD	Seizures (age of onset)	Hypotonia	Elevated CK	chondrodysplasia	Scoliosis	Cardiac abnormalities	Renal abnormalities	Structural brain abnormalities
1	M, 10y	Nonsense; de novo	Normal	+	−	−	+	−	−	−	+, 8y	−	−	−	−	−	−	−
2	M, 11y	Deletion, frameshift; de novo	Mild	+	+	−	+	−	−	−	−	−	−	−	−	+	−	+
3 (Ververi #3)	F, 9y	In-frame indel, nonsense; de novo	Mild	−	+	+	+	+	+	−	−	−	+	−	−	−	−	−
4 (Baruch #1)	M, 4y	Deletion, frameshift; de novo	Mild	−	−	+	+	−	+	+	−	−	−	−	−	−	−	−
5	M, 22m	Splice-site; Paternal	Normal	−	+	−	+	+[a]	+	−	−	+	−	−	−	−	−	−
6	F, 10y	Nonsense; de novo	Normal	−	+	+	+	−	−	−	−	+	−	−	+	−	−	−
7	F, 3y	Missense; de novo	Severe	−	+	+	+	−	−	−	−	−	−	−	−	−	−	+
8	F, 13y	Nonsense; de novo	Mild	−	+	+	+	−	−	−	−	+	−	−	+	−	+	−
9 (Föhrenbach #2)	F, 15.25y	Deletion, frameshift; de novo	Normal	−	+	+	+	−	−	−	−	−	−	−	+	−	−	+
10	M, 11y	Missense; de novo	Mod	+	+	+	+	−	−	−	+, 5y	−	−	−	−	−	−	−
11	M, 26y	Duplication, frameshift; Maternal	Mild	+	+	+	+	−	+	−	−	−	−	−	−	−	−	−
12	F, 13y	Deletion, frameshift; Paternal	Mild-mod	−	−	−	+	−	+	−	+, 6y	−	−	−	−	−	−	−
13	M, 13y	Deletion, frameshift; de novo	Mod-severe	-	-	+	+	+	−	−	−	−	−	−	−	−	−	+
14	M, 7y	Deletion, frameshift; de novo	Mild	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−
15	M, 15y	Missense; de novo	Mild	+	+	+	+	−	−	+	+, 14y	+	+	−	−	−	−	−

TABLE 1 (Continued)

Individual number (reference)	Age and gender at evaluation	Variant type and inheritance	ID or GDD	Autism	Motor delay	Language delay	Facial dysmorphism	Short stature (height < −2SD)	Low weight (weight < −2SD)	Feeding difficulties/ GORD	Seizures (age of onset)	Hypotonia	Elevated CK	chondrodysplasia	Scoliosis	Cardiac abnormalities	Renal abnormalities	Structural brain abnormalities
16	F, 11y	Nonsense; de novo	Severe	−	+	+	+	+[b]	+	−	+, 6m	+	−	−	+	−	−	+
17	F, 14y	Frameshift; de novo	Normal	−	−	+	+	+	+	−	−	−	−	−	+	−	−	−
18	F, 6y	Missense; de novo	Normal	−	−	+	+	−	+	−	−	−	−	−	−	+	−	−
19 (Cope #1)	F, 15y	Nonsense; de novo	Mild	−	+	−	+	−	−	+	+, 6y	−	−	−	−	+	+	+
20 (Lui #2)	M, 11y	Nonsense; de novo	Mild	−	+	+	+	−	−	−	−	−	−	+	−	+	−	−
21	M, 6y	Missense; de novo	Mild	−	+	+	+	+	+	−	−	+	−	-	−	−	−	−
22 (Lui #1)	F, 8y	Nonsense; de novo	U	−	+	−	+	+	+	−	−	+	−	+	+	−	−	−
23	M, 15y	Deletion, frameshift; de novo	Mod	−	+	+	+	−	−	−	−	−	−	−	−	−	−	+
24	F, 6y	Missense; de novo	Normal	−	+	+	+	+[a]	−	−	−	+	−	−	−	−	−	−
25	M, 8y	Frameshift; Paternal	Mod	−	−	+	+	+	−	−	−	−	−	−	−	−	−	−
26	M, 14y	Missense; de novo	Normal	+	+	+	−	−	−	−	+, 9y	−	−	−	−	−	−	−
27	M, 3y	Splice-site; de novo	U	−	+	−	+	+	−	−	−	−	−	−	−	−	−	−
28	M, 3y	Missense; de novo	Normal	+	+	+	−	−	−	−	+	+	−	−	−	−	−	−
29 (Föhrenbach #4)	M, 12.5y	Deletion, frameshift; de novo	Normal	−	+	+	+	−	+	−	−	+	−	−	−	−	−	−
30	M, 2y	Missense; de novo	Normal	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−
31	F, 11y	Splice-site; de novo	Mild	−	+	−	+	−	−	+	−	+	−	−	−	+	−	+

(Continues)

TABLE 1 (Continued)

Individual number (reference)	Age and gender at evaluation	Variant type and inheritance	ID or GDD	Autism	Motor delay	Language delay	Facial dysmorphism	Short stature (height < −2SD)	Low weight (weight < −2SD)	Feeding difficulties/ GORD	Seizures (age of onset)	Hypotonia	Elevated CK	chondrodysplasia	Scoliosis	Cardiac abnormalities	Renal abnormalities	Structural brain abnormalities
32 (Ververi #2)	F, 14y	Duplication, frameshift; de novo	Mod	−	+	+	+	−	−	−	−	+	+	−	−	−	−	−
33	F, 4y	Nonsense; unknown	Mod	+	−	+	+	−	−	−	+, 18m	+	−	−	−	−	+	+
34	F, 4y	Nonsense; de novo	Mild	−	+	+	+	−	−	+	−	+	−	−	−	−	−	−
35 (Ververi #1)	M, 8y	Nonsense; de novo	Mild	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−
36 (Föhrenbach #3)	M, 5.5y	Nonsense; de novo	U	−	+	+	+	−	−	−	−	+	−	−	+	−	+	+
37 (Föhrenbach #1)	F, 2.75y	Missense; de novo	Mod	−	−	+	+	+	−	+	−	−	−	−	−	−	−	−
38	M, 21m	Nonsense; de novo	Mild	−	+	+	+	+	−	+	−	−	−	−	−	−	−	−
Total	−	−	27	11	28	30	35	12	11	7	9	15	3	2	7	5	4	10

Abbreviations: CK, creatinine phosphokinase; F, female; GDD, global developmental delay; GORD, gastro-oesophageal reflux disease; ID, intellectual disability; m, months; M, male; Mod, moderate; Pt, patient; U, unspecified; y, years.

[a]Growth hormone responsive.

[b]Growth hormone unresponsive.

TABLE 2 *QRICH1* variants and their predicted effects

gDNA chr3 Chr3(GRCh37)	cDNA NM_017730.3	Exon/intron	Predicted effect on QRICH1	QRICH1 domain	Predicted coding effect	CADD Score	Inheritance	ACMG/AMP	ID of the affected individual	Publication
g.49114405G>A	c.46C>T	3	p.(Arg16*)	CARD	Nonsense	37	*dn*	P	1	Present study
g.49114316del	c.136del	3	p.(Gln46Serfs*200)	CARD	Frameshift	30	*dn*	P	2	Present study
g.49114312_49114313delinsAA	c.138_139delinsTT	3	p.(Gln46_Gln47delinsHis*)	CARD	Nonsense	34	*dn*	P	3	Ververi 2017
g.49114205del	c.246del	3	p.(Ser83Valfs*163)		Frameshift	29.9	*dn*	P	4	Baruch 2021
g.49095325T>G	c.310-2A>C	IVS3	p.?[a]		Splice-site	24	pat/fam	P	5	Present study
g.49095092G>A	c.541C>T	4	p.(Gln181*)	Gln-Rich	Nonsense	36	*dn*	P	6	Present study
g.49094877C>A	c.756G>T	4	p.(Met252Ile)	Gln-Rich	Missense	23	*dn*	LP	7	Present study
g.49094810G>A	c.823C>T	4	p.(Gln275*)	Gln-Rich	Nonsense	36	*dn*	P	8	Present study
g.49094800_49094801del	c.832_833del	4	p.(Ser278Leufs*25)	Gln-Rich	Frameshift	33	*dn*	P	9	Föhrenbach 2020
g.49094782G>A	c.851C>T	4	p.(Pro284Leu)	Gln-Rich	Missense	28	*dn*	LP	10	Present study
g.49094719dup	c.914dup	4	p.(Gly306Argfs*48)	Gln-Rich	Frameshift	33	mat/fam	LP	11	Present study
g.49094677del	c.961del	4	p.(Asp321Thrfs*47)	Gln-Rich	Frameshift	33	pat/fam	LP	12	Present study
g.49094650del	c.985del	4	p.(His329Thrfs*39)	Gln-Rich	Frameshift	32	*dn*	P	13	Present study
g.49094487_49094490del	c.1147_1150del	4	p.(Leu383Phefs*6)	Gln-Rich	Frameshift	33	*dn*	P	14	Present study
g.49094453G>C	c.1180C>G	4	p.(His394Asp)	Gln-Rich	Missense	24	*dn*	LP	15	Present study
g.49094375G>A	c.1258C>T	4	p.(Gln420*)	Gln-Rich	Nonsense	38	*dn*	P	16	Present study
g.49094341dup	c.1292dup	4	p.(Pro432Thrfs*23)	Gln-Rich	Frameshift	32	*dn*	P	17	Present study
g.49094329T>C	c.1304A>G	4	p.(Gln435Arg)	Gln-Rich	Missense	9	*dn*	VUS	18	Present study
g.49084640G>A	c.1378C>T	5	p.(Gln460*)	Gln-Rich	Nonsense	39	*dn*	P	19	Present study
g.49083998G>A	c.1531C>T	6	p.(Arg511*)		Nonsense	44	*dn*	P	20	Lui 2018
g.49083950C>T	c.1579G>A	6	p.(Gly527Arg)		Missense	34	*dn*	LP	21	Present study
g.49083923G>A	c.1606C>T	6	p.(Arg536*)		Nonsense	42	*dn*	P	22	Lui 2018
g.49083905del	c.1626del	6	p.(Tyr543Metfs*23)		Frameshift	25	*dn*	P	23	Present study
g.49083880T>C	c.1649A>G	6	p.(Tyr550Cys)		Missense	29	*dn*	LP	24	Present study
g.49083877del	c.1655del	6	p.(Phe552Serfs*14)		Frameshift	33	pat	LP	25	Present study
g.49081889A>C	c.1720T>G	7	p.(Tyr574Asp)		Missense	29	*dn*	LP	26	Present study

(Continues)

TABLE 2 (Continued)

gDNA chr3 Chr3(GRCh37)	cDNA NM_017730.3	Exon/intron	Predicted effect on QRICH1	QRICH1 domain	Predicted coding effect	CADD Score	Inheritance	ACMG/AMP	ID of the affected individual	Publication
g.49070663T>C	c.1787-2A>G	IVS7	p.?		Splice-site	25	*dn*	P	27	Present study
g.49070641C>A	c.1807G>T	8	p.(Val603Leu)	DUF3504	Missense	34	*dn*	LP	28	Present study
g.49070635_49070636del	c.1812_1813del	8	p.(Glu605Glyfs*25)	DUF3504	Frameshift	34	*dn*	P	29	Föhrenbach 2020
g.49070564G>C	c.1884C>G	8	p.(Phe628Leu)	DUF3504	Missense	29	*dn*	LP	30	Present study
g.49070208T>C	c.1896-2A>G	IVS8	p.?		Splice-site	25	*dn*	P	31	Present study
g.49070149dup	c.1953dup	9	p.(Arg652Alafs*9)	DUF3504	Frameshift	33	*dn*	P	32	Ververi 2017
g.49070148G>A	c.1954C>T	9	p.(Arg652*)	DUF3504	Nonsense	40	uk[b]	P	33	Present study
							dn	P	34	Present study
							dn	P	35	Ververi 2017
							dn	P	36	Föhrenbach 2020
g.49068009C>T	c.2207G>A	11	p.(Ser736Asn)	DUF3504	Missense	28	*dn*	LP	37	Föhrenbach 2020
g.49068000C>T	c.2216G>A	11	p.(Trp739*)	DUF3504	Nonsense	39	*dn*	P	38	Present study

Abbreviations: ACMG/AMP, variant classification according to ACMG/AMP (American College of Medical Genetics and Genomics/Association for Molecular Pathology) criteria; CARD, caspase activation recruitment domain; del, deletion; delins, deletion-insertion; dn, de novo; dup, duplication; DUF3504, Domain of unknown function; fs, frame shift; Gln-Rich, glutamine-rich domain; IVS, intervening sequence (intron); LP, likely pathogenic; mat, maternal; pat, paternal; P, pathogenic; uk, unknown; VUS, variant of unknown significance.

[a]Further analyses revealed skipping of exon 4 predicted to result in an inframe deletion, p.(Val104_Ser446del), in the individual and his father.

[b]Parents were not available for testing.

*Translation termination codon.

TABLE 3 Common clinical features noted in individuals with *QRICH1* variants

	Missense (*n* = 10)		LOF (*n* = 28)		Total (*n* = 38)		
	#	%	#	%	#	%	*p* values[a]
Intellectual disability/GDD	5	50	22	79	27	71	ns
Mild	2	20	13	46	15	39	ns
Moderate	2	20	5	18	7	18	ns
Severe	1	10	1	4	2	5	ns
Unspecified	0	0	3	11	3	8	ns
Motor delay	7	70	21	75	28	74	ns
Language delay	10	100	20	71	30	79	ns
Regression	1	10	3	11	4	11	ns
Facial dysmorphism	7	70	28	100	35	92	**0.0142**
Broad forehead	0	0	7	25	7	18	ns
Frontal bossing	1	10	2	7	3	8	ns
Hypertelorism	3	30	6	21	9	24	ns
Downslanting palpebral fissures	1	10	5	18	6	16	ns
Prominent nose	1	10	13	46	14	37	ns
Bulbous/broad nasal tip	4	40	13	46	17	45	ns
Broad/full nasal bridge	1	10	5	18	6	16	ns
Ear anomalies	2	20	17	61	19	50	ns
Thin upper lip	3	30	17	61	20	53	ns
Wide mouth	1	10	16	57	17	45	**0.0124**
High arched palate	0	0	7	25	7	18	ns
Small/recessed chin	0	0	5	18	5	13	ns
Short stature (height <−2*SD*)	3	30	9	32	12	32	ns
Low weight (weight <−2*SD*)	2	20	9	32	11	29	ns
ASD	5	50	6	21	11	29	ns
Seizures	4	40	5	18	9	24	ns
Hypotonia	4	40	11	39	15	39	ns
Elevated CK	1	10	2	7	3	8	ns
Feeding difficulties/reflux	2	20	5	18	7	18	ns
Chondrodysplasia	0	0	2	7	2	5	ns
Scoliosis	0	0	7	25	7	18	ns
Cardiac abnormalities	1	10	4	14	5	13	ns
Renal abnormalities	0	0	4	14	4	11	ns
Brain MRI abnormalities	1	10	9	32	10	26	ns

Note: Three individuals with short stature include Individual 24 who responded to growth hormone therapy; %, frequency of the individuals with the given feature; #, number of individuals with the given feature; ns, not significant; the significant *p* values (< 0.05) are given in bold.

Abbreviations: CK, creatine kinase; GDD, global developmental delay; LOF, loss-of-function; MRI, magnetic resonance imaging; No., number.

[a]*p* values: comparison of missense variants and loss of function variants using two-tailed Fisher's exact test.

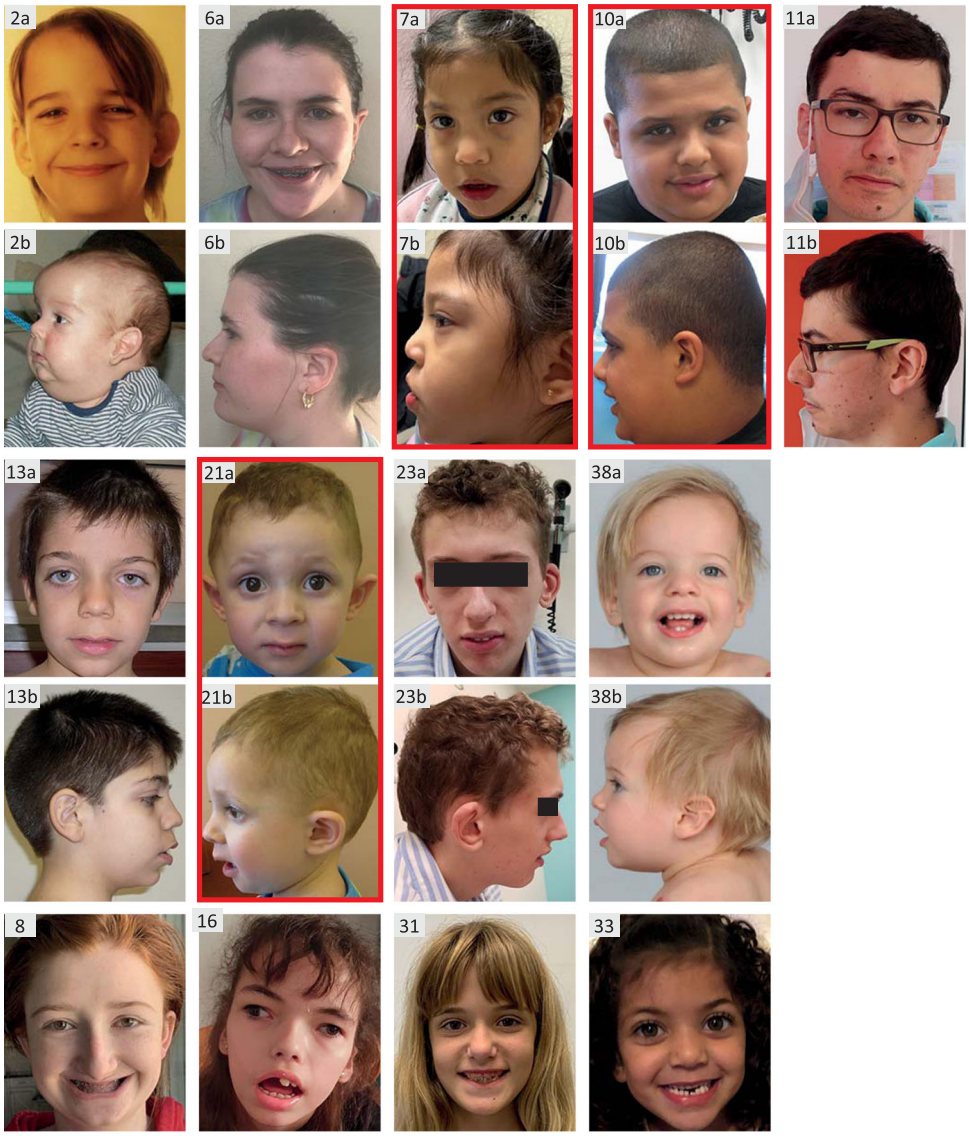

FIGURE 2 Facial photographs. No identifiable facial gestalt is shared by all cases, but there are some common features. Number in the top left corner of each frame corresponds to individual case number. Red boxes denote individuals with a missense variant. Hypertelorism in Individuals 7, 8, 16, and 38. Downslanting palpebral fissures in Individuals 3, 13, 23, 38. High anterior hairline or tall broad forehead in Individuals 3, 16, 38. Thin upper vermillion in Individuals 3, 6, 8, 11, 13, 20, 31, 33, 38. Relatively short philtrum in Individuals 8, 10, 16, 23, 31. Relatively long philtrum in Individuals 13, 21. Wide mouth in Individuals 3, 6, 8, 11, 13, 16, 23, 31, 33, 38. Prominence of the nasal tip or bulbous nasal tip in Individuals 6, 8, 10, 11, 13, 21, 23, 31, 33. Dysplastic ears in Individuals 3, 10, 11, 16, 21, 23, 38

SD for corrected age and birth lengths from −2.7 to +2.8 *SD* for corrected age.

The most consistent feature reported was developmental delay and/or ID in 27 individuals (27/38; 71%). In this group, 5/27 were too young for cognitive testing, and a clinical diagnosis of global developmental delay (GDD) was made. The remaining 22 were diagnosed with ID through either formal IQ (intelligence quotient) testing (6/27) or clinical assessment (16/27). For 10 of these 16 individuals early GDD was also reported. The severity of ID or GDD was mild in 15 (56%), moderate in seven (26%), and severe in two (7%) individuals. In the remaining three individuals, formal IQ testing or developmental assessment was not provided, but there was a history of delayed early developmental milestones. Eleven individuals (11/38; 29%) were reported to have normal IQ, however, only four of these individuals had formal IQ assessments. Learning difficulties, provision of additional educational supports or a history of early developmental delay were reported in 10 of these 11 individuals suggesting subtle cognitive problems. The two individuals with severe ID (individuals 7 and 16) demonstrated additional factors that likely contributed to their more severe phenotype. Individual 7 has a blended phenotype with a homozygous pathogenic variant in *AP4B1* causing autosomal recessive spastic paraplegia. Individual 16 had septicemia and subsequent developmental regression at age 6 years, on a backdrop of pre-existing developmental delay and known structural cerebral abnormalities.

Language development was highly variable, with average age of speech acquisition at 18.25 months. Two individuals, both aged 11 years, were nonverbal. Mild delays in early motor development were common, noted in 28/38 (74%) individuals. The average age of rolling was 6 months, sitting was 8.5 months and unassisted walking was 18 months. Four individuals displayed signs of developmental regression, occurring from the age of 1 year in the youngest individual. Two of these individuals had regression in language only. One who had pre-existing developmental delay (Individual 16), reported language regression following an episode of severe septic shock, which may have led to the loss of skills. Another experienced motor regression following the onset of seizures at age three (Individual 28). None of the reported individuals with developmental regression had features suggestive of ASD.

Additional neurological findings were nonspecific and included generalized hypotonia (15/38; 39%) and seizures (9/38; 24%). Age of seizure onset was between 6 months to 14 years. Seizure types varied, including infantile spasms, absence seizures and drop attacks, all with associated generalized EEG (electroencephalogram) abnormalities. The implementation and response to antiepileptic therapy was also variable. Brain imaging was performed in 19/38 (50%) of individuals. Nine individuals (10/19; 52%) demonstrated a minor anomaly on imaging, including nonspecific periventricular white matter abnormalities, arachnoid cysts, and pineal cysts.

Features of ASD were reported in 11 of 38 individuals (29%) and a range of behavioral problems and specific character traits were noted in 16/38 (42%). These included anxiety, attention or concentration difficulties, and shy or quiet personality traits.

Elevated CK was present in two previously reported individuals (Individuals 3 and 32) (Ververi et al., 2018). Of the currently investigated patients, 10 had CK testing and one new individual had significantly elevated CK levels without any specific neurological or neuromuscular features (Individual 15). Altogether, 3/10 (30% of those tested) demonstrated CK elevation. One individual, who also harbors a heterozygous variant in *RYR1*, had significant muscle fatigue and mild proximal weakness (Individual 6). She demonstrated a normal muscle biopsy and CK level and responded symptomatically to treatment with pyridostigmine.

Facial dysmorphism was reported in a significant proportion of individuals (35/38; 92%), (Figure 2). The most consistently noted facial features were a broad, bulbous nasal tip (17/38; 45%), a thin upper lip (20/38; 53%), ear anomalies including large/prominent ears, increased folding or cup shaped ears (19/38; 50%), and a wide mouth in 17/38 (45%). High anterior hairline was apparent in four individuals (2, 16, 32, and 38).

Twelve individuals (12/38; 32%) had short stature, with height at or below −2*SD*. Three individuals (5, 16, 24) with short stature were treated with growth hormone, with improvement in growth in two and without effect in the third (Individual 16).

Only two previously reported individuals had growth plate irregularities and short upper limbs (Individuals 20 and 22); limb abnormalities or radiological evidence of chondrodysplasia were not identified in other individuals. Chondrodysplasia was therefore relatively rare (2/11; 18%); however, this was not evaluated in most individuals ($n = 27$). Scoliosis was documented in seven individuals (7/38; 18%). Four new individuals (6, 11, 13, and 24) and a previously reported individual (22)[5] had delayed bone age. No other specific radiological abnormalities were identified.

Eleven individuals had weights at or below −2*SD*. Six of these individuals had corresponding short stature. Aside from Individual 11, body mass index was within the normal range for all individuals with short stature and/or poor growth. Several newly reported individuals had significant feeding difficulties and gastrointestinal reflux that potentially have influenced poor weight gain. Two individuals required gastrostomy insertion for feeding difficulties.

Structural organ malformations were infrequent. Cardiac anomalies were identified in five (5/38; 13%) and renal anomalies in four (4/38; 11%). Of those four, two individuals had renal cysts (Individuals 19 and 33), one individual had nephroblastoma (Individual 37), and one had significant bilateral cystic renal disease due to a concomitant pathogenic *PKD1* variant (Individual 8). Individual 27 also showed a possible blended phenotype, with a likely pathogenic monoallelic *TNFRSF13B* variant that may potentially explain the reported immune deficiency.

For four individuals the variants were inherited (Individuals 5, 11, 12, and 25), and three variants (of Individuals 5, 11, and 12) were familial - either genetically confirmed or clinically suspected (Pedigrees can be found in Figure S2). Sequence data did not suggest mosaicism in any of the parents. The variant was paternally inherited in three individuals (5, 12, 25) and maternally inherited in one (Individual 11). Limited clinical information was available about the carrier parents or family members. The father of Individual 5 (carrier of the variant) had planning and organization difficulties, the paternal grandmother had severe dyslexia and difficulties in school, and paternal grandaunt had dyslexia (the two former are carriers of the variant). The mother of Individual 11 was reported to have learning difficulties (carrier of the variant) and the maternal aunt to have ID (not genetically verified). A paternal history of ADHD and learning disability as well as a family history of ID was reported for Individual 12. The father of Individual 25 had writing difficulties (variant verified).

4.2 | Spectrum of QRICH1 variants

Among the 38 individuals, 35 different variants were identified (Tables 1 and 2, Figure 1). One recurrent nonsense variant, c.1954C>T, was identified in four unrelated individuals. One variant (c.914dupC) was maternally, and three variants (c.961del, c.1655del, c.310-2A>C) were paternally inherited. All the other variants were found to be de novo, except for the recurrent variant c.1954C>T in Individual 33, for whom the parents were not available for segregation analysis.

Among the 35 different variants, ten were missense while the remaining were predicted to be protein truncating variants: nine nonsense variants, three single-nucleotide duplications, one four-nucleotide, two two-nucleotide, and six single-nucleotide deletions, one two-nucleotide indel leading to a stop codon and three splice site variants (Figure 1). Only one nonsense variant, c.2216G>A, located in the last exon was predicted to escape

NMD, while all the other protein truncating variants were predicted to be subject to degradation by NMD.

Only one splice site variant (c.310-2A>C, identified in Individual 5) was reported in the gnomAD database (https://gnomad.broadinstitute.org/) with an allele frequency of 0.000003997 (1/250,190 alleles). This variant was familial (verified in the father and paternal grandmother and is possibly present in the paternal grandaunt) and the carriers had some mild symptoms as described above. This variant was initially classified as variant of unknown significance. However, targeted RNA sequencing revealed skipping of exon 2 both in the proband and the father (Figure S3), and this variant is now classified as pathogen. Material was not available for the two other individuals (11 and 12) to evaluate RNA skipping, but these variants were not reported in gnomAD database, and were classified as likely pathogen. Apart from the missense variant identified in Individual 18, all the other variants were classified as pathogenic or likely pathogenic according to ACMG/AMP criteria.

None of the missense variants were predicted to be highly likely to affect splicing, using Splice AI. Four missense variants were localized to the glutamine-rich domain (Gln-rich) and three variants in the domain of unknown function (DUF3504), while no missense variants were found to be localized to the CARD.

5 | DISCUSSION

Previously, very few individuals with *QRICH1* variants have been reported in the literature, which has limited understanding of the phenotypic and genotypic spectrum of this condition. We describe 38 individuals (11 previously published, one with sparse clinical information) with *QRICH1* variants, and confirm that the phenotype is relatively nonspecific, including variable neurodevelopmental features, hypotonia, short stature, poor growth and minor facial dysmorphisms.

The individuals demonstrated mild to moderate developmental delay in infancy. Two individuals with severe ID had additional factors and variants in other genes. The finding of four variants inherited from parents who were mildly affected or with few difficulties, further supports the conclusion that the impact on cognition is generally mild. s. Gross motor milestones were mildly delayed or within the later range of normal in most individuals. Although developmental regression was reported in four individuals, additional contributing factors were present in two of them, and isolated language regression was reported in the other two. These data do not suggest that *QRICH1* associated related neurodevelopmental disorder is regressive.

A few *QRICH1* variants have previously been identified in large cohorts with ASD (De Rubeis et al., 2014; Feliciano et al., 2019). No additional phenotypic information is available about these individuals, and only one is reported with cognitive impairment (Feliciano et al., 2019). Approximately 75% of individuals with ASD have disabilities that require substantial social and educational support (Mefford et al., 2012), indicating common co-occurrence of ID and ASD. Our data support ASD as a common feature of this disorder, as all but one individual with ASD had comorbid ID. A recent study has shown involvement of QRICH1 in ER homeostasis (You et al., 2021). It is thus plausible that *QRICH1* variants lead to neurodevelopmental features through dysregulation of ER stress responses, as impairment of the secretory pathway has been suggested to impair synapse formation and/or function during neurodevelopment (Martínez et al., 2018; Mignogna et al., 2015). We suggest that *QRICH1* variants predispose to both ID and ASD, like many other genes involved in neurodevelopmental disorders.

Short stature, chondrodysplasia, scoliosis, and delayed bone age demonstrates that QRICH1 protein plays a role in regulating skeletal growth and development. Short stature was identified in 33% of newly reported individuals, concordant with earlier reports (Föhrenbach et al., 2021; Ververi et al., 2018) of short stature or stature within the lower range of normal in three individuals with *de novo QRICH1* variants (Individuals 3, 22, and 37 in this series). Lui et al. (2019) identified chondrodysplasia in two previous individuals and demonstrated that decreased *Qrich1* expression in mouse models produces downregulation of several genes involved in chondrocyte hypertrophic differentiation, leading to impaired longitudinal bone growth. In this series we found that 18% of the examined individuals had chondrodysplasia. However, we are limited in our ability to draw meaningful conclusions about the frequency of chondrodysplasia as only 11 individuals of the present cohort had hand X-rays, suggesting this feature may be under ascertained. Furthermore, chondrodysplasia may be an age-related feature, varying with the degree of skeletal maturation, making comparison between individuals difficult. Further radiographic investigations are required to determine the specificity of chondrodysplasia in this condition. Two of three individuals treated with growth hormone had a response to this intervention, which suggests that growth hormone therapy may be able to ameliorate the skeletal impact of complete or partial loss of QRICH1. However, larger interventional studies are required before definitive conclusions can be drawn about the impact of growth hormone on individuals with *QRICH1* associated neurodevelopmental disorder. The identification of scoliosis or lumbar hyperlordosis in this cohort is new, and likely underappreciated. This new clinical information further expands the skeletal phenotype associated with *QRICH1*, and we suggest that monitoring for scoliosis should be considered as part of comprehensive pediatric care for individuals with this condition.

Feeding difficulties in *QRICH1* associated neurodevelopmental disorder are relatively frequent and may lead to poor weight gain as well as short stature. Our data support inclusion of poor weight gain, failure to thrive, and short stature, as part of the phenotype.

Dysmorphic features were frequently found in the individuals included in this study. We find supportive evidence for the previously reported features of prominent nose, large or dysplastic ears, wide mouth and thin upper vermillion border as part of the facial phenotype. However, a unifying facial gestalt was not apparent. In comparing facial features between those with missense and LoF variants, we were able to review facial photographs of only three individuals

with missense variants, compared to ten individuals with LoF variants. This limits our conclusions as sample size is inadequate to determine whether true differences exist between individuals with missense and LoF variants. Although predominant expression of *Qrich1* has been demonstrated in the maxilla and mandible of mouse embryos (Lui et al., 2019), none of the individuals had striking lower craniofacial or palatal malformations.

Elevated CK was reported in three individuals, but most individuals in this series did not have CK level tested. Furthermore, two individuals with large chromosomal deletions, including *QRICH1* (Decipher database [https://www.deciphergenomics.org/, Firth et al., 2009]) were reported to have CK elevation. Based on currently available data, further evidence is required to definitively implicate QRICH1 in muscle metabolism.

Structural cardiac anomalies were noted in five individuals. Homozygous knock out *Qrich1* mice develop congenital heart disease, cleft palate and renal anomalies (San Agustin et al., 2016). One of five individuals reported in the Decipher database with a heterozygous *QRICH1* deletion is reported to have congenital heart disease (transposition of the great arteries and atrial septal defect). It is possible that heterozygous loss of function variants have a morphological impact on the development of midline structures. Hypertelorism and pineal cysts are other features that support this hypothesis. Formal evaluation of pituitary function is needed to determine whether defects in the pituitary-hypothalamic axis, frequently associated with midline defects, contribute to the short stature.

Several individuals underwent methylation studies for Russell Silver syndrome, *SHOX* gene sequencing or investigation for possible Noonan syndrome with a rasopathy gene panel, with the clinical indication of short stature likely to influence the selection of these investigations. The generally mild developmental delay in conjunction with subtle and variable dysmorphism in individuals with *QRICH1* variants mean that the differential diagnosis is broad. Exome or genome sequencing, as opposed to single gene or panel testing, is likely to be a more efficient diagnostic strategy in these individuals.

To investigate how missense variants could affect the protein function, we attempted to carry out protein modelling. It was not possible to generate a 3D model of sufficient quality to analyze the effect of the missense variants. Sequence-structure relationships (Phyre2 [Kelley et al., 2015]) predicted the existence of secondary structure from residues 1–64 (CARD domain) as well as from residues 460 to the end of the protein (including the DUF3504 domain) (Figure 1). The confidence score for the sequence-structure homology between residues 460–600, harboring three missense variants, with several other DNA-binding proteins was significant (more than 80%). It has been previously suggested that the DUF3504 domain could belong to a family of "domesticated" tyrosine-recombinase transposons present in jawed vertebrates (Kojima & Jurka, 2011). The results of our sequence-structure relationship analysis suggested a high similarity between this domain and the N-terminal domain of the yeast Ndc10 protein (protein data bank code 4ACO, https://www.rcsb.org/), which is evolutionarily related to tyrosine recombinases (Perriches & Singleton, 2012). Tyrosine recombinases recognize and bind to specific DNA segments leading to genomic rearrangements, which may be a function of the QRICH1 protein. The missense variants reported were all considered likely to alter the interaction of the protein with its purported DNA binding function. However, this hypothesis necessitates further functional studies of the protein to be confirmed.

We compared specific phenotypic features amongst individuals with missense variants to individuals with LoF variants, in an attempt to identify genotype-phenotype correlations. No significant differences were identified regarding neurodevelopmental, growth or skeletal features (Table 3). Those with LoF variants were more likely to display facial dysmorphism (7/10 missense; 28/28 LoF variants; $p = .014$) particularly a wide mouth (1/10 missense; 16/28 LoF; $p = .012$). However, we are cautious in interpretation of these data, with results potentially skewed due to the relatively small sample size and inconsistent reporting across clinicians. Notably, *QRICH1* is not only predicted to be intolerant to missense and LoF variants (gnomAD), but very recently also predicted to be triplosensitive (Collins et al., 2021).

6 | CONCLUSION

Heterozygous variants in *QRICH1* cause a mild neurodevelopmental disorder with variable ID, developmental delay, hypotonia, short stature, poor weight gain and subtle facial dysmorphism. Even though pathogenic *QRICH1* variants are likely cause a neurodevelopmental phenotype, it should be considered that this may be an ascertainment bias as all the individuals were referred to genetic testing mainly because of this phenotype. Associated features requiring further clarification regarding clinical significance include elevated CK level, structural cardiac defects and nonspecific structural neurological and skeletal abnormalities including chondrodysplasia. Scoliosis may be an important clinical feature and individuals may benefit from surveillance. The variant spectrum includes LoF ($n = 28$) and missense variants ($n = 10$), which are de novo in most individuals. Transmitting parents presented with mild symptoms, suggest clinical variability, which is important for reproductive counselling. Strong genotype-phenotype relationships were not found but may emerge as more individuals with *QRICH1* variants are identified. This manuscript, including 27 previously unreported individuals, recruited via international collaborative efforts, enhances our understanding of this recently identified condition. We respectfully suggest that "*QRICH1* associated neurodevelopmental disorder" may be an appropriate term to apply to individuals with this condition.

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CONFLICT OF INTERESTS

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DATA AVAILABILITY STATEMENT

The variant and phenotype data compiled are included in the tables or supplementary material and variant data is submitted to LOVD-database (https://databases.lovd.nl/shared/genes/QRICH1). All data which are not already included in the Supporting Informatio Material are available upon request.

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SUPPORTING INFORMATION

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