



HAL
open science

The clinical and molecular spectrum of QRICH1 associated neurodevelopmental disorder

Smitha Kumble, Amanda Levy, Jaya Punetha, Hua Gao, Nicholas Ah Mew, Kwame Anyane-Yeboah, Paul Benke, Sara Berger, Lise Bjerglund, Belinda Campos-Xavier, et al.

► **To cite this version:**

Smitha Kumble, Amanda Levy, Jaya Punetha, Hua Gao, Nicholas Ah Mew, et al.. The clinical and molecular spectrum of QRICH1 associated neurodevelopmental disorder. *Human Mutation*, 2022, 43 (2), pp.266-282. 10.1002/humu.24308 . hal-03519376

HAL Id: hal-03519376

<https://hal-univ-rennes1.archives-ouvertes.fr/hal-03519376>

Submitted on 26 Jan 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial | 4.0 International License

Anne-Sophie Denommé-Pichon ORCID iD: 0000-0002-8986-8222

Alice Fiévet ORCID iD: 0000-0003-2096-8708

Kosuke Izumi ORCID iD: 0000-0002-7922-7480

Jennifer Posey ORCID iD: 0000-0003-4814-6765

Jill Rosenfeld ORCID iD: 0000-0001-5664-7987

Zeynep Tümer ORCID iD: 0000-0002-4777-5802

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

Smitha Kumble¹, Amanda M. Levy², Jaya Punetha^{3,4}, Hua Gao⁵, Nicholas Ah Mew⁶, Kwame Anyane-Yeboah⁷, Paul J. Benke⁸, Sara M. Berger⁷, Lise Bjerglund⁹, Belinda Campos-Xavier^{10,11}, Michael Ciliberto¹², Julie S. Cohen^{13,14}, Anne M. Comi^{13,15}, Cynthia Curry¹⁶, Lena Damaj¹⁷, Anne-Sophie Denommé-Pichon^{18,19}, Lisa Emrick^{20,3}, Laurence Faivre^{21,22}, Mary Beth Fasano²³, Alice Fiévet^{24,25}, Richard S. Finkel^{26,27}, Sixto García-Miñaur^{28,29}, Amanda Gerard^{3,30}, Paulino Gomez-Puertas³¹, Maria J. Guillen Sacoto³², Trevor L. Hoffman³³, Lillian Howard¹², Alejandro D. Iglesias³⁴, Kosuke Izumi³⁵, Austin Larson³⁶, Anja Leiber³⁷, Reymundo Lozano⁴, Iñigo Marcos-Alcalde^{31,38}, Cassie S. Mintz⁴, Sureni V. Mullegama³², Rikke S. Møller^{39,40}, Sylvie Odent⁴¹, Henry Oppermann⁴², Elsebet Ostergaard^{43,44}, Marta Pacio-Míguez²⁸, Maria Palomares-Bralo^{28,29}, Sumit Parikh⁴⁵, Anna M. Paulson¹², Konrad Platzer⁴², Jennifer E. Posey³, Lorraine Potocki^{3,30}, Anya Revah-Politi^{46,47}, Marlene Rio⁴⁸, Alyssa L. Ritter³⁵, Scott Robinson⁷, Jill A. Rosenfeld^{3,49}, Fernando Santos-Simarro^{28,29}, Sérgio B. Sousa^{10,50}, Undiagnosed

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/humu.24308.

Diseases Network, Mathys Wéber²¹, Yili Xie³², Wendy K. Chung⁷, Natasha J.

Brown^{1,51,#}, Zeynep Tümer^{2,44,#,\$}

1. Victorian Clinical Genetics Services, Murdoch Children's Research Institute,
Melbourne, Australia

2. Kennedy Center, Department of Clinical Genetics, Copenhagen University Hospital,
Rigshospitalet, Copenhagen, Denmark

3. Department of Molecular & Human Genetics, Baylor College of Medicine, Houston,
TX, USA

4. Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount
Sinai, New York, NY 10029, USA

5. Department of Review Analysis, GeneDx LLC, Maryland, USA

6. Rare Disease Institute, Children's National Hospital, Washington, DC 20010 USA

7. Department of Pediatrics, Columbia University Irving Medical Center, New York,
New York, USA

8. Division of Genetics, Joe DiMaggio Children's Hospital, Hollywood, FL 33021 USA

9. Department of Pediatrics, University Hospital Hvidovre, Denmark

10. Medical Genetics Unit, Hospital Pediátrico, Centro Hospitalar e Universitário de
Coimbra

11. Division of Genetic Medicine, Lausanne University Hospital and University of Lausanne (CHUV), Lausanne, Switzerland
12. Stead Family Department of Pediatrics, University of Iowa, IA, USA
13. Department of Neurology and Developmental Medicine, Kennedy Krieger Institute, Baltimore, Maryland, USA
14. Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA
15. Departments of Neurology and Pediatrics, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA
16. Genetic Medicine, Dept of Pediatrics, UCSF/Fresno, Fresno, CA, USA
17. Service de pédiatrie et de génétique clinique, CHU Rennes, France
18. INSERM UMR 1231 Equipe GAD, Université de Bourgogne, Dijon, France
19. Unité Fonctionnelle Innovation en Diagnostic génomique des maladies rares, FHU-TRANSLAD, CHU Dijon Bourgogne, Dijon, France
20. Division of Neurology and Developmental Neuroscience, Department of Pediatrics, Baylor College of Medicine, Houston, TX, USA
21. Centre de Référence Anomalies du Développement et Syndromes Malformatifs, FHU TRANSLAD, Hôpital d'Enfants, CHU Dijon, Dijon, France

22. Inserm UMR1231 GAD, Génétique des Anomalies du Développement, Université de Bourgogne, Dijon, France
23. Internal Medicine & Pediatrics, University of Iowa Carver College of Medicine, IA, USA
24. Laboratoire de biologie médicale multisites Sequoia – FMG2025, Paris, France
25. Service Génétique des Tumeurs, Gustave Roussy, Villejuif, France
26. Nemours Children's Hospital, Orlando, FL, USA
27. Center for Experimental Neurotherapeutics, St. Jude Children's Research Hospital, Memphis, TN, USA
28. Institute of Medical and Molecular Genetics (INGEMM), La Paz University Hospital, Idipaz, Madrid, Spain
29. Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER, U753), Instituto Carlos III, Madrid, Spain
30. Texas Children's Hospital, Houston, TX, USA
31. Molecular Modelling Group, Severo Ochoa Molecular Biology Centre (CBMSO, CSIC-UAM), Madrid, Spain
32. Clinical Genomics Program, GeneDx, Maryland, USA
33. Regional Department of Genetics, Southern California Kaiser Permanente Medical Group, Pasadena, CA, USA

34. Division of Clinical Genetics, Department of Pediatrics, Columbia University Irving Medical Center, New York, NY, USA
35. Division of Human Genetics, Department of Pediatrics, Children's Hospital of Philadelphia, Philadelphia, USA
36. Section of Genetics, Department of Pediatrics, University of Colorado School of Medicine, CO, USA
37. Department of neuropaediatrics, Childrens Hospital of Eastern Switzerland, St. Gallen, Switzerland
38. Biosciences Research Institute, School of Experimental Sciences, Universidad Francisco de Vitoria, Pozuelo de Alarcón, Madrid, Spain
39. Department of Epilepsy Genetics and Personalized Treatment, The Danish Epilepsy Centre, Dianalund, Denmark
40. Department of Regional Health Research, University of Southern Denmark, Odense, Denmark
41. CHU Rennes, Hôpital Sud, Service de Génétique Clinique, Univ Rennes, CNRS IGDR UMR 6290, Centre de référence Anomalies du développement CLAD-Ouest, ERN ITHACA, 35203 Rennes, France
42. Institute of Human Genetics, University of Leipzig Medical Center, Leipzig, Germany

43. Department of Clinical Genetics, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark

44. Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

45. Mitochondrial Medicine & Neurogenetics, Cleveland Clinic, Cleveland, USA

46. Institute for Genomic Medicine, Columbia University Medical Center, New York, New York, USA

47. Precision Genomics Laboratory, Columbia University Irving Medical Center, New York, New York, USA

48. Service de Génétique, Hôpital Necker-Enfants Malades, Assistance Publique-Hôpitaux de Paris (APHP), 75015 Paris, France

49. Baylor Genetics Laboratories, Houston, TX, USA

50. University Clinic of Genetics, Faculty of Medicine, University of Coimbra, Coimbra, Portugal

51. University of Melbourne Department of Paediatrics, Royal Children's Hospital, Melbourne, Australia

Shared senior authorship

§ Correspondence to

Zeynep Tümer

Kennedy Center, Department of Clinical Genetics

Copenhagen University Hospital, Rigshospitalet

Gl. Landevej 7, 2600 Glostrup, Denmark

Tel. +45 20294855

E-mail: Zeynep.tumer@regionh.dk

Funding information

National Human Genome Research Institute (NHGRI) and National Heart, Lung, and Blood Institute (NHLBI)'s grant, grant number: UM1 HG006542; Rashid Family Fund; Centro Portugal Regional Operational Programme (CENTRO 2020) through the European Regional Development Fund (ERDF); Spanish Ministry of Science/State Research Agency projects: RTC-2017-6494-1, RTI2018-094434-B-I00, and DTS20-00024; European Commission JPIAMR projects CONNECT and AEPIC; Raregenomics network through the Consejería de Educación de la C. de Madrid, grant number: S2017/BMD-3721; European Social Fund to María Palomares-Bralo; ISCIII, Ministerio de Ciencia e Innovación, grant number: PI19/01681; National Human Genome Research Institute (NHGRI), grant number: K08 HG008986; National Center for Advancing Translational Sciences, National Institutes of Health, grant number: UL1TR001873; Undiagnosed Diseases Network; NIH Common Fund through the Office of Strategic

Coordination/Office of the NIH Director, grant number: U01HG007709; SFARI and JPB Foundation.

Abstract

De novo variants in *QRICH1* (Glutamine-rich protein 1) has recently been reported in 11 individuals with intellectual disability. The function of *QRICH1* is largely unknown but it is likely to play a key role in the unfolded response of endoplasmic reticulum (ER) 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/intellectual disability (71%), non-specific facial dysmorphism (92%) and hypotonia (39%). Additional findings included poor weight gain (29%), short 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.

Key words

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

Introduction

Recent advances in sequencing technologies have enabled identification of multiple genes associated with rare intellectual disability 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, (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, 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.

Materials and methods

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.

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 Supp. Table 1). 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).

Methods

QRICH1 variants were identified in the probands using massively parallel sequencing (next generation sequencing, NGS) 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 ACMG/AMP (American College of Medical Genetics and Genomics / Association for Molecular Pathology) 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 HGVS (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).

Results

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 Supp. Table 1. 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 Supp. Figure 1. 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 intra-uterine 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 $+1.27$ 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 intellectual disability 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 intellectual disability (ID) through either formal IQ (intelligence quotient) testing (6/27) or clinical assessment (16/27). For ten 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 ten of these 11 individuals suggesting subtle cognitive problems. The two individuals with severe intellectual disability (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 non-verbal. 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 one 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 non-specific 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 abnormalities. The implementation and response to anti-epileptic 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 non-specific 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 creatine phosphokinase (CK) was present in two previously reported individuals (Individuals 3 and 32) (Ververi et al., 2018). Of the currently investigated patients, ten 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 -2SD. 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) had delayed bone age, which had been previously noted in Individual 22⁵. No other specific radiological abnormalities were identified.

Eleven individuals had weights at or below -2SD. Six of these individuals had corresponding short stature. Aside from Individual 11, body mass index (BMI) 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 Supp. Figure 2). 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 intellectual disability (not genetically verified). A paternal history of ADHD and learning disability as well as a family history of intellectual disability was reported for Individual 12. The father of Individual 25 had writing difficulties (variant verified).

Spectrum of QRICH1 variants

Among the 38 individuals, 35 different variants were identified (Table 1, Table 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 nonsense-mediated decay (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 VUS (variant of unknown significance). However, targeted RNA sequencing revealed skipping of exon 2 both in the proband and the father (Supplementary figure 3), 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 caspase activation recruitment domain (CARD).

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 non-specific, 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 intellectual disability 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. 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 intellectual disability and ASD. Our data support ASD as a common feature of this disorder, as all but one individual with ASD had comorbid intellectual disability. 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 intellectual disability 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 thirty-three percent 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.* 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 (Lui et al., 2019). 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 in order 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 creatine phosphokinase (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 to 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 in order 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; $p=0.014$) particularly a wide mouth (1/10 missense; 16/28 LoF; $p=0.012$). 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, *QRICHI* 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).

Conclusion

Heterozygous variants in *QRICHI* cause a mild neurodevelopmental disorder with variable intellectual disability, developmental delay, hypotonia, short stature, poor weight gain and subtle facial dysmorphism. Even though pathogenic *QRICHI* 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 non-specific 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 *QRICHI* 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 “*QRICHI* associated neurodevelopmental disorder” may be an appropriate term to apply to individuals with this condition.

Acknowledgements

We thank Frederic Tran-Mau-Them (Inserm UMR1231 GAD, Génétique des Anomalies du Développement, Université de Bourgogne, Dijon, France) for the RNA splicing experiments performed for individual 5. Jette Bune Rasmussen (Department of Clinical Genetics, Copenhagen University Hospital - Rigshospitalet) is acknowledged for her graphical support. Kathleen Pope (MD, Division of Genetics, Nemours Children's Hospital, USA) is acknowledged for the clinical information of individual 6 and Eléonore Viora-Dupont (Medical student, Centre de Génétique Clinique, CHU de Dijon) for the further clinical information of individuals 5 and 11. Part of this work has been generated within the European Reference Network on Rare Congenital Malformations and Rare Intellectual Disability (ERNITHACA) [EU Framework Partnership Agreement ID: 3HP-HP-FPA ERN-01-2016/739516]. This study was supported by the National Human Genome Research Institute (NHGRI) and National Heart, Lung, and Blood Institute

(NHLBI)'s grant (UM1 HG006542), the Rashid Family Fund, the Centro Portugal Regional Operational Programme (CENTRO 2020) through the European Regional Development Fund (ERDF), the Spanish Ministry of Science/State Research Agency projects (RTC-2017-6494-1, RTI2018-094434-B-I00, and DTS20-00024), the European Commission JPIAMR projects CONNECT and AEPIC, the Raregenomics network through the Consejería de Educación de la C. de Madrid (S2017/BMD-3721), the European Social Fund to María Palomares-Bralo, ISCIII, Ministerio de Ciencia e Innovación (PI19/01681), the National Human Genome Research Institute (NHGRI) (K08 HG008986), the National Center for Advancing Translational Sciences, National Institutes of Health (UL1TR001873), the Undiagnosed Diseases Network, the NIH Common Fund through the Office of Strategic Coordination/Office of the NIH Director (U01HG007709), and the SFARI and JPB Foundation.

Members of the Undiagnosed Diseases Network

Maria T. Acosta, Margaret Adam, David R. Adams, Pankaj B. Agrawal, Mercedes E. Alejandro, Justin Alvey, Laura Amendola, Ashley Andrews, Euan A. Ashley, Mahshid S. Azamian, Carlos A. Bacino, Guney Bademci, Eva Baker, Ashok Balasubramanyam, Dustin Baldrige, Jim Bale, Michael Bamshad, Deborah Barbouth, Pinar Bayrak-Toydemir, Anita Beck, Alan H. Beggs, Edward Behrens, Gill Bejerano, Jimmy Bennet, Beverly Berg-Rood, Jonathan A. Bernstein, Gerard T. Berry, Anna Bican, Stephanie Bivona, Elizabeth Blue, John Bohnsack, Carsten Bonnenmann, Devon Bonner, Lorenzo Botto, Brenna Boyd, Lauren C. Briere, Elly Brokamp, Gabrielle Brown, Elizabeth A. Burke, Lindsay C. Burrage, Manish J. Butte, Peter Byers, William E. Byrd, John Carey, Olveen Carrasquillo, Ta Chen Peter Chang, Sirisak Chanprasert, Hsiao-Tuan Chao, Gary

D. Clark, Terra R. Coakley, Laurel A. Cobban, Joy D. Cogan, Matthew Coggins, F. Sessions Cole, Heather A. Colley, Cynthia M. Cooper, Heidi Cope, William J. Craigen, Andrew B. Crouse, Michael Cunningham, Precilla D'Souza, Hongzheng Dai, Surendra Dasari, Joie Davis, Jyoti G. Dayal, Matthew Deardorff, Esteban C. Dell'Angelica, Shweta U. Dhar, Katrina Dipple, Daniel Doherty, Naghmeh Dorrani, Argenia L. Doss, Emilie D. Douine, David D. Draper, Laura Duncan, Dawn Earl, David J. Eckstein, Lisa T. Emrick, Christine M. Eng, Cecilia Esteves, Marni Falk, Liliana Fernandez, Carlos Ferreira, Elizabeth L. Fieg, Laurie C. Findley, Paul G. Fisher, Brent L. Fogel, Irman Forghani, Laure Fresard, William A. Gahl, Ian Glass, Bernadette Gochuico, Rena A. Godfrey, Katie Golden-Grant, Alica M. Goldman, Madison P. Goldrich, David B. Goldstein, Alana Grajewski, Catherine A. Groden, Irma Gutierrez, Sihoun Hahn, Rizwan Hamid, Neil A. Hanchard, Kelly Hassey, Nichole Hayes, Frances High, Anne Hing, Fuki M. Hisama, Ingrid A. Holm, Jason Hom, Martha Horike-Pyne, Alden Huang, Yong Huang, Laryssa Huryn, Rosario Isasi, Fariha Jamal, Gail P. Jarvik, Jeffrey Jarvik, Suman Jayadev, Lefkothea Karaviti, Jennifer Kennedy, Dana Kiley, Shilpa N. Kobren, Isaac S. Kohane, Jennefer N. Kohler, Deborah Krakow, Donna M. Krasnewich, Elijah Kravets, Susan Korrick, Mary Koziura, Joel B. Krier, Seema R. Lalani, Byron Lam, Christina Lam, Grace L. LaMoure, Brendan C. Lanpher, Ian R. Lanza, Lea Latham, Kimberly LeBlanc, Brendan H. Lee, Hane Lee, Roy Levitt, Richard A. Lewis, Sharyn A. Lincoln, Pengfei Liu, Xue Zhong Liu, Nicola Longo, Sandra K. Loo, Joseph Loscalzo, Richard L. Maas, John MacDowall, Ellen F. Macnamara, Calum A. MacRae, Valerie V. Maduro, Marta M. Majcherska, Bryan C. Mak, May Christine V. Malicdan, Laura A. Mamounas, Teri A. Manolio, Rong Mao, Kenneth Maravilla, Thomas C. Markello, Ronit Marom, Gabor

Marth, Beth A. Martin, Martin G. Martin, Julian A. Martínez-Agosto, Shruti Marwaha, Jacob McCauley, Allyn McConkie-Rosell, Colleen E. McCormack, Alexa T. McCray, Elisabeth McGee, Heather Mefford, J. Lawrence Merritt, Matthew Might, Ghayda Mirzaa, Eva Morava, Paolo M. Moretti, Deborah Mosbrook-Davis, John J. Mulvihill, David R. Murdock, Anna Nagy, Mariko Nakano-Okuno, Avi Nath, Stan F. Nelson, John H. Newman, Sarah K. Nicholas, Deborah Nickerson, Shirley Nieves-Rodriguez, Donna Novacic, Devin Oglesbee, James P. Orengo, Laura Pace, Stephen Pak, J. Carl Pallais, Christina GS. Palmer, Jeanette C. Papp, Neil H. Parker, John A. Phillips III, Jennifer E. Posey, Lorraine Potocki, Bradley Power, Barbara N. Pusey, Aaron Quinlan, Wendy Raskind, Archana N. Raja, Deepak A. Rao, Genecee Renteria, Chloe M. Reuter, Lynette Rives, Amy K. Robertson, Lance H. Rodan, Jill A. Rosenfeld, Natalie Rosenwasser, Francis Rossignol, Maura Ruzhnikov, Ralph Sacco, Jacinda B. Sampson, Susan L. Samson, Mario Saporta, C. Ron Scott, Judy Schaechter, Timothy Schedl, Kelly Schoch, Daryl A. Scott, Vandana Shashi, Jimann Shin, Rebecca Signer, Edwin K. Silverman, Janet S. Sinsheimer, Kathy Sisco, Edward C. Smith, Kevin S. Smith, Emily Solem, Lilianna Solnica-Krezel, Ben Solomon, Rebecca C. Spillmann, Joan M. Stoler, Jennifer A. Sullivan, Kathleen Sullivan, Angela Sun, Shirley Sutton, David A. Sweetser, Virginia Sybert, Holly K. Tabor, Amelia L. M. Tan, Queenie K.-G. Tan, Mustafa Tekin, Fred Telischi, Willa Thorson, Audrey Thurm, Cynthia J. Tiff, Camilo Toro, Alyssa A. Tran, Brianna M. Tucker, Tiina K. Urv, Adeline Vanderver, Matt Velinder, Dave Viskochil, Tiphonie P. Vogel, Colleen E. Wahl, Stephanie Wallace, Nicole M. Walley, Chris A. Walsh, Melissa Walker, Jennifer Wambach, Jijun Wan, Lee-kai Wang, Michael F. Wangler, Patricia A. Ward, Daniel Wegner, Mark Wener, Tara Wenger, Katherine

Wesseling Perry, Monte Westerfield, Matthew T. Wheeler, Jordan Whitlock, Lynne A. Wolfe, Jeremy D. Woods, Shinya Yamamoto, John Yang, Muhammad Yousef, Diane B. Zastrow, Wadih Zein, Chunli Zhao, and Stephan Zuchner.

Conflicts of interest

The Department of Molecular and Human Genetics at Baylor College of Medicine receives revenue from clinical genetic testing completed at Baylor Genetics Laboratories. HG, MJGS, SM, and YX are employees of GeneDx, Inc. The other authors otherwise do not have any conflict of interest.

Availability of data and material

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 supplementary material are available upon request.

WEB Sources

Data Bank database: <https://www.rcsb.org/>

Decipher database: <https://www.deciphergenomics.org/>

ERT-ITHACA network: <https://ern-ithaca.eu/>

GeneMatcher: <https://genematcher.org/statistics>

gnomAD: <https://gnomad.broadinstitute.org/>

This article is protected by copyright. All rights reserved.

GraphPad: <https://www.graphpad.com>

GTEx portal: <https://www.gtexportal.org/home/>

HGVS: <https://varnomen.hgvs.org/>

LOVD, Leiden Open Variation Database: <https://databases.lovd.nl/shared/genes/QRICH1>

Mutalyzer: <https://mutalyzer.nl/>

NCBI: <https://www.ncbi.nlm.nih.gov/gene/54870>

NMDEscPredictor tool: <https://nmdprediction.shinyapps.io/nmdescpredictor/>

Phyre2 server: <http://www.sbg.bio.ic.ac.uk/phyre2>

RCSB Protein data bank: <https://www.rcsb.org/>

SpliceAI: <https://github.com/Illumina/SpliceAI>

SwissModel server: <https://swissmodel.expasy.org/>

Author contributions

Conceptualization: SK, NJB, ZT

Data curation – genetic analyses: JP, HG, LB, BCX, ADP, AF, RSF, MJGS, TLH, SVM,

RSM, HO, MPM, KP, JEP, ARP, YX, NJB, ZT

Clinical investigation: SK, NAM, KAY, SMB, LB, MC, JSC, AMC, CC, LD, LE, LF, MBF, RSF, SGM, AG, TLH, LH, ADI, KI, AL, AL, RL, CSM, SVM, RSM, EO, SP, LP, ARP, MR, ALR, SR, JAR, SBS, UDN, MW, WKC, NJB

Project administration: AML, NJB, ZT

Visualization: AML, ZT

Writing – original draft: SK, NJB, ZT, AML

Writing – review and editing: All the authors

References

Baruch, Y., Horn-Saban, S., Plotsky, Y., Bercovich, D., & Gershoni-Baruch, R. (2021).

A case of Ververi-Brady syndrome due to QRI1 loss of function and the literature review. *American Journal of Medical Genetics, Part A*, 1–5.

<https://doi.org/10.1002/ajmg.a.62184>

Bouchier-Hayes, L., & Martin, S. J. (2002). CARD games in apoptosis and immunity.

EMBO Reports, 3, 616–621. <https://doi.org/10.1093/embo-reports/kvf139>

Collins, R. L., Glessner, J. T., Porcu, E., Niestroj, L., Ulirsch, J., Kellaris, G., ...

Talkowski, M. E. (2021). A cross-disorder dosage sensitivity map of the human genome. *MedRxiv*. <https://doi.org/https://doi.org/10.1101/2021.01.26.21250098>

Cope, H., Spillmann, R., Rosenfeld, J. A., Brokamp, E., Signer, R., Schoch, K., ...

Shashi, V. (2020). Missed diagnoses: Clinically relevant lessons learned through

medical mysteries solved by the Undiagnosed Diseases Network. *Molecular Genetics and Genomic Medicine*, 8, e1397. <https://doi.org/10.1002/mgg3.1397>

De Rubeis, S., He, X., Goldberg, A. P., Poultney, C. S., Samocha, K., Cicek, A. E., ... Buxbaum, J. D. (2014). Synaptic, transcriptional and chromatin genes disrupted in autism. *Nature*, 515, 209–215. <https://doi.org/10.1038/nature13772>

Deciphering Developmental Disorders, S. (2017). Prevalence and architecture of de novo mutations in developmental disorders. *Nature*, 542, 433–438. <https://doi.org/10.1038/nature21062>

Feliciano, P., Zhou, X., Astrovskaya, I., Turner, T. N., Wang, T., Brueggeman, L., ... Chung, W. K. (2019). Exome sequencing of 457 autism families recruited online provides evidence for autism risk genes. *Npj Genomic Medicine*, 4, 19. <https://doi.org/10.1038/s41525-019-0093-8>

Firth, H. V., Richards, S. M., Bevan, A. P., Clayton, S., Corpas, M., Rajan, D., ... Carter, N. P. (2009). DECIPHER: Database of Chromosomal Imbalance and Phenotype in Humans Using Ensembl Resources. *American Journal of Human Genetics*, 84, 524–533. <https://doi.org/10.1016/j.ajhg.2009.03.010>

Föhrenbach, M., Jamra, R. A., Borkhardt, A., Brozou, T., Muschke, P., Popp, B., ... Redler, S. (2021). QRICH1 variants in Ververi-Brady syndrome—delineation of the genotypic and phenotypic spectrum. *Clinical Genetics*, 99, 199–207. <https://doi.org/10.1111/cge.13853>

Kelley, L. A., Mezulis, S., Yates, C. M., Wass, M. N., & Sternberg, M. J. E. (2015). The

Phyre2 web portal for protein modeling, prediction and analysis. *Nature Protocols*, 10, 845–858. <https://doi.org/10.1038/nprot.2015.053>

Kojima, K. K., & Jurka, J. (2011). Crypton transposons: Identification of new diverse families and ancient domestication events. *Mobile DNA*, 2, 12. <https://doi.org/10.1186/1759-8753-2-12>

Lui, J. C., Jee, Y. H., Lee, A., Yue, S., Wagner, J., Donnelly, D. E., ... Baron, J. (2019). QRICH1 mutations cause a chondrodysplasia with developmental delay. *Clinical Genetics*, 95, 160–164. <https://doi.org/10.1111/cge.13457>

Marcos-Alcalde, Í., Mendieta-Moreno, J. I., Puisac, B., Gil-Rodríguez, M. C., Hernández-Marcos, M., Soler-Polo, Di., ... Gómez-Puertas, P. (2017). Two-step ATP-driven opening of cohesin head. *Scientific Reports*, 7(1). <https://doi.org/10.1038/s41598-017-03118-9>

Martínez, G., Khatiwada, S., Costa-Mattioli, M., & Hetz, C. (2018). ER Proteostasis Control of Neuronal Physiology and Synaptic Function. *Trends in Neurosciences*, 41, 610–624. <https://doi.org/10.1016/j.tins.2018.05.009>

Mefford, H. C., Batshaw, M. L., & Hoffman, E. P. (2012). Genomics, Intellectual Disability, and Autism. *New England Journal of Medicine*, 366, 733–743. <https://doi.org/10.1056/nejmra1114194>

Mignogna, M. L., Giannandrea, M., Gurgone, A., Fanelli, F., Raimondi, F., Mapelli, L., ... D'Adamo, P. (2015). The intellectual disability protein RAB39B selectively regulates GluA2 trafficking to determine synaptic AMPAR composition. *Nature*

Communications, 6, 6504. <https://doi.org/10.1038/ncomms7504>

Perriches, T., & Singleton, M. R. (2012). Structure of yeast kinetochore Ndc10 DNA-binding domain reveals unexpected evolutionary relationship to tyrosine recombinases. *Journal of Biological Chemistry*, 287, 5173–5179. <https://doi.org/10.1074/jbc.C111.318501>

Richards, S., Aziz, N., Bale, S., Bick, D., Das, S., Gastier-Foster, J., ... Rehm, H. L. (2015). Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genetics in Medicine*, 17, 405–424. <https://doi.org/10.1038/gim.2015.30>

San Agustin, J. T., Klena, N., Granath, K., Panigrahy, A., Stewart, E., Devine, W., ... Pazour, G. J. (2016). Genetic link between renal birth defects and congenital heart disease. *Nature Communications*, 7, 11103. <https://doi.org/10.1038/ncomms11103>

Sobreira, N., Schiettecatte, F., Valle, D., & Hamosh, A. (2015). GeneMatcher: A Matching Tool for Connecting Investigators with an Interest in the Same Gene. *Human Mutation*, 36, 928–930. <https://doi.org/10.1002/humu.22844>

Ververi, A., Splitt, M., Dean, J. C. S., & Brady, A. F. (2018). Phenotypic spectrum associated with de novo mutations in QRICH1 gene. *Clinical Genetics*, 93, 286–292. <https://doi.org/10.1111/cge.13096>

Wang, S., Mandell, J. D., Kumar, Y., Sun, N., Morris, M. T., Arbelaez, J., ... Sul, J. H. (2018). De Novo Sequence and Copy Number Variants Are Strongly Associated with

Tourette Disorder and Implicate Cell Polarity in Pathogenesis. *Cell Reports*.
<https://doi.org/10.1016/j.celrep.2018.08.082>

You, K., Wang, L., Chou, C. H., Liu, K., Nakata, T., Jaiswal, A., ... Xavier, R. J. (2021).
QRICH1 dictates the outcome of ER stress through transcriptional control of proteostasis.
Science, 371, eabb6896. <https://doi.org/10.1126/science.abb6896>

Tables

Table 1- Phenotypic features

Table 2- Variants and predicted effects

Table 3- Frequency of clinical features

Figures

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.

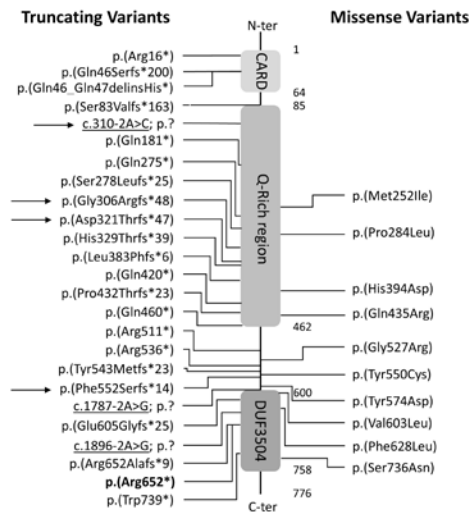


Figure 2– Facial photographs

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

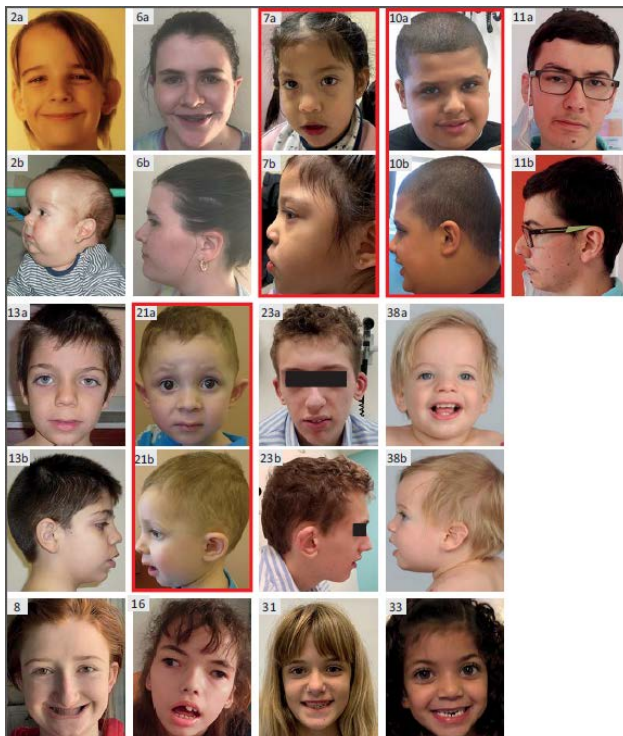


Table 1 – Phenotypic features in individuals with *QRICH1* variants.

Individual number (reference)	Age & Gender at Evaluation	Variant Type & 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	-	+	-	+	+, †	+	-	-	+	-	-	-	-	-	-
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	+	+	-	-	-	-	-
16	F, 11y	Nonsense; de novo	Severe	-	+	+	+	+, †	+	-	+, 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	-	+	+	+	†	-	-	-	+	-	-	-	-	-	
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	-	+	-	+	-	-	+	-	+	-	-	-	+	+	
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

ID: Intellectual disability; GDD: global developmental delay; Mod: moderate; U: Unspecified; †: growth hormone responsive; ‡: growth hormone unresponsive; y: years; m: months; M: Male; F: Female; Pt: Patient; GORD: gastro-oesophageal reflux disease; CK: creatinine phosphokinase

TABLE 2 – *QRICH1* variants and their predicted effects

gDNA chr3	cDNA	Ex on / Intron	Predicted effect on <i>QRICH1</i>	QRICH1 domain	Predicted coding effect	CA DD Score	Inheritance	AC MG / AMP	ID of the affected individual	Publication
g.49114405G>A	c.46C>T	3	p.(Arg16*)	CARD	Nonse nse	37	<i>dn</i>	P	1	Present study
g.49114316del	c.136del	3	p.(Gln46Serfs*200)	CARD	Frame shift	30	<i>dn</i>	P	2	Present study
g.49114312_49114313delinsAA	c.138_139delinsTT	3	p.(Gln46_Gln47delinsHis*)	CARD	Nonse nse	34	<i>dn</i>	P	3	Ververi 2017
g.49114205del	c.246del	3	p.(Ser83Valfs*163)		Frame shift	29.9	<i>dn</i>	P	4	Baruch 2021
g.49095325T>G	c.310-2A>C	IVS3	p.?		Splice -site	24	pat/fam	p ^a	5	Present study
g.49095092G>A	c.541C>T	4	p.(Gln181*)	Gln-Rich	Nonse nse	36	<i>dn</i>	P	6	Present study
g.49094877C>A	c.756G>T	4	p.(Met252Ile)	Gln-Rich	Misse nse	23	<i>dn</i>	LP	7	Present study
g.49094810G>A	c.823C>T	4	p.(Gln275*)	Gln-Rich	Nonse nse	36	<i>dn</i>	P	8	Present study
g.49094800_49094801del	c.832_833del	4	p.(Ser278Leufs*25)	Gln-Rich	Frame shift	33	<i>dn</i>	P	9	Föhrenbach 2020
g.49094782G>A	c.851C>T	4	p.(Pro284Leu)	Gln-	Misse	28	<i>dn</i>	LP	10	Presen

				Rich	nse						t study
g.49094719dup	c.914dup	4	p.(Gly306Argfs*48)	Gln-Rich	Frame shift	33	mat/fam	LP	11		Present study
g.49094677del	c.961del	4	p.(Asp321Thrfs*47)	Gln-Rich	Frame shift	33	pat/fam	LP	12		Present study
g.49094650del	c.985del	4	p.(His329Thrfs*39)	Gln-Rich	Frame shift	32	dn	P	13		Present study
g.49094487_49094490del	c.1147_1150del	4	p.(Leu383Phefs*6)	Gln-Rich	Frame shift	33	dn	P	14		Present study
g.49094453G>C	c.1180C>G	4	p.(His394Asp)	Gln-Rich	Misense	24	dn	LP	15		Present study
g.49094375G>A	c.1258C>T	4	p.(Gln420*)	Gln-Rich	Nonse nse	38	dn	P	16		Present study
g.49094341dup	c.1292dup	4	p.(Pro432Thrfs*23)	Gln-Rich	Frame shift	32	dn	P	17		Present study
g.49094329T>C	c.1304A>G	4	p.(Gln435Arg)	Gln-Rich	Misense	9	dn	VUS	18		Present study
g.49084640G>A	c.1378C>T	5	p.(Gln460*)	Gln-Rich	Nonse nse	39	dn	P	19		Present study
g.49083998G>A	c.1531C>T	6	p.(Arg511*)		Nonse nse	44	dn	P	20		Lui 2018
g.49083950C>T	c.1579G>A	6	p.(Gly527Arg)		Misense	34	dn	LP	21		Present study
g.49083923G>A	c.1606C>T	6	p.(Arg536*)		Nonse nse	42	dn	P	22		Lui 2018

g.49083905del	c.1626del	6	p.(Tyr543Metfs*23)		Frame shift	25	<i>dn</i>	P	23	Present study	
g.49083880T>C	c.1649A>G	6	p.(Tyr550Cys)		Misense	29	<i>dn</i>	LP	24	Present study	
g.49083877del	c.1655del	6	p.(Phe552Serfs*14)		Frame shift	33	<i>pat</i>	LP	25	Present study	
g.49081889A>C	c.1720T>G	7	p.(Tyr574Asp)		Misense	29	<i>dn</i>	LP	26	Present study	
g.49070663T>C	c.1787-2A>G	IVS7	p.?		Splice-site	25	<i>dn</i>	P	27	Present study	
g.49070641C>A	c.1807G>T	8	p.(Val603Leu)	DUF3504	Misense	34	<i>dn</i>	LP	28	Present study	
g.49070635_49070636del	c.1812_1813del	8	p.(Glu605Glyfs*25)	DUF3504	Frame shift	34	<i>dn</i>	P	29	Föhrenbach 2020	
g.49070564G>C	c.1884C>G	8	p.(Phe628Leu)	DUF3504	Misense	29	<i>dn</i>	LP	30	Present study	
g.49070208T>C	c.1896-2A>G	IVS8	p.?		Splice-site	25	<i>dn</i>	P	31	Present study	
g.49070149dup	c.1953dup	9	p.(Arg652Alafs*9)	DUF3504	Frame shift	33	<i>dn</i>	P	32	Ververi 2017	
g.49070148G>A	c.1954C>T	9	p.(Arg652*)	DUF3504	Nonsense	40		<i>uk^b</i>	P	33	Present study
								<i>dn</i>	P	34	Present study

							<i>dn</i>	P	35	Ververi 2017
							<i>dn</i>	P	36	Föhrenbach 2020
g.49068009C>T	c.2207G>A	11	p.(Ser736Asn)	DUF3504	Missense	28	<i>dn</i>	LP	37	Föhrenbach 2020
g.49068000C>T	c.2216G>A	11	p.(Trp739*)	DUF3504	Nononsense	39	<i>dn</i>	P	38	Present study

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

Table 3 – Common clinical features noted in individuals with *QRICH1* variants.

	Missense (n=10)		LOF (n=28)		Total (n=38)		<i>P-Values*</i>
	#	%	#	%	#	%	
	Intellectual disability/GDD	5	50%	22	79%	27	
Mild	2	20%	13	46%	15	39%	<i>ns</i>
Moderate	2	20%	5	18%	7	18%	<i>ns</i>
Severe	1	10%	1	4%	2	5%	<i>ns</i>
Unspecified	0	0%	3	11%	3	8%	<i>ns</i>

Motor delay	7	70%	21	75%	28	74%	<i>ns</i>
Language delay	10	100%	20	71%	30	79%	<i>ns</i>
Regression	1	10%	3	11%	4	11%	<i>ns</i>
Facial Dysmorphism	7	70%	28	100%	35	92%	0.0142
Broad forehead	0	0%	7	25%	7	18%	<i>ns</i>
Frontal bossing	1	10%	2	7%	3	8%	<i>ns</i>
Hypertelorism	3	30%	6	21%	9	24%	<i>ns</i>
Downslanting palpebral fissures	1	10%	5	18%	6	16%	<i>ns</i>
Prominent nose	1	10%	13	46%	14	37%	<i>ns</i>
Bulbous/broad nasal tip	4	40%	13	46%	17	45%	<i>ns</i>
Broad/full nasal bridge	1	10%	5	18%	6	16%	<i>ns</i>
Ear anomalies	2	20%	17	61%	19	50%	<i>ns</i>
Thin upper lip	3	30%	17	61%	20	53%	<i>ns</i>
Wide mouth	1	10%	16	57%	17	45%	0.0124
High arched palate	0	0%	7	25%	7	18%	<i>ns</i>
Small/recessed chin	0	0%	5	18%	5	13%	<i>ns</i>
Short stature (Height < -2SD)	3	30%	9	32%	12	32%	<i>ns</i>

Low weight (Weight < -2SD)	2	20%	9	32%	11	29%	<i>ns</i>
ASD	5	50%	6	21%	11	29%	<i>ns</i>
Seizures	4	40%	5	18%	9	24%	<i>ns</i>
Hypotonia	4	40%	11	39%	15	39%	<i>ns</i>
Elevated CK	1	10%	2	7%	3	8%	<i>ns</i>
Feeding Difficulties/Reflux	2	20%	5	18%	7	18%	<i>ns</i>
Chondrodysplasia	0	0%	2	7%	2	5%	<i>ns</i>
Scoliosis	0	0%	7	25%	7	18%	<i>ns</i>
Cardiac abnormalities	1	10%	4	14%	5	13%	<i>ns</i>
Renal abnormalities	0	0%	4	14%	4	11%	<i>ns</i>
Brain MRI abnormalities	1	10%	9	32%	10	26%	<i>ns</i>

**p-values: comparison of missense variants and loss of function variants using 2-tailed Fisher's exact test. ns, not significant; #, number of individuals with the given feature; %, frequency of the individuals with the given feature; CK, creatine kinase; MRI, magnetic resonance imaging; SD, standard deviation; GDD, global developmental delay; LOF, loss-of-function; No., number. Note three individuals with short stature include Individual 24 who responded to growth hormone therapy.*