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QRICH1 Mutations cause a Chondrodysplasia with Developmental Delay

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Abstract

In many children with short stature, the etiology of the decreased linear growth remains unknown. We sought to identify the underlying genetic etiology in a patient with short stature, irregular growth plates of the proximal phalanges, developmental delay, and mildly dysmorphic facial features. Exome sequencing identified a *de novo*, heterozygous, nonsense mutation (c.1606C>T:p.R536X) in *QRICH1*. *In vitro* studies confirmed that the mutation impaired expression of the QRICH1 protein. siRNA-mediated knockdown of *Qrich1* in primary mouse epiphyseal chondrocytes caused downregulation of gene expression associated with hypertrophic differentiation. We then identified an unrelated individual with another heterozygous *de novo* nonsense mutation in *QRICH1* who had a similar phenotype. A recently published study identified *QRICH1* mutations in three patients with developmental delay, one of whom had short stature. Our findings indicate that *QRICH1* mutations cause not only developmental delay but also a chondrodysplasia characterized by diminished linear growth and abnormal growth plate morphology due to impaired growth plate chondrocyte hypertrophic differentiation.

Graphical Abstract

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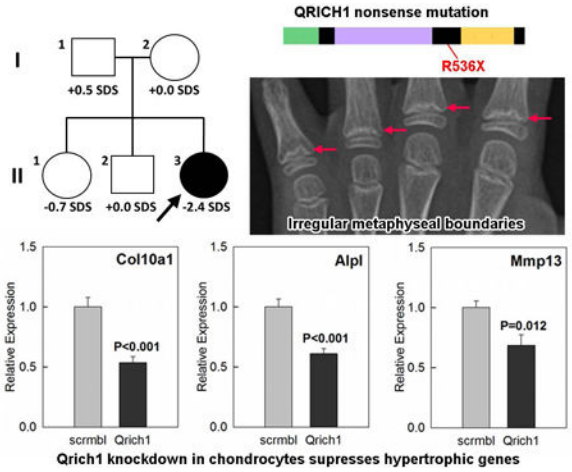

Keywords

short stature; growth; chondrogenesis

Introduction

Genetic defects that impair growth plate chondrogenesis cause short stature. However, in many children who present with short stature, the genetic etiology remains unknown [1]. Recently, exome sequencing of affected families has been applied to discover new monogenic causes of short stature. However, it is often difficult to identify the causative variant from the many benign variants and to confirm the causal role, for example by functional studies. Here, we report a patient with short stature, abnormal growth plate morphology on bone age radiograph, developmental delay, and dysmorphic facies and a second patient with a similar phenotype except height in the lower portion of the normal range. In both individuals, exome sequencing revealed *de novo*, heterozygous nonsense mutations in *QRICH1*, a gene whose function is unknown. A recent study reported mutations in *QRICH1* as a cause of developmental delay [2]. Here we show evidence that these mutations can also cause a subtle chondrodysplasia, and consequently that these mutations should be considered in the differential diagnosis of short stature. We also show evidence that the chondrodysplasia arises from impaired growth plate chondrocyte hypertrophic differentiation, a process necessary for normal linear growth.

Subjects and Methods

This study was approved by the Institutional Review Board of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, and written informed consent was obtained from the participant and legal guardians. SNP array was performed by the Cancer Genetics and Comparative Genomics Branch at National Human Genome Research Institute (NHGRI) using the Infinium HumanOmniExpressExome DNA analysis kit (Illumina, USA) according to the manufacturer's instruction. Exome sequencing was performed by the NIH Intramural Sequencing Center (NISC) as previously described [3]. Methods for plasmid preparation, site-directed mutagenesis, western blot, chondrocyte

isolation and transfection, ^{3}H-thymidine uptake, RNA extraction and purification, quantitative RT-PCR, and whole-mount embryo in situ hybridization are provided in the Supporting Information.

Results

The initial subject was an 8-year-old girl with a history of short stature (Figure 1A). Her birth weight and length were normal. She has a history of single umbilical artery and difficulty feeding in the newborn nursery. During her infancy and early childhood, she showed a delay in acquisition of motor skills, which required physical and occupational therapy. At 18 months of age, she had not begun speaking any words and has received speech therapy since age 2 years.

During early childhood, she showed mild delays in acquisition of motor skills and expressive speech. Other family members were unaffected (Figure 1B). The proband's height SDS was −2.5 and weight SDS was −2.02. Her arm span was 7 cm shorter than her height, but her sitting to standing height ratio was normal. She had large ears, mildly high-arched palate, mild scoliosis, bilateral 5th clinodactyly and decreased muscle tone. Her bone age was 5 years 9 months at chronological age 7 years 8 months and 7 years 10 months at 10 years 7 months. The radiograph revealed irregular metaphyseal boundaries of the proximal phalangeal growth plates (Figure 1D).

A de novo heterozygous nonsense mutation in QRICH1

SNP array confirmed paternity and did not identify any significant copy number variation. Exome sequencing of the patient, parents, and siblings was performed. Sequence variants identified were filtered for low population frequency (< 2%), pathogenicity predicted by 4 silico analyses (CADD score, Polyphen2, SIFT and MutationTaster), and fitting the Mendelian inheritance or *de novo* occurrence. This approach yielded only one candidate sequence variant in the proband, which was a nonsense mutation in *QRICH1* (NM_001320585.1: c.1606C>T (p.R536X), chr3:49083923 G>A, Supplementary Figure 2A). It was not present in the parents or siblings. The variant was not found in gnomAD and was confirmed by Sanger sequencing (Figure 1C). QRICH1 has a Residual Variation Intolerance Score (RVIS) of 5.3% (http://genic-intolerance.org) suggesting mild selective pressure against mutations in the gene.

The nonsense mutation decreases QRICH1 protein level

The human *QRICH1* gene contains 10 exons, and the nonsense mutation in our patient was in exon 5. We used site-directed mutagenesis to introduce the mutation into the human *QRICH1* cDNA and expressed in HEK293 cells. Western blot showed that transfection of wild-type *QRICH1* gene increased expression of QRICH1 protein compared to transfection of empty vector, whereas transfection of nonsense *QRICH1* mutant showed expression levels similar to empty vector (Supplementary Figure 1). A band at lower molecular weight compared to endogenous QRICH1 was observed after mutant transfection, which might represent a truncated QRICH1 protein. Our data therefore suggest that this *QRICH1*

nonsense mutation leads to decreased protein expression, either by producing a truncated product and/or by nonsense-mediated mRNA decay

Knockdown of Qrich1 in chondrocytes downregulates genes associated with hypertrophic differentiation

Because the patient showed short stature, disproportionate shortening of the upper extremities, and radiological growth plate abnormalities, we hypothesized that the mutation directly affected growth plate chondrocyte function. To test this hypothesis and explore the molecular pathogenesis, we used siRNA to knockdown *Qrich1* expression in primary mouse epiphyseal chondrocytes. Proliferation was not affected. However, several genes involved in chondrocyte hypertrophic differentiation, including collagen X (*Col10a1*), alkaline phosphatase (*Alpl*), and *Mmp13*, were downregulated (Figure 2). Other genes, such as *Col2a1* and *Sox9*, which are expressed more generally in chondrocytes, were not affected. These findings suggest that decreased *Qrich1* expression impairs chondrocyte hypertrophy, a process required for normal longitudinal bone growth.

Cellular and organismal distribution of QRICH1

The physiological and molecular functions of *QRICH1* are largely unknown. We expressed *QRICH1* in HEK293 cells and found QRICH1 protein in the cytoplasmic, cell membrane, and nuclear fractions (Supplementary Figure 2B). An additional band at approximately 10kDa was detected in the chromatin fraction, suggesting that a cleaved fragment of QRICH1 may interact with chromatin.

To analyze the organismal distribution of *Qrich1*, in situ hybridization using whole-mount E12.5 embryos (Supplementary figure 2C) was performed and showed greater *Qrich1* mRNA signal in the prefrontal cortex, maxilla, mandible, and limbs. Quantitative real-time PCR in 1-week old mice showed expression of *Qrich1* in all major tissues studied (Supplementary figure 2D), with slightly higher expression in the brain. These findings suggest that haploinsufficiency of *QRICH1* could affect multiple organ systems, including brain and bone.

An additional subject with a truncating *QRICH1* mutation

In the DECIPHER database [4], we identified another subject with a *de novo* nonsense mutation in *QRICH1*. He was an 11-year-old boy with stature in the lower portion of the normal range (−1.3 SD score), mild delays in motor and speech development, and transposition of the great vessels, which was successfully repaired at 4 weeks of age. Mother's height SDS was 0.3 and father's height SDS was −1.2. Considering the parents' heights, this child's height does not appear to be severely affected by the QRICH1 mutation. The proband had a wide mouth with thin upper lip, mildly bulbous nose, large ears, and bilateral clinodactyly. His bone age radiograph revealed irregular metaphyseal boundaries of the proximal phalangeal growth plates (Figure 1E), similar to the female subject described above. Exome sequencing, confirmed by Sanger sequencing, identified a *de novo*, heterozygous, nonsense mutation (1531C>T(Arg511X), chr3:49083998 G>A) in *QRICH1*. The variant was not found in gnomAD.

Discussion

We studied a child who presented with short stature, irregular proximal phalangeal growth plates, mild developmental delay, and mild dysmorphic facial features. Exome sequencing identified a heterozygous *de novo* nonsense mutation in the *QRICH1* gene, which was confirmed by Sanger sequencing. *In vitro* studies indicated that the mutation impaired expression of the QRICH1 protein. We then identified a second individual with a similar phenotype including height in the lower portion of the normal range, irregular proximal phalangeal growth plates, mild developmental delay, and mild dysmorphic facial features who had a different heterozygous *de novo* nonsense mutation in *QRICH1*, confirming the association.

SiRNA-mediated knockdown of *Qrich1* in primary mouse epiphyseal chondrocytes resulted in decreased expression of genes involved in hypertrophic differentiation, which is required for normal longitudinal bone growth and therefore normal height gain. The physiological and molecular function of QRICH1 remains to be elucidated, but we found *Qrich1* expression in brain and limbs of mouse embryos by in situ hybridization and more general expression in postnatal mice by real-time RT-PCR.

These findings indicate that heterozygous truncating mutations in *QRICH1* cause a subtle chondrodysplasia which manifests as diminished linear growth and abnormal radiological growth plate morphology. Studies in cultured growth plate chondrocytes suggest that the chondrodysplasia results from impaired growth plate chondrocyte hypertrophic differentiation, which is critical for longitudinal bone growth.

A recent study described three patients with *de novo*, heterozygous, nonsense or frameshift mutations in *QRICH1*[2]. The phenotypic description focused on the mild to moderate developmental and speech delay and mild dysmorphic facial features. The height of one subject was below normal (−3 SDS) and the height of the other two were in the lower half of the normal range (−0.9 and −0.7 SDS). No other evaluation of growth was reported. Thus, the current study adds to the previous report to indicate that heterozygous truncating mutations in *QRICH1* cause not only developmental delay but also a subtle chondrodysplasia, which can present to the clinician as short stature.

It is often difficult to prove unequivocally that specific genetic variants found in exome sequencing are causally related to the phenotype of the patients [5]. In our subject, several lines of evidence support causality. First, the *de novo* occurrence of the mutation matches the pedigree, explaining why the parents and siblings were unaffected. Second, the mutation creates a premature stop codon which is predicted to cause early truncation of the protein. *In vitro* studies confirmed the decrease in expression. Thus, the mutation likely causes haploinsufficiency for *QRICH1*. Third, the mutation is extremely rare in a large database of individuals. Fourth, siRNA-mediated knockdown of *Qrich1* expression to approximately 50% of control levels, mimicking haploinsufficiency, in primary mouse chondrocytes caused downregulation of genes important for hypertrophic differentiation which is a process required for normal growth plate chondrogenesis. Fifth, a second subject was identified with another nonsense mutation in *QRICH1* and a similar phenotype.

The findings of irregular proximal phalangeal growth plates and of short arm span, combined with our in vitro findings in chondrocytes, indicate that haploinsufficiency of *QRICH1* affects linear growth through a local effect on the growth plate. In addition, the radiological finding and the disproportion may provide useful clinical clues to the diagnosis. However, additional subjects are needed to determine the sensitivity and specificity of these findings for the diagnosis. In the current study, radiological abnormalities were only observed in the metaphyses of the proximal phalanges. The finding of decreased linear growth suggests that other long bones are affected and therefore might also show radiological abnormalities. However, the families did not consent to radiological imaging other than the bone age image. Therefore, additional studies in other children with *QRICH1* mutations will be needed to evaluate the full radiological features of this subtle chondrodysplasia.

Taken together, the evidence suggests that both the developmental and statural phenotypes can be mild and variable, which could explain why *QRICH1* is only modestly intolerant to mutations. Thus, there may be individuals with heterozygous variants that cause loss of protein function but such mild clinical manifestations that they are unlikely to be evaluated for a genetic disorder.

In conclusion, our findings provide strong evidence that haploinsufficiency for *QRICH1* cause not only developmental delay but also a chondrodysplasia which manifests as diminished stature and irregular proximal phalangeal growth plates. We found evidence that the bone abnormalities are caused by an impairment in growth plate chondrocyte hypertrophic differentiation. Thus, mutations in *QRICH1* should be considered in the differential diagnosis of short stature. The QRICH1 syndrome should be suspected particularly when the child shows developmental delay or the bone age radiograph shows irregular metaphyseal margins of the growth plate.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgement:

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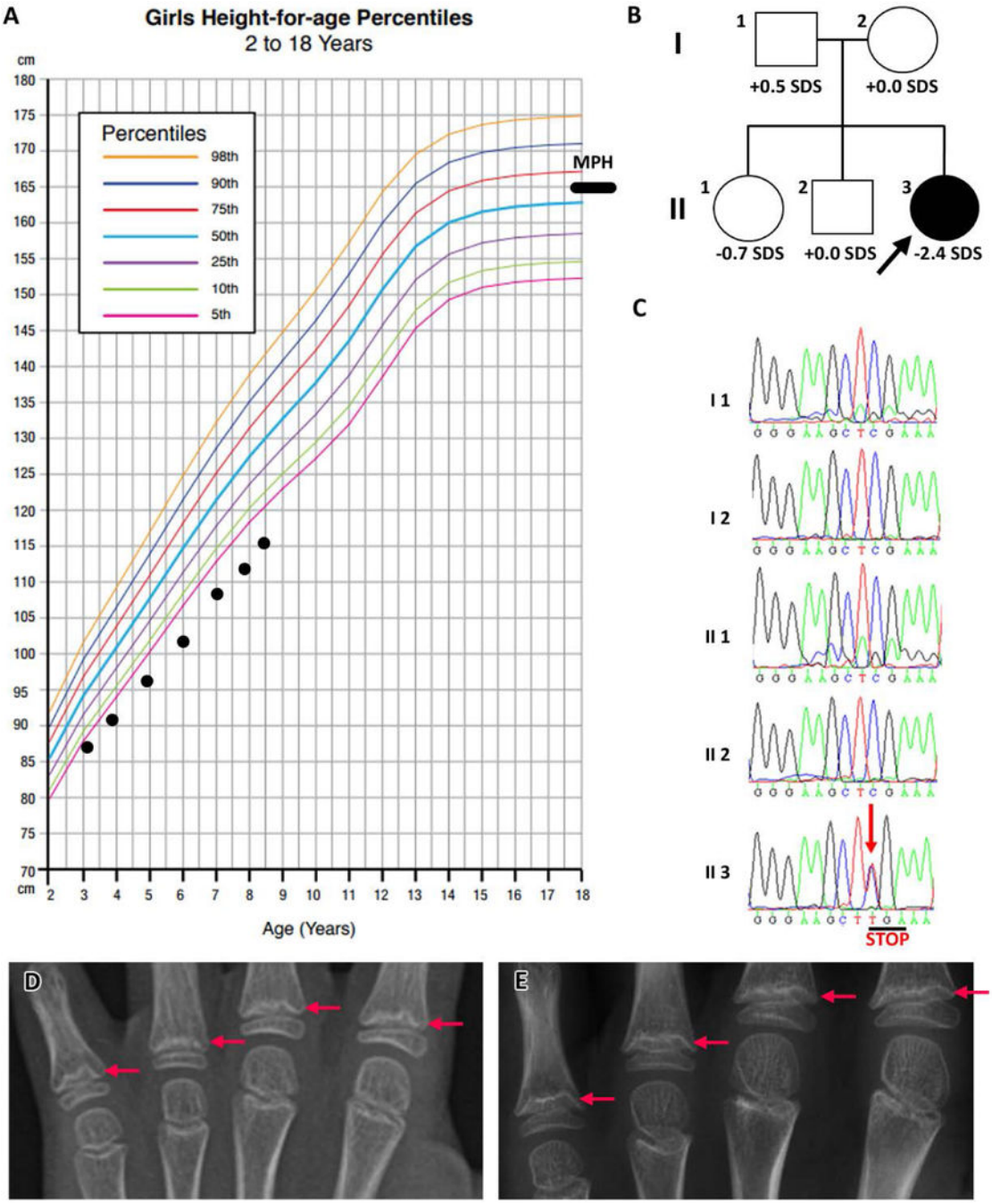

Fig 1. Heterozygous *de novo* nonsense variant in *QRICH1* in a patient with short stature and developmental delay.

(A) Growth chart (based on 2000 CDC growth data) of proband showing height for age (filled circles). MPH, adjusted mid-parental height. **(B) Family** pedigree. Arrow indicates proband. Solid symbol, heterozygous *QRICH1* mutation; open symbols, absence of mutation. Height SDS is indicated below each symbol. **(C)** Heterozygous nonsense mutation of *QRICH1* was confirmed by Sanger sequencing in the proband but not parents or siblings. **(D)** Bone age radiograph of the proband, showing irregular metaphyseal boundaries of

proximal phalangeal growth plates (red arrows). **(E)** Bone age radiograph of an additional individual with a *de novo* nonsense mutation in *QRICH1* showing similar irregular metaphyseal boundaries.

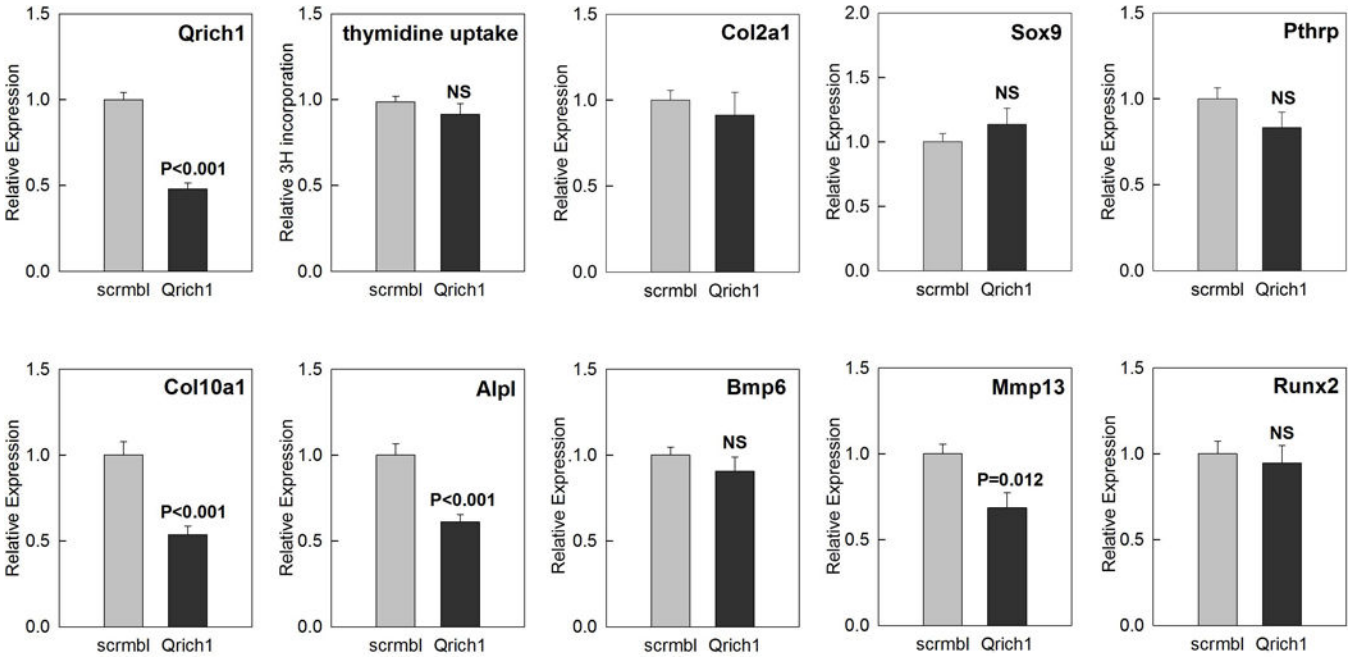

Fig 2. Knockdown of *Qrich1* in chondrocytes downregulates genes associated with hypertrophic differentiation.

In primary epiphyseal chondrocytes cultured from 1-wk old mouse tibias, transfection with *Qrich1*-targeted siRNA suppressed *Qrich1* expression by approximately 50% relative to scrambled siRNA. Knockdown of *Qrich1* did not significantly affect proliferation, (assessed by tritiated thymidine uptake) but downregulated expression (assessed by real-time PCR, normalized to 18SRNA) of *Col10a1*, *Alpl*, and *Mmp13* which are associated with chondrocyte hypertrophic differentiation. Data are presented as mean ± SEM