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A novel *COL12A1* variant expands the clinical picture of congenital myopathies with extracellular matrix defects

Jaya Punetha, MS^{1,2}, Akanchha Kesari, PhD¹, Eric P. Hoffman, PhD^{1,2}, Monika Gos, PhD³, Anna Kami ska, MD, PhD⁴, Anna Kostera-Pruszczyk, MD, PhD⁴, Irena Hausmanowa-Petrusewicz, MD, PhD^{5,*}, Ying Hu, MS⁶, Yaqun Zou, MD⁶, Carsten G. Bönnemann, MD⁶, and Maria J drzejowska, MD, PhD^{5,7}

¹Research Center for Genetic Medicine, Children's National Medical Center, Washington, DC

²Department of Integrative Systems Biology, The George Washington University School of Medicine and Health Sciences, Washington, DC ³Department of Medical Genetics, Institute of Mother and Child, Warsaw, Poland ⁴Department of Neurology, Medical University of Warsaw, Warsaw, Poland ⁵Neuromuscular Unit, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland ⁶National Institute of Neurological Disorders and Stroke/NIH, Porter Neuroscience Research Center, Bethesda, MD ⁷Department of Medical Genetics, The Children's Memorial Health Institute, Warsaw, Poland

Abstract

Introduction—Mutations in the *COL12A1* gene have been described in a milder Bethlem-like myopathy in 6 patients from 3 families (dominant missense), and in a severe congenital form with failure to attain ambulation in 2 patients in a single pedigree (recessive loss-of-function).

Methods—We describe an 8-year old girl of Polish origin who presented with profound hypotonia and joint hyperlaxity at birth after a pregnancy complicated by oligohydramnios and intrauterine growth retardation.

Results—We identified a novel, potentially pathogenic heterozygous missense *COL12A1* c. 8329G>C (p.Gly2777Arg) variant using a targeted sequencing panel. Patient fibroblast studies confirmed intracellular retention of the *COL12A1* protein, consistent with a dominant-negative mutation.

Conclusions—As our patient showed a more intermediate phenotype, this case expands the phenotypic spectrum for *COL12A1* disorders. So far, *COL12A1* disorders seem to cover much of the severity range of an Ehlers-Danlos/Bethlem-like myopathy overlap syndrome associated with both connective tissue abnormalities and muscle weakness.

Corresponding author: Maria J drzejowska, Neuromuscular Unit, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland, mjedrzejowska@imdik.pan.pl, Tel: +48 22 60 86 408, Fax: +48 22 60 86 631.
*Deceased

Conflict of Interest

The authors have no conflict of interest to report.

Keywords

Collagen XII; collagen VI; hypotonia; floppy infant; congenital myopathy; Ullrich congenital muscular dystrophy; Bethlem myopathy; targeted sequencing

Introduction

Collagen XII is a member of the family of fibril-associated collagens with interrupted triple helix (FACIT). It helps preserve muscle and bone architecture through collagen fibril organization.^{1,2} Mutations in *COL12A1* have been recently described to cause a blended muscle and connective tissue phenotype that is reminiscent of collagen VI related myopathies. The typical clinical picture of collagen XII related myopathy includes distal joint hyperlaxity, mild muscle weakness, and joint contractures, with different ages of onset and severity. Two siblings with recessive loss of function mutations in the *COL12A1* gene have been reported to have a severe congenital myopathy phenotype compared to a milder Bethlem-like myopathy seen in 6 patients from 3 families with dominant missense mutations.^{3,4} *COL12A1* knockout mice also display connective tissue disorders with variable muscle weakness.⁴

In this report, we describe a patient with a novel mutation in the *COL12A1* gene with a severe clinical course in the neonatal period and subsequent improvement.

Materials and Methods

The patient was recruited under protocol 2405 approved by the Office for the Protection of Human Subjects at Children's National Medical Center, Washington, DC. Informed consent for molecular testing was obtained. Genomic DNA was extracted from peripheral blood, and target genes were amplified using the RDT1000 system (RainDance Technologies, Lexington, MA). Target genes were a custom myopathy gene panel of 81 genes previously associated with muscle disease phenotypes. Amplified exons (n=1852) were then sequenced on the Illumina MiSeq (Illumina, San Diego, CA) system following manufacturer protocols. Alignment, variant calling, and annotation were performed using NextGene (SoftGenetics, State College, PA) software. Variants identified in *COL12A1* were validated via Sanger sequencing. The primers for the amplification and sequencing were designed using Primer3web online tool (<http://primer3.ut.ee/>).

A muscle biopsy was obtained from the left quadriceps (vastus lateralis) and flash frozen. Cryosections were stained with hematoxylin and eosin (H&E), NADH dehydrogenase (DPNH diaphorase, oxidative myofibers), and ATPase after alkaline pre-incubation at pH 9.4. A fibroblast cell line was derived from a skin biopsy. Immunocytochemical analysis of dermal fibroblasts derived from the patient was performed as described in Zou et al.⁴

Case report

An 8-year old girl, the first child of unrelated parents (Figure 1), was born at 39 weeks gestation by elective Caesarian section due to oligohydramnios, intrauterine growth

retardation, and a breech position. Her birth weight was 2820g (<third percentile), she was 57 cm long (fiftieth to seventy-fifth percentile) and had an Apgar score of 10. After birth, she was noted to have severe hypotonia, weak spontaneous movements, scoliosis, proximal contractures, facial asymmetry with skull flattening, and dysmorphic features (micrognathia, short nose, big dysplastic ears, high-arched palate, pectus excavatum and long slender fingers). Deep tendon reflexes were preserved. She sucked well, and no respiratory problems were present apart from inspiratory stridor, diagnosed as laryngomalacia by videolaryngoscopy. She gained weight poorly and did not exceed the third percentile in the first year of life. Motor milestones were delayed. She was able to sit at age 1 year and walked unaided at age 3 years. She had a progressive S-shape scoliosis, which was partially controlled with a brace. At age 7 years, she was able to walk but fell frequently. She got out of a squatting position and climbed stairs with support. Neurological examination revealed mild dysmorphic features (Figure 1), a high-arched palate with teeth that had not erupted properly, slight global muscle weakness (weak grip, difficulty raising her legs against gravity), brisk tendon stretch reflexes, marked joint hypermobility, and significant scoliosis with a kyphotic hump (Cobb angle of 52° and 43° at D3-D12 and D12-L5, respectively). No contractures were observed. Her cognitive development was within normal limits, however the onset of speech was age 4 years. At age 8, she underwent scoliosis surgery with reduction of the Cobb angle to 17° and 25°, respectively (Figure 1).

An MRI scan of the brain and spinal cord, performed at age 1 year, showed S-shaped scoliosis of the thoraco-lumbar segment with normal spine structure. The brain MRI showed mild ventricular widening, but no other structural abnormalities and no disruption in myelination. Echocardiography revealed a patent foramen ovale that did not require intervention. Gastrointestinal examination showed no abnormalities. Electromyography and nerve conduction studies were normal. Serum creatine kinase levels were normal. Biopsy of the left vastus lateralis muscle performed at age 4 years showed mild nonspecific myopathic changes, with increased variability in fiber shape and diameter, and increased connective tissue (Figure 2).

Motor milestones in the child's mother were also delayed. She started to walk at age 2 years and had poor physical performance at school. At age 32 years, her neurological examination was normal. There was no information about the child's father or his family. An initial diagnosis of a congenital Bethlem-like myopathy was proposed.

Molecular diagnosis

Targeted sequencing using a custom panel of 81 candidate myopathy genes (n=1852 exons) identified a heterozygous *COL12A1* c.8329G>C (p.Gly2777Arg) variant (variant named according to NCBI reference sequences NM_004370.5 and NP_004361.3). The variant was verified via Sanger sequencing and was absent in the proband's mother. Possible tissue somatic mosaicism was excluded in analysis of DNA extracted from blood, buccal swabs, and hair follicles. The father of the affected child was not available for molecular analysis.

The p.Gly2777Arg missense change results in substitution of the glycine with an arginine in the collagen triple helical domain, breaking the Gly-X-Y motif, and is predicted to be pathogenic with bioinformatics tools (Mutation Taster⁵, PROVEAN⁶, SIFT⁷, PolyPhen-2⁸).

This variant is absent from the NHLBI exome sequencing project⁹ and the ExAC¹⁰ databases. We did not find other variants likely to be pathogenic in other Bethlem myopathy related genes (*COL6A1*, *COL6A2*, and *COL6A3*) in the proband.

Fibroblast studies

Fibroblasts were grown from her dermal biopsy to check for collagen XII protein expression (Figure 3). With collagen XII stain, we found that the amount of formed collagen XII extracellular matrix was reduced. Evidence for intracellular retention was observed upon staining in the presence of triton, for cell permeation. Thus, the fibroblast culture studies are consistent with a dominant negative mode of action of the mutation, which leads to formation of abnormal COL12A1 homotrimers. These abnormal homotrimers are incapable of assembling a stable matrix, while at the same time they lead to intracellular protein retention, presumably by interfering with the normal assembly and secretory process. Collagen VI immunocytochemical studies were normal.

Discussion

Collagen XII related myopathy is a muscle and connective tissue disorder recently described in 8 patients from 4 families (Supplementary Table S1, available online).^{3,4} The clinical picture of our patient was intermediate compared to previously reported patients. The clinical features of congenital profound muscle weakness, distal joint laxity, proximal contractures, feeding difficulties, and kyphoscoliosis, combined with mild facial dysmorphism (long face, micrognathia, high-arched palate) resembled those of the *COL12A1* affected siblings reported by Zou et al.⁴ Flattening of the skull and asymmetry may be a consequence of the *COL12A1* mutation; however the interpretation of causality is complicated by oligohydramnios during gestation in the patient, along with reduced fetal movements. Similarly, possible hypoxia associated with the complicated pregnancy could have led to the observed delayed speech development. Muscle strength improvement with achievement of the ability to walk was similar to the improvement reported in patients with dominant *COL12A1* mutations associated with milder phenotypes.^{3,4} This clinical variability suggests that collagen XII- related disorders, similar to collagen VI- related disorders, can have recessive as well as dominant inheritance and lead to a wide spectrum phenotypes ranging from severe Ullrich congenital muscular dystrophy-like forms to intermediate forms to milder Bethlem-like myopathies.¹¹

The collagen XII protein has been shown to interact specifically with fibers containing collagen I and proteins such as decorin or tenascin X.¹² It is also involved in the response to mechanical stress, matrix deformability, and matrix reorganization during development, especially during osteoblast development and bone formation.¹³ The collagen XII protein contains 2 triple helical domains that are responsible for homotrimer formation and contain specific repetitive Gly-X-Y sequences (glycine – mainly proline – mainly hydroxyproline) which are indispensable for correct homotrimer formation. The *COL12A1* mutation in our patient results in breaking of the Gly-X-Y motif in the collagen triple helical domain in the collagen XII protein, as is often seen in collagen disorders, and is consistent with a dominant-negative biochemical defect.^{14,15} This dominant-negative biochemical model was

supported by *in vitro* patient fibroblast studies that showed diminished formation of a stable collagen XII matrix while also showing evidence for intracellular retention of collagen XII protein. These findings are consistent with a dominant-negative mode of action of the mutation. The mutant chain is incorporated in the homotrimeric structure, which is then unable to form a stable matrix and is also prone to be delayed in the secretory process and leads to retention in the cells. While the *in vitro* fibroblast studies of the patient supported potential pathogenicity of the identified c.8329G>C (p.Gly2777Arg) variant, lack of information from the patient's father is a potential limitation in assessing variant pathogenicity. Nevertheless, the c.8329G>C variant does not seem to be a polymorphism, as it has not been described in population databases (1000genomes¹⁶, NHLBI/EVS⁹, and ExAC¹⁰), including our in-house database (Children's National) and the in-house database of the Institute of Mother and Child, Warsaw, Poland (unpublished data). Therefore, collagen XII-related disorders should be included in the differential diagnosis of patients with an overlapping phenotype that combines both muscle and connective tissue defects.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

COL12A1	Collagen, Type XII, Alpha 1
MRI	magnetic resonance imaging
CNS	central nervous system
H&E	hematoxylin and eosin
DPNH-D	DPNH dehydrogenase
ATP-ase pH 9.4	ATPase after preincubation at pH 9.4
NCBI	National Center for Biotechnology Information

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Figure 1.

Proband at age 7 years shows mild dysmorphic features (long, expressionless face and open mouth, micrognathia, big dysplastic ears), scoliosis, long fingers and toes with a wide sandal gap, and valgus feet. The patient's back after scoliosis surgery at age 8 is shown. Distal joint laxity with hyperextension of the thumb to the forearm is observed, along with long slender fingers.

Figure 2.

Biopsy of the vastus lateralis muscle shows mild variation in fiber size (H&E X 100) and a normal pattern of metabolic muscle fiber differentiation (DPNH X 100 and ATPase, pH 9.4 X 100).

Figure 3.

Collagen XII staining in fibroblasts derived from a normal control and the patient show that collagen XII matrix staining without triton-X 100 (extracellular) is reduced in the patient. Intracellular Collagen XII (with addition of triton-X 100) is more prominent in the patient. Collagen VI immunoreactivity is normal for both the control and the patient.