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Ehlers-Danlos/myopathy overlap syndrome caused by a large de novo deletion in COL12A1

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Abstract

Autosomal dominant and recessive mutations in COL12A1 cause the Ehlers-Danlos/myopathy overlap syndrome. Here, we describe a boy with fetal hypokinesia, severe neonatal weakness, striking hyperlaxity, high arched palate, retrognathia, club feet, and pectus excavatum. His motor development was initially delayed but muscle strength improved with time while hyperlaxity remained very severe causing recurrent joint dislocations. Using trio exome sequencing and a copy number variation (CNV) analysis tool, we identified an in-frame de novo heterozygous deletion of the exons 45 to 54 in the COL12A1 gene. Collagen XII immunostaining on cultured skin fibroblasts demonstrated intracellular retention of collagen XII, supporting the pathogenicity of the deletion. The phenotype of our patient is slightly more severe than other cases with dominantly acting mutations, notably with the presence of fetal hypokinesia. This case highlights the importance of CNVs analysis in the COL12A1 gene in patients with a phenotype suggesting Ehlers-Danlos/myopathy overlap syndrome.

Keywords: CNV; COL12A1; collagen XII; copy number variation; dominant; myopathy.

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Title: Ehlers-Danlos/myopathy overlap syndrome caused by a large *de novo* deletion in *COL12A1*

Running title: EDS/myopathy overlap syndrome caused by a large deletion in *COL12A1*

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Abstract

Autosomal dominant and recessive mutations in *COL12A1* cause the Ehlers-Danlos/myopathy overlap syndrome. We describe a boy with fetal hypokinesia, severe neonatal weakness, striking hyperlaxity, high arched palate, retrognathia, club feet and pectus excavatum. His motor development was initially delayed but muscle strength improved with time while hyperlaxity remained very severe causing recurrent joint dislocations. Using trio whole exome sequencing and a copy number variation (CNV) analysis tool, we identified an in-frame *de novo* heterozygous deletion of the exons 45 to 54 in the

COL12A1 gene. Collagen XII immunostaining on cultured skin fibroblasts demonstrated intracellular retention of collagen XII, supporting the pathogenicity of the deletion. The phenotype of our patient is slightly more severe than other cases with dominantly acting mutations, notably with the presence of fetal hypokinesia. This case highlights the importance of the analysis of CNV in the *COL12A1* gene in cases with a phenotype suggesting Ehlers-Danlos/myopathy overlap syndrome.

Keywords: myopathy, collagen XII, COL12A1, dominant, copy number variation, CNV

Main text

1) Introduction

Collagen XII is a homotrimer composed by three $\alpha 1$ chains, that are encoded by the *COL12A1* gene. Collagen XII is a nonfibrillar collagen that belongs to the fibril-associated collagens with interrupted triple helices (FACIT) family. (Shaw and Olsen, 1991) The collagen XII homotrimer is composed by two short triple helical domains (COL1 and COL2) interrupted by two short non-triple-helical domains (NC1 and NC2) and prolonged by three long “finger-like” non-collagenous domains (NC3), each of them comprising 18 fibronectin type 3 (FN3) domains, four von Willebrand factor A (vWA) domains and a laminin-G like domain (LGL) (Yamagata et al., 1991). Collagen XII is expressed in tendons, cartilage, bone and dermis and can be found in two different isoforms, generated by alternative mRNA splicing. Compared to the long isoform, the short isoform lacks eight FN3 and two vWA domains (Koch et al., 1995).

Mutations in *COL12A1* cause Ehlers-Danlos/myopathy overlap syndrome. Both recessive and dominant mode of inheritance have been described. Recessive inheritance is associated with biallelic loss-of-function variants and severe congenital weakness precluding the acquisition of walking. Dominant inheritance is described for variants with a dominant

negative effect on the collagen XII homotrimer assembly and leads to a milder phenotype (Zou et al., 2013; Hicks et al., 2014).

2) Case report

2.1 Clinical findings and initial workup

The affected boy, born on term after a pregnancy characterised by reduced fetal movements, presented with severe neonatal hypotonia muscle weakness and weak cry. Generalized muscle atrophy and striking distal and proximal joint hyperlaxity were present. Birth weight and height were of 3.350 grams and 48cm. High arched palate, retrognathia, club feet and pectus excavatum were noted. He was able to feed independently but was fatigable during breastfeeding. Karyotype and molecular genetic testing for Prader-Willi syndrome, myotonic dystrophy 1 and spinal muscular atrophy were normal. Echocardiography disclosed atrial and ventricular septal defects that resolved spontaneously on control echocardiography.

Motor development was delayed. Independent sitting acquired at 11 months, crawling at 2 years and independent walking at 2,5 years. Muscle strength improved over time. Genu valgum, pes planus valgus with prominent calcaneum were noted on further examinations. Marked muscle wasting in the shoulder girdle, persistent joint hyperlaxity and spontaneous scapular dislocation were also present. Pulmonary function testing at 8 years showed a forced vital capacity of 1,36 liters (73% of the predicted values) and a total lung capacity of 2,37 liters (90% of the predicted values). Echocardiography and pulmonary function testing remained stable at the last control. Muscle biopsy, performed at 8 years, showed nonspecific changes with very mildly increased fiber size variability. Sanger sequencing of the coding sequence of the *COL6A1*, *COL6A2* and *COL6A3* genes did not reveal any pathogenic mutation. Immunocytochemistry for collagen VI on skin fibroblast cultures

showed a normal pattern. Muscle MRI disclosed an increase of T1 weighted signal in the distal part of the vastus intermedius and in the region surrounding the posterior part of the flexor digitorum longus et anterior part of the soleus. (Fig.1)

2.2 Whole exome sequencing

Informed consent for Whole Exome Sequencing (WES) was received from the family in compliance with protocols approved by the ethics committee of Erasme Hospital, Brussels (#P2015/158). Trio-WES was performed at the Broad Institute of MIT and Harvard's Genomic Platform (Cambridge, Massachusetts, USA) on a HiSeq 4000 (Illumina) after library preparation with the Nextera Rapid Capture Exome Kit 38Mb (Illumina). Analysis with *Seqr* (Center for Mendelian Genomics at the Broad Institute) of rare sequence variation (minor allele frequency < 1% in gnomAD) in muscle disease genes did not show any convincingly pathogenic variant. The GATK gCNV tool (BABADI et al.) was then used in *Seqr* to look for copy number variations (CNVs) in muscle disease genes. A *de novo* heterozygous deletion of the exons 45 to 54 of the *COL12A1* gene was identified (chr6:75,105,109-75,119,571 (hg38)) in the proband (Fig.2A). At the cDNA level, this deletion is predicted to lead to an in-frame deletion of 1179 bases. At the protein level, this deletion is predicted to remove the last half of the fourth vWA domain, the entire laminin-G domain and the proximal part of the COL2 domain (including the first three glycines) (Fig.2B).

2.3. Immunohistochemistry on skin fibroblasts

Skin fibroblasts, from a thigh skin biopsy, were cultured for 9 days in Dulbecco's modified Eagle medium with 10% fetal calf serum, 225µM Ascorbic acid phosphate and 125µM L-Ascorbate. Cells were fixed with Methanol-Acetone 1:1 at -20°C for 5 minutes, permeabilized with 0,1% Triton, blocked at room temperature for one hour (PBS/ 2% BSA/ 0,2% Tween) and incubated with primary antibodies at 4°C overnight (anti-collagen (KR75,

1:200) or anti-Tenascin-X (KR86, 1:2000)) and then with secondary antibody (anti-rabbit Alexa Fluor 488) at room temperature for one hour. Nuclear staining was performed with DAPI 0,4 µg/ml for 10 minutes. Slides were imaged with an Axiophot Zeiss microscope with the NIS Elements F Imaging software.

Collagen XII staining demonstrated the absence of collagen XII in the extracellular matrix and an increased intracellular staining. Tenascin-X staining was not affected. (Fig.3)

3) Discussion

We have identified a *de novo* heterozygous in-frame deletion of the exons 45 to 54 of *COL12A1* in a boy with fetal hypokinesia, severe neonatal hypotonia, weakness and joint hyperlaxity. Muscle strength improved over time, but joint hyperlaxity remained severe with recurrent joint subluxations. Muscle biopsy showed only mild myopathic changes. Immunocytochemistry demonstrates intracellular retention of collagen XII in the skin fibroblast culture, supporting the pathogenicity of the deletion.

Currently, 26 cases with 14 unique dominant variants in *COL12A1* have been described. Missense variants include substitutions of highly conserved glycine residues and arginine to cysteine substitutions. Splice site variants leading to an in-frame single exon skipping (exons 52, 53, 54 or 56) or copy number variant (exon 52 deletion) are also acting dominantly. Intracellular retention of collagen XII in skin fibroblast cultures was demonstrated for many dominantly acting variants, contrasting with the total absence of collagen XII staining observed in cases harboring biallelic loss-of-function variants (Hicks et al., 2014; Zou et al., 2013; Witting et al., 2018; Mohassel et al., 2019; Delbaere et al., 2019; Jezela-Stanek et al., 2019).

The phenotype of our case is slightly more severe than other cases with dominantly acting mutations. Indeed, fetal hypokinesia has never been described and neonatal hypotonia is only present in a subset of cases with dominant mutations. Muscle weakness detected during childhood, delayed motor milestones and improvement of muscle strength with age were almost always present. Micrognathia and high arched palate were noted in a few cases. Joint hyperlaxity was frequent, but shoulder subluxation has not been described earlier. When available, muscle biopsy usually showed mild myopathic features or mild fibrosis, similarly to our case. Respiratory involvement was absent or very mild. Cardiac involvement was consistently absent (Hicks et al., 2014; Zou et al., 2013; Witting et al., 2018; Mohassel et al., 2019; Delbaere et al., 2019; Jezela-Stanek et al., 2019). It is difficult to know if the occurrence of atrial and ventricular septal defects in our case a coincidental finding or a direct consequence of the collagen XII defect is. The increased muscle MRI signal in the perimysium surrounding the flexor digitorum longus and the anterior part of the soleus is somewhat similar to that described in another family (Mohassel et al., 2019). In two other families, muscle MRI disclosed a severe atrophy of the rectus femoris muscle in several patients, that is not present in our case (Hicks et al., 2014; Witting et al., 2017).

To our knowledge, this is the largest pathogenic intragenic CNV described in *COL12A1*. An in-frame single exon 52 deletion in *COL12A1* has been described in a family with dominantly inherited congenital muscle weakness and proximo-distal hyperlaxity (Mohassel et al., 2019). It was suggested that the dominantly inherited deletion of exon 52, encoding part of the laminin-G domain, could disrupt the stability of the collagen XII homotrimer. Indeed the laminin-G domain contains several cysteine residues, and leaving some unpaired cysteine residues on the collagen XII chain encoded by the normal *COL12A1* allele may lead to protein misfolding (Mohassel et al., 2019). In our case, the deletion also

encompasses exon 54 that encodes the first three glycine residues of the COL2 domain. Substitution of glycine residues are well known to impair the triple helix assembly in collagens.

This case also highlights the importance of analyzing CNVs in patients with muscle disease. (Välipakka et al., 2017; Zenagui et al., 2018). Specific bioinformatic tools have been developed to analyze CNVs directly from next-generation sequencing data by comparing the read depth in each region of a sample to multiple control samples sequenced on the same platform. If a deletion is present, the read depth is expected to be decreased (Zhao et al., 2013). Genome sequencing combined with muscle RNA-seq could also be helpful to detect deep intronic variants influencing splicing, that are not detected by WES (Cummings et al., 2017).

In conclusion, *COL12A1*-related myopathy should be suspected in patients with early-onset muscle weakness, joint hyperlaxity and improvement of strength with age. Collagen XII immunocytochemistry on skin fibroblast cultures can indicate collagen XII intracellular retention. Copy number variants analysis could help to identify cases without sequence variants in *COL12A1*.

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Figure legends

Figure 1. Axial T1-weighted imaging of the lower limbs of the proband showing an increase of signal in the distal part of the vastus intermedius and in the region surrounding the posterior part of the flexor digitorum longus et anterior part of the soleus.

Figure 2. (A) IGV visualization of the heterozygous deletion of exons 45 to 54 of *COL12A1* in the proband (red line) and his healthy parents (blue lines). The grey lines represent other samples in the same batch of CNV calling and show the average amount of noise across the region. The y-axis denotes the copy number as inferred by gCNV, and the x-axis shows the position along the gene in kilobases. The proband's sample appears to fall at a copy number

of 1, while both parent samples fall around a copy number of 2, suggesting a *de novo* heterozygous deletion of exons 45 to 54. (B) Schematic representation of protein domains of the collagen XII monomer. The black line represents the region of the collagen XII monomer that is removed by the deletion of the exons 45 to 54 of the *COL12A1* gene. Domain nomenclature is as follows: NC1-2-3: non collagenous domains 1-2-3, COL1-2: collagenous domains 1-2, vWA4: fourth von Willebrand factor A domain of the NC3 domain

Figure 3. Immunofluorescence of cultured skin fibroblasts obtained from the patient compared to a control. Collagen XII staining (green) reveals a normal collagen XII network in the extracellular matrix of the control in contrast to a near complete intracellular retention of collagen XII in the patient. Tenascin-X staining (green) is not significantly different in the patient and in the control. Magnification 20x.