

Dysspondyloenchondromatosis Without *COL2A1* Mutation: Possible Genetic Heterogeneity

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Dysspondyloenchondromatosis is a rare form of generalized enchondromatosis associated with spinal involvement. This skeletal dysplasia is characterized by multiple enchondromas present in vertebrae as well as in metaphyseal and diaphyseal parts of the long tubular bones, post-natal short stature, and early development of kyphoscoliosis. A novel heterozygous missense mutation in *COL2A1* was recently identified in a patient with dysspondyloenchondromatosis. This suggests that dysspondyloenchondromatosis might expand the already broad spectrum of type II collagenopathies. Here, we report on a young girl with features of dysspondyloenchondromatosis, specifically short stature, thoracoscoliosis, and generalized enchondromas lesions. Sanger sequencing failed to detect a mutation in *COL2A1*. We therefore suggest that dysspondyloenchondromatosis is a genetically heterogeneous condition.

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Key words: dysspondyloenchondromatosis; *COL2A1* gene; skeletal dysplasia

INTRODUCTION

Dysspondyloenchondromatosis (DSC or spondyloenchondromatosis) is a rare form of generalized enchondromatosis associated with spinal involvement. This entity differs from other forms of enchondromatosis by the severe spinal deformities mimicking vertebral malsegmentation [Pansuriya et al., 2010]. Dysspondyloenchondromatosis belongs to the group of spondylometaphyseal dysplasias [Warman et al., 2011]. It was initially described by Freisinger et al. [1993] and subsequently four additional patients were reported

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[Kozłowski et al., 1994]. This disease is characterized by post-natal short stature and multiple enchondromas in the vertebral bodies and the metaphyseal or diaphyseal parts of the long tubular bones. These enchondromas lead to severe and early-onset kyphoscoliosis, and unequal limb length. In patients with DSC, the dysplastic process

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usually affects one or more adjacent vertebral bodies to the extent that severe structural changes simulate a segmentation anomaly [Freisinger et al., 1993; Kozlowski et al., 1994]. To the best of our knowledge, 14 patients have been reported in the literature [Mainzer et al., 1971; Spranger et al., 1978; Azouz, 1987; Lerman-Sagie et al., 1987; Freisinger et al., 1993; Kozlowski et al., 1994; Haga et al., 1998; Nakane et al., 2011; Kenis et al., 2013]. Recently Nakane et al. [2011] described a boy with a novel heterozygous missense mutation in the *COL2A1* gene, leading to the substitution of a bulky amino acid by a glycine.

In this paper, we report on a young girl with clinical and radiological features of DSC with no mutation in the coding sequences of *COL2A1*.

CLINICAL REPORT

The patient was the only child of unrelated healthy parents originating from France. She was born at 34 weeks of gestation after an uneventful pregnancy. Birth parameters were in the normal range (length 44 cm, -2.9 SD; weight 2,475 g, -0.3 SD; OFC 35 cm, $+1.6$ SD). She was referred to the Genetic Department for severe post-natal short stature (length 79.5 cm, -4 SD at 3 years and 4 months). Her motor, language and intellectual development was reported to be normal, but was not formally evaluated. She had hip claudication diagnosed at the age of two due to transient synovitis.

At the most recent examination, at four years of age, her height was 84.5 cm (4 SD); weight was 11.5 kg (-2.5 SD); and OFC was

53 cm ($+2.5$ SD). She presented with a short thorax, pectus carinatum, and severe thoracoscoliosis associated with hyperlordosis (Fig. 1a and b). Examination of joints showed elbow limitation. Urinary organic acid chromatography was also normal excluding metaphyseal enchondromatosis with 2-hydroxyglutaric aciduria (MC-HGA, OMIM 271550). Abdominal, renal and heart ultrasounds were also normal. Skeletal X-rays showed spondylometaphyseal dysplasia with generalized enchondromatosis. On the vertebral column, there was spinal involvement with severe thoracic scoliosis and hyperlordosis associated with enchondromatosis of vertebral bodies mimicking vertebral malsegmentation. Vertebral enchondromas were confined to the thoracic and lumbar spine. Less severe enchondromas were also observed both on distal radius and ulna and on proximal tibia (Fig. 2). In addition, no asymmetry of the limbs was observed.

Genetic Analysis

Genomic DNA was isolated from peripheral EDTA anti-coagulated whole blood samples of the patient and her healthy parents using QIAamp DNA Blood Midi Kit (QIAGEN, Courtaboeuf, France) according to the supplier's protocol. PCR amplifications of the 54 exons and the intron-exon junctions of *COL2A1* gene (NG.008072.1-NM_001844.4) were performed using two pairs of primers (sequences available upon request), using the Promega PCR Master Mix, according to the manufacturer's protocol (Promega, Madison, WI). Mutation screening was performed by direct bi-

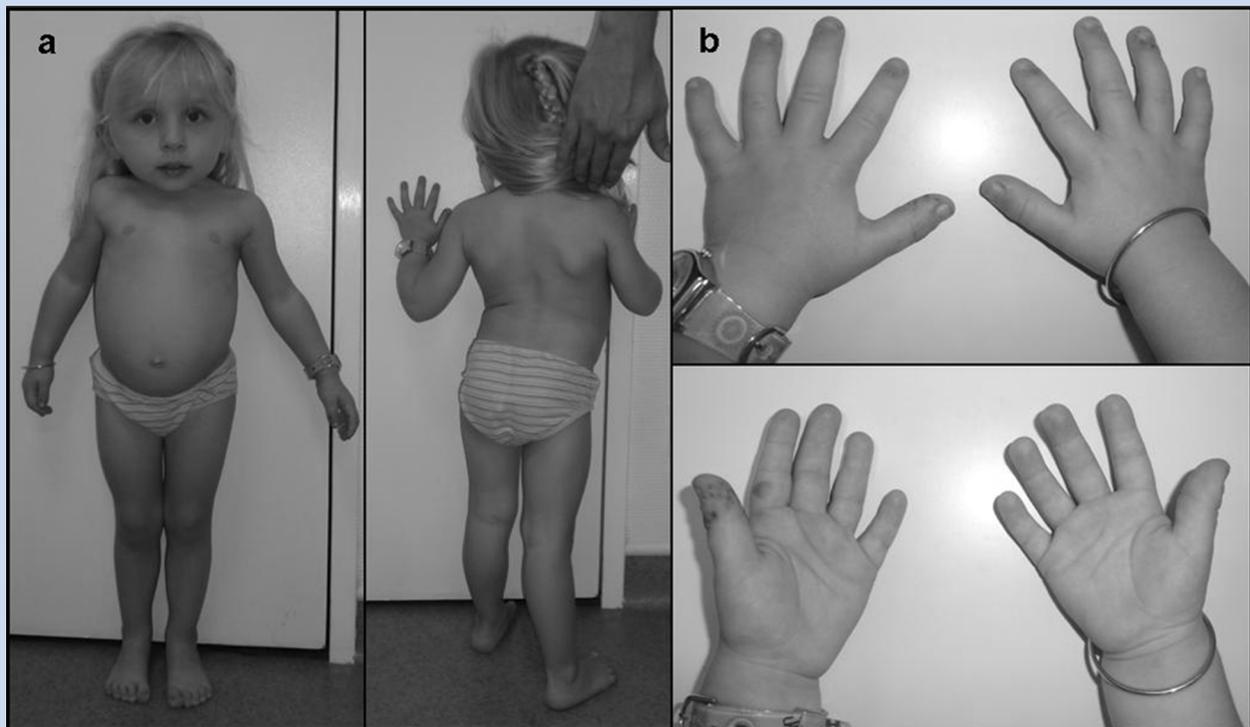


FIG. 1. a: Front and dorsal view of the patient at 4 years of age: note her short stature, thoracoscoliosis and hyperlordosis. b: Anterior and posterior views of her hands showing short hands with broad terminal phalanges and persistence of foetal fingertip pads.



FIG. 2. Skeletal X-rays at 4 years of age. Note severe thoracolumbar scoliosis with enchondroma lesions of vertebral bodies leading to a malsegmentation-like aspect and enchondroma lesions localised on the metaphyse of the radius and distal ulna, and on proximal metaphyse of the tibia.

directional sequencing, using the BigDye terminator cycle sequencing kit (BDT v3.1), followed by electrophoresis of the amplicons on an ABI 3100XL Genetic Analyzer, according to the manufacturer's recommendations (Applied Biosystems, Foster City, CA). The obtained sequences were aligned to the reference DNA sequence of the *COL2A1* gene (NG_008072.1) using the Seqscape 2.6 Software (Applied Biosystems). DNA sequence information referred to the public UCSC database GRCh37 (hg19).

We identified several nucleotide variations, among which a variant of unknown significance (VoUS), c.610-7G>A in intron 8. The proband inherited this heterozygous VoUS from her healthy mother. The patient's karyotype was normal (46,XX).

DISCUSSION

Skeletal dysplasia with enchondromatosis manifests both phenotypic and genetic heterogeneity. The first classification of enchondromatosis was proposed by Spranger et al. [1978]. The authors classified enchondromatosis with irregular vertebral lesions as type V, later coined DSC [Pansuriya et al., 2010]. More recently, DSC was classified as type VII enchondromatosis by Superti-Furga et al. [2012]. Among the subtypes of generalized enchondromatosis with spinal involvement, three distinct entities are described: (1) spondylenchondrodysplasia (SPENCD, OMIM 271550) characterized by intellectual disability, cerebral calcification, autoimmunity and mild to moderate vertebral involvement, which is due to mutations in *ACP5* [Superti-Furga et al., 2012]; (2) metaphyseal enchondromatosis with 2-hydroxyglutaric aciduria (MC-HGA,

OMIM 271550) characterized by intellectual deficiency, vertebro-metaphyseal involvement and hydroxyglutaric aciduria, which is due to mutations in *IDH1* or *IDH2* [Superti-Furga et al., 2012] and (3) DSC. Dysspondyloenchondromatosis is characterized by enchondromatosis and severe involvement of vertebrae leading to radiological aspects similar to those observed in vertebral segmentation anomaly.

The clinical and radiological features of the 14 previously reported patients as the present patient are summarized in Table I. Most patients were born at term. Their birth lengths were all below 50 cm, ranging from 39.5 cm (−6 SD) to 49 cm (mean) with an average at 43.4 cm (−4 SD). Length or height at last examination ranged from −3 to −9.8 SD (median, −5.6 SD), suggesting that severe short stature is a component of this disorder. The severe short stature could in part be due to the severe and frequent scoliosis. Indeed, all patients presented with scoliosis except patient 3 described by Kozłowski et al. [1994]. A second element that might be responsible for this severe short stature is the limb asymmetry. All patients, except for the patient reported here, presented with limb asymmetry detected in the first years of life. Asymmetric limbs might be the result of asymmetric localization on femoral and tibial enchondroma growth plates that could lead to limb length inequality. The patient reported here did not have major femoral or tibial enchondromas that could explain the absence of limb asymmetry.

A patient with DSC was recently reported with a novel heterozygous missense mutation in *COL2A1*. It was an apparently de novo heterozygous c.2258G>A (p.Gly753Asp) mutation in exon 34. It

TABLE I. Clinical and Radiological Features in Reported Patients With Dyspondylenchondromatosis

	Mainzer et al. [1971]		Spranger et al. [1978]		Azouz [1987]		Lerman-Sagie et al. [1987]		Freisinger et al. [1993]			Kozlowski et al. [1994]			Haga et al. [1998]		Nakane et al. [2011]		Kenis et al. [2013]		Present report	
									Patient 1	Patient 2	Patient 3	Patient 1	Patient 2	Patient 3	Patient 4							
Gender	F	M	M	F	M	M	M	M	M	M	F	M	M	F	M	M	M	M	M	M	F	F
BW, g	NR	2,400	2,500	2,450	2,950	3,450	3,450	3,500	3,045	3,500	2,100	3,500	3,500	3,650	3,650	2648	2648	2648	NR	NR	2,475	2,475
BL, cm (SD)	NR	40 (-6)	NR	NR	40.5 (-5.5)	43 (-4)	41 (-5)	46 (-2)	46 (-2)	47.7 (-1.5)	39.5 (-6)	46 (-2)	46 (-2)	46 (-2)	46 (-2)	41 (-5)	41 (-5)	41 (-5)	NR (-3)	NR (-3)	44 (-2.5)	44 (-2.5)
Age at diagnosis	18 months	4 years	3 years	2 years and 7 months	3 years and 6 months	13 months	2 years and 6 months	1st day	1st day	1st day	4th day	1st day	1st day	5th day	5th day	3 y	3 y	3 y	NR (-3)	NR (-3)	5 years	5 years
Age at last examination	20 years	4 years	3 years	2 years and 7 months	3 years and 6 months	13 months	2 years and 7 months	5 years	4 years and 6 months	15 months	3 years and 9 months	3 years and 6 months	4 years and 6 months	3 years and 9 months	3 years and 9 months	14 y	14 y	14 y	5 years	5 years	5 years	5 years
Weight, kg (SD)	NR	NR	NR	11.25 (-1.2)	9 (-4.5)	8.2 (-1.7)	7.5 (-3)	NR	NR	NR	NR	NR	NR	10, (-3)	NR	21	21	21	NR	NR	11.5	11.5
Height, cm (SD)	NR	82.5 (-6)	NR	80 (-3)	69 (-6)	63 (-5)	63.5 (-5.5)	NR	NR	NR	NR	NR	NR	72 (-6)	NR	(-3, 4)	(-3, 4)	(-3, 4)	NR	NR	(-2, 5)	(-2, 5)
DFC, cm (SD)	NR	51.2 (+1.2)	NR	Me	51 (+0.5)	NR	48 (Me)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	53	53
Intellectual development ^a	Above average	Normal	NR	Delayed	NR	NR	Normal	Normal	NR	Normal	Delayed	Normal	Normal	Normal	NR	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Asymmetric limbs (detected age)	+ At birth	+4 years	+3 years	+2 years and 7 months	+At birth	+13 months	+17 months	+At birth	+At birth	+2 years and 6 months	+At birth	+At birth	+2 years and 6 months	+5 weeks	+1 year	+3 years	+3 years	+3 years	+5 years	+5 years	-	-
Vertebral malsegmentation or hypodysplasia	+	++	+++	+	++	+++	+++	++	++	++	++	++	++	++	+	+	+	+	+++	+++	+	+
Scoliosis	+++	++	+++	+++	+++	++	++	++	+	++	None at 15 months	++	++	+++	+++	+++	+++	+++	++	++	+++	+++
Enchondromata	+	+++	++	++	++	+++	++	++	++	++	++	++	++	+++	+	++	++	++	++	++	+	+

BL, birth length; BW, birth weight; F, female; M, male; Me, mean; m, months; y, years; NR, not reported; SD, standard deviation; y, years.

+ mild; ++ moderate; +++ severe involvement.

^aClaims for intellectual development are estimates, as formal testing was not performed.

suggests that DSC might expand the broadspectrum of type II collagenopathies [Nishimura et al., 2005; Nakane et al., 2011].

Type II collagenopathies result from mutations in the *COL2A1* gene, which are responsible for several skeletal dysplasias [Spranger et al., 1994; Warman et al., 2011]. In addition to the classical skeletal anomalies observed in Type II collagenopathies Walter et al. [2007] described four individuals with *COL2A1* mutation and metaphyseal involvement with enchondroma-like lesions extending from the metaphysis to the diaphysis.

However, the patient reported here carries several frequent *COL2A1* SNPs and a maternally inherited VoUS, c.610-7G>A, with no clear pathogenic mutation. Moreover, we have identified this VoUS in other patients with type 2 collagenopathies, but it was often inherited through asymptomatic parents.

We report a girl who fulfills the DSC criteria namely short stature, scoliosis, enchondromas, vertebral involvement, and apparently normal intellectual development and we show that she did not have mutation in *COL2A1*. We propose that patients with DSC should be screened for *COL2A1* mutations, to determine if DSC is a genetically heterogeneous condition.

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