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Phenotype of COL3A1/COL5A2 deletion patients☆

Marlies JE. Kempers [a,*], Marja Wessels [b], An Van Berendoncks [c], Ingrid MBH. van de Laar [b], Nicole de Leeuw [d], Bart Loeys [a,e]

[a] Department of Clinical Genetics, Radboud University Medical Center, Nijmegen, the Netherlands
[b] Department of Clinical Genetics, Erasmus MC, University Medical Center Rotterdam, Rotterdam, the Netherlands
[c] Department of Cardiology, Antwerp University Hospital/University of Antwerp, Antwerp, Belgium
[d] Department of Human Genetics, Radboud University Medical Center, Nijmegen, the Netherlands
[e] Center for Medical Genetics, Antwerp University Hospital/University of Antwerp, Antwerp, Belgium

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ABSTRACT

Introduction: The diagnosis of Ehlers-Danlos syndrome is usually based on well-defined diagnostic criteria and the result of DNA investigation. Classical (cEDS) and vascular type (vEDS) are the most prevalent subtypes and are caused by heterozygous pathogenic variants in COL5A1, COL5A2, COL1A1 or, respectively, in COL3A1. We describe 3 cases with contiguous deletions resulting in haploinsufficiency of both genes with relative mild features of connective tissue disease.

Patients and methods: Information on medical history, physical information, genetic results (CNV-analysis) and imaging were obtained from the medical file.

Results: The first patient was a 31 yr old female, diagnosed during pregnancy after the NIPT result showed an interstitial deletion of 2.3 Mb on chromosome 2q32.2, confirmed by XON array. She had normal aortic diameters. She had no signs of cEDS or vEDS except for a relatively thin skin with increased visibility of the veins. Her father died suddenly of a type A/B dissection at the age of 62 years. The second patient was diagnosed at the age of 10 years after she was referred because of her intellectual disability, autism and constipation. She was known with a thin and vulnerable skin and had a bleeding after tooth extraction. Array showed a 14,5 Mb deletion of 2q31.3q32.3 (de novo). Imaging (latest age 17 years) did not show any abnormalities. The third patient, aged 28 years, was diagnosed during pregnancy with an interstitial deletion of circa 6 Mb on chromosome 2q31.1q32.2 3, previously shown in the fetus with bilateral club feet and hydronephrosis. She had no vEDS facial features and the skin was relatively thin. She has thoracolumbar scoliosis and dural ectasia. Imaging did not reveal any vascular abnormalities. Her son, born at 37 weeks 3 days. had club feet but not other clinical signs suggestive of classical or vascular EDS.

Discussion: Three patients are described with a contiguous deletion of varying size encompassing the COL3A1 and COL5A2 gene. Due to the mild phenotype a diagnosis of EDS was not suspected and was found coincidental. Since two of the patients were pregnant without major complications these patients may require a less defensive, approach to pregnancy/delivery.

1. Introduction

The Ehlers-Danlos syndromes are a group of hereditary connective tissue disorders comprising several subtypes (Beighton et al., 1988, 1998; Malfait et al., 2017). Usually patients have joint problems (hypermobility, dislocations), skin features (hyperelasticity, abnormal scarring) and some degree of vascular fragility (bleeding tendency, easy bruising). Depending on the subtype, patient characteristics may differ substantially in type and severity and several other features may occur as well (e.g. hernias and organ prolapse, eye disease, cardiovascular disorders, and life-threatening ruptures of intestines, blood vessel or uterus).

All subtypes of Ehlers-Danlos syndrome, except hypermobile EDS, are genetically characterized. Diagnosis is usually made based on well-

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* Corresponding author.
E-mail address: marlies.kempers@radboudumc.nl (M.JE. Kempers).

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defined diagnostic criteria and the result of DNA investigation. Among the genetic types of EDS the classical (cEDS) and vascular type (vEDS) are the most well-known with a prevalence of 1:20,000 for cEDS (P et al., 2001) and at least 1:200,000 for vEDS (Byers et al., 1993).

The molecular diagnosis of cEDS is established in a patient with the identification of a heterozygous pathogenic variant in *COL5A1*, *COL5A2*, or (less commonly) *COL1A1*; in patients with vEDS heterozygous pathogenic variants in *COL3A1* are most commonly found. The *COL5A2* and *COL3A1* gene are in close proximity on chromosome 2q32.2. In the literature one 3.5-Mb deletion that includes *COL3A1* and *COL5A2* is reported (Meienberg et al., 2010).

Here we describe 3 additional cases with contiguous deletions resulting in haploinsufficiency of both genes with relative mild features of connective tissue disease.

2. Patients and Methods

For all probands clinical information was obtained from the medical file. Information on medical history, physical information, genetic results and imaging were recorded.

Informed consent from the probands was obtained in accordance to local medical ethical requirements.

3. NIPT (Non-invasive prenatal testing)

NIPT was performed using Next Generation Sequencing (NGS) on cell free DNA from blood of a pregnant mother as previously described by van der Meij et al. (Am J Hum Genet. 2019 Dec 5; 105 (6):1091–1101). Blood draw for NIPT is scheduled at or after 11 + 0 weeks of gestation. Blood is drawn in two 10 mL Cell-Free DNA BCT CE tubes (Streck). Clinical Genetic laboratories from three university medical centers (Amsterdam UMC location VUMC, Rotterdam Erasmus MC, and Maastricht UMC+) perform NIPT in The Netherlands, including DNA isolation, library preparation, next-generation sequencing (NGS), data analysis, interpretation, and reporting. Cell-free DNA (cf.DNA) is isolated from plasma through the use of QIAsymphony Circulating DNA Kits (QIAgen). DNA libraries are prepared for genome-wide shallow sequencing (0.2 × ; 51bp single-end), which was performed with either

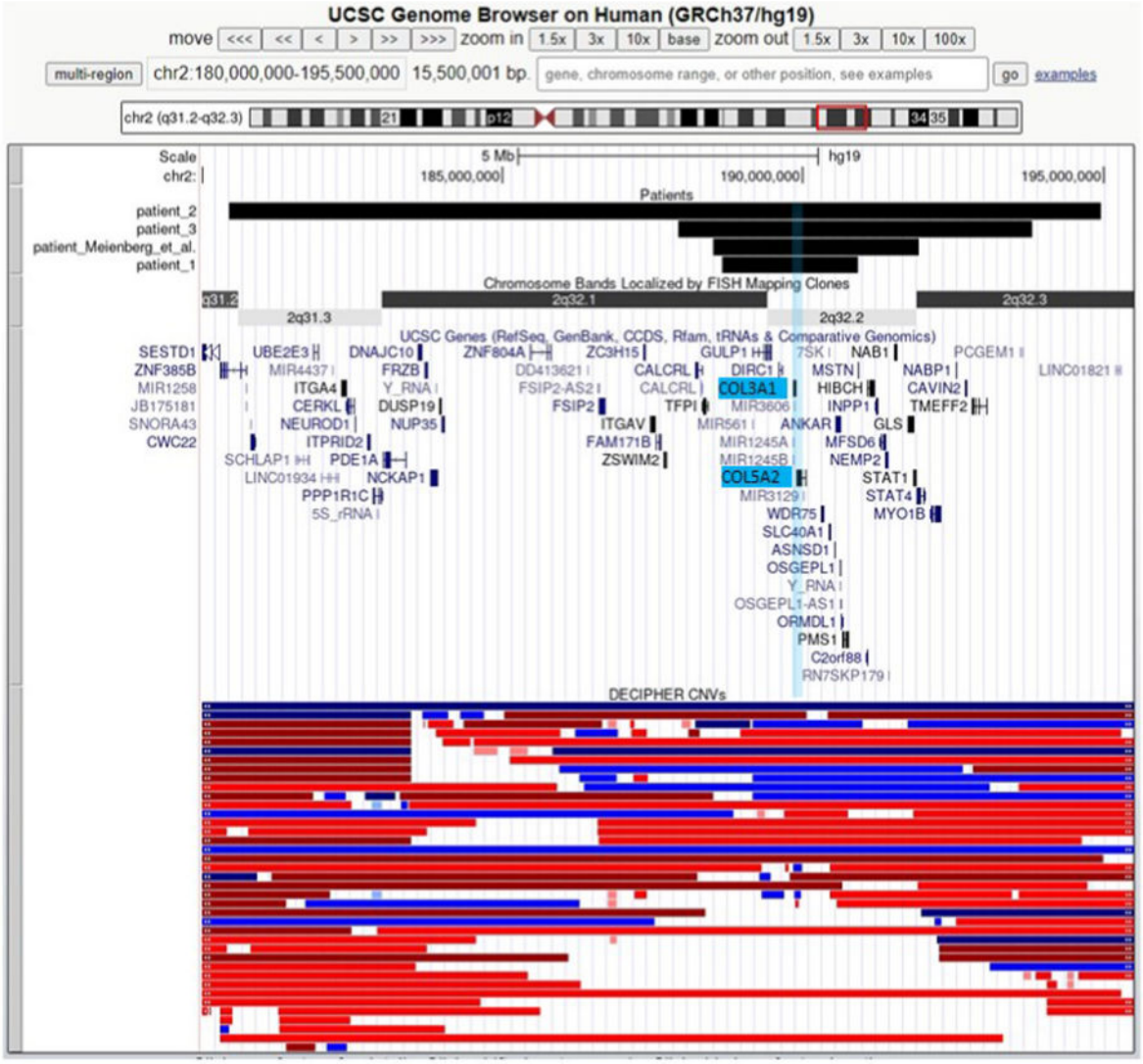

Fig. 1. Deletions in 2q31.2q32.3
Overview of the deletions in the 3 patients and the patient described by Meienberg et al. (Meienberg et al., 2010), in order of the size of the deletion.
The deletions of these 4 patients (solid black bars) are presented in the UCSC genome browser (http://genome.ucsc.edu/).
The genes in this region are shown in the lower part, including the *COL3A1* and *COL5A2* gene which are highlighted in blue. In the lowest part the DECIPHER CNVs are shown. . (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

the Illumina HiSeq4000 or the NextSeq500 sequencer (Illumina). Bioinformatic analysis was performed using the WISECONDOR (v2.0.1) algorithm under standard settings to call aneuploidy and other unbalanced chromosomal aberrations (Straver).

4. CNV analysis

The deletions were detected by whole-genome array analysis with different platforms. In family 1, 100 ng DNA from peripheral blood of the index patient was analysed using the CytoScan XON array platform (Thermo Fisher Scientific, Waltham, MA, USA), that contains 6.85 million empirically selected probes for whole-genome CNV analysis (with a practical resolution of ~100 kb) and which has excellent exon coverage for the reliable detection of intragenic CNVs. Eighty ng DNA from her diseased father was obtained from paraffin embedded tissue and analysed using the OncoScan array platform (Thermo Fisher Scientific, Waltham, MA, USA), that has an average genome wide resolution of ~300 kb. Data analysis was performed using the Chromosome Analysis Suite software version 4.2 (Thermo Fisher Scientific, Waltham, MA, USA). A CNV was called if at least 10 contiguous probes showed an abnormal log2 ratio.

In family 2 and 3, DNA analysis was performed using the CytoSNP12v2.1 chip (Illumina). Data analysis was performed by Nexus Copy Number Discovery v6.1 (patient 2) and CNV-Webstore 2.0 (patient 3) with genome wide resolution of 180 kb.

The CNV coordinates were mapped to the NCBI Human Genome Build GRCH36/(patient 2) and GRCh37/hg19 (patient 3) (Fig. 1).

5. Results

5.1. Family 1

The proband in Family 1 is a female of 31 years, who was referred by her midwife to the Clinical Genetics Department of the Radboudumc, because of an abnormal result of the NIPT. She was G1P0, the NIPT was performed at gestational age (GA) 13 + 4 weeks. The result of chromosomes 13,18,21 was normal but an interstitial deletion of 2.3 Mb on chromosome 2q32.2 was found.

She was seen at the Clinical Genetics Department at GA 15 + 1 weeks. She had a previous diagnosis of local scleroderma. She had no hypermobility, no joint problems or dislocation, except for the jaw, no abnormal scarring or problems with sutures, vascular events, ruptures, or hernias; there was a slightly increased tendency for ecchymosis. At physical examination she had no signs of cEDS or vEDS except for a relatively thin skin with increased visibility of the veins and acrogeria.

She has a healthy brother; her father died suddenly of a type A/B dissection at the age of 62 years; he was known with hypertension and sarcoidosis; the paternal grandmother died of a stomach bleeding at the age of 73 years.

The proband was seen by a cardiologist at 18 + 4 weeks GA. Ultrasound showed normal dimensions of the aorta (sinus 28.6 mm (z-0.53), ascendens 27.7 mm, arch 22.3 mm, abdominal 13.8 mm). No valvular abnormalities, stenosis or insufficiency were observed. Celioprolol was started at 200 mg once a day.

At GA 27 + 5 weeks echocardiography showed an aorta sinus of 29 mm (z-0.41), ascendens 30 mm.

Pregnancy was uncomplicated and at GA 37 weeks a caesarean section was performed because of maternal vEDS. She had a suboptimal response to pain relief medication. A healthy daughter was born, BW 3155 g. Total blood loss 750 mL. One month after delivery magnetic resonance angiography (MRA) showed normal aortic diameters (sinus 27 mm, ascendens 29 mm, arch 24 mm, abdominal 15 mm). The pulmonary artery was 37 mm.

The XON array confirmed the deletion in the proband. Consequently, paraffine embedded tissue of the father was analysed and the deletion was detected in the deceased father as well. The proband's healthy brother does not carry the deletion., the result of the paternal brother is pending and the proband's daughter has not been tested yet.

5.2. Family 2

This girl was known from birth on because of developmental problems and severe constipation. Her TIQ, measured at the age of 4 years was 55 (maternal IQ 95, paternal IQ 80); a diagnosis of Hirschsprung disease could not be established.

At the age of 10 years she was referred to the Genetics Department of the ErasmusMC because of her intellectual disability, autism and constipation. At age 8 years she had a percutaneous endoscopic gastrostomy tube and later Mic-key button for daily administration of laxatives. She was known with a thin and vulnerable skin. On physical examination a thin and vulnerable skin was noted.

DNA investigation of FMR1, FMR2, FLNA and metabolic investigation showed no abnormalities.

Array showed a 14,5 Mb deletion of 2q31.3q32.3 (de novo) and a maternally inherited 15q13.3 duplication.

The large deletion, containing 74 genes, was considered causal for the developmental delay. The 15q13.3 duplication is associated with a higher risk of (mild) intellectual disability/learning problems.

At the age of 15 years the girl suffered from bleeding after tooth extraction and she had ongoing gastro-intestinal problems.

MRA at the age of 10 years showed normal large and middle size arterial vessels, including normal cerebral vessels. Cardiac ultrasound at the age of 17 years showed no aortic dilatation; neither did CT angiography.

5.3. Family 3

The proband of Family 3 is a pregnant 28-years old woman, who was referred by obstetrician to the Center for Medical Genetics of the Antwerp University Hospital, because of prenatal ultrasound finding of bilateral club feet and hydronephrosis (right > left) in her male fetus. She was G1P0, the amniocentesis was performed at GA 17 + 4 weeks. The result of array analysis revealed an interstitial deletion of circa 6 Mb on chromosome 2q31.1q32.2 (minimal size 187.934.476–193.825.169 on built GRCh37). Further analysis revealed maternal inheritance of this deletion. She was clinically evaluated: there was no history of joint hypermobility, subluxation or dislocations, vascular events, or hernias and no abnormal scarring or problems with wound healing. At physical examination she had no vEDS facial features and the skin was relatively thin but no hyperelasticity, abnormal scarring or acrogeria. She has a thoracolumbar scoliosis. Subsequent work up revealed normal aortic measurements upon echocardiography (sinus 29 mm, ascending aorta 30 mm) and post-partum MRA of brain vessels and the thoracic and abdominal aorta with side branches did not reveal any vascular abnormalities. In addition to the scoliosis, dural ectasia was noted.

A babyboy was born after an uneventful primary caesarian section at 37 weeks 3 days. Birth weight was 3.070 g, length of 49 cm and occipitofrontal circumference 34 cm. Surgical correction of club feet was performed and antibiotic prophylaxis with Trimethroprim was initiated for bilateral vesico-urethral reflux (grade 5 right, grade 3 left). No clinical signs suggestive of classical or vascular EDS were observed upon physical examination.

Family history revealed a history of club feet in a sister of the paternal grandmother. Segregation analysis revealed absence of the chromosomal deletion in the proband's healthy brother but presence of the deletion in the proband's 63-year old mother. The latter did not have a vascular event by age 63; she declined further vascular imaging. Two brothers (65 and 68 years old) of the mother did not have the familial deletion. Both maternal grandparents of the proband died at old age without a history of vascular ruptures.

6. Discussion

We described three families with a contiguous deletion of 2q32.2 encompassing both the *COL3A1* and *COL5A2* gene. In all three probands the phenotype was absent or mild, consistent with the fact that the diagnosis of cEDS/vEDS was coincidental and not based on clinical suspicion. In one family member a Type A/B aortic dissection had occurred; no other vascular events or organ ruptures were described.

To our knowledge a comparable deletion of 2q32.2 has only been described once in literature by Meienberg et al. (2010). In the DECIPHER database some patients are enrolled with overlapping deletions, but the description of clinical manifestations is absent or limited. Meienberg et al. described 1 family with 8 affected family members. In this family abdominal aortic dissection at age 34 years, 1 death at 51 year with previous dissections at age 43 and 48 yrs and 1 bladder rupture were noted as well as thin translucent skin, hypermobility, easy bruising and early onset varicose veins in some of the family members. In family 3 it is unclear whether the club feet in the fetus could be attributed to the deletion of *COL3A1* gene as there is also a family history of club feet on the paternal side of the family and to our knowledge club feet have not been associated with *COL3A1* haploinsufficiency (in contrast to glycine substitutions in *COL3A1*).

The minimal overlapping region of the 2q32.2 deletion in the three families encompasses 17 genes, of which 4 are OMIM-genes *COL3A1, COL5A2, SLC40A1* and *MSTN*. The *SLC40A1* gene is related to hemochromatosis type 4 (#606069) with autosomal dominant inheritance with heterogeneous phenotypical manifestations. Ferritine measured in the index of Family 1 was normal. The *MSTN* gene (#614160) has been related to muscular hypertrophy, probably inherited in an autosomal recessive pattern (Schuelke et al., 2004).

The *COL3A1* and *COL5A2* gene are related to different subtypes of Ehlers-Danlos syndrome.

Pathogenic variants in *COL5A2* are related to classic Ehlers-Danlos syndrome, and are usually splice site variants that result in exon skipping or missense variants that result in substitution of glycine in the triple-helical region of the collagen molecule. Although haploinsufficiency is commonly seen in cEDS due to pathogenic variants in *COL5A1*, null alleles for *COL5A2* have not been described as such.

Most pathogenic variants found in *COL3A1* are heterozygous missense substitutions affecting one of the glycine residues of the Gly-X-Y repeat within the triple helical region of type III collagen. These missense substitutions, and more rarely, heterozygous splice-site variants leading to in-frame exon-skipping, are thought to act as dominant negative variants. Pathogenic variants resulting in frameshifts and nonsense likely result in functional haploinsufficiency, whereas whole gene deletions result in true haploinsufficiency. In vEDS the severity of the phenotype seems to be related to the type of variant; those resulting in a null allele seem to be milder with later onset of symptoms (Byers et al., 1993; Meienberg et al., 2010; Schuelke et al., 2004; Frank et al., 2019); (Pepin et al., 2014). However, the other described family with a contiguous deletion had serious vascular events at relatively young age.

Pregnancy in vEDS should be approached with consideration because of the relatively high reported risks of mortality and morbidity. A mortality rate up to 6% in a large series of pregnancies in vEDS has been reported, as well as life-threatening complications such as arterial dissection/rupture, uterine rupture, and other pregnancy-related complications such as lacerations and preterm delivery (Murray et al., 2014). A large series of 132 women with vEDS of whom 56% were at least pregnant once (160 pregnancies), reported on complications at delivery or the post-partum period in 36% of patients (Frank et al., 2015).

In *COL3A1* pathogenic variants leading to haploinsufficiency seem to have a more favourable outcome as compared to missense variants since in 565 deliveries of 256 women with pathogenic protein-altering pathogenic variants 30 pregnancy-related deaths were reported but none in 27 women (51 pregnancies) with pathogenic variants leading to a null allele.

In our 3 pregnant patients, no complications were observed. Imaging results were normal, when performed, but this is probably not unusual since in the majority of vEDS patients with COL3A1 ″structural" variants, arterial aneurysms were not found (Kerwin et al., 2008).

Whether the relative mild presentation in our probands is related to their genotype remains to be proven. The number of reports on phenotype in *COL3A1* haploinsufficiency is relatively small. This might imply that in the majority of haploinsufficient patients symptoms are so mild that they do not come to medical attention (reduced penetrance). On the other hand in the family reported by Meienberg et al. (2010) with a 3.4-Mb deletion encompassing *COL3A1*, the phenotype was quite severe with aortic dissections occurring at a relatively young age. Maybe, the age of our probands was relatively young and an event may develop over time.

Either way it is likely that also in *COL3A1* variants leading to haploinsufficiency phenotypic severity has a spectrum with milder presentations on one side, but with maybe rare but serious outliers at the severe end of the spectrum.

According to VASCERN guidelines (https://vascern.eu/what-we-do/dos-donts-factsheets-for-rare-vascular-disease-patients/) the 3 pregnant women delivered by caesarean section. The mode of delivery deserves a personal approach as well, since both a C-section as well as vaginal delivery can be complicated; hemorrhage risk of 33% has been described for C-section, and the occurrence of perineal tears is associated with vaginal delivery (Frank et al., 2015). Caesarean delivery cannot always prevent uterine rupture or (fatal) vascular complications since both events can occur during pregnancy, labor at term, and after caesarean delivery (Murray et al., 2014).

In our reported pregnant women, no vEDS related (vascular) complications were present before during and after pregnancy.

If, indeed, pregnancy does not alter survival of affected women beyond the underlying effects of vEDS itself and since haploinsufficient patients seem to have the mildest phenotype and because C-section itself can have complications as well, it might be that not all vEDS patients should be advised against vaginal delivery. However, the decision to support vaginal delivery in women with *COL3A1* variants leading to haploinsufficiency should not solely be based upon the fortunate history of our described cases, but also the patients history, phenotype, family history and the patient's and doctor's preferences and hospital facilities should be taken into account.

In conclusion, we described 3 patients with a contiguous deletion of varying size encompassing the *COL3A1* and *COL5A2* gene. Although in general, a definite diagnosis of EDS for all subtypes but hypermobile EDS, relies on molecular confirmation of a clinically suspected diagnosis, the deletion was found coincidental and due to the mild phenotype a diagnosis of EDS was not suspected (or considered). All three pregnancies were without major complications which invites us to consider whether patients with *COL3A1* variants leading to haploinsufficiency require a different, less defensive, approach to pregnancy/delivery.

CRediT authorship contribution statement

Marlies JE. Kempers: Conceptualization, Methodology, Data collection, Writing – original draft. **Marja Wessels:** Data collection, Writing – review & editing. **An Van Berendoncks:** Data collection, Writing – review & editing. **Ingrid MBH. van de Laar:** Data collection, Writing – review & editing. **Nicole de Leeuw:** Data curation, Resources, Writing – review & editing. **Bart Loeys:** Conceptualization, Data collection, Writing – review & editing, Supervision.

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