Severe Phenotype of Cutis Laxa Type 1B with Antenatal Signs due to a Novel Homozygous Nonsense Mutation in *EFEMP2*

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Established Facts

- Autosomal recessive cutis laxa type 1B (ARCL1B) is a rare multisystem disease affecting skin, skeleton, and vascular structures. Severity of the disease ranges from perinatal death to manifestations compatible with survival.
- ARCL1B is due to mutations in the *EFEMP2* gene (alias *FBLN4*). To date, only a few postnatal cases have been described.

Novel Insights

- The 2 related patients described here are the first prenatal presentations of ARCL1B. They have a particularly severe phenotype, leading to termination of pregnancy.
- We give a detailed histology of arterial tortuosity.
- The c.639C>A mutation (p.Cys213*) is the first nonsense mutation reported in *EFEMP2*.

Keywords

Arterial tortuosity · Autopsy · Autosomal recessive cutis laxa type 1B · Bone fractures · *EFEMP2* · Elastic tissue · *FBLN4* · Fetus · Fibulin-4 · Prenatal diagnosis

Abstract

EFEMP2 mutations are known to be responsible for autosomal recessive cutis laxa type 1B (ARCL1B), a rare multisystem disease affecting skin, skeleton, and vascular structures. We report 2 additional related cases of ARCL1B of particular severity leading to termination of pregnancy. Cardinal signs of this connective tissue disease were already seen during the

second trimester of pregnancy, then confirmed and clarified at autopsy. Anomalies included cutis laxa, arachnodactyly, clubfoot, wormian bones, moderate bowing of long bones with slender bone trabeculae, rib fractures, undermuscularized diaphragm, hiatal hernia, and arterial tortuosity with thick vascular walls and disorganized elastic fibers. Sequencing of the *EFEMP2* gene revealed a novel homozygous nonsense mutation: c.639C>A (p.Cys213*). We performed a thorough histological analysis and discuss differential diagnoses, genotype-phenotype correlations, and the challenge of prenatal diagnosis of this disease.

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Mutations in the *EFEMP2* (alias *FBLN4*, OMIM 604633) gene, located on chromosome 11q13.1, which encodes the EGF-containing fibulin-like extracellular matrix protein 2 (also named fibulin-4 protein) lead to autosomal recessive cutis laxa type 1B (ARCL1B, OMIM 614437). This rare disease is characterized by cutis laxa, arterial involvement (arterial tortuosity, aneurysms, or stenosis), respiratory involvement (emphysema, diaphragmatic hernia, or hypoplasia), craniofacial involvement (midface retrusion, retrognathia, widely spaced eyes, and high palate), and other features of connective tissue disorders (e.g., joint laxity and arachnodactyly). The *EFEMP2* protein seems to play an important role in arterial elastic fibers assembly and maturation of smooth

muscle cells in the aortic wall [Huang et al., 2009]. In the literature, 46 patients are described with *EFEMP2*-related cutis laxa [Huchtagowder et al., 2006; Dasouki et al., 2007; Hoyer et al., 2009; Renard et al., 2010; Erickson et al., 2012; Iascone et al., 2012; Kappanayil et al., 2012; Sawyer et al., 2013; Hebson et al., 2014; Rajeshkannan et al., 2014], and 9 Arabic patients only present with arterial manifestations; cutis laxa was not found [Al-Hassnan et al., 2012]. Severity of the disease ranges from perinatal death to manifestations compatible with survival. Even within sibships, clinical heterogeneous presentation was described [Sawyer et al., 2013]. Among previously published patients, disease manifestations were not seen in antenatal period, and diagnosis could not be suspected. In a few cases, literature reports nonspecific features such as oligohydramnios or overgrowth during pregnancy [Huchtagowder et al., 2006; Hoyer et al., 2009; Erickson et al., 2012].

This report describes 2 sibs: 1 fetus with *EFEMP2*-related cutis laxa and specific antenatal disease manifestations leading to termination of pregnancy and 1 fetus with early molecular prenatal diagnosis.

Patients and Results

Patient 1

A male fetus weighing 1,004 g was examined at autopsy. He was the first conceptus of a 23-year-old Romanian

Fig. 1. Radiological and macroscopic features of patient 1. Note **A, E** excess of soft tissue (arrow in **A**); **B, E** clenched hand; **C, E** clubfoot; **D, F** moderate bowing of long bones (femur shown in **D**); **F** rib fractures indicated by arrows; **G, H** tortuosity and sinuosity of aorta and coronary arteries and elongated ductus arteriosus (arrows).

Fig. 2. Histological features of patient 1. For bone examination, note slender trabeculae (A) compared to a control (B), and wormian bones (C) compared to a control (D). E For arterial examination, note thickened arterial walls (mediastinal vessel with hematoxylin and eosin stain) compared to a control (F), and G elastic fiber irregularity and disorganization, indicated by arrows (mediastinal vessel with orcein stain), compared to a control (H). Scale bars, 100 μ m.

woman and her 33-year-old Romanian husband. No consanguinity was reported. Early pregnancy was unremarkable. Ultrasonographic examinations at 18 and 23 weeks of gestation (WG) revealed excess of soft tissue, clenched hands, clubfoot, cranial vault deformation, bowing of long bones, atrial dilatation, tortuosity of the aorta and umbilical arteries, suspicion of pulmonary arteriovenous fistula, and suspected left-sided diaphragmatic hernia (Fig. 1A–D). Because of these features, amniocentesis was performed. The prenatal karyotype and BACs-on-Beads (BoBs)TM were normal. Pregnancy was terminated at 24 WG.

On external examination, the fetus was slender with multiple congenital anomalies. We observed cutis laxa, arachnodactyly, clinodactyly, camptodactyly, clubfoot, and micrognathia (Fig. 1E).

X-ray examination revealed general demineralization, wormian bones, moderate bowing of long bones, and rib fractures of different ages with bony calluses (Fig. 1F).

Autopsy confirmed diaphragm anomalies with under-muscularization and hiatal hernia. Pyelum of kidneys and ureters were dilated. The aorta, coronary, and umbilical arteries were tortuous and dilated, whereas heart architecture was normal. Ductus arteriosus was elongated (Fig. 1G, H). Histological examination showed a fragmentation of skull bones (wormian bones), femur with fewer and rather slender bone trabeculae (Fig. 2A–D), and arteries with very thick walls containing many smooth muscle cells as well as fragmented and disorganized elastic fibers (Fig. 2E–H), compared with age-matched controls.

Taking into consideration the various anatomical, radiological and histopathological findings, the diagnosis of EFEMP2-related cutis laxa was suspected, and molecular testing was performed. Genomic DNA was extracted from fetal frozen lung. Mutation analysis of *EFEMP2* (NM_016938.4) revealed homozygosity for a nonsense mutation in exon 7: c.639C>A, leading to p.Cys213* in the EFEMP2 protein (ENST00000307998.10). Both parents were heterozygous for this mutation (Fig. 3A). For their second pregnancy, parents were lost to follow-up, and a boy was born at term without problems. Results for patient 1 were announced to the parents after this birth.

Patient 2

For the third pregnancy of this couple, an ultrasound-guided chorionic villi biopsy was performed at 10 WG to carry out early prenatal diagnosis. Molecular analysis showed the homozygous c.639C>A mutation in the fetus and pregnancy was terminated at 16 WG. Ultrasonographic examination performed at 11 WG was normal. Autopsy was authorized by the parents and results are similar to the first sibling: slender male fetus with arachnodactyly, defect of ossification of the cranial vault, very elongated ductus arteriosus, and tortuous aorta and coronary arteries (Fig. 4). Vascular anomalies at histological analysis were also present in this patient.

Discussion

Clinical Spectrum of ARCL1B

ARCL1B is a rare entity with rather limited description in the literature. Thus, the full range of the severity of the clinical spectrum is still unknown. Here, we report 2 cases with clinical features consistent with ARCL1B but with

Fig. 4. Clinical and radiological features of patient 2. Note long trunk (**A**), crane vault deformation, slight bowing of long bones (**B**), tortuosity and/or sinuosity of aorta, coronary and umbilical arteries, and elongated ductus arteriosus (**C–E**).

severe phenotype. Diagnosis was not suspected in ante-natal period because of its rarity, but major features were present in patient 1. First of all, cutis laxa was described as “soft tissue outgrowth” by sonographers; it is difficult to detect during pregnancy and requires a trained eye.

Secondly, arterial tortuosity appeared as “varicose veins” in the umbilicus. Additionally, mediastinal vessels were termed as “abnormal”. Thirdly, respiratory involvement is usually a major criterion, including emphysema, diaphragmatic hernia or hypoplasia. Emphysema cannot be

Fig. 5. Kaplan-Meier survival analysis curve calculated on 26 patients with *EFEMP2* mutations and available data about survival. Survival time is given in months. p value <0.0001 ; $\chi^2 = 18.4$ with log-rank test.

Table 1. Primers used to evaluate expression of *EFEMP2* in qPCR

Gene	Forward	Reverse
<i>EFEMP2.1</i>	TTCTGAAGAGCCCGACAGCTAC	TGGTCAGACACTCGTTGACATCC
<i>EFEMP2.2</i>	GTGCCTACAATGCCTTTTCAGATCC	GCGCTGACGTTGTTGATTTGCC
<i>B2M</i>	TGCTGTCTCCATGTTTGATGTATCT	TCTCTGCTCCCCACCTCTAAGT
<i>SDHA</i>	TGGGAACAAGAGGGCATCTG	CCACCACTGCATCAAATTCATG
<i>YWHAZ</i>	ACTTTTGGTACATTGTGGCTTCAA	CCGCCAGGACAAACCAGTAT

Two different primer pairs were developed to check *EFEMP2* expression. *EFEMP2* transcription levels were normalized by 3 stably expressed reference genes (*B2M*, *SDHA*, and *YWHAZ*).

seen before birth. In our case, ultrasonographic examination suspected a left-sided diaphragmatic hernia which was actually a hiatal hernia. In addition, visceral examination revealed an undermuscularized diaphragm. Moreover, the craniofacial involvement with midface hypoplasia, retrognathia, ocular hypertelorism, and high-arched palate was not easily accessible to prenatal diagnosis.

Differential Diagnoses of *ARCL1B*

There are other syndromes that could be suspected with the features mentioned. The most important differential diagnoses include Loeys-Dietz syndrome (LDS) and arterial tortuosity syndrome (ATS). LDS is an autosomal dominant disorder most commonly caused by *TGFBR1* and *TGFBR2* mutations, whereas ATS is inherited as an autosomal recessive trait with mutations in the *SLC2A10* gene. LDS is characterized by arterial tortuosity and aneurysms, hypertelorism, and cleft palate or bifid uvula. The skin can be translucent and velvety. ATS features include elongation, tortuosity and aneurysms of

major arteries, cutis laxa, joint laxity, hernias, long slender face, and high-arched palate. In a few cases of LDS with *TGFBR1* or *TGFBR2* missense mutations, ultrasound prenatal findings have been reported [Valenzuela et al., 2017]. These findings include cardiologic and/or musculoskeletal involvement (pulmonary artery aneurysm, aortic aneurysm, and/or arthrogryposis) without fatal outcome. For ATS, only 1 case with antenatal signs (abnormal elongation of the pulmonary arteries, tortuous and elongated abdominal aorta, oligohydramnios, hypertelorism, and scrotal mass) has been suspected [Aslan et al., 2008]. At birth, the infant had severe hypotonia, inguinal hernia, keratoconus, skin and joint laxity, and died in the early neonatal period due to respiratory failure. Autopsy was refused by the parents, and no molecular diagnosis was performed.

Skeletal Involvement in *ARCL1B*

Skeletal involvement is not a cardinal sign of *ARCL1B*, but is present in our 2 related cases and reported 3 times

in the literature: one patient with multiple fractures at birth [Huchtagowder et al., 2006], a stillborn baby with fibular fracture, bowing long bones and soft relocatable cranial bones [Hoyer et al., 2009], and monozygotic twins with multiple fractures and wormian bones at birth [Erickson et al., 2012]. Interestingly, the diagnosis of osteogenesis imperfecta type IIC was initially suspected for these twins. A possible link between EFEMP2 and bone development could be lysyl-oxidase, an enzyme that interacts with EFEMP2 and plays a pivotal role in cross-linking of collagen and elastin [Horiguchi et al., 2009; Sasaki et al., 2016].

Genotype-Phenotype Correlations

Among patients with ARCL1B described in the literature, the report of the twin patients with a homozygous truncating mutation c.85del (p.Asp29Ilefs*31) [Erickson et al., 2012] is very reminiscent to the present fetus with truncating mutation c.639C>A (p.Cys213*). The twins had a severe phenotype and died a few minutes after birth (delivery term at 31 WG). Postmortem features included cutis laxa, hypoplastic right diaphragm, arterial tortuosity, small lungs with atelectasis, hypertelorism, and multiple fractures. Unfortunately, prenatal care and ultrasound examinations were limited, and no antenatal sign, except oligohydramnios, had been described.

Sequencing of cDNA obtained from RNA extracted from frozen lung tissue of patient 1 revealed a c.639C>A (p.Cys213*) nonsense mutation. This nucleotide variation is a newly reported EFEMP2 mutation and the first EFEMP2 nonsense mutation (Fig. 3B). Because this mutation results in a premature stop codon, which usually leads to nonsense-mediated mRNA decay, it was not expected to observe the mutant sequence. Therefore, we hypothesized that the mutant mRNA is expressed and partially escapes nonsense-mediated mRNA decay. Using quantitative RT-PCR analysis, we were able to observe a

decreased expression of EFEMP2 in patient 1 with a residual expression of about 10% (Fig. 3C). Primers used for this experiment are shown in Table 1.

We performed Kaplan-Meier survival analysis for patients born alive comparing 2 groups: patients with 2 missense mutations (first group, $n = 22$) and patients with at least 1 truncating mutation (second group, $n = 4$). Using log-rank test, we showed that the second group has a shorter median survival compared to the first group of patients (9.45 months vs. 36 months; $p < 0.0001$) (Fig. 5) confirming the severity of EFEMP2 truncating mutations.

Conclusion

To the best of our knowledge, we report the first case of prenatal presentation of ARCL1B and the first EFEMP2 nonsense mutation. We enlarged the clinical spectrum of ARCL1B with a particularly severe phenotype. However, antenatal diagnosis of ARCL1B remains difficult because this multisystem disease is not well-known, and the antenatal signs are not usually as severe as those described here. Unfortunately, differential diagnoses and molecular analyses of such connective tissue disorders are usually not possible during the time allowed in pregnancy.

Statement of Ethics

Informed consent for DNA analysis was given by the parents according to the french ethical standards.

Disclosure Statement

The authors declare no conflicts of interest.

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