Recessive multiple epiphyseal dysplasia – Clinical characteristics caused by rare compound heterozygous SLC26A2 genotypes

Mehran Kausar\textsuperscript{a,b}, Riikka E. Mäkitie\textsuperscript{b}, Sanna Toiviainen-Salo\textsuperscript{c}, Jaakko Ignatius\textsuperscript{d}, Mariam Aneesa,\textsuperscript{e} Outi Mäkitie\textsuperscript{b,e,f,g,*}  
\textsuperscript{a}Department of Biochemistry, Quaid-i-Azam University, Islamabad, Pakistan 
\textsuperscript{b}Folkhälsan Institute of Genetics and University of Helsinki, Helsinki, Finland 
\textsuperscript{c}Department of Pediatric Radiology, HUS Medical Imaging Centre, University of Helsinki and Helsinki University Hospital, Helsinki, Finland 
\textsuperscript{d}Department of Clinical Genetics, University of Turku and Turku University Hospital, Turku, Finland 
\textsuperscript{e}Children's Hospital, University of Helsinki and Helsinki University Hospital, Helsinki, Finland 
\textsuperscript{f}Department of Molecular Medicine and Surgery and Center for Molecular Medicine, Karolinska Institutet, Stockholm, Sweden 
\textsuperscript{g}Department of Clinical Genetics, Karolinska University Hospital, Stockholm, Sweden

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ABSTRACT  
Pathogenic sequence variants in the solute carrier family 26 member 2 (SLC26A2) gene result in lethal (achondrogenesis Ib and atelosteogenesis II) and non-lethal (diastrophic dysplasia and recessive multiple epiphyseal dysplasia, rMED) chondrodysplasias. We report on two new patients with rMED and very rare compound heterozygous mutation combinations in non-consanguineous families. Patient I presented in childhood with waddling gait and joint stiffness. Radiographs showed epiphyseal changes, bilateral coxa plana–deformity and knee valgus deformity, for which he underwent surgeries. At present 33 years his height is 165 cm. Patient II presented with cleft palate, small jaw, short limbs, underdeveloped thumbs and on radiographs, cervical kyphosis with an underdeveloped C4. He also developed severe scoliosis but has grown at \(-2.9\) SD curve. Molecular analysis revealed that patient I is heterozygous for two known pathogenic variants in SLC26A2, a splice site variant c.-26+2T > C and a missense variant c.1957T > A (p.Cys653Ser), while patient II is compound heterozygous for missense variants c.835C > T (p.Arg279Trp) and c.1535C > A (p.Thr512Lys). These patients further elucidate the variability of the phenotypic and genetic presentations of rMED.

1. Introduction  
Autosomal recessive multiple epiphyseal dysplasia (rMED; OMIM \#226900) is a rare and relatively mild form of chondrodysplasia caused by mutations in SLC26A2 (OMIM 606718) (Superti-Furga et al., 1999) (Mäkitie et al., 2003). Solute carrier family 26 member 2 (SLC26A2) is a transmembrane sulfate carrier protein that partakes in sulfation of proteoglycans by transporting sulfate into the cartilage matrix. Mutations in SLC26A2 result in inappropriately constructed cartilage matrix; undersulfated proteoglycans hamper bone development especially at long bones epiphyses, large joints, and metacarpals and metatarsals. To date, 51 different SLC26A2 mutations have been reported to associate with four chondrodysplasias: achondrogenesis Ib, atelosteogenesis II, diastrophic dysplasia (DTD) and rMED. The most frequent SLC26A2 mutation in the Finnish population, where DTD is exceptionally common, is c.-26+2T > C, also known as the Finnish founder mutation (Hastbacka et al., 1999). In other populations, the most prevalent mutation is c.862C > T (p.Arg279Trp) (Syvanen et al., 2013). The phenotype of rMED varies according to the type of SLC26A2 mutation (Czarny-Ratajczak et al., 2010). The main clinical features include normal to mildly short stature, joint contractures, clubfeet, shortening of limbs and waddling gait; radiographs show epiphyseal changes, bilateral coxa plana–deformity and knee valgus deformity, for which he underwent surgeries. At present 33 years his height is 165 cm. Patient II presented with cleft palate, small jaw, short limbs, underdeveloped thumbs and on radiographs, cervical kyphosis with an underdeveloped C4. He also developed severe scoliosis but has grown at \(-2.9\) SD curve. Molecular analysis revealed that patient I is heterozygous for two known pathogenic variants in SLC26A2, a splice site variant c.-26+2T > C and a missense variant c.1957T > A (p.Cys653Ser), while patient II is compound heterozygous for missense variants c.835C > T (p.Arg279Trp) and c.1535C > A (p.Thr512Lys). These patients further elucidate the variability of the phenotypic and genetic presentations of rMED.

* Corresponding author. Folkhälsan Institute of Genetics, P.O.Box 63, FIN-00014, University of Helsinki, Finland.  
E-mail address: outi.makitie@helsinki.fi (O. Mäkitie).

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2. Clinical report

This retrospective study is part of an ongoing research program on epidemiological, clinical and genetic characteristics of skeletal dysplasia in Finland, carried out at Children's Hospital, Helsinki University Hospital, Finland. The protocol was approved by the Institutional Research Ethics Committee. The two patients had been followed at various clinics at Helsinki University Hospital (Patient I) and Turku University Hospital (Patient II) and were referred to the Clinical Genetics Department, Helsinki University Hospital in 2017 for further evaluation.

The patients' data from hospital records were reviewed for clinical presentation, disease progression, anthropometry and radiographic skeletal manifestations. Growth data were compared with Finnish growth standards. Genetic studies were performed from peripheral blood genomic DNA; for patient I, a gene panel including SLC26A2 was performed at Blueprint Genetics (Helsinki, Finland) and the identified SLC26A2 variants were confirmed by Sanger sequencing. For patient II, SLC26A2 was screened at HUSLAB (Helsinki, Finland) for two Finnish major mutations and subsequently for all exons.

2.1. Patient I

This presently 33-year-old man was born at full term to non-consanguineous parents who were healthy and normal in height. The birth measurements were normal: length 49 cm (−1.2 standard deviation units, SDS), weight 3845 g (+0.3 SDS), and head circumference 36.5 cm (+0.8 SDS). He was treated for bilateral hip dysplasia after birth. He walked at 12 months but due to abnormal gait, he underwent clinical investigations at 2 years. He was noticed to have diffuse skeletal epiphyseal changes that were most prominent in his lower limbs; he had bilateral coxa plana deformity and his knees were in valgus position. Despite normal spine, he was diagnosed with spondylo-epiphyseal dysplasia; no molecular diagnosis was performed. During childhood he underwent surgeries to correct his knee valgus deformity and hip and knee surgeries are presently considered because of joint pain. Other joints and the spine are less severely affected and only exhibit mild stiffness and restricted range of motion. His hands and feet are slightly wide and stubby and both fourth metacarpals and metatarsals are short. There is no clubfoot deformity. He has no apparent facial dysmorphism, extra-skeletal abnormalities or dental problems, and has normal vision, hearing and intelligence. He has no scoliosis and has reached an adult height of 165 cm (−2.5 SDS).

Radiographs between 6 and 33 years of age were reviewed (Fig. 1). At 6 years, he had flat crescent-shaped femoral epiphyses, abnormally broad and short femoral necks, and a dysplastic right acetabulum. Epiphyses and metaphyses of the knees were abnormal. The double layer patellae were laterally displaced. All metacarpals, especially the fourth, were short. Epiphyses of both radius and ulnae were slightly flat. Lumbar vertebrae were of normal height and shape. At 18 years he had developed bilateral severe hip arthritis and the right femoral head was cranially dislocated. At 33 years the patient had marked arthritis in both hips and knees.

2.2. Patient II

This presently 5-year-old boy was born by elective Cesarian section at 38 + 6 gestational weeks from breech position measuring 47 cm (−1.9 SDS) and 3230 g (−0.8 SDS); his head circumference was 35.5 cm (+0.3 SDS). The parents were non-consanguineous, healthy and normal in height. Routine ultrasounds during pregnancy showed normal morphology and growth. Immediately after birth, he was noticed to have a wide cleft palate exposing inferior nasal structures, small jaw suggesting Pierre Robin–sequence, overall short limbs and length, spontaneous palmar ulnar deviation and undeveloped thumbs (Fig. 2). Ultrasound showed femoral heads to be abnormal in shape. Cervical radiographs indicated kyphosis with an underdeveloped C4. Cleft palate was closed at 1 year and his jaw has grown spontaneously. He developed severe progressive scoliosis during the second year and was treated with casting and a corset until present age.

Presently, at 5 years, his height is 98 cm (−3.0 SDS) and weight 19 kg (0 SDS) (Fig. 2). He has developed significant valgus deformity at knees. His ankles are stiff but there is no clubfoot deformity. Joint stiffness is also present in upper limbs and he has contractures in both third fingers. Both earlobes are slightly thick and low set. His head circumference is normal (−0.5 SDS).

On radiographs, at age 2 days, both upper and lower limbs showed broad metaphyses and elbow contractures (Fig. 3). Cervical kyphosis with underdeveloped fourth cervical vertebrae was present at 3 months but was mostly resolved spontaneously by 5 years. Thoracic and lumbar vertebrae were of normal shape but there was significant scoliosis. Lower extremities at 5 years showed bilateral coxa vara with abnormally broad and short femoral necks and flat epiphyses, and both knees were in valgus position. Knee MRI demonstrated bilateral laterally displaced double layer patellae.

2.3. Genetic findings

Patient I had been previously diagnosed as having spondyloepiphyseal dysplasia but as the characteristics were atypical for COL2A1-related skeletal dysplasia, a bone dysplasia gene panel was performed at Blueprint Genetics, which indicated heterozygosity for two known SLC26A2 pathogenic variants: c.26 + 2T > C (rs386833492) and c.1957T > A (p.Cys653Ser, rs104893924) (Fig. 4). The former is a well-known disease-causing splice donor site mutation and recognized as the Finnish founder mutation for DTD (Hastbacka et al., 1999). The latter is a missense variant with pathogenic predictions by SIFT and PolyPhen and a rather low allele frequency in databases. Homozygous p.Cys653Ser has previously been described in relatively mild rMED (Mäkitie et al., 2003). The parents were healthy and were not available for genetic testing.

Genetic testing for Patient II for suspected SLC26A2-related disease was performed in the first days postpartum at HUSLAB (Helsinki, Finland) for the Finnish major mutations and subsequently for the whole SLC26A2 gene. The patient was found to harbor two heterozygous missense mutations in SLC26A2 (NM_000112.3): the common variant c.835C > T (p.Arg279Trp, rs104893915; paternally inherited) and a rare mutation c.1535C > A (p.Thr512Lys, rs121908078; maternally inherited), which has only been found in the Finnish population (submitted to LOVD, accession number 00181232) (Fig. 4). His clinical characteristics, particularly his mildly short stature, and genetic findings were not typical for DTD and his diagnosis was regarded as rMED or variant of DTD.

3. Discussion

We describe two patients with unusual combinations of SLC26A2 mutations leading to an rMED phenotype. These patients elucidate the genetic and clinical variability of this entity and further underscore the overlap between rMED and DTD. Further, as seen in Patient II, some of these manifestations may be severe and require prompt treatment (e.g. severe cervical kyphosis, early-childhood onset scoliosis) and therefore careful follow up of these patients is indicated.

The SLC26A2 gene comprises three exons and encodes a 739 amino acid transmembrane sulfate carrier protein (SLC26A2) (Hastbacka et al., 1994). Pathogenic sequence variants in SLC26A2 cause various forms of recessive chondrodysplasias with mild to severe phenotypes including achondrogenesis type 1B (OMIM 600972), atelosteogenesis type II (OMIM 256050), diastrophic dysplasia (OMIM 222600) and recessive MED (OMIM 226900). There are S1 SLC26A2 mutations listed in the Human Gene Mutation Database professional (www.hgmd.cf.ac.uk): 29 are missense mutations, three nonsense mutations, two splice...
site mutations and 16 frameshift mutations. ClinVar (www.ncbi.nlm.nih.gov/clinvar) reports 58 SLC26A2 variants that are classified as pathogenic or likely pathogenic. Several of these mutations have been reported as causing rMED (Table 1), the most common genotype in rMED being homozygosity for c.835C > T (p.Arg279Trp) (Bonafe et al., 2002; Barbosa et al., 2011). The phenotypic severity can be correlated with the residual activity of the sulfate transporters resulting from different mutant alleles (Rossi and Superti-Furga, 2001; Syvanen et al., 2013).

A previous report indicated that patients with rMED may have similar cervical kyphosis as seen in DTD (Mäkitie et al., 2015). This was also seen in our Patient II. Here we further report the presence of severe...
scoliosis in a patient with rMED. Furthermore, the patient also had a very small jaw with cleft palate, reminiscent of Pierre Robin–sequence, which was also considered as his diagnosis. In 2013, Zechi-Ceide et al. reported on two siblings with compound heterozygosity for SLC26A2 mutations p.Arg279Trp and the Finnish founder mutation (c.-26+2T > C) and Robin sequence, and a phenotype intermediate between DTD and rMED (Zechi-Ceide et al., 2013). Interestingly, in our patient the disproportionately small jaw has normalized by age 5 years.

Our Patient I had received a diagnosis of spondylo-epiphyseal dysplasia, which is an autosomal dominant disease with a 50% risk of recurrence in the offspring. Careful clinical and radiographic review, combined with genetic testing, confirmed the correct diagnosis to be rMED, with remarkably lower inheritance risk even in the Finnish population with higher than normal carrier frequency for SLC26A2 mutations.

In summary, our report further elucidates the phenotypic and genotypic variability of rMED. The severe manifestations in one of our patients highlight the need for careful clinical follow up of patients with dysplasia, which is an autosomal dominant disease with a 50% risk of recurrence in the offspring. Careful clinical and radiographic review, combined with genetic testing, confirmed the correct diagnosis to be rMED, with remarkably lower inheritance risk even in the Finnish population with higher than normal carrier frequency for SLC26A2 mutations.

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### Table 1
List of known mutations in SLC26A2 causing rMED.

<table>
<thead>
<tr>
<th>Allele A</th>
<th>Allele B</th>
<th>Phenotype</th>
<th>Reported by</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.862C &gt; T</td>
<td>p.Arg279Trp</td>
<td>c.862C &gt; T</td>
<td>p.Arg279Trp</td>
</tr>
<tr>
<td>c.-26 + 2T &gt; C</td>
<td>IVS1 + 2T</td>
<td>c.794T &gt; C</td>
<td>p.Phe256Ser</td>
</tr>
<tr>
<td>c.-26 + 2T &gt; C</td>
<td>IVS1 + 2T</td>
<td>c.1984T &gt; A</td>
<td>p.Cys653Ser</td>
</tr>
<tr>
<td>c.862C &gt; T</td>
<td>p.Arg279Trp</td>
<td>c.1562C &gt; A</td>
<td>p.Thr512Lys</td>
</tr>
<tr>
<td>c.-26 + 2T &gt; C</td>
<td>IVS1 + 2T</td>
<td>c.1562C &gt; A</td>
<td>p.Thr512Lys</td>
</tr>
<tr>
<td>c.835C &gt; T</td>
<td>p.Arg279Trp</td>
<td>c.1565C &gt; T</td>
<td>p.Ser522Phe</td>
</tr>
<tr>
<td>c.-26 + 2T &gt; C</td>
<td>IVS1 + 2T</td>
<td>c.1957aT &gt; A</td>
<td>p.Cys653Ser</td>
</tr>
<tr>
<td>c.-26 + 2T &gt; C</td>
<td>IVS1 + 2T</td>
<td>c.1957aT &gt; A</td>
<td>p.Cys653Ser</td>
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<td>c.835C &gt; T</td>
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</table>

* NM_000112.3 (Reference sequence differ from older version).*

rMED. Furthermore, we emphasize the importance of correct molecular diagnosis in skeletal dysplasias as this has a major impact on genetic counseling.

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### Conflicts of interest
All authors declare no conflicts of interest.

### Appendix A. Supplementary data
Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmg.2018.11.007.

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