Rcessive multiple epiphyseal dysplasia (rMED): phenotype delineation in eighteen homozygotes for $\text{DTDST}$ mutation R279W

D Ballhausen, L Bonafé, P Terhal, S L Unger, G Bellus, M Classen, B C Hamel, J Spranger, B Zabel, D H Cohn, W G Cole, J T Hecht, A Superti-Furga

**LETTER TO JMG**

**M**ultiple epiphyseal dysplasia (MED) is a generalised skeletal dysplasia that although relatively mild is associated with significant morbidity. Joint pain, joint deformity, waddling gait, and short stature are the main clinical signs and symptoms. In the past, the disorder was subdivided into the milder Ribbing type, usually with flattened epiphyses, and the more severe Fairbank type with round epiphyses, but many cases were not classifiable as clearly either type. MED can be caused by mutations in at least six separate genes: COMP, collagen IX ($\text{COL9A1}$, $\text{COL9A2}$, and $\text{COL9A3}$), matrilin 3 ($\text{MATN3}$), and the sulphate transporter, DTDST ($\text{DTDST/SLC26A2}$). We have previously reported an adult with a recessively inherited form of MED (rMED) characterised by club feet, double layered patellae, and normal stature, who was homozygous for the mutation 862c>t/R279W in the $\text{DTDST}$ gene, previously associated with the achondrogenesis 1B-atelosteogenesis 2-diastrophic dysplasia spectrum. We now report on a group of 18 subjects who are homozygous for this point mutation, allowing a comprehensive assessment of this particular MED phenotype. Distinction of rMED is important because of its recessive inheritance (unlike other MED types) and genetic counselling implications. The frequency of the R279W mutation and the number of subjects with molecularly proven rMED identified since its description suggest that rMED may be more common than hitherto assumed.

**MATERIAL AND METHODS**

Blood or genomic DNA was sent to the Zurich centre for $\text{DTDST}$ mutation analysis because of clinical and radiographic signs similar to the reported case of rMED, or because of a clinical diagnosis of MED and negative mutation analysis of COMP or collagen IX genes. Genomic DNA was extracted from blood leucocytes using standard protocols and was subjected to $\text{DTDST}$ mutation analysis. The fragment of interest, that is, the 5′ part of exon 3, was amplified according to the procedure previously described. The PCR fragment was digested with the restriction endonuclease StyI. Results were visualised on agarose gels in the presence of positive and negative controls.

As the nucleotide change 862c>t (leading to amino acid substitution R279W) creates a new restriction site for StyI, heterozygous and homozygous patients can be distinguished from wild type homozygous subjects. In all subjects with a positive StyI digestion, the genotype was confirmed by direct sequencing of the fragment amplified in a second PCR. The ABI Prism Big Dye Terminator Ready Reaction Kit and protocol were used for sequencing in an ABI Prism 310 Genetic Analyzer. In all subjects who were not homozygous for R279W, a complete $\text{DTDST}$ mutation screening was performed to test for the presence of other mutations.

When R279W homozygosity was identified, the referring physician was asked to participate in this study by sending photographs and radiographs, as well as by completing a questionnaire about family and personal history, symptoms leading to diagnosis, clinical course, present complaints, and anthropometric data. Informed consent for use of personal clinical and radiographic data in this study was obtained from all subjects in compliance with local regulations of clinical centres. When possible, parents and sibs were also genotyped in order to confirm recessive inheritance.

**RESULTS**

A total of 21 patients from 15 families were found to be homozygous for the R279W mutation, including three sibs reported in 1995 by Deere et al as recessive MED unlinked to COMP, as well as the original case reported. For two subjects, the complete clinical information requested was not available; a third subject did not give permission to include his data in this study. Seven additional patients with clinical and radiographic signs compatible with rMED were found to carry two $\text{DTDST}$ mutations but were not homozygous for R279W; three were homozygous for mutation C653S and one each was observed with the genotypes G237V/R279W, N77H/R279W, and P160L/R279W.

**Key points**

- DTDST mutations have been identified in 28 subjects with recessive MED (rMED). Twenty-one were homozygous for R279W and seven had other genotypes.
- From the 21 R279W homozygotes, 18 were included in a retrospective data collection study including a questionnaire to the referring physician and radiograph review.
- Abnormal clinical findings were present at birth in only 8/18 subjects. The diagnosis of a skeletal dysplasia was made in late childhood or thereafter in the majority.
- Symptoms leading to diagnosis were chronic joint pain, mild deformity of the hands and feet, scoliosis, or joint contractures.
- The height of affected subjects ranged from slightly below the 3rd centile to the 90th centile for age throughout the age range. Short stature was not a frequent finding.
- The main radiographic findings were flat proximal femoral epiphyses, mild brachydactyly, and double layered patellae on lateral knee films.
- The frequency of the R279W mutation and the number of subjects with molecularly proven rMED identified since its description suggest that it may be more common than hitherto assumed.
R279W/"Finnish", and “Finnish”/C635S. The phenotype of these patients was similar in all respects to that of the R279W homozygote group (including a double layered patella in all three patients in whom lateral knee films were available), but we preferred to investigate a homogeneous group in order to estimate phenotypic variability given the shared DTDST mutation, and we did not include them in our detailed analysis. Thus, the final study sample consisted of 18 R279W homozygotes from 12 families.

**Gender and ethnic distribution**

Of the eighteen R279W homozygotes, nine were female and nine male (table 1). The age at evaluation varied from 9 to 49 years. All patients were of Europid origin, but there were no recognisable ethnic or geographical associations. This is in accordance with the lack of ethnic association of R279W in our earlier studies.

**Clinical findings at birth**

Abnormal findings at or shortly after birth were reported in eight out of 18 cases: 4/18 club foot, 1/18 cleft palate, 1/18 cystic ear swelling, 1/18 clinodactyly. None showed the “hitch-hiker” thumbs typical of diastrophic dysplasia. The diagnosis of a “skeletal dysplasia”, without further specification, had been made at birth in three out of 18 cases.

**Clinical course**

The majority of patients were diagnosed with a skeletal disorder in childhood. Reasons for seeking medical assistance were joint pain (8/18 cases), hand/foot deformities (6/18), affected sibs (2/18), scoliosis (1/18), genua vara (1/18), and fractures (1/18). The most frequently reported complaint was chronic joint pain in the hips, knees, wrists, and fingers. Limitation in joint movements was present in some subjects. A rheumatoid disorder was suspected in one case because of the combination of joint pain and fusiform finger swelling (fig 1C). Only one patient (patient 1 in table 1 and fig 2) had received a diagnosis of diastrophic dysplasia variant; this girl was one of the few patients whose stature was below the 3rd centile.

**Surgical interventions**

Patient 2 (table 1) had varisation osteotomies of both femoral necks performed at 8 years. Patient 6a had club foot surgery at ages 6, 8, and 13 years. Patient 8 had seven operations for club foot correction. Patient 9 had club foot surgery at ages 1, 2, 8, 9, and 13 years. None of the patients has undergone hip replacement so far.

**Height**

Fig 2 shows the height of individual patients plotted as age related centiles. Reduced stature (below –2 SD for age) was present in five out of 18 patients. There was no association between age and the height centile score, suggesting that in most cases there was no downward crossing of the growth curve during development. Median age related height was on the 10th centile, but two subjects were of above average height.

**Radiographic findings**

**Hands** (fig 3)

In contrast to other types of MED, skeletal maturation (bone age) is not delayed. There is an apparent dissociation between phalangeal epiphyses and the carpal
bones, with carpal bones showing rather advanced ossification as compared to bone age standards, leading to a reduced carpal width in patients around the age of 10 and older. The phalanges tend to be broad and short giving moderate brachydactyly. The phalangeal epiphyses tend to be small, simulating delayed bone development. Their shape was normal in most patients; a single patient had cone shaped epiphyses of the proximal phalanges (fig 3).

Pelvis (fig 4)
The most frequent and suggestive finding in the pelvic x rays was abnormal capital femoral epiphyses. In childhood there

Figure 1  [A] Patient 7 at 10 years of age. Her stature was at the 10th centile for age. There are bilateral genua valga and the fingers are moderately short, without hitch-hiker thumb. Her habitus is otherwise unremarkable and not suggestive of a skeletal dysplasia. The ears are not deformed. [B, C] Patient 2 at the age of 13 years. The face (B) is typical of DTD with a long and bulbous nose and distinct from the flat bridged nose seen in other skeletal dysplasias. Note the ears lacking the “cauliflower” deformity typical of diastrophic dysplasia. The hands (C) show slight contractures of the fingers as well as the “bracket” clinodactyly of digits 2 and 5 (ulnar deviation of 2 and radial deviation of 5) which is typical of, although not exclusive to, rMED. These changes, together with hip pain, had suggested a diagnosis of a rheumatoid disorder. There is no hitch-hiker thumb. The feet (C) show slight broadening of the space between the first and second toes but no pes adductus or frank club foot deformity.

Figure 2  Height of R279W homozygotes shown in relation to age related centiles (adult centiles for patients over 18 years of age).
may be epiphyseal flattening without frank dysplasia (fig 4A), but in subjects aged 10 or older the epiphyses are thin and crescent shaped. At the same time, the femoral neck tends to become broad and to assume a valgus position (fig 4B shows pronounced changes seen in patient 8 at the same age as that of (A)). These changes are rather characteristic of the earlier “Ribbing type” MED. In adult patients, where the thin epiphyses are no longer discernible, the femoral neck appears short.

Knees (fig 5)
A lateral x ray of the knee was performed in 10 of 18 patients; seven (two-thirds) of them showed a double layered patella. Their age range was between 8 and 28 years. Thus, unlike the double layered sternum seen only in young children with DTD, the double layered patella persists into early adulthood. This probably indicates that it is caused not merely by an abnormal ossification pattern but is a true anatomical variant, with two ossific nuclei instead of one. Although we cannot be sure how many rMED patients have a double layered patella (diagnostic sensitivity), specificity of this sign may be high, as sure how many rMED patients have a double layered patella with two ossific nuclei instead of one. Although we cannot be sure how many rMED patients have a double layered patella (diagnostic sensitivity), specificity of this sign may be high, as 

Spine
Changes in the spine were least marked. Platypondyly or other structural changes of the vertebral bodies were not observed. Mild lumbar scoliosis was observed occasionally, but this may have been the consequence of lower limb and foot deformity. In most patients, the interpedicular distance in the L1 to L5 segment remained constant or decreased, as usually observed in diastrophic dysplasia.

DISCUSSION
Following the identification of the DTDST gene, molecular diagnosis of subjects affected by DTD, AO2, or ACG1B has shown that c862X/R279W is the most frequent DTDST mutation, but it was never found in the homozygous state. Homozygosity for R279W was finally observed in a patient who had double layered patella, club foot, and normal stature and had received the diagnosis of multiple epiphyseal dysplasia.37 Following the description of this person, several other homozygotes were found in a relatively short time.38,39,40 In this study, we have delineated the clinical and radiographic phenotype of 18 R279W homozygotes. Subjects with the same pathogenic genotype are rare in the skeletal dysplasias (with the notable exception of achondroplasia). This gave us the opportunity of defining the phenotype and of clarifying the 

The results of this study confirm the existence of a recessive form of MED situated at the mild end of the dysplasia spectrum that includes achondrogenesis 1B, atelosteogenesis 2, and diastrophic dysplasia. The finding of normal stature and the rarity of abnormal findings at birth (particularly of cleft palate, hitch-hiker thumbs, and cystic ear swelling) clearly distinguishes rMED from diastrophic dysplasia and justifies the descriptive term “multiple epiphyseal dysplasia”, as has been applied to several patients in the past.28 The diagnosis of rMED is usually made in childhood because of joint complaints and mild hand and foot deformity. Stature is slightly reduced on average, with some patients below the normal mean and others above.

Because of the relatively mild radiographic findings, differential diagnosis of the various MED types is difficult. However, this differential diagnosis is important, as it should form the basis of further molecular diagnostic studies. Unger et al41 have delineated radiographic criteria to distinguish COMP associated MED from collagen 9 associatedMED, where hip involvement more pronounced than knee involvement) from collagen 9 associated MED (where the hips are relatively spared).42 The hip changes in rMED, with broad and flat epiphyses, are quite consistent in our series and easy to distinguish from those in COMP MED, with small and round epiphyseal nuclei. On hand radiographs, rMED patients may be identified by slightly flattened epiphyses sitting on relatively broad metacarpal ends. The extent of brachydactyly is similar to that seen in COMP MED and greater than that in collagen 9 MED. The finding of even mild club foot deformity (present in five of 18 patients in this series) is suggestive of rMED as it is not commonly seen in other MED types. The observation of a double layered patella on lateral radiographic films is strongly suggestive of rMED; unfortunately, the non-systematic nature of skeletal surveys in our patient series does not allow statements on the sensitivity of this sign on the time frame in which it can be observed. It is surprising that in spite of the evident changes at the hips, no subject has undergone hip replacement surgery. This is in contrast with the findings in COMP associated MED, where hip surgery is common. The reason for this difference might be the ligamentous laxity and joint instability in COMP MED as opposed to the tendency to joint contractures in rMED.

The stature range of R279W homozygotes was even larger than that of the reference population, with some of them being below the 3rd centile for height and others being tall-normal. While the median height is below the normal mean, most patients fall within the low normal range. This wide stature range is at odds with the expectation of a skeletal dysplasia having a major impact on stature. The reasons for this variability are unclear. Parental height is a major determinant of individual height; unfortunately, this information was
rMED should be studied more accurately, as adult stature is an important general outcome criterion in skeletal dysplasias.

Following the description of the first R279W homozygote, we put forward the hypothesis that DTDST mutations are responsible for a subset of cases of idiopathic club foot. Indeed, Huber et al described four R279W homozygotes in two families who had presented at birth with “apparently isolated” club foot, and in whom later examination had shown rMED. We tested a large sample of families with familial idiopathic talipes equinovarus for the presence of DTDST mutations but did not find any. Interestingly, though, we found heterozygosity for R279W in two unaffected subjects, probably as a chance finding (see below). In the present series, club foot was seen in six out of 18 subjects, confirming that it is a frequent but not obligatory feature of rMED. It seems prudent to conclude that children with bilateral club foot should be examined for the presence of rMED but that the contribution of DTDST mutations to the pathogenesis of club foot as a whole is probably small.

Unlike all other MED types known so far, this type of MED is inherited as a recessive trait (rMED), which has implications for genetic counselling. Recurrence probability in sibs is 25%. For an affected subject, the probability of transmitting the disorder to offspring is not 50% but significantly lower. For such a subject, the risk of having a child with a DTD skeletal dysplasia (rMED, DTD, AO2, or ACG1B) may be around 1 in 200 (assuming a carrier frequency of around 1 in 100 for DTDST mutations altogether). In spite of the relatively low risk, screening of the partner for the most common DTDST mutations (including R279W) is an option that is frequently used.

It would be of interest to estimate what proportion of MED cases might be ascribed to DTDST associated rMED. The incidence of rMED, and the proportion of MED cases that is accounted for by rMED, cannot be determined with precision by this retrospective case collection. Nonetheless, some indications can be derived from this study and from published reports. Occurrence of c862t/R279W at a CpG dinucleotide might be consistent with recurrent mutational events. R279W is the only DTDST mutation that has been found fortuitously in the heterozygous state in unaffected subjects (two of 207 samples in one study). Homozygosity may thus be more common than hitherto recognised. Since we have observed the rMED phenotype in seven other subjects with DTDST genotypes other than R279W homozygosity, rMED is not exclusively associated with R279W, although this genotype appears to be the most frequent. Altogether, rMED has been proven by mutation analysis in a total of 28 subjects in our laboratory. Czarny-Ratajczak et al screened a large sample of MED patients and found three instances of COMP mutations, one of COL9A1, none of COL9A2 or COL9A3, and two unrelated R279W homozygous probands; more probands in that series had a double layered patella but other DTDST mutations were not tested for. Huber et al reported four R279W homozygous rMED subjects. Thus, DTDST associated rMED constitutes at present the largest subgroup of molecularly defined MED patients. These observations suggest that rMED may not be uncommon. Given the variability observed in our series, it is conceivable that some patients with rMED might never be diagnosed with a generalised skeletal disorder or might even escape medical attention. The data presented here can help increase recognition of rMED by clinicians.

ACKNOWLEDGEMENTS

This work was supported by the Swiss National Foundation (grant 31-57272.99) and the National Institutes of Health (grant HD 22657). We are grateful to Outi Makitie and Ravi Savarirayan for sharing information on non-R279W rMED patients, and to Leena Ala-Kokko and Beat Steinmann for constructive discussions and for reviewing the manuscript.
Figure 5  Lateral knee x-ray of (A) patient 3a at 20 years and (B) patient 5b at 28 years showing a double layered patella. Persistence of this finding into adulthood indicates a true anatomical variant and is a useful diagnostic clue.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Overview on molecular defined types of multiple epiphyseal dysplasia</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIM number</td>
<td>Gene</td>
</tr>
<tr>
<td>EDM1</td>
<td>*600310</td>
</tr>
<tr>
<td>EDM2</td>
<td>600204</td>
</tr>
<tr>
<td>EDM3</td>
<td>600969</td>
</tr>
<tr>
<td>EDM4</td>
<td>226900</td>
</tr>
<tr>
<td>EDM5</td>
<td>*602109</td>
</tr>
</tbody>
</table>


Recessive multiple epiphyseal dysplasia (rMED): phenotype delineation in eighteen homozygotes for DTDST mutation R279W

D Ballhausen, L Bonafé, P Terhal, S L Unger, G Bellus, M Classen, B C Hamel, J Spranger, B Zabel, D H Cohn, W G Cole, J T Hecht and A Superti-Furga

doi: 10.1136/jmg.40.1.65