

## Short reports

## Recessively inherited multiple epiphyseal dysplasia with normal stature, club foot, and double layered patella caused by a *DTDST* mutation

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### Abstract

We have observed over 25 different mutations in the diastrophic dysplasia sulphate transporter gene (*DTDST*) in association with the recessive disorders achondrogenesis 1B, atelosteogenesis 2, and diastrophic dysplasia. The c862t (R279W) transition is the most common mutation in non-Finnish patients, but in these disorders it is usually combined with other *DTDST* mutations. We had not seen a case of homozygosity for c862t (R279W) until we analysed DNA from a 36 year old male with tall-normal stature (180 cm) who asked for genetic counselling for suspected multiple epiphyseal dysplasia. He was treated for club foot and hip dysplasia at birth. Skeletal changes consistent with multiple epiphyseal dysplasia, with the peculiar finding of a double layered patella, were recognised during childhood. Cleft palate, swelling of the ear pinna, and hitch hiker thumb were absent. He was found to be homozygous, and both healthy parents heterozygous, for the R279W mutation in *DTDST*, and his fibroblasts showed a sulphate incorporation defect typical of *DTDST* disorders. Counselling was given for a recessive disorder, thereby considerably reducing the probability of affected offspring.

Multiple epiphyseal dysplasia is more frequently caused by dominant mutations in the *COMP* (EDM1, McKusick 132400) and *COL9A2* genes (EDM2, McKusick 600204). A few other patients and families with features similar to our proband have been described previously and considered to have autosomal recessive MED (EDM4, McKusick 226900). This observation confirms the existence of this entity and assigns it to the phenotypic spectrum associated with mutations at the *DTDST* locus. (J Med Genet 1999;36:621-624)

Keywords: multiple epiphyseal dysplasia; *DTDST*; double layered patella

Recessive mutations in the diastrophic dysplasia sulphate transporter gene (*DTDST*) are the cause of a continuous spectrum of bone

dysplasia which includes, as "landmark" phenotypes, achondrogenesis 1B, atelosteogenesis 2, and diastrophic dysplasia.<sup>1-4</sup> In the course of molecular characterisation of patients and their families for confirmation of diagnosis, genetic counselling, and prenatal diagnosis, we have observed over 25 different *DTDST* mutations.<sup>1-2,5-8</sup> Among these mutations, c862t (R279W) is the single most common in non-Finnish patients, accounting for approximately a quarter of disease alleles. Its presence in patients from different ethnic backgrounds and its occurrence at a CpG dinucleotide is consistent with independent mutation events. In spite of its original observation in a patient with AO2,<sup>6</sup> this mutation is mostly associated with a non-lethal phenotype. In fact, in spite of its relative frequency, we have so far observed this mutation only in the heterozygous state, in combination with other *DTDST* mutations, and never in the homozygous state. We now report the first instance of homozygosity for the c862t/R279W mutation in an adult whose phenotype was different from DTD and its variants. Findings in this subject expand the spectrum of *DTDST* associated disorders and support the notion that the c862t/R279W mutation may be a relatively "mild" mutation.

### Case report

This 36 year old German engineer requested counselling for a presumed dominant form of multiple epiphyseal dysplasia. The family history was unremarkable and parental consanguinity was denied. Bilateral club foot had been surgically corrected in childhood and "hip dysplasia" had been treated with bandaging. Small femoral heads were observed in childhood. Lateral knee x rays at 8 years of age showed bilateral double layered patella. At puberty, brachydactyly became apparent.

On physical examination at the age of 36 years, he was 180 cm tall, the same as his father; his mother was 174 cm and a brother 183 cm tall. Neither his parents nor his brother had brachydactyly or foot deformities. The patient's body habitus was unremarkable. He had discrepancy in leg length, which may have resulted from early ankle and foot surgery for club foot (see below). The facies was unremarkable, the palate was neither cleft nor high arched, and the external ears were well formed

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Figure 1 (A) The proband aged 36 years. Except for mild valgus deformity at the knees, the body habitus is unremarkable. (B) Left hand with mild brachydactyly and clinodactyly of the fifth finger. (C) Adduction of the right forefoot with wide spacing between the first and second toes. The left foot shows only mild shortening of the first toe. (D) Radiograph of the left hand showing moderate shortening of the metacarpals as well as of middle phalanges II and V.

(no swelling reported). Body proportions were within the normal range. Left convex scoliosis was present in the lumbar region. The lower arms were slightly bowed and elbow flexion limited. The hands showed mild brachydactyly, although overall hand length was normal (fig 1B). The index fingers were unable to be bent completely at the metacarpophalangeal joint, but the mobility of the other finger joints was not limited. There was no hitch hiker thumb deformity. The feet and ankles showed some deformity resulting from surgical correction of congenital club foot, with more pronounced valgus position at the right ankle and hypotrophy of leg and foot muscles. The right big toe was deviated medially. The proband complained of pain in the hips, knees, and feet after physical exercise.

Review of the *x* rays obtained during childhood showed inappropriately small epiphyses at several sites including the proximal femora (fig 2A), knees, humeri, distal radii, and ulnae. Lateral views of the knees at the age of 8 years, obtained because of complaints of restricted movement, showed an unusual finding consisting of two separate anterior and posterior ossification layers of the patella, the so-called double layered patella (fig 2C). In adulthood, there was marked flattening of proximal femoral epiphyses (fig 2B), mild shortening of the ulna, mild shortening of the metacarpals and medial phalanges II and V, and pronounced lumbar scoliosis apparently secondary to degenerative changes at L5/S1. On lateral projection, the vertebral bodies showed no platyspondyly nor significant dysplasia.

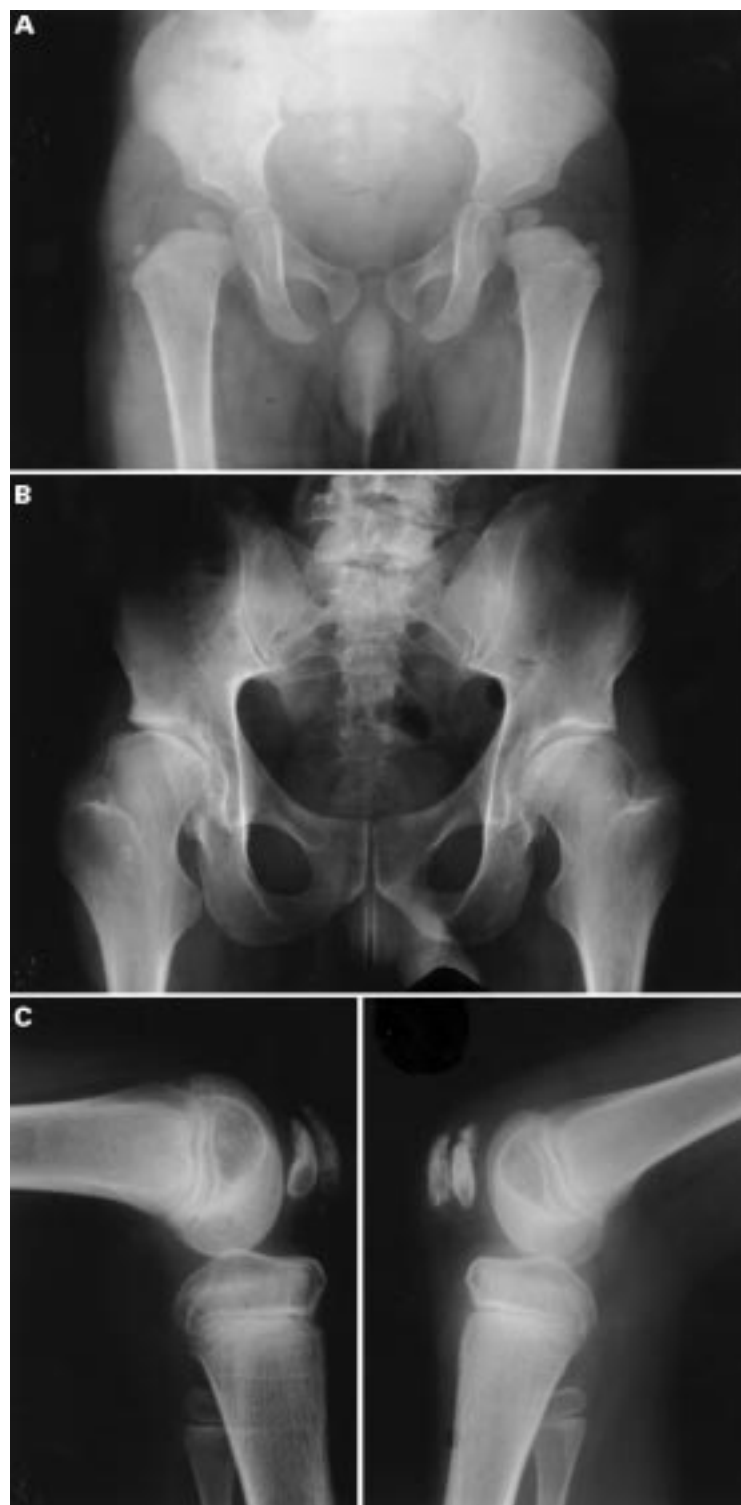


Figure 2 (A) Radiograph of the pelvis at the age of 3 years. Note small ossification centres of the proximal femoral epiphyses and the trochanter major. The femoral necks are short. (B) Follow up of the pelvis at the age of 36 years. The femoral necks are short and there is significant flattening of the proximal femoral epiphyses. The trochanters are of normal size at this stage. There also is localised degeneration of vertebral body S1 with lateral tilt of L5. (C) Lateral view of the knees at 8 years showing double layered patellae.

#### Laboratory investigations and results

Leucocyte DNA of the proband and of both parents was screened for mutations in the coding region of *DTDST* by genomic PCR and SSCP<sup>5-7</sup> followed by direct PCR sequencing in both directions using dye terminators and an ABI 310 apparatus. The mutation status was

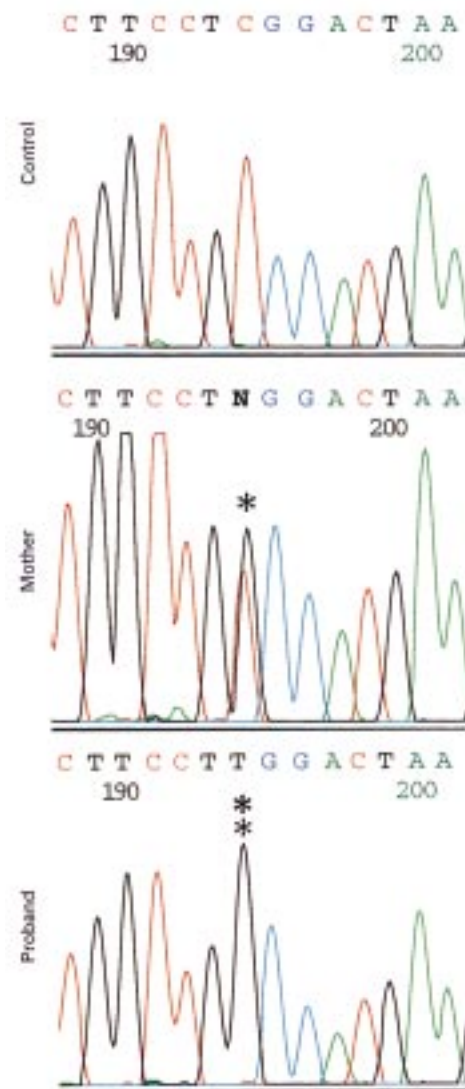


Figure 3 DNA sequence of genomic PCR products of a portion of the *DTDST* gene showing the c862t/R279W mutation in the homozygous state in the proband and in the heterozygous state in the mother (the father, not shown, was also heterozygous). The transition converts codon 279 from arginine to tryptophan.

confirmed by restriction enzyme analysis. The proband was homozygous, and the healthy parents heterozygous, for the c862t transition in *DTDST* predicting the substitution R279W in the fifth extracellular loop of the transporter protein (fig 3). No other mutation was observed in the coding region of *DTDST*. Since functional proof of the causative role of the c862t/R279W mutation is lacking, the proband consented to undergo a skin biopsy for fibroblast culture. Sulphate incorporation in his fibroblast cultures was reduced to a level similar to other patients with diastrophic dysplasia<sup>8</sup> (not shown), suggesting that the R279W mutation was indeed pathogenic, and confirming that his skeletal dysplasia was related to the *DTDST* bone dysplasia family. It must be noted that the sulphate incorporation assay is useful for diagnostic purposes but does not allow confident distinction between severe and milder variants.<sup>8</sup>

## Discussion

Double layered patella is a rare and peculiar radiological finding which has been observed mostly in association with multiple epiphyseal dysplasia (MED).<sup>9-12</sup> Patients with MED may also have forefoot adduction or club foot.<sup>9 13 14</sup> Multiple epiphyseal dysplasia had therefore been diagnosed in this patient in his teens. Delayed development of the epiphyses without gross structural alterations, as seen in the radiograph of the pelvis taken at 3 years of age (fig 2A), is compatible with the diagnosis of MED. Normal stature is also a feature of milder forms of MED.

The clinical and radiological findings were re-evaluated when the proband requested genetic counselling for MED, which, in the known variants caused by mutations in the *COMP* gene (EDM1, MIM 132400) or the *COL9A2* gene (EDM2, MIM 600204), is inherited as an autosomal dominant trait. The aim was to focus on a potential candidate locus for prenatal diagnosis. However, since club feet are not usually seen in MED caused by *COMP* or *COL9A2* mutations, but are a feature of diastrophic dysplasia, evidence for a DTDST disorder was searched for. Homozygosity for a known DTDST mutation and reduced sulphate incorporation in cultured fibroblasts confirm that the condition seen in the proband belongs to the DTDST bone dysplasia family (in spite of the absence of cleft palate, hitch hiker thumbs, changes in the external ears, and normal stature).

We have previously shown that the R279W mutation appears to result in DTDST mRNA of normal stability, as opposed to DTDST mRNA harbouring a premature stop codon.<sup>7</sup> The findings in this proband also suggest that the c862t/R279W mutation, which affects an amino acid residue in an extracellular loop of the transporter protein, is relatively "mild" and explains why we had not observed homozygosity in the patient population we had studied before, a population ascertained for the three known DTDST phenotypes, ACG1B, AO2, and DTD. It seems likely that this mutation allows for some residual activity of the transporter protein, with an additive effect in homozygotes accounting for the mild phenotype.

Although variability in diastrophic dysplasia has been recognised for a long time, none of the subjects with the "diastrophic variant" reported by Horton *et al*<sup>13</sup> and Lachman *et al*<sup>16</sup> reached a final stature of 150 cm or more, and a DTDST related condition with tall-normal stature has not been predicted. Since c862t/R279W appears to be the most frequent DTDST mutation in Europe (except for Finland), homozygotes may not be particularly rare. The diagnostic value of the double layered patella and, perhaps more importantly, of club foot remains to be established. Identification of the molecular defect was good news for our proband, as the estimation of the risk of having affected offspring could be reduced from the 50% of a presumed dominant condition to the much lower risk of a recessive condition.

The McKusick catalogue contains an entry on autosomal recessive multiple epiphyseal

dysplasia (\*226900) based on the description of three affected sibs by Juberg and Holt.<sup>13</sup> Spranger<sup>17</sup> has suggested that these cases were instances of "diastrophic variants". The sibs described by Juberg and Holt<sup>13</sup> had moderately reduced stature (adult stature around 150 cm), but the clinical and radiographic findings were similar to those seen in our proband (including a double layered patella), supporting Spranger's suggestion. The three sibs with "double patellae and multiple epiphyseal dysplasia" reported by Hodkinson<sup>9</sup> also had forefoot adduction; their adult stature was around 150 cm. In spite of the tall stature in the subject reported here contrasting with these observations, it does not appear appropriate to create yet another different entity, since we are dealing with a continuous spectrum of related conditions. We therefore suggest that MIM entry 226900 may be definitively assigned to "autosomal recessive multiple epiphyseal dysplasia" caused by DTDST mutations. Within this recessive form of MED associated with DTDST mutations, the relative frequency of normal versus moderately reduced stature and the diagnostic value of double layered patella remains to be established.

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- Superti-Furga A, Rossi A, Steinmann B, Gitzelmann R. A chondrodysplasia family produced by mutations in the diastrophic dysplasia sulfate transporter gene: genotype/phenotype correlations. *Am J Med Genet* 1996;63:144-7.
- Hästbacka J, de la Chapelle A, Mahtani MM, et al. The diastrophic dysplasia gene encodes a novel sulfate transporter: positional cloning by fine-structure linkage disequilibrium mapping. *Cell* 1994;78:1073-87.
- Superti-Furga A. A defect in the metabolic activation of sulfate in a patient with achondrogenesis type IB. *Am J Hum Genet* 1994;55:1137-45.
- Superti-Furga A. Defects in sulfate metabolism and skeletal dysplasias. In: Scriver R, Beaudet AL, Sly A, Valle D, eds. *The metabolic and molecular bases of inherited disease*. 8th ed. New York: Academic Press, 1999 (in press).
- Superti-Furga A, Hästbacka J, Wilcox WR, et al. Achondrogenesis type IB is caused by mutations in the diastrophic dysplasia sulphate transporter gene. *Nat Genet* 1996;12:100-2.
- Hästbacka J, Superti-Furga A, Wilcox WR, Rimoin DL, Cohn DH, Lander ES. Atelosteogenesis type II is caused by mutations in the diastrophic dysplasia sulphate transporter gene (DTDST): evidence for a phenotypic series involving three chondrodysplasias. *Am J Hum Genet* 1996;58:255-62.
- Rossi A, van der Harten H, Beemer FA, et al. Phenotypic and genotypic overlap between atelosteogenesis type 2 and diastrophic dysplasia. *Hum Genet* 1996;98:657-61.
- Rossi A, Kaitila I, Wilcox WR, et al. In vivo and in vitro proteoglycan sulfation in sulfate transporter chondrodysplasias: relationship to clinical severity and indications on the role of intracellular sulfate production. *Matrix Biol* 1998;17:361-9.
- Hodkinson HM. Double patellae in multiple epiphyseal dysplasia. *J Bone Joint Surg Br* 1962;44:569-72.
- Dahners LE, Francisco WD, Halleran WJ. Findings at arthrotomy in a case of double-layered patellae associated with multiple epiphyseal dysplasia. *J Pediatr Orthop* 1982;2:67-70.
- Sheffield EG. Double-layered patella in multiple epiphyseal dysplasia: a valuable clue in the diagnosis. *J Pediatr Orthop* 1998;18:123-8.
- Yochum TR, Sprowl CG, Barry MS. Double patella syndrome with a form of multiple epiphyseal dysplasia. *J Manipul Physiol Therap* 1995;18:407-10.
- Juberg RC, Holt JF. Inheritance of multiple epiphyseal dysplasia, tarda. *Am J Hum Genet* 1968;20:549-63.
- Kozłowski K, Fellmann K, Senger A, Prokop E, Kuczynski W. Spondylo-epiphysäre Dysplasie mit Valgusdeformität der Kniegelenke. *Fortschr Röntgenstr* 1971;115:287-95.
- Horton WA, Rimoin DL, Lachmann RS, et al. The phenotypic variability of diastrophic dysplasia. *J Pediatr* 1978;93:609-13.
- Lachman R, Sillence D, Rimoin D, et al. Diastrophic dysplasia: the death of a variant. *Radiology* 1981;140:79-86.
- Spranger J. The epiphyseal dysplasias. *Clin Orthop* 1976;114:46-60.