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Sulphate transporter gene mutations in apparently isolated club foot

EDITOR—Diastrophic dysplasia was originally ascribed to sulphate transporter gene (*DTDST*) mutations. The *DTDST* gene is now also known to account for a variety of bone dysplasias including diastrophic dysplasia, atelosteogenesis type II (AO2), and achondrogenesis Ib.[1–3] Abnormally sulphated cartilage proteoglycans with deficient cartilage sulphate content has been reported in these conditions,[4] suggesting that a variable residual *DTDST* activity probably accounts for the broad spectrum of clinical phenotypes at this locus.[5]

While a predominant founder mutation in the splice donor site of a previously undescribed 5' untranslated exon accounts for the disease in Finland, more than 30 mutations have been reported so far world wide[6] and compound heterozygosity for variably deleterious mutations probably explains the broad spectrum of clinical phenotypes at the *DTDST* locus.[2 7 8] The R279W mutation is the most common mutation in non-Finnish patients and, apart from its original report in an AO2 patient, compound heterozygosity for this mutation has been consistently associated with a non-lethal phenotype.

Recently, Superti-Furga *et al*[9] reported homozygosity for the R279W mutation in an adult affected with multiple epiphyseal dysplasia, normal stature, club foot, and double layered patella. Here, we add further support to this view and report on the association of apparently isolated club foot with the homozygous R279W *DTDST* mutation in two unrelated sibships of western French ancestry (Brittany).

In family 1, dizygotic twins, a boy and a girl, aged 14 years, were referred for moderate hand stiffness. They were born after a normal pregnancy (birth weight 2650 g and length 48 cm for the boy and birth weight 2700 g and length 47 cm for the girl). At birth, the girl had a metatarsus varus of the left foot which rapidly recovered after physiotherapy. She developed normally and at 14 years of age she had normal stature (height 158 cm), no facial dysmorphism or abnormal external ears, and did not complain of hip, knee, or ankle pain after physical exercise. At birth, her brother had bilateral club foot which required surgery with persistence of retraction on the left side. His stature was normal (height 160 cm at 14 years). There was no ear swelling, hitch hiker thumb deformity, or limitation of full extension of the elbow and his face and palate were normal. At birth, *x* rays showed a normal spine and lower limbs, but a bone age advanced by one year was observed when the boy was 2 year old. At 14 years, *x* rays showed enlarged metaphyses of the phalanges and shortened metacarpals (fig 1A). When the radiographs were re-examined, mild epiphyseal dysplasia of the upper femoral neck (fig 1B) was noted but no knee abnormality or double layered patella were observed. The parents originated from the same region of Brittany but no consanguinity was known.

In family 2, the first child, a boy (birth weight 4650 g and length 52 cm) had bilateral club foot diagnosed during pregnancy by ultrasound examination. Associated with

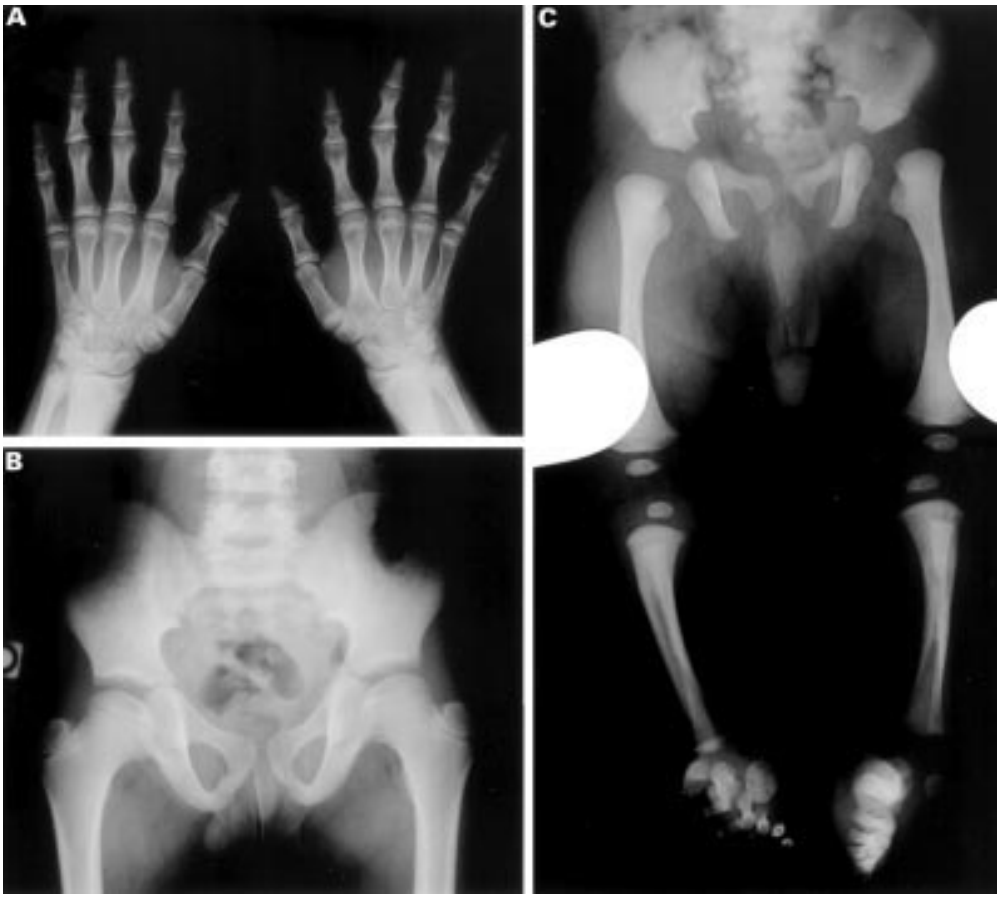

Figure 1 (A) Case 1, aged 14, showing enlarged metaphyses of the phalanges and metacarpals. (B) Case 1, aged 14, showing flattened femoral epiphyses. (C) Case 2, newborn, note the mild enlargement of the femoral metaphysis and the club foot.

this, mild micromelia with a small chin and mouth was noted at birth. Neither ear swelling nor hand abnormalities were detected, but pronosupination of the elbows was limited. He grew slowly (height 90 cm at 3 years, 25th centile) and *x* rays of the long bones were considered to be normal (fig 1C). Retrospectively, however, a mild flaring of the metaphyses of the long bones with delayed ossification of the epiphyses of the hip were noted at 3 years of age. Vertebral bodies were normal. During the second pregnancy, short femora were detected antenatally. The baby, a girl (birth weight 3250 g, length 46 cm at 37 weeks of pregnancy), had micromelia with brachymesophalangy of the fifth finger and a narrow thorax. The thumbs and feet were normal but a subluxation of the left hip was detected on echography at 2 months and treated by abduction splinting. Radiographs of the upper limb showed two ossified carpal bones and a dislocated elbow at 4 months. Her stature at 9 months was 67 cm (25th centile). There was no functional deficit or pain in the two children. Their parents originated from two villages 10 kilometres apart, but no consanguinity was known.

Because a mild form of diastrophic dysplasia was considered, leucocyte DNA from the probands were screened for mutations in the coding region of the *DTDST* gene. Direct PCR sequencing was performed in both directions using Big Dye terminators (Perkin- Elmer) on an Applied Biosystem 373A apparatus using the two following primers (forward: DTD5: TGC TCT GAT GAT ATG TCT CCA TGC and reverse: DTD8: TAT TCG ATC TAC AGC CAC ACT).[6] Interestingly, the four probands were homozygous for the mutation R279W in the fifth extracellular loop of the sulphate transporter protein.

Club foot is a common malformation, either isolated or associated with various neurological syndromes or chondrodysplasias, including diastrophic dysplasia, spondyloepimetaphyseal dysplasia with joint laxity, Kniest disease, and multiple epiphyseal dysplasia (MED). Isolated club foot (or metatarsus varus with no other clinical abnormality or short stature) is currently regarded as a malformation with a multifactorial mode of inheritance with a recurrence risk of approximately 10%.[10 11] In family 1, club foot was originally regarded as isolated, especially as the two affected children did not complain of functional deficit. They only complained of stiffness of the fingers and the diagnosis of MED was eventually considered after *x* ray discovery of small upper femoral epiphyses. At that time, the association of club foot (metatarsus varus), MED, and stiffness of the hand with short metacarpals and enlarged metaphyseal phalanges prompted us to consider and eventually confirm the diagnosis of a diastrophic dysplasia variant,[12] despite the absence of the double layered patella. The diagnosis of MED has been difficult to reach in family 2 as well, because the two children were too young. However, the delayed ossification of the femoral epiphyses of the hips might have suggested this diagnosis to us earlier.

The association of MED with the homozygous *DTDST* R279W mutation has been previously noted and ascribed to the normal stability of the mutant *DTDST* mRNA.[8] Recently, Superti-Furga *et al*[9] reported that autosomal recessive multiple epiphyseal dysplasia could result from homozygous *DTDST* R279W mutations. The patients reported here add to the view that the clinical spectrum of *DTDST* mutations is much broader than originally believed and includes apparently isolated club foot. However, the molecular mechanisms underlying the intrafamilial or interfamilial clinical variability of the homozygous R279W mutation remain unexplained.[5]

Based on these observations, we suggest giving consideration to *DTDST* mutation analysis in apparently isolated club foot, especially in cases of familial recurrence. Skeletal *x* rays and molecular investigations should help to confirm this diagnosis and determine the actual incidence of *DTDST* mutations in apparently isolated club foot.

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Development and application of linkage analysis in genetic diagnosis of familial hypertrophic cardiomyopathy

EDITOR—Familial hypertrophic cardiomyopathy (FHC) is a prevalent dominantly inherited disease characterised by unexplained hypertrophy of the heart muscle. The clinical manifestations are heterogeneous and the disease is a leading cause of sudden cardiac death among young, otherwise healthy people.[1] More than 120 different mutations have been reported in the following eight genes encoding sarcomeric polypeptides given in parentheses: *TNNT2* (troponin T), *MYL3* (essential myosin light chain), *MYH7* (β myosin heavy chain), *MYBPC3* (myosin binding protein C), *MYL2* (regulatory myosin light chain), *TPM1* (α tropomyosin), *ACTC* (α cardiac actin), and *TNNI3* (troponin I).[2 3] Furthermore, a disease locus on chromosome 7 has been linked to FHC, but the gene has not yet been identified.[4] Additional disease genes probably remain to be discovered since two recent studies found that it was only possible to genotype 50-60% of the FHC population by mutation analyses of seven disease genes.[5 6] In order to optimise risk stratification and management of FHC patients, it is important to identify all disease carriers, which is difficult by physical examination because of the age dependent penetrance of the disease. However, disease carriers may be identified by use of genetic diagnosis, although it is laborious because of the large number of disease genes and the pronounced allelic heterogeneity of the disease loci, with the majority of affected families having their own "private" missense mutation.[7] In addition, genetic diagnosis is complicated by the fact that several amino acid polymorphisms occur in most of the FHC genes[8 9] (unpublished observations). Given this complex genetic background, the use of linkage analysis can be beneficial as it may identify the most likely disease gene and provide evidence for exclusion of some or all of the other candidate disease loci even in small families.[10]

It was the aim of the present study to develop a firm basis for efficient use of linkage analysis in genetic diagnosis of FHC by a well founded selection of polymorphic markers defining nine FHC loci, including a refined genetic mapping of the troponin T gene in a 4 cM interval. For rapid analysis, multiplex PCR panels were developed comprising all markers selected. The feasibility of the method was evaluated by identification of mutations in three families of varying size.

The genetic mapping of *TNNT2* was based on analysis of six informative CEPH pedigrees (obtained from CEPH, France: Nos 102, 884, 1347, 1362, 1413, 1416; 102 subjects) using a previously published intragenic insertion/deletion polymorphism localised in intron 4 (*TNNT2*-Ins/Del).[11] A 340 bp fragment of *TNNT2* including exons 3, 4, and *TNNT2*-Ins/Del (base position 181-520) was amplified using the primers: forward (F) 5'-GTGGCAGGCAGCGTGACTCCAC-3' (the primer sequence was modified in accordance with our own unpublished sequencing results of intron 3 by omitting Gs in position 184, 190, and 198), reverse (R) 5'-CAGGATTTCCACATTGCTGA-3'. PCR was carried out as previously described[3] with primer concentrations given in table 1 and an annealing temperature of 62°C. Multipoint linkage analyses were carried out using chromosome 1 DNA markers in the region previously reported to harbour *TNNT2*[12 13] and the CEPH database version v8.1,[14] essentially as previously described.[15] A 16 point reference map was chosen in accordance with the Généthon genetic map where the marker loci are ordered with odds of at least 1000:1.[16] CMAP analysis was carried out calculating the likelihood for any position of *TNNT2* with respect to the fixed map. The location score curve showed a peak location score of 92.9 between D1S2716 and D1S504, which corresponded to a multipoint lod score of 20.2. The location of *TNNT2* in a 4 cM interval between D1S2716 and D1S504 was favoured

Table 1 Composition of multiplex PCR panels used for linkage analysis in FHC pedigrees

Multiplex name (No PCR cycles)	*Marker data*					*Primer concentration (pmol/μl)*	
	Marker name (gene)	*Length (bp)*	*Fluorescent dye*	*HZ index*	*No of alleles*	*Forward*	*Reverse*
MP1 (35 cycles)	D11S1993, (*MYBPC3*)	224–245	HEX	75	8	0.2	0.2
	D7S661, (*FHC locus*)	252–282	TET	76	10	0.4	0.4
MP2 (30 cycles)	D3S3685, (*MYL3*)	195–221	TET	89	13	0.2	0.2
	D3S3564, (*MYL3*)	204–220	HEX	79	7	0.3	0.3
	D3S3582, (*MYL3*)	220–236	FAM	68	7	0.2	0.2
MP3 (30 cycles)	MYO I-CA, (*MYH7*)	90–102	FAM	66	6	0.2	0.2
	MYO II-CA, (*MYH7*)	108–132	HEX	81	13	0.2	0.2
MP4 (30 cycles)	D1S2622, (*TNNT2*)	165–189	TET	79	11	0.2	0.2
	TNNT2 Ins/Del, (*TNNT2*)	335–339	FAM	50	2	0.1	0.1
	D1S2872, (*TNNT2*)	187–207	FAM	76	11	0.4	0.4
MP5 (30 cycles)	D12S1583, (*MYL2*)	219–247	HEX	87	14	0.1	0.1
	D15S153, (*TPM1*)	194–226	TET	86	12	0.1	0.1
	D15S1036, (*TPM1*)	118–140	TET	85	10	0.2	0.2
MP6 (35 cycles)	D12S1343, (*MYL2*)	190–216	FAM	70	9	0.2	0.2
	D12S84, (*MYL2*)	199–219	TET	83	9	0.2	0.2
	HTMα-CA, (*TPM1*)	108–120	TET	60	6	0.1	0.1
MP7 (35 cycles)	D7S505, (*FHC locus*)	262–278	FAM	69	8	0.2	0.2
	D7S483, (*FHC locus*)	166–188	HEX	81	10	0.2	0.2
MP8 (35 cycles)	D3S1578, (*MYL3*)	140–166	FAM	87	14	0.2	0.2
	MYBPC3-CA, (*MYBPC3*)	282–290	HEX	52	5	0.2	0.2
MP9 (35 cycles)	D11S1785, (*MYBPC3*)	268–276	FAM	70	5	0.15	0.15
	D11S903, (*MYBPC3*)	99–109	FAM	74	6	0.2	0.2
	D11S1344, (*MYBPC3*)	273–293	HEX	80	11	0.3	0.3
	D11S4191, (*MYBPC3*)	111–135	TET	87	11	0.2	0.2
MP10 (35 cycles)	ACTC-CA, (*ACTC*)	70–96	TET	86	12	0.2	0.2
	D19S887, (*TNNI3*)	246–262	HEX	72	8	0.3	0.3
	D19S254, (*TNNI3*)	110–150	TET	60	5	0.4	0.4
	D19S210, (*TNNI3*)	165–177	TET	73	6	0.2	0.2

Primer sequences of the polymorphic markers were obtained from the Genome Database (GDB)[37] except for *MYBPC3-CA* where primers were designed in accordance with the published genomic sequence (base position 11 473–11 756 (F-5'-GATTTATTTGGCCAGGGAACC-3'; R-5'-TTTCAAAACTGGGGGAACATC-3')).[26]