DTDST mutations are not a frequent cause of idiopathic talipes equinovarus (club foot)

L Bonafé, S H Blanton, A Scott, S Broussard, C A Wise, A Superti-Furga, J T Hecht

PHILOM, I diophathic talipes equinovarus (ITEV) or isolated club foot deformity is a common birth defect having an average birth prevalence of 1 per 1000 live births. However, the birth prevalence of ITEV varies among different populations, ranging from 0.39-7 per 1000 live births, with the highest rate in the Hawaiian and Maori populations. ITEV occurs more frequently in males and the skewed 2:1 ratio is consistent across all ethnic groups. Bilateral deformity is seen in more than half of the cases regardless of race and unilateral defects occur more often on the right side.

ITEV is an isolated congenital deformity of the foot and lower leg occurring when the foot is plantar flexed and inverted. ITEV is just one of the many types of foot deformities that are referred to as club foot. Club foot is often used loosely to describe a variety of different abnormalities that are morphologically similar but aetiologically distinct. Both metatarsus adductus and talipes calcaneovalgus are considered mild and usually self-correcting abnormalities. While many refer to these mild conditions as club foot, talipes equinovarus (TEV) is suggested to be the only true club foot and generally requires serial manipulations and castings, followed by one or more surgical procedure(s). Whereas TEV is part of many genetic syndromes, ITEV occurs as an isolated birth defect.

Epidemiological studies have attempted to define the aetiology of ITEV and none has shown a significant association with socioeconomic factors or teratogenic exposures in non-Hispanic white, Hawaiian, African American, and Oriental populations. Theories surrounding the aetiology of ITEV can be grouped into several categories: extrinsic prenatal influences, intrinsic anatomical factors, and genetic factors. The oldest theory of ITEV causation is the uterine compression theory has been supported through centuriety and then by the presence or absence of a family history of ITEV.

Material and Methods

Probands with ITEV were ascertained at Shriners Hospital for Children in Houston and Scottish Rite Hospital for Children, Dallas, Texas. Subjects were excluded from the study if they had TEV associated with other anomalies or syndromes. All cases of ITEV were interviewed and the diagnosis was confirmed either by examination or by review of medical records. Two generation pedigrees were collected on all participants and the probands were recorded as having a positive or negative family history. This information was used in the analysis. For probands without a family history of ITEV, blood samples for DNA were obtained from only the nuclear family (triad). For those with a family history, blood samples were obtained from all of the relatives. DNA was made using GenePure kit (Genta, Minneapolis, MN).

Since the DTDST gene does not have an intragenic short tandem repeat marker, two tightly linked flanking markers, D5S1507 and D5S1469, were tested. These markers were PCR amplified at an annealing temperature of 55°C and genotyped using the Gelcode silver stain system to visualise the alleles.

The genotyping data were analysed using the TDT option of GENEHUNTER. The flanking markers were analysed together and individually. Families were grouped first by ethnicity and then by the presence or absence of a family history of ITEV; p values were evaluated using a permutation test. For this, the transmitted and non-transmitted alleles are switched at random in 50% of the data. This is done at a specified number of times (1000 in this case) and the number of times that a p value of the same level or less is obtained is recorded.

Analysis of the R279W mutation was performed following the procedure of Superti-Furga et al. The coding sequence of the DTDST gene, as well as the region containing the IVS1+2>T>C mutation, were amplified in a set of 10 overlapping fragments.

Results

One hundred and twenty-five ITEV probands and their parents were genotyped for D5S1507 and 155 for D5S1469. Linkage and association results obtained with GENEHUNTER for D5S1507 were not significant. Results for D5S1469 showed that in all groups, except for the Hispanic familial group, the 4 allele was transmitted nearly twice as often as not and yielded a slightly significant p value (table 1). However, the permutation test to determine the robustness of the p values indicates that these were not significant.

None of the known pathogenic mutations were found in the DNA from 10 ITEV probands who received the 4 allele of D5S1469. Sequencing of the whole coding region excluded the presence of any new, previously unknown mutations.

Abbreviations: ITEV, isolated talipes equinovarus; TEV, talipes equinovarus; MED, multiple epiphyseal dysplasia

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DNA samples from 207 probands were tested for the R279W mutations and two probands, 6448 and 7517, showed heterozygous mutations. Proband 6448 had severe, bilateral ITEV that was treated by serial casting and surgical correction. Family history was negative for ITEV and neither parent had the R279W mutation. Proband 7517 had a right ITEV that also required surgical correction after serial casting. His mother has the R279W mutation and he has a positive family history of ITEV in a maternal cousin. DNA samples from the other family members were not available for testing.

**DISCUSSION**

Homozygotes for the mild R279W mutation in **DTDST** may present with ITEV as the only clinical abnormality at birth, although their later clinical history shows additional abnormalities typical of multiple epiphyseal dysplasia. Moreover, Huber et al. recently suggested that “apparently isolated clubfoot” may be a presenting sign of MED. These observations led us to test the hypothesis that the **DTDST** gene may play a role in the causation of ITEV. Testing for linkage and association to the **DTDST** gene in a cohort of subjects with ITEV gave positive results. Although this was not significant, we pursued sequencing of the gene and functional analysis. Ten probands with a positive family history and receiving the “4” allele were sequenced and no alterations in the coding region were identified. Mutation screening detected two heterozygotes, one who had inherited the change and the other who occurred as a new mutation. Interestingly, the inherited R279W mutation was in a family in which ITEV had previously occurred in a relative. However, the R279W mutation occurs in 1% of a control sample and the results of this study found the same frequency. This suggests that the R279W mutation is not aetiological.

Huber et al. reported homozygosity for the R279W gene in two pairs of siblings with apparently isolated clubfoot (ITEV). The clinical descriptions suggest that these subjects do not have “apparent” ITEV as each affected person had additional malformations consistent with a diagnosis of autosomal recessive mild multiple epiphyseal dysplasia. As Huber et al. suggested, the “apparent” diagnosis of ITEV had to be revised when the additional features of recessive MED were identified.

None of our probands, selected for having only ITEV, was homozygous for R279W. Moreover, heterozygous R279W mutations have not been reported to cause a pattern of malformations, specifically ITEV, in parents of children with diastrophic dysplasia. Alterations in the coding region were not identified in 10 probands with ITEV and a positive family history of ITEV, suggesting that this gene does not play an important aetiological role in these subjects. Altogether, these results suggest that the R279W mutation is no more frequent in this population of ITEV probands than in controls.

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**REFERENCES**


Table 1

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<th>Population</th>
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<th>Transmitted</th>
<th>Not transmitted</th>
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<th>Permutation*</th>
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*p Number of times out of 1000 that the simulation had a maximum result as high as in the initial data set.

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