**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

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**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.[20] The genotyping data were analysed using the TDT option of GENEHUNTER.[21] 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.

**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.

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**Abbreviations:** ITEV, isolated talipes equinovarus; TEV, talipes equinovarus; MED, multiple epiphyseal dysplasia
Table 1 DSS1469 TDT results

<table>
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<th>Population</th>
<th>Allele</th>
<th>Transmitted</th>
<th>Not transmitted</th>
<th>p value</th>
<th>Permutation*</th>
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<td>39</td>
<td>26</td>
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<td>12</td>
<td>0.06</td>
<td>131</td>
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</tbody>
</table>

*Number of times out of 1000 that the simulation had a maximum result as high as in the initial data set.

DNA samples from 207 probands were tested for the R279W mutations and two probands, 6448 and 7517, showed heterogeneous mutations. Proband 6448 had severe, bilateral IITEV that was treated by serial casting and surgical correction. Family history was negative for IITEV and neither parent had the R279W mutation. Proband 7517 had a right IITEV that also required surgical correction after serial casting. His mother has the R279W mutation and he has a positive family history of IITEV 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 IITEV as the only clinical abnormality at birth, although their later clinical history shows additional abnormalities typical of multiple epiphyseal dysplasia. As 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 IITEV. Testing for linkage and association to the DTDST gene in a cohort of subjects with IITEV gave positive results. Although this was not significant, we pursued sequencing the gene and mutational 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 with the R279W mutation. Proband 7517 had a right IITEV that also required surgical correction after serial casting and surgical correction. His mother has the R279W mutation and he has a positive family history of IITEV in a maternal cousin. DNA samples from the other family members were not available for testing.

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