A common DLX3 gene mutation is responsible for tricho-dento-osseous syndrome in Virginia and North Carolina families

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Abstract
Tricho-dento-osseous syndrome (TDO) is characterised by a variable clinical phenotype primarily affecting the hair, teeth, and bone. Different clinical features are observed between and within TDO families. It is not known whether the variable clinical features are the result of genetic heterogeneity or clinical variability. A gene for TDO was localised recently to chromosome 17q21 in four North Carolina families, and a 4 bp deletion in the human distal-less 3 gene (DLX3) was identified in all affected members. A previous genetic linkage study in a large Virginia kindred with TDO indicated possible linkage to the ABO, Gc, and Kell blood group loci. To examine whether TDO exhibits genetic heterogeneity, we have performed molecular genetic analysis to determine whether affected members of this Virginia kindred have the DLX3 gene deletion identified in North Carolina families. Results show that affected subjects (n=3) from the Virginia family have the same four nucleotide deletion previously identified in the North Carolina families. A common haplotype for three genetic markers surrounding the DLX3 gene was identified in all affected subjects in the North Carolina and Virginia families. These findings suggest that all people with TDO who have been evaluated have inherited the same DLX3 gene deletion mutation from a common ancestor. The variable clinical phenotype observed in these North Carolina and Virginia families, which share a common gene mutation, suggests that clinical variability is not the result of genetic heterogeneity at the major locus, but may reflect genetic heterogeneity at other epigenetic loci or contributing environmental factors or both.

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Tricho-dento-osseous syndrome (TDO) is a highly penetrant, autosomal dominant trait characterised by abnormalities of the hair, teeth, and bone. The phenotypic features include kinky/cruly hair at birth, enamel hypoplasia, taurodontism (enlarged pulp chambers), and thickening or increased density of the cortical bones of the skull. Other phenotypic features reported to be associated with TDO include flat fingernails and altered craniofacial morphology, including macrocephaly and dolichocephaly. The reported diversity of the TDO phenotype and the apparent segregation of specific clinical traits within some families resulted in classification of TDO into three subtypes. It is not known whether these different TDO clinical subtypes result from genetic heterogeneity involving non-allelic mutations, allelic mutations, or variable expression of a single gene mutation.

Recently, genetic linkage was identified on chromosome 17q21 for TDO in four North Carolina families. Subsequent haplotype analysis and mutational studies showed that all affected subjects (n=46) in these four families and the affected subjects from two additional small North Carolina families (n=7) shared a common haplotype for three polymorphic markers flanking the DLX3 gene. A 4 bp deletion in the DLX3 gene (GenBank AF028233) was subsequently identified in all affected members of the six families. The common haplotype and gene mutation suggest that

Figure 1 Pedigree showing subjects examined and studied in the current report and their relationship to the original Virginia kindred reported by Lichtenstein and Warner. Subjects with TDO are shaded. Numbered subjects were examined in the present study.
MOLECULAR GENETIC ANALYSIS
DNA was isolated from buccal swabs as previously described.\(^6\) The six family members were genotyped for three highly polymorphic genetic markers flanking the DLX3 gene (D17S941, DLX3, and D17S943) by standard methods.\(^6\) To test for the presence of the DLX3 deletion found in the six North Carolina TDO families, the six Virginia family members were analysed for the DLX3 exon 3 deletion using PCR amplification.\(^8\) Briefly, primers flanking the 4 bp DLX3 gene deletion were used to PCR amplify genomic DNA. The normal allele results in a 145 bp product and the deleted allele results in a 141 bp product. The PCR products of each subject were subcloned into pGEM-T Easy (Promega, Madison, WI) and six individual clones were sequenced by the Wake Forest University Medical Center Sequencing Core facility using a Prism 377 automated sequencer (Applied Biosystems, Foster City, CA).

Results
CLINICAL FINDINGS
The six family members studied are shown in relation to the kindred reported by Lichtenstein et al.\(^1\) (fig 1). All affected subjects were reported to have kinky/curly hair in infant photographs, while none of the unaffected subjects had kinky or curly hair. The affected woman (V.11) and her affected son (VI.1) both showed marked osseous changes radiographically compared with the unaffected father. Affected subjects also exhibited obliteration of diploe and a complete lack of visible mastoid pneumatization. Although these osseous changes were evident in the 15 year old proband, they were much more pronounced in his mother (fig 2). The proband had teeth with small clinical crowns and thin enamel. Radiographically, taurodontism was present in all the molars (fig 3). His affected mother was edentulous, and his unaffected father had normal dentition. Teeth are missing in affected subjects secondary to dental abscess formation treated by extraction and not to developmental hypodontia.

MOLECULAR FINDINGS
All three affected subjects carried the same haplotype for the three polymorphic markers (D17S941, DLX3, D17S941) flanking the DLX3 locus that have been identified as common to all TDO affected subjects (n=46) from the North Carolina families.\(^*\)

PCR deletion analysis showed that the three affected subjects studied have the normal 145 bp allele and the deleted 141 bp allele (fig 4A). The three unaffected subjects only exhibited the normal 145 bp allele. The PCR products from an affected subject were subcloned and six individual clones were sequenced, clearly showing the 4 bp deletion.

The 4 bp deletion results in a frameshift of the 3' terminal portion of DLX3 (fig 4B), but leaves the homeodomain intact. A premature termination codon is present in the deletion allele, resulting in a mutant protein which is 32 amino acids shorter that the wild type DLX3 protein. The 4 bp deletion was observed in only

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Figure 2  A marked increase in bone density and thickness is seen in this adult female with TDO (pedigree V.11).

TDO in these six families was inherited from a common ancestor.

The observation of a common gene mutation in the North Carolina families suggests that variable expression of a single gene mutation is responsible for the differences in clinical characteristics observed between and within these six families. To date, the only other genetic linkage study for TDO was performed by Rivas et al\(^2\) in a large Virginia kindred. Results of this linkage analysis using polymorphic protein markers suggested possible linkage with the ABO, Gc, and Kell blood group loci (for the ABO locus, Zmax=0.70, \(\theta=0.00\)). However, recent evaluation of genetic polymorphisms located near these loci excluded linkage of TDO in four large North Carolina families (all lod scores \(<-2.0\)).\(^8\) These results argue against a TDO gene modifying gene(s) genetically linked to the ABO, Gc, or Kell loci. The purpose of this study was to evaluate affected and unaffected subjects from the large Virginia family originally reported by Lichtenstein and Warson\(^1\) to determine whether the genetic basis for TDO in this kindred is the same DLX3 gene mutation common to the six North Carolina families.

Methods
FAMILY ASCERTAINMENT
We have identified, clinically examined, and sampled six subjects related to the TDO kindred of 169 people originally reported by Lichtenstein and Warson\(^1\) (fig 1). Three of these subjects were examined clinically and radiographically as previously described.\(^8\)

Figure 3  Taurodontism of the posterior teeth and enlarged pulp chambers of the anterior teeth are evident in this 15 year old male with TDO (pedigree VI.1).
from the six North Carolina families previously reported by Price et al and the large Virginia kindred previously reported by Lichtenstein et al. Additionally, the affected subjects from this Virginia kindred have the same haplotype region found in all those with TDO who have been tested to date. This finding suggests all affected subjects from these families inherited the mutant DLX3 allele from a common ancestor.

To date there have been four large populations in the United States identified with TDO. Three of these populations are located in geographical proximity to each other (fig 5). The kindred reported by Melnick et al is derived from one of the North Carolina kindreds. The results of the present study suggest that the concentration of people with TDO in this geographical region results from a founder effect and that these kindreds share a common ancestry.

Phenotypic diversity that appeared to show familial segregation resulted in the suggestion that TDO has three subtypes. Careful characterisation of the clinical phenotype in the North Carolina families documents variable expression of TDO even within nuclear families. Shapiro et al suggested that the kindred reported by Lichtenstein et al can be classified as TDO type I based primarily on a lack of calvarial changes seen in the proposed TDO types II and III. While Lichtenstein and Warson did report increased bone density, they did not describe an increased thickness of the calvaria or obliteration of diploe. In the North Carolina families increased thickness of the calvarial bone and obliteration of diploe were seen in 65% and 68% of affected subjects, respectively. In the original reports by Lichtenstein and Warson and Lichtenstein et al, only 10 affected subjects were examined radiographically with half of those examined being below the age of 18 years. It is possible with this limited sample and the variable expression of bone changes in TDO families that these changes were present but not observed. The present study clearly shows that members of the Virginia kindred can have increased bone density as has been described for TDO types II and III.

Dysplastic dentin was reported as a unique feature for 'TDO type II. Studies of teeth from the North Carolina families showed that approximately 64% had irregular dentin seen primarily in the region coronal to the pulp. Thus, dentinal changes appear to be variably expressed in subjects with TDO. It is not known whether dentinal changes seen in affected teeth result from expression of the mutant DLX3 gene or occur secondary to thin enamel and excess attrition. The variability of TDO phenotypes in these families having the same DLX3 gene mutation and sharing a common haplotype for genetic STRP loci surrounding the DLX3 gene argue against the existence of multiple TDO subtypes based on phenotypic variation.

In summary, it appears that the TDO syndrome segregating in these North Carolina and Virginia families has a common origin. All
affected subjects share the same DLX3 gene deletion mutation and appear to have inherited the gene "identical by descent" from a common ancestor. The variable clinical phenotype found in these North Carolina and Virginia families suggests that clinical variability is not the result of genetic heterogeneity at the major locus, but may reflect genetic heterogeneity at other epigenetic loci or contributing environmental factors. It is unlikely that modifier genes exist close to the ABO, Gc, or Kell loci, as linkage for TDO to genetic markers in these regions was excluded for the North Carolina families. Subclassification of the TDO syndrome based upon reported clinical phenotypes does not appear consistent with the genetic aetiology of the condition in these families.

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