CRANIOFACIAL VARIATIONS IN THE TRICHO-DENTO-OSSEUS SYNDROME

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A thesis submitted to the faculty of the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Master of Science in the School of Dentistry (Orthodontics).

Chapel Hill
2008

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ABSTRACT

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(Under the direction of Dr. J Timothy Wright)

Tricho-dento-osseous (TDO) syndrome is an autosomal dominant trait characterized by curly kinky hair at birth, enamel hypoplasia, taurodontism, thickening of cortical bones and variable expression of craniofacial morphology. Genetic studies have identified a 4 bp deletion in the *DLX*3 gene that is associated with TDO; however, phenotypic characterization and classification of TDO remains unclear in the literature. This study compares the craniofacial variations between 53 TDO affected subjects and 34 unaffected family members. Standardized cephalograms were obtained and digitized. Cephalometric measures were analyzed using a general linear model with family as a random effect. Many of the craniofacial measurements in both groups showed marked variability. TDO affected subjects showed smaller SNB, ANB angles, longer mandibular corpus length (GoGn) and shorter ramus height ($p<0.05$).

ACKNOWLEDGEMENTS

I would like to thank the following for their contribution, support and dedication:

Dr. J Timothy Wright, for his mentorship, support and encouragement throughout the development and completion of this project; for making what could be a very stressful process so enjoyable.

Dr. Ceib Phillips, for her tremendous help and advice; for her insight and expertise with statistical analysis.

Dr. Sylvia Frasier-Bowers, for her advice, input and insight.

Mr. Zakaria Khonder, for his assistance with the statistical analysis. Your time and effort is much appreciated.

Ms. Debbie Price, for your time and assistance with data input.

Mom and dad, for always providing me with their love, assistance, encouragement, wisdom and guidance.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

I LITERATURE REVIEW

TRICHO-DENTO-OSSEOUS SYNDROME

Tricho-dento-osseous syndrome is an autosomal dominant disorder with complete penetrance characterized by abnormalities involving hair, bone and teeth. It was first identified in 1966 by Robinson (1). He presented 5 cases from a single pedigree in which all affected family members displayed enamel hypoplasia and curly kinky hair. He showed an autosomal dominant inheritance pattern over four generations and suggested that this condition was distinct from amelogenesis imperfecta due to the association of both enamel hypoplasia and hair characteristics. In 1970, Crawford reported a similar population of subjects exhibiting taurodontism in addition to enamel hypoplasia and curly hair (2). It was not until 1971 that TDO was characterized as a distinct condition by Lichtenstein et al. (3). He traced six generations of an Irish-American family residing in the mountains of Washington County Virginia, along the Holston River. The kindred displayed kinky hair at birth, enamel hypoplasia, taurodontism and sclerotic bone inherited in an autosomal dominant pattern. He reported that affected subjects had increased susceptibility to dental caries, gingival abscess, brittle nails, increased cortical bone density of the skull and altered craniofacial morphology including dolichocephaly, frontal bossing and a square jaw.

More cases of TDO were identified in the following years, yet the nature of the genetic defect responsible for TDO remained unknown. In 1973, Jorgenson and Warson re-

examined Lichtenstein's TDO population and suggested that the three dental phenotypes, taurodontism, enamel hypoplasia and dental impactions, observed in TDO were a direct result of defects in dental epithelia (4). He noted that taurodontism is due to a defect in Hertwig's epithelial root sheath. The sheath consists of an inner and outer dental epithelium which surrounds the base of the developing crown and grows inward to produce multiple roots. Failure of the epithelium to invaginate at the correct horizontal level results in taurodontism. He cited Weinmann's study (5) which showed defects in the inner dental epithelium during the formative stage resulted in enamel hypoplasia, while defects in the maturation stage resulted in enamel hypocalcification. He suggested that defective reduced enamel epithelia, a derivative of dental epithelium, were unable to disorganize the tissue overlying the crown, an event that is necessary for the eruption process. Based on these observations, he proposed that a single gene mutation in dental epithelia was responsible for TDO.

Melnick, argued that it was mesenchymal tissues, not epithelia, that were responsible for all the reported features of TDO (6). He examined extracted teeth from affected subjects under light and scanning electron microscopy and discovered peri-radicular sheath, membranes that surrounds the open apices and extended part way up the root, containing collagen fibers. He cited the works of Kollar (7, 8), who demonstrated that dental papilla was responsible for induction of radicular development, and Croissant (9), who showed that odontoblasts play a critical role in the initiation of enamel matrix. He noted other predominant TDO features such as osteosclerosis of the skull and involvement of hair and nail were mesenchymal in nature. Together with his microscopy findings, it lent support to a mesenchymal involvement.

In 1983, another kindred with TDO was discovered along the Holston River, in Tennessee, by Quattromani and Shapiro (10). In addition to the fuzzy kinky hair and enamel hypoplasia, they found affected individuals also displayed sclerotic and thickened calverium and long bones that showed subtle undertubulation but no sclerosis. In light of the variable phenotypic expression and close proximity between the kindreds, Shapiro et al hypothesized that differences in the phenotypic subtypes were due to distinct genetic entities and argued for the classification of TDO subtypes, TDO-I and TDO-II (11). TDO Type I exhibits the typical kinky curly hair, enamel hypoplasia, delayed teeth eruption, thickened chondrocranium, but normal calvarial density and thickness. TDO Type II displayed sparse kinky hair that was easily detachable, nail changes, precocious tooth eruption and thickening and sclerosis of the calveria. This created controversy among researchers as to whether the variance in the TDO phenotype was a result of genetic heterogeneity or variable expression of a single gene mutation. This subclassification of TDO was influenced in part by the works of Rivas et al (12). Using polymorphic protein markers, they performed a linkage analysis on the Virginia kindred and suggested possible linkage between the TDO-I locus and the ABO, GC and Kell blood group loci. However, a later study by Hart et al showed that this linkage was incorrect (13).

The TDO controversy continued in the 1980's as many authors questioned whether TDO was a distinct syndrome or merely a subdivision of amelogenesis imperfecta hypomaturation- hypoplasia (AIHHT). In 1990, Crawford and Alfred (14) performed a critical review of the TDO literature and concluded that TDO syndrome was different from AIHHT with taurodontism but suggest that the two disorders may represent variable clinical expression of a common gene mutation. They supported Witkop's proposal (15) that the

minimal diagnostic criteria for TDO should include enamel hypoplasia, posterior teeth with taurodontism, autosomal dominant inheritance pattern, and tightly curly hair at birth and/or radiographic evidence of bone sclerosis. They concluded that nail and bony defects were not always present and that many early cases of TDO were mistakenly reported as AIHHT. Seow believed that TDO and AIHHT with taurodontism are distinct entities and suggested that the two could be delineated by dental phenotype (16). She observed that all reported cases of TDO exhibited severe taurodontism of the mandibular first permanent molars and suggested that it be used as a criteria to diagnose TDO syndrome (17). This would later be refuted by Wright et al who showed taurodontism of mandibular first molars was highly variable (13).

In 1997, Wright identified on three new TDO kindreds in Alamance County of Western North Carolina and established a link between one of North Carolina family and that of the Midwest TDO family reported by Melnick (6). Their phenotypic analysis of this TDO population revealed marked variability in clinical characteristics (18). Enamel hypoplasia and taurodontism showed full penetrance in affected individuals while hair and bone characteristics were variably expressed. Kinky curly hair at birth was found in 85% of the affected population. However, 55% was developed straight hair by childhood. Wright proceeded to examine the hair follicles of TDO affected subjects under electron microscopy and found that hair shaft for TDO syndrome subjects were smaller and tended to have central shaft depression (19). All TDO individuals had generalized thin and/or pitted enamel hypoplasia. Taurodontism was present in all affected individual, but it did not always affect the first permanent molars and ranged from mild to severe. Skeletal involvement was more prevalent in the North Carolina TDO population (97%) compared to unaffected family

members (30%). Affected individuals showed a greater prevalence of obliterated diploe, lack of frontal sinus pneumatization and lack of mastoid pneumatization. In 1996, Kula et al characterized craniofacial features of TDO syndrome in the North Carolina kindred (20). They reported that the affected individuals had significantly longer anterior cranial base length (SN), greater cranial base angle (BaSN), longer mandibular body length (GoPg) and a more obtuse gonial angle in TDO affected adults compared to unaffected adults. They measured cortical bones of skulls on cephalograms and found that TDO affected individuals had thicker intramembranous bones, although it was unclear whether the thicker cortical bone was a product of increased bone deposition on the external surface or decreased bone resorption on the inner surfaces. Islam et al reported similar findings in 2005 (21). They reported three new cases of TDO from a large family and saw an increase in bone density in long bone, increased thickness and density of skull bone especially skull base and mandibles that are in the "upper normal size limit" with increased trabeculation and bone density in TDO affected individuals. Haldeman et al performed dual-energy x-ray absorptiometry (DEXA) scans at fours common standardized test regions. They found a marked increase in bone mineral density of TDO affected subjects with no associated systemic pathology. This increase in bone mineral density occurred in intramembranous as well as endochondral bone suggesting the role of *DLX3* in bone formation and/or homeostasis of the appendicular skeleton (22).

The late 1990's heralded the genotypic characterization of TDO. Hart et al evaluated the North Carolina families and using a genome-wide search strategy. They obtained evidence for linkage of the TDO syndrome locus to a marker on chromosome 17q21 (D17S791) (13). However, there were hundreds of expressed sequences, including several

candidate genes that could account for the phenotypic traits of TDO, located in the interval at 17q21. Price et al. narrowed their search to two specific members of the Distal-Less homeobox gene family located in this region, *DLX3* and *DLX7*. They cloned and sequenced both genes and identified a 4 base pair deletion in human *DLX3* which correlated with the TDO syndrome phenotype (23). The 4 base pair deletion causes a frameshift mutation leading to the formation of a termination codon. The resulting truncated protein can potentially still bind to DNA, but due to the intact homeodomain region, but is functionally altered. While the *DLX* gene family has been shown to be important in tooth and bone development in the murine model, this was the first human study to show their importance in the development of hair, teeth and bones. Price et al also evaluated the Virginia TDO kindred reported by Lichtenstein. They found the same 4 base pair base deletion mutation previously reported in the North Carolina kindred (24). Additionally, the affected Virginia TDO subjects had the same haplotype found in the affected North Carolina TDO subjects suggesting the *DLX3* deletion mutation was inherited from a common ancestor. They further suggested that the clinical variability observed in the Virginia and North Carolina families was not the result of genetic heterogeneity at the multiple loci, but reflected genetic heterogeneity at other epigenetic loci and/or contributing environmental factors.

In 1999, Price et al re-examined the association between TDO and amelogenesis imperfecta hypomaturation- hypoplasia type (AIHHT). Armed with the knowledge that TDO is caused by a *DLX3* mutation deletion, they performed mutational analysis and sequencing studies on a family with AIHHT. Neither the affected nor unaffected AIHHT subjects possessed the 4 base pair *DLX3* mutation present in the known TDO affected subjects. The authors concluded that TDO and AIHHT were genetically distinct conditions (25). In 2005,

Dong et al renewed the debate with their published study of an Australian "AIHHT" family (26). The affected subjects all showed reduced enamel thickness and enlarge pulps. No family members had curly hair and skull radiograph of affected individuals showed no evidence of bone sclerosis. The authors excluded the diagnosis of TDO due to a lack of bone and hair involvement. However, it is interesting to note that 55% of TDO affected subjects hairs straighten throughout childhood (18) and DEXA scans were not performed on the Australian members in the study to truly rule out bone involvement. They identified a novel 2 base pair CT deletion *DLX3* mutation. This new mutation was located within the homeodomain of *DLX3* and produces an altered protein truncated by 88 amino acids at the COOH terminus compared the 32 amino acid truncation found in the TDO *DLX3* mutation. The authors concluded that TDO and some forms of AIHHT are allelic and suggest that this new *DLX3* mutation affects the formation of enamel and teeth, but lacks the defects of bone and hair. Recently, Wright et al identified another group of TDO kindred in Switzerland with the same 2 base pair (c561_562delCT) deletion mutation. All affected subjects had diminished hair shaft diameter and morphology similar to the c.571_574delGGGG *DLX3* deletion. They all had reduced enamel thickness, although tooth size and severity of taurodontism were less severe compared to the 4G deletion mutation. While the Switzerland family did not exhibit sclerotic bone on skull radiograph, they all displayed a slight increase in bone mineral density based on DEXA evaluation. The authors conclude that this new 2 base pair deletion causes attenuated phenotype of TDO syndrome and not AIHHT (27).

DLX 3

Dlx genes are vertebrates homologous to the *Distal-Less (DII)* gene originally characterized in *Drosophila*. To date six *DLX* homedomain genes have been identified and organized into 3 pairs (*DLX1/ DLX2*) located on chromosome 2, *DLX5/ DLX6* located on chromosome 7 and *DLX3/ DLX7* located on chromosome 17q21) (28). These homeodomain proteins act as transcription factors that regulate many developmental processes, ranging from organizing body segments to differentiation of individual tissues. *DLX* genes are thought to regulate cell differentiation in the skeleton including bone, cartilage and tooth. In the forebrain, *DLX1*, *DLX2*, *DLX5* and *DLX6* have overlapping spatial expression and a distinct temporal pattern of expression (29)

Unlike the other members of the *DLX* family, *DLX3* is not expressed in central nervous system (30). Early *DLX3* expression is localized in specific areas of the first and second branchial arches including neural crest cells that will give rise to odontoblast and craniofacial structure. Later in development, *DLX3* is expressed in structures with epithelial mesenchymal interaction such as skin, hair follicles, otic and olfactory placodes, limb buds, placenta, tooth germs (29, 30, 31, 32). Attempts at targeted deletion of the *DLX3* gene in mouse result in embryonic death due to failure of the placenta to undergo proper morphogenesis (32).

In the skin, *DLX3* is expressed in stratified epidermis and the matrix cells at the base of hair follicles and thought to play a key role in murine keratinocyte differentiation (33). Transcription of *DLX3* is initiated in the suprabasal cells as they start their migration outwards and begin terminal differentiation. In addition, transgenic over-expression of *DLX3*

in basal cell layer produces abnormal epidermal phenotype including cessation of cellular proliferation and premature differentiation of basal cell (31). Morasso et al. outlined a possible pathway for the regulation of *DLX3* in keratinocytes. Using a series of in vitro experiments, they showed that bone morphogenic protein (BMP-2) induced *DLX3* expression through phosphorylation of Smad1/ Smad4 proteins (34). Recently, *DLX3* was shown to be regulated by p63, a gene associated with ectodermal dysplasia (EDs) (35).

Robinson and Mahon first described *DLX3* expression in the developing murine dentition in 1994 (29). Using radioactive in situ hybridization, they localized *DLX3* expression to dental mesenchyme early in development. A later study by Zhao et al (36) produced conflicting results. They found *DLX3* was expression in dental epithelium throughout tooth development and argued that Robinson's radioactive in situ hybridization technique had lower cellular resolution. They reported that *DLX2* and *DLX3* are the only *DLX* genes expressed in the lamina of molar and incisor primordial during embryonic stages that correlate to tooth initiation. Citing a study by Mina and Kollar (37) which showed that tooth development is initiated by signals from dental epithelium, the authors suggest that *DLX3* plays a role in tooth initiation. Their study also showed *DLX3* expression in the early cap and enamel late bell stage of tooth development. Ghoul-Mazgar reported that *DLX3* is expressed in differentiating ameloblast and down-regulated during odontoblast polarization suggesting a role in tooth morphogenesis (38). This would be consistent with altered tooth morphology exhibited in TDO patients.

Identification of a *DLX3* mutation in people with TDO revealed a link between bone development the *DLX* gene family (23). *DLX3* mutation in humans result in altered osteogenesis and increase in mineral density of bone matrix suggesting a role in

endochrondral ossification (22). In vivo experiments in human and murine embryonic tissues showed that *DLX3* protein expression varied specifically with terminal differentiation of ameloblast, odontoblast, osteoblast and chondoblasts (38). The authors reported *DLX3* protein expression in chondrocytes in the prehypertrophic cartilage zone and differentiating osteoblasts of metaphyseal periosteum. It is interesting to note that *DLX3* protein expression correlated well temporally and spatially with Indian Hedgehog (Ihh), a gene associated with chondrogenesis and osteogenesis. Hassan et al examined the regulatory role of *DLX3* in osteoprogenitors cells (39). They showed recruitment of DLX3 proteins to the osteocalcin (OC) promoter region during osteoblast differentiation. *DLX3* association with the OC promoter region decreased while Dlx5 recruitment increased during the mineralization stage of osteoblast differentiation. Recently, Choi et al tested the effects of the *DLX3* 4 base pair mutation in preosteoblastic and multipotent mesenchymal cells (40). The 4 bp deletion mutation produced mesenchymal differentiation to an osteoblastic lineage instead of a myoblastic lineage. It also accelerated osteoprogenitor cells differentiation to osteoblast.

Much has been learned about the function and regulation of *DLX3* in the past few years. However, the early lethality of *DLX3* knock-out in mouse limits our ability to study the role of *DLX3* in late development. TDO offers a unique genetic model to exam the role of *DLX3* in craniofacial development. The tissues affected by TDO (hair, teeth and bone) correlate with the known expression *DLX3* in different tissues (hair matrix cells, dental epithelium, odontoblast, and neural crest cell). By characterizing the phenotypic expression of TDO, we can continue to uncover the role of *DLX*3 in human craniofacial development.

II INTRODUCTION

Tricho-dento-osseous syndrome (TDO) is an autosomal dominant disorder with complete penetrance characterized by abnormalities involving hair, teeth and bone (10). Early reports of TDO subject showed marked phenotypic variability of the tissues involved including changes in fingernails, enamel hypoplasia, taurodontism, doliocephaly, cranial sclerosis and mandibular prognathism (4).

The identification of a large group of TDO individuals in North Carolina led to the discovery of the molecular etiology of TDO (19). Using a genome-wide search strategy, TDO was mapped to a locus on chromosome 17q21, a region known to contain members of the distal-less homeobox gene family including *DLX*3 and *DLX7* (13). Molecular analysis of individuals with TDO revealed a 4 bp deletion mutation of *DLX*3 (c.571_574delGGGG). This deletion produces a frame-shift mutation resulting in early termination of the *DLX*3 protein (23). A re-examination of the original Lichtenstein TDO population indicated that all affected members carry the same 4 bp *DLX3* mutation suggesting that the phenotypic variations observed in TDO subjects are not the result of genetic heterogeneity at multiple loci, but reflect genetic heterogeneity at other epigenetic loci and/or contributing environmental factors.

DLX3 is a member of the homeobox gene family homologous to the *Distal-Less* (*DII*) gene of Drosophila. *DLX* genes act as transcription factors that regulate many developmental processes, ranging from organizing body segments in Drosophila, to differentiation of

individual tissues including bone cartilage and tooth (29, 41). Early *DLX3* expression is localized in specific areas of the first and second branchial arches including neural crest cells that will give rise to odontoblast and craniofacial structures. Later in development, *DLX3* is expressed in structures with epithelial mesenchymal interaction such as skin, hair follicles, otic and olfactory placodes, limb buds, placenta, tooth germs (29, 31).

Attempts at targeted deletion of the *DLX3* gene in mice result in embryonic death due to failure of the placenta to undergo proper morphogenesis (32) limiting our ability to study the role of the gene in later development. TDO offers a unique genetic model to exam the role of *DLX3* in craniofacial development. The tissues observed to be affected by TDO (hair, teeth and bone) are associated with some of the tissues showing expression of *DLX3* (hair matrix cells, dental epithelium, odontoblast, and neural crest cell).

Kula et al measured variations in craniofacial features between TDO and unaffected family members (20) They reported an increase in bone density and thickness of the chondrocranium and calveria, lack of pneumatization of the mastoids, frontal sinus and diploe, greater cranial base length and cranial base angle, longer mandibular body length and less obtuse gonial angle in TDO individuals. However, this study had a limited sample size with TDO status determined by phenotypic traits rather genetic testing. Affected individuals also had a marked increase in bone density in both endochondral and intramembranous bone formation with no associated systemic pathology suggesting an association between TDO syndrome and bone formation and homeostasis (22). With the marked variations of bone craniofacial phenotype displayed by TDO affected subjects, studies that examine craniofacial morphology must be large enough that to rule out possible familial traits. Our current study contains the largest sample of size of TDO affected and unaffected family members

evaluated to date and characterizes the craniofacial features of TDO syndrome to help elucidate the role of *DLX*3 in human craniofacial development.

III MATERIALS AND METHODS

Subjects

Eight families from a single kindred were identified for clinical and radiographic signs of TDO. The study was approved by the Biomedical Institutional Review board and informed consent was obtained from all participants. DNA was extracted from peripheral blood samples using standard DNA isolation techniques (Qiagen, Valencia, CA). *DLX3* gene was sequenced using primers for all three exons and splice sites. Amplicons were sequenced using ABI 770 and verified (24). Subjects were screened for TDO by genetic testing for the mutations in the *DLX*3 gene. Allocation of subjects to affected or unaffected TDO groups was done based on genetic results for *DLX*3 mutations. The demographic characteristics of the TDO and unaffected family members are shown in Table 1.

Cephalometric Analysis

Cephalograms were taken at a standardized distance with a magnification of 12%. All subjects were positioned in natural head position. Edentulous subjects were recorded without prostheses with their mandible in rest position. Cephalograms were scanned and imported into Dolphin Imaging software version 9.0 (Chatworths, CA). Digitization and cephalometric analysis were performed by one examiner (TN). Fourteen linear and angular measurements were recorded (Figure 1 and Table 2). Bilateral anatomic structures were

bisected. Composite cephalograms were generated for the 53 TDO affected and 34 unaffected subjects using the protocol described by Proffit et al (42). Superimpositions were performed along the Sella-Nasion (S-N) line with registration at Sella (s) and the inferior border of the mandible with registration on Menton (Me) (Figure 2).

Reliability of the Measurements

The intraclass correlation statistic was used to assess the reliability of the measurements using ten randomly selected cephalograms that were traced and digitized on three occasions over a two week intervals by the same observer.

Statistical Analysis

The influence of the presence of TDO on craniofacial cephalometric measures was assessed adjusting for the effect of age, gender, and edentulous status using a general linear model with age, sex, edentualism, and TDO status as main effects. Family was considered a random effect. Alternative covariance structures were examined to control for the expected correlation among subjects within the family unit. Variance component was selected due to the non-convergence of the models when more complex covariance structures were used. Variance component divides the overall error variance into two components- variation between families and variation between subjects within families. The model for each measure was fit with a random intercept for each family. Significance level was set at $P<0.05$.

IV RESULTS

Intra-class correlation coefficient (ICC) ranged from 87% to 99.5% for the intra-observer reliability. Midface length (Co-A) had the lowest ICC of 87%. All other variables had ICCs above 90% implying high intra-observer consistency for all other cephalometric measures.

Marked variation was observed in most of the cranial features measured in this study. Summary of the results after adjusting for age, gender and edentulous status is shown in Table 3 & 4. Affected individuals varied from severe Class III appearances to moderate Class II with mandibular retrusion. The average midface length (Co-A) was similar between the two groups, but average maxillary protrusion (SNA) was significantly less ($P<0.001$) in the affected group compared to the unaffected group. Average ANB ($p = 0.0018$) was also smaller in the affected group. Mandibular protrusion (SNB) was comparable between the two groups. There was no statistical significant difference in cranial base length (N-Ba, S-N, S-Ba) or cranial base angle (Ba-S-N) between affected and unaffected subjects.

There was no statistically significant difference in total face height (N-Me), upper face height, (N-ANS) or lower face height (ANS-Me) between the TDO affected group and unaffected control. Mandibular body length (Go-Gn) was significantly longer ($p = 0.009$) in the TDO affected individuals with a mean difference of 3.6mm when compared with the unaffected individuals. Ramus height (Ar-Go) was significantly shorter ($p = 0.05$) in

affected individuals, however, there was no difference in gonial angle (Ar-Go-Me) between affected and unaffected individuals.

Superimpositions of composite tracings representing the population samples of TDO affected and unaffected individuals are shown in Figure 2. Superimposition performed at the cranial based showed that TDO affected group tended to have a more anterior positioned Nasion (N) and slightly posterior positioned Basion (Ba). A point (A) and anterior nasal spine (ANS) was slightly retrusive in the TDO affected group when compared to the unaffected controls. Superimposition of the composite tracing at the inferior border of the mandible showed that the TDO affected group had a longer mandibular body length and shorter ramus height. In addition the condyles of the TDO group were positioned more inferior and posterior when compared the unaffected group.

V DISCUSSION

Early reports of TDO in the literature noted variable craniofacial features including doliocephalic facial pattern, square jaw and mandibular prognathism (1, 3, 4). However, these studies often had small sample size and relied on clinical signs for classification of TDO which may not be as accurate as genotypic classification. In this study, we present the largest reported sample of TDO affected individuals confirmed for the *DLX3* mutation by genetic testing.

Kula et al. (20) reported that TDO affected individuals had a more obtuse cranial flexure and increased cranial base length compared with unaffected individuals. Shapiro et al. (11) also noted flattened cranial bases and dolichocephalic appearances, but no cephalometric values were reported. We found no statistical significant difference in cranial base length or cranial base angle although our cranial base length means were comparable to those reported by Kula et al. Our study also found no statistical significant difference in total face height, upper or lower face height. While Kula reported no difference in face height, they noted that both groups tended to have a greater total and lower face height than standards. While we did not compare individual face height values to Bolton and Behrent's standards (43), our mean face height values for TDO affected and unaffected groups were similar to those published by Behrents (43) when matched for mean age of each group. A possible explanation is that in clinical studies, subjects with drastic phenotypic traits are often

first identified and enrolled. As more subjects are recruited and examined, a once dramatic trait could normalize to the mean especially with the level of phenotypic variance in craniofacial expression exhibited in TDO individuals.

The position of the maxilla in relation to the cranial base (SNA) was more retrusive in TDO affected individual compared to unaffected individuals, although there was no difference in maxillary unit length (Co-A) between the groups. The composite superimposition (Figure 2) showed that TDO affected individuals tended to have a more anteriorly positioned nasion (N) and upper face rather than an absolute retrusion of the midface when compared to unaffected family members. It is interesting to note that both groups showed slightly smaller maxillary length (Co-A) when compared to standards. This coincides with early case reports citing maxillary deficiency in TDO subjects (1, 3, 16). Kula et al. (20) reported no significant difference in SNA or CoA measurements in her study, but noted that 84% of the TDO affected and 80% of non-affected populations had smaller maxillas compared to Bolton standards. Further studies are needed to determine if this slight maxillary retrusion found in both groups is due to a familial trait. The Intraclass Correlation in our study was lowest (87%) for condylion (Co) which could explain why no significance difference in midface length was observed in between the groups.

Patients with TDO are often described as having prognathic mandibles (1, 3, 16). Our study showed no difference in mandibular protrusion (SNB), but due to a retrusive maxilla, the relative position of the mandible to the maxilla (ANB) appears more prognathic with a Class III appearance. Whether this Class III skeletal pattern observed in TDO individuals is due to familial influences or mutation in the *DLX3* gene remains to be determined and further studies are needed to test this. TDO individuals had significantly

longer mandibular body length (Go-Gn), shorter ramus height (Ar-Go) with no difference in gonial angle (Ar-Go-Me). A shorter ramus height can mask mandibular projection even with a significant increase in body length. Previous studies by Robinson (1) and Lichtenstein (3) suggest that a more obtuse gonial angle is characteristic of TDO whereas the present study revealed a normal gonial angle. These studies were descriptive in nature and had a high percentage of edentulous patients. The edentulous mandible undergoes bone deposition at the inferior border of the corpus and resorption in the antegonial region giving the appearance of a more obtuse gonial angle (44).

Skeletal patterning in the vertebrate system is a complex process involving numerous molecular pathways, transcription factors and regulatory proteins. *DLX3* has been shown to a plays a role in osteogenesis and bone formation (38, 39). While our study shows that TDO affected individuals display considerable phenotypic variation, there does appears to be an association between *DLX3* mutation and craniofacial dysmorphology. Future studies of *DLX3* mutation and thorough phenotyping of TDO syndrome will help advance our understanding of craniofacial development and bone formation.

VI CONCLUSIONS

1. TDO affects subjects display relatively retrusive maxilla (SNA) and decreased SNB compared to unaffected family members.
2. *DLX3* gene mutation resulting in TDO syndrome is associated with a statistically significant increase in mandibular body length (Go-Gn).
3. *DLX3* gene mutation resulting in TDO syndrome is associated with a statistically significant decrease in ramus height (Ar-Go).

VII. TABLES

Table 1. Demographic Characteristics of the TDO Affected and Unaffected Groups

	Mean Age	Gender	Edentulous
TDO n = 53	31.7 (5.0 - 79.3)	Females = 25 Males = 28	26%
Unaffected n = 34	37.1 (5.8 - 76.0)	Females = 18 Males = 16	6%

Table 2. Cephalometric Measurements

Linear measurements

Cranial base (N-Ba)

Anterior cranial base (S-N)

Posterior cranial base (S-Ba)

Total face height (N-Me)

Upper face height (N-ANS)

Lower face height (ANS-Me)

Mandibular ramus height (Ar-Go)

Maxillary unit length (Co-A)

Mandibular unit length (Co-Gn)

Mandibular body length (Go-Gn)

Angular measurements

Cranial base angle (Ba-S-N)

Gonial angle (Ar-Go-Me)

SNA

SNB

ANB

Table 3. **Mean Cephalometric Values and Standard Errors**

Variables	TDO		Unaffected	
	TDO Mean	SEM	Unaffected Mean	SEM
SNA	78.84	0.45	81.50	0.53
SNB	77.58	0.51	78.22	0.62
ANB	1.27	0.39	3.29	0.3
N-Ba	113.09	0.98	110.79	1.05
S-N	75.96	0.67	75.01	0.7
S-Ba	40.15	0.78	38.51	0.85
Ba-S-N	129.42	0.58	128.72	0.81
N-Me	122.15	1.71	122.88	1.88
N-ANS	53.40	0.7	54.05	0.79
ANS-Me	69.55	1.29	70.29	1.37
Ar-Go	45.58	0.91	48.05	1.09
Ar-Go-Me	128.18	0.8	128.14	1.26
Co-A	91.47	0.87	91.14	1.14
Co-Gn	126.65	1.25	124.70	1.25
Go-Gn	82.47	0.95	78.87	1.25

Table 4. P values Associated with the Variance Component General Linear Model.

Variables .	P-value for TDO	P-value for Age	P-value for Gender	P-value for Edent
SNA	**<0.001***	0.3	**0.01**	0.24
SNB	0.1986	0.19	**0.01**	0.63
ANB	**0.002***	0.77	0.2	0.13
N-Ba	0.11	**0.02**	**<0.001**	0.50
S-N	0.32	**0.01**	**<0.001**	0.60
S-Ba	0.28	0.38	**<0.001**	0.84
Ba-S-N	0.58	0.97	**0.01**	0.19
N-Me	0.69	**<0.001**	**<0.001**	0.18
N-ANS	0.8	**<0.001**	**<0.001**	0.61
ANS-Me	0.93	**0.01**	**0.01**	0.36
Ar-Go	**0.05***	**<0.001**	**<0.001**	0.89
Ar-Go-Me	0.87	0.99	0.22	0.78
Co-A	0.76	**0.02**	**<0.001**	0.87
Co-Gn	0.07	**<0.001**	**<0.001**	0.87
Go-Gn	**0.01***	**0.01**	**<0.001**	0.85

Table 4. P values for cephalometric variables with the variance component general linear model. Adjusting for age, gender and edentulous status, TDO status significantly affected SNA, ANB, ArGo and GoGn.

VIII. FIGURES

Figure 1. Representative Cephalometric Tracing

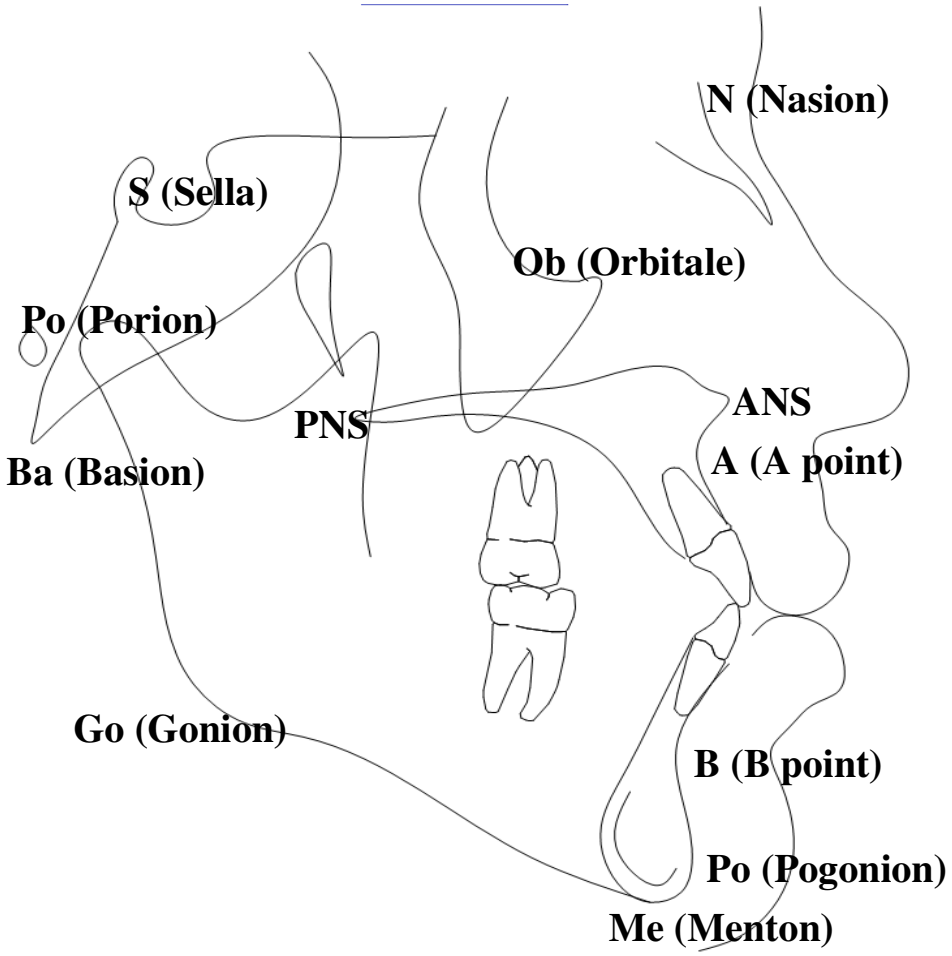

Figure 2 Superimposition of Composite Tracings for TDO Affected and Unaffected

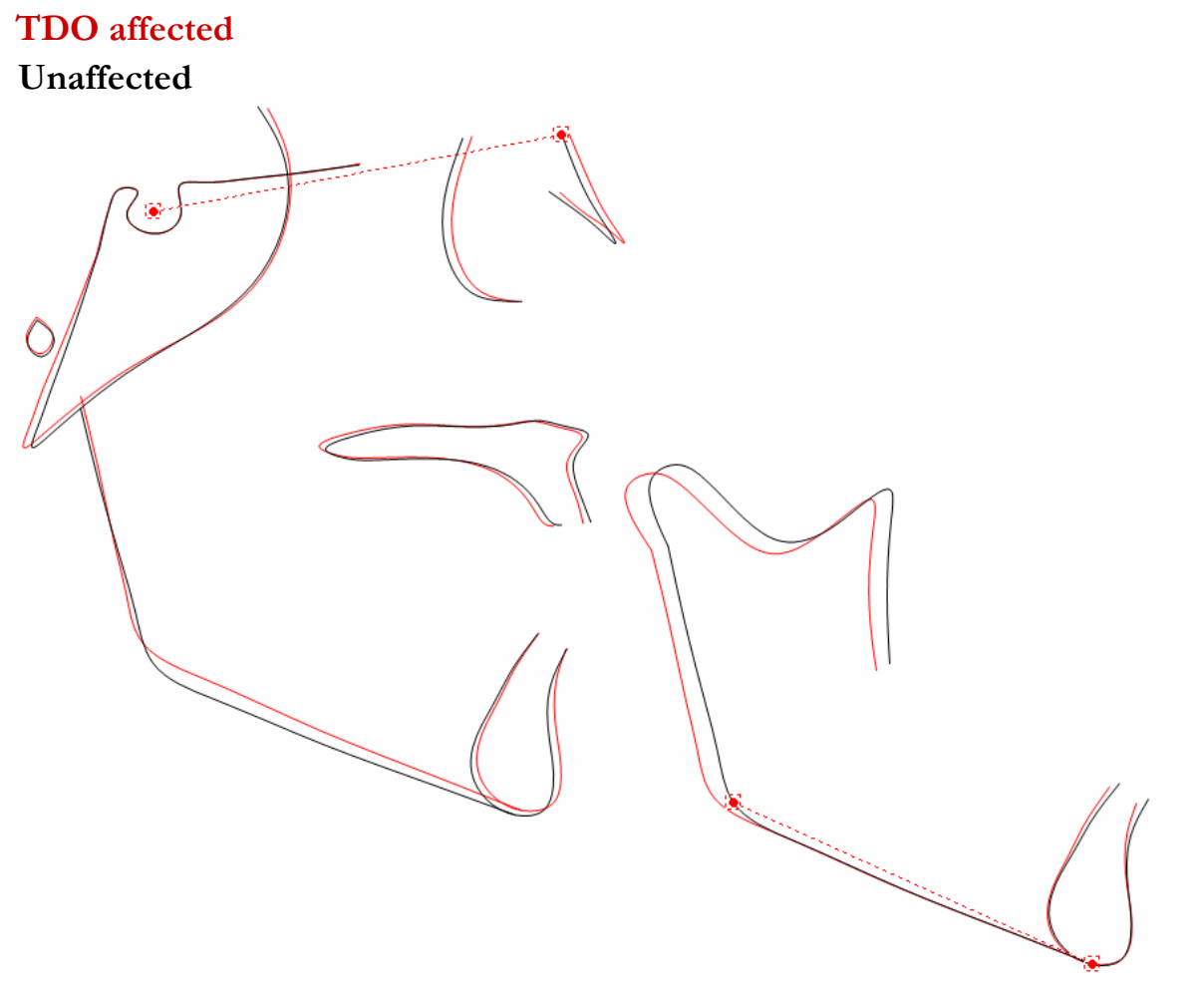

Figure 2. Superimposition of composite tracings generated from 53 TDO affected (red) and 34 unaffected family members (black). Superimpositions were performed along the Sella-Nasion (S-N) line with registration at Sella (s) and the inferior border of the mandible with registration on Menton (Me).

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