Dentinogenesis Imperfecta: Relationship of genotype with clinical and radiographic features

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ABSTRACT

S. J. Shahangian: Dentinogenesis Imperfecta- Relationship of genotype with clinical and radiographic features
(Under the direction of J. Timothy Wright, DDS, MS)

Dentinogenesis Imperfecta (DGI) is the most common hereditary anomaly of human dentin. It is typically diagnosed by clinical and radiographic features. It occurs in isolation or in conjunction with the syndrome osteogenesis imperfecta. Insufficient understanding of its pathophysiology, phenotype-genotype relationships, and variation in disease severity make diagnosis and treatment of DGI a challenge. **Objectives:** To characterize the phenotype and instigate evaluation of phenotype-genotype correlations in DGI. **Methods:** Study participants were diagnosed based on major and minor phenotypic features. Phenotyping was completed with clinical and radiographic examination to objectively asses occlusal relations, tooth size and morphology, tooth color, x-ray absorption property, attrition, enamel defect and fracturing. **Results:** Subjects were classified based on genetic defect (*COL1A1/A2*, *DSPP*, controls*)*. Genotype-phenotype correlations were found between the groups. DGI teeth were found to be smaller and more bulbous. Other differences exist between the groups in their dental and skeletal morphology, degree of morbidity and dental shade.

Dedicated to Roya F. Shahangian born June 21, 2008

A special thanks to my wife and parents for their relentless support and encouragement over the years;

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CEJ	Cemento-enamel Junction
CC	Cervical Constriction
DD	Dentin Dysplasia
DD-II	Dentin Dysplasia Type II
DEJ	Dentin-enamel Junction
DGI-I	Dentinogenesis Imperfecta Type I
DGI-I	Dentinogenesis Imperfecta Type II
DGI-II	Dentinogenesis Imperfecta type III
DSP	Dentin Sialoprotein
DPP	Dentin Phosphoprotein
DSPP	Dentin Sialophosphoprotein
HOC	Height of Contour
OI	Osteogenesis Imperfecta\
PCO	Pulp Canal Obliteration

Dentinogenesis Imperfecta- Relationship between genotype with dentoskeletal morphology and morbidity

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LITERATURE REVIEW

Normal Dentin:

Normal mineralized human tissues include bone, cartilage, and teeth. The mineralized constituents of a tooth include three specialized tissues: enamel, dentin, and cementum. Dentin dominates as its most abundant component and is critical in its form and function. Dentinogenesis is the process of dentin formation that involves terminally differentiated dentin producing cells (odontoblasts) that create and secrete the extracellular matrix that mineralizes and becomes dentin. These cells are formed from dental papilla mesenchymal cells that interact with the epithelial cells (future ameloblasts) of the developing tooth. These epithelial-mesenchymal interactions trigger cytodifferentiation of the specialized odontogenic cells. Odontoblasts secrete organic components similar to bone forming osteoblsts[1]. Of all calcified human tissues, dentin resembles bone the most in its basic composition and many of its genetic and cellular pathways[2].

Dentin Structure and Composition:

Dentin is porous and made of seventy percent inorganic hydroxyapatite and non-crystalline amorphous calcium phosphate, twenty percent organic material, and ten percent water by weight[3]. Its main functions include providing structural support for enamel as well as immunologic, physical, and thermal protection of the pulp. Its yellow color shines through the translucent enamel playing a major role in defining the intrinsic shade of a tooth. Tubules that are remnants of the dentin producing cells' (odontoblast) processes, transverse the dentin from the pulp to radiate out to the dentin-enamel junction (DEJ). The density and number of dentinal tubules increases towards the pulp with an approximately 1:5 ratio on the opposite ends[4]. The lumen however, gets larger towards the pulp and follows an S-shaped curvature that is characteristic of mammalian dentin[5]. Other than their original function in dentinogenesis, these tubules continue playing a role in communication of pulp afferent nerves, and biological response against environmental insults including caries invasion[6].

Types of Dentin:

Three types of dentin named, primary, secondary, and tertiary dentin have been described. Primary dentin makes up the majority of the dentin compartment by volume and includes all the dentin formed to the point of closure of the root apex. The dentin compartment can be subdivided into two subcomponents including mantle dentin and circumpulpal dentin. Mantle dentin is the very first layer of dentin produced adjacent to the basal lamina and inner enamel epithelium. It is only about 20 micrometers thick and is the first tissue to mineralize on the dentin side of the DEJ[4]. It lacks phosphoryn and is less dense in collagen fibrils and mineral content, rendering it more elastic than the rest of the primary dentin, typically referred to as circumpulpal dentin. Secondary dentin, or dentin formed after the tooth root has fully formed and apexified, is laid down at a lower rate throughout life and its composition is generally similar to primary dentin. This process causes a decrease in pulp chamber size with advancing age. Tertiary dentin, also called reparative dentin, is formed in response to external stimuli such as caries or trauma. Its architectural irregularity varies but includes few dentinal tubules with an irregular course, depending on the type and duration of insult. Tertiary dentin is generally relatively poorly organized and less mineralized than secondary dentin and is the tooth's mechanism for walling off the pulp from noxious stimuli like dental caries or tooth fractures.

Dentin Genes and Matrix:

Dentinogenesis is a highly regulated and genetically driven process. Major genes expressed by odontoblasts during tooth formation include *COL1A1*, *COL1A2*, and *DSPP*[7]. The most abundant protein in dentin is type I collagen, comprising nearly 90% of its organic extracellular matrix[8]. Other minor dental collagenous proteins include type I trimmer, type III, V, and VI collagen. Type I procollagen forms from intertwining of two pro-alpha1 and a single pro-alpha2 chains. Multiple posttranslational modifications such as hydroxylation of certain amino acids promote intermolecular cross-linking and stabilize the heterotrimer to construct an insoluble fiber with high tensile strength. Inadequate quantity or quality of collagen can lead to abnormal function and properties in collagen-rich organs.

In addition to collagens, non-collagenous proteins (NCPS) are believed to play a key role in the mineralization process. The most abundant non-collagenous proteins in dentin are classified as small integrin-binding ligand N-linked glycoproteins (SIBLINGS)[9]. They consist of dentin matrix protein 1 (DMP1), matrix extracellular phosphoglycoprotein (MEPE), osteopontin (OPN), bone sialoprotein (BSP), and dentin sialophosphoprotein (DSPP). While both odontoblasts and osteoclasts secrete collagen and SIBLINGS during formation and mineralization of dentin and bone respectively, the quantity of OPN, MEPE, BSP, DMP1, and DSPP that is secreted is very different. The major SIBLINGS proteins present in dentin include DSPP, which is cleaved into its functional domains of dentin sialoprotein (DSP), dentin phosphoprotein (DPP) and dentin glycoprotein (DGP) [10]. DSP is a proteoglycan at the N-terminus, DGP is the middle component, and DPP is a highly phosphorylated protein at the C-terminus. DSP is believed to play a role in synthesis of dentin matrix formation, while DPP is involved in nucleation and control of dentin matrix mineralization. Abnormalities in this latter protein lead to the classic clinical trait of dentin known as dentinogenesis imperfecta (DGI) [1, 11]. The role of DGP is even less clear but it too may play a role in dentin biomineralization[12].

Abnormal Dentin Formation:

Developmental aberrations in dentinogenesis were first identified decades ago. These anomalies may be identified on the spectrum spanning from biochemical to the macroscopic level. On the broadest level, dentinal anomalies can be classified as either isolated to the tooth (non syndromic) or in association with anomalies in other organs (syndromic). Bailleul-Forestier et al. have compiled a list of the most commonly reported syndromic and non-syndromic anomalies of dentin[13 14]. The non syndromic forms of hereditary dentinal anomalies include dentinogenesis imperfect type II (DGI-II) and dentin dysplasia (DD). The syndromic forms include DGI with osteogenesis imperfecta (DGI-I), Ehler-Danlos syndrome (EDS) OMIM #130000, Goldblatt syndrome OMIM 184260, Schimke immunoosseous dysplasia OMIM 242900, Hyperphosphatemic familial tumoral calcinosis (HFTC) OMIM 211900, Familial hypophosphatemic vitamin D-resistant rickets OMIM #307800, and Seckel syndrome OMIM 210600. Tables 9a, 9b, 9c and 9d summarize these conditions.

Cardinal clinical and radiographic features of common hereditary dentinal defects included dental discoloration, cervical crown constriction, variations in pulp canal/chamber size and morphology, enamel fracturing and exaggerated attrition, as well as variations in root morphology. Histologically, the dental tubules may be less dense and more irregular[15] and frequently include vesicular structures and inclusions [16]. Kinney et al have suggested that some subtypes have dentin that lacks intrafibrillar mineralization[17].

Classification of Hereditary Dentin Anomalies:

Two systems of classification for the most common inherited defects of dentin have been commonly cited. One was proposed by Shields[18] and the other by Witkop [19]. Given the limitations of a classification system that is based on clinical findings, many deviations have arisen over the years and each subtype of this condition has earned multiple alternative names. Much of this deviation is attributed to the fact that very little was known about the molecular etiology at the time of their proposal [20]. Currently, due to extensive phenotypic variability and genetic heterogeneity, the characterization of DD, DGI and OI w/ DGI has proven to be a challenge and no universally accepted comprehensive classification exists. While new genetics-based classification's have been proposed, the most common classification system used in the dental literature is strictly phenotype based[9]. It divides inherited dentin defects into two groups (DGI types I OMIM #166240, II OMIM #125490, and III OMIM #125500) and dentin dysplasia (DD types I OMIM %125400, II OMIM #125420). Conversely, the medical literature describes OI subtypes that are associated with DGI as subtype A, and those without DGI as subtype B. See table 8 for summary of OI and DGI. DGI-I encompasses the most common syndromic dentinal defect which is associated with osteogenesis imperfect. The advancements in our understanding of the genetic etiology of non-syndromic heritable dentin defects suggest that many of the DGI and DD subtypes appear to be manifestation of the same disease with a wide spectrum of severity[9]. Dentin dysplasia type II, DGI-II and DGI-III are caused by allelic variations of DSPP gene[21, 22]. The genetic etiology of dentin dysplasia type I, an exceedingly rare condition, remains elusive. For the purpose of this thesis, we will call all DGI cases associated with

OI as DGI-I (and will specify subtype of OI in parentheses if necessary); we will call all non-OI associated cases (including DD II) DGI-II. DD-I will be specified as such.

Genetics of Common Hereditary Dentinal Disorders:

Currently, nearly all heritable dentin structural disorders with known genetic patterns are inherited by an autosomal mechanism, the majority of which are dominant traits. Known causative genes of the most commonly inherited dentin defects include *COL1A1*, *COL1A2*, and *DSPP*. These genes are located on human chromosomes 17q21.33, 7q21.3, and 4q22.1 respectively. To date, approximately 800 collagen mutations, and 15 *DSPP* mutations have been reported[23-25]. While all DSPP mutations result in dental aberrations, many of the collagen defects do not appear to produce a clinically discernable tooth phenotype. This may be a result of under diagnosis of mildly affected or sub-clinically affected dentitions where collagen mutation phenotypes are below our visual or radiographic level of detection. This could lead to patients with OI having subclinical ultrastructural changes in dentin yet not be diagnosed as having DGI [26, 27]. Alternatively, this observation could be a function of the complex genetic interactions and redundancies in dental genesis whereby individuals with type I collagen mutations and brittle bones truly have normal dentin. Most dentinal disorders of DSPP mutations appear to be non syndromic, and so far, the only possible associated non dental finding reported in a few families with DGI-II has been hearing loss[28]. Analysis of known DSPP mutations suggests that when DSPP secretion is decreased by half, DGI-II is the phenotype, whereas, when the reduction is less, DD-II phenotype results[21]. DGI-II and DD-II appear related to alterations in the DSP while DGI-III is caused by mutation in the DPP region of dentin sialophosphoprotein[1]. However, all known dentinal conditions with collagen mutations affect multiple organs. The most prevalent of these syndromes is osteogenesis imperfect. This finding is most likely due to the fact that type I collagen is the major matrix macromolecule of both bone and dentin[29]. Table 9d (*FGF23* mutation) gives us a sampling of non-collagen mutations affecting dentinogenesis as well as other organs[30-32]. The present study investigates the most common hereditary dentinal disorder of

dentinogenesis imperfect and its allelic variant, dentine dysplasia type II.

DGI Tooth Composition and Structure:

The dental trait seen in DGI patients has been studied by multiple investigators at the molecular, histological, and clinical level. Knowing the complexity of this genetic condition, it is imprudent to assume that findings from any one study are an accurate representation of the entire DGI population. Variations exist amongst different mutations, families, and even tooth types within the same patient. With some reservation these studies can be insightful as the majority of clinical, radiographic, and histological manifestations reported for DG-I and II are similar[18, 33, 34].

Composition- Enamel from DGI-II has a normal chemical composition while the dentin has increased water (by 60%) and decreased inorganic content (by 10%)[35]. Some Electron microprobe studies report calcium and phosphorus percentages are near normal[35]. In contrast, others report an increase in Ca/P ratio and overall reduction in Ca, P, and Mg[36]. On physical testing, the dentin has a decreased density, x-ray absorption value and microhardness, all consistent with a decrease in mineralization [35].

Histological- Enamel and cementum from DGI-II teeth appear mostly normal with minor abnormalities such as accentuated Striae of Retzius with prism bending and discontinuity[37]. Developmental defects in enamel have been attributed to irregular epithelial-mesenchymal interaction, although DSPP is known to be expressed at least transiently in pre-ameloblasts[38]. Some have reported mantle dentin to be normal in DGI patients regardless of type[34, 39-41], yet others have found histological abnormalities in this layer of dentin[37, 42, 43]. Similarly, there are contradicting reports on the morphology of the DEJ and whether or not it lacks scalloping[35, 39, 44]. There is more agreement in reports on the remaining "non-mantle" dentin which lacks structural regularity with a marked decrease in number of tubules that are also short and misshapen. The atypical granular dentin matrix frequently has interglobular calcifications. The odontoblasts too are atypical as they sparsely line the pulp surface and may be entrapped within defective dentin[45].

Gross- It is worthwhile noting that the expression of DGI type I is more varied than DGI-II. Clinical and radiographic signs of DGI are more pronounced in primary than permanent teeth[46, 47]. Although some have found that histologically, this generalization does not apply and attribute the marked primary tooth discoloration and attrition to thinner enamel in all primary teeth[48].

Craniofacial and Dental Features in OI:

Some dental findings have been associated with OI patients who do not have DGI. Class III malocclusion, openbite, crossbite, and increased impaction of teeth and dentigerous cysts are more common in OI patients regardless of their DGI status. Other reported dental abnormalities include agenesis, apically extended pulp chambers, invagination, and denticles[33, 34, 46, 49, 50]. Additionally, subtle, subclinical histologic manifestations of DI such as dysplastic changes in dentin are more common in patients with OI that do not present with classical features of DGI[51].

Purpose of Study and Significance:

Due to the diverse spectrum of dental defects in DGI, and the paucity of available information regarding predictors of dental morbidity (e.g., attrition, enamel fracturing, and caries) in teeth with DGI, clinicians are often hesitant to intervene with treatment until gross and apparent defects of dental tissues have occurred. This seems to be especially true in the pediatric population. We hypothesize that information that is readily clinically evaluable can provide insight as to the likelihood of enamel loss and subsequent attrition that is commonly seen in severe DI cases. In this study, we correlate characteristics of the DGI trait that are readily evaluable by clinicians (e.g. dental shade, radiographic dentinal density), and the degree of tooth destruction. We feel it is imperative to establish a standardized method of objective phenotype based diagnosis for DGI-I and DGI-II and their respective subtypes. Defining such quantitative diagnostic criteria for DGI could facilitate the identification of subtypes of both osteogenesis imperfecta and dentinogenesis imperfecta[1]. The importance of such subtyping is illustrated by Paterson who demonstrated that, within their OI population, patients with associated DGI-I had a more severe expression of OI anomalies, a greater fracture rate and increased likelihood of growth impairment, when compared to those without DGI[52,

[53]. Furthermore, it is known that in assessing OI, though the expressivity of dental aberrations may vary within the same family, its presence or absence exhibits close to 100% penetrance, while other non-dental clinical features can vary[42, 53, 54]. Hence, dental evaluations are commonly critical in diagnosis of mild forms of OI[54]**.** This knowledge also can contribute to the goal of establishing clear guidelines for diagnosis and treatment of those affected by DGI.

Therefore, the over-riding goal of the present study was to establish objective and quantitative measure of the prominent clinical and radiographic features and correlate these phenotypes with the specific causative genotypes.

MANUSCRIPT

INTRODUCTION

The oral and systemic manifestations of osteogenesis imperfecta (OI) and dentinogenesis imperfecta (DGI) can be devastating. DGI is characterized by a marked decrease in dentin mineralization in both the primary and permanent dentition due to mutations in the *COLIA1, COLIA2, DSPP* and other genes critical for normal tooth and/or bone mineralization. Individuals with DGI type I (DGI-I) are, by definition, also diagnosed with OI, or brittle bone disease, whereas those with DGI type II (DGI-II) are generally free of non-dental anomalies, although altered hearing has been reported[55]. Those with DGI-I can have any of the OI subtypes with varying degrees of bone fragility and deformity. Other common findings associated with OI are joint hyper-extensibility, blue sclera, and hearing loss[56]. The frequency of DGI is highly variable depending on population and subtype of OI; on the other hand, not all individuals with OI have clinically discernable dental anomalies. The reported incidence of OI ranges between 6 to 20 in 100,000 individulas[57, 58]. While 20-50,000 individuals suffer from OI, the prevalence of DGI-I has not been well established in the United States. DGI-II or non-syndromic DI is believed to occur in 1 in 6-8000 US children[59]. In both DGI-I and DGI-II, primary teeth are more severely affected compared with the permanent dentition, making this study particularly important to the oral health care providers of infants and children. With the realization that DGI is the most common hereditary oral disorder and the complexities it presents for patients and care providers alike, the American Academy of Pediatric Dentistry (AAPD) recently published "Guideline for Oral Health/Dental Management of Heritable Dental Developmental Anomalies"[60].

The severity and character of DGI associated dental aberrations is highly variable. This variability is a reflection of the complexity of genes and cellular interactions involved in the development of the human dentition. The many reciprocal interactions between the neural crest originated cells (ectomesenchyme) and oral epithelium eventually pave the way for the genesis of the highly specialized odontoblasts that synthesize unmineralized organic matrix to form predentin, comprised mainly of type I collagen. Non-collagenous proteins such as dentin sialoprotein (DSP) and

dentin phosphoprotein (DPP) are produced to facilitate the collagen mediated mineralization of dentin as calcium and other ions are accumulated to form the mineralized dentin. The function and organic secretions of the odontoblasts resemble that of the bone synthesizing osteoblasts, and their common genetic pathways explain the clinically similar dental findings between some OI and DGI patients. Also, gross morphologic variations such as altered crown morphology and cervical constriction may be attributed to direct or indirect disruption of these cell-signaling pathways involved in morphodifferentiation. The variability in the severity of signs and symptoms of DGI is now believed to be a manifestation of variable expressivity in a disease that represent a continuum of phenotype from very mild to severe [9].

Clinical Phenotype

The classic clinical presentation of DGI is described as an opalescent discoloration of the teeth with enamel that can crack and fracture from the dentin. The hypomineralized exposed dentin then undergoes attrition as it fails to endure the forces of normal mastication, resulting in loss of function and vertical dimension [55, 61]. However, tooth discoloration and rapid breakdown of enamel and dentin is not unique to DGI and in isolation cannot serve as diagnostic or prognostic indicators for the clinician. On the other hand, certain variations such as dentin dysplasia type II can have such mild or subtle discoloration and clinical presentation, especially in the permanent dentition, that careful study of radiographs and family history plays a key role in their diagnosis.

As previously mentioned, the odontoblasts differentiate from mesenchymal cells of the dental papilla early on. Although most directly involved in the formation of dentin, the odontoblast are also major players in the histodifferentiation of the young dental papilla and the eventual formation the dental enamel by the ameloblast [62]. Ameloblasts lay down the matrix that eventually mineralizes into mature enamel and determines the outline of the clinical crown. Ethnic and gender dependent variations in tooth crown morphology have been described by anthropologists [63]. Patients affected by hereditary dental conditions such as DGI are also known to have unique variations in their crown anatomy. Other than dental shade, the majority of these variations may not be readily discernable at

the clinical level and have not been well characterized. However, our anecdotal observation is that in addition to cervical constriction (discussed below), the crown size in DGI patients appears to be smaller than their unaffected counterparts. To date, no one has reported evaluating possible variations in crown dimension and morphology. If so, this finding would have significant implications in the orthodontic and operative aspects of clinical care for the DGI patients.

The healthy Asian population appears to have more bulbous crowns than some other ethnic groups and a range of bulbous crown from is expected as normal variation in the non-DGI dentition[64]. However, exaggerated cervical constriction is a classic feature reported in DGI patients[18]. We are unaware of whether this feature is a function of a narrower crown circumference at the level of the CEJ or an illusion caused by shorter, narrower roots against a normal sized crown. Accurate data on the etiology of cervical constriction can shed some light on which stage and cellular processes have been primarily altered by the DGI mutation.

Radiographic Phenotype

Radiographically the DGI dentition is described as having bulbous crown morphology (cervical constriction), pulp chamber and canals that are often narrow or completely obliterated, and/or abnormal roots that are frequently narrow and blunted[18, 61, 65]. Careful analysis of radiographic features is critical in the diagnosis of DGI, especially milder allelic variations such as dentin dysplasia II [21]. Objective radiographic phenotyping of DGI is lacking in the current literature.

The aim of this study was to define objective major and minor phenotypic features to diagnose DGI; and to compare the dental morphology of different subtypes of DGI on radiographs as compared to controls. We hypothesize that abnormal DSPP and type I collagen expression can alter the size and morphology of teeth and that presence of DGI can be predictive of the genotype in OI patients.

METHODS

This was a prospective cross-sectional clinical study which was approved by University of North Carolina IRB and the National OI Foundation Registry. Assent was obtained from children

under 17 years of age or decisionally-impaired adults, while consent was obtained from adults enrolling in the study or the parent/legal guardian for children. Of note, participants did not have to partake in all components to be considered. If a patient did not assent/consent to or could not participate in any single part of the study (e.g. phlebotomy, radiographs), they were excluded only from that part in data analysis. Study participants were recruited through multiple clinics including the UNC Pediatric Dental Clinic, UNC Pediatric Endocrinology/Bone Subspecialty Clinic, and UNC Pediatric Genetics Clinic. A recruitment email was sent to all pediatric dentists in North Carolina and this project was approved and distributed to members of the National OI Foundation.

To participate one must either have had a confirmed or probable diagnosis of OI or DGI. The OI diagnosis and classification were made by the patient's physician based on either genetic testing or clinical major and minor criteria as described in the literature[24, 66]. When an individual's diagnosis was confirmed according to study protocol (see below), then his/her family members were also invited to participate in order to determine if they possessed a previously undiagnosed (likely milder) forms of DGI or were unaffected (Controls).Individuals having any systemic disease or syndrome known to affect the dentition other than OI were excluded from the study. There was no age, sex, or racial exclusion criterion.

Recruitment, Diagnosis, and Examination

The family history and medical history was evaluated via questionnaires inquiring about their health, hereditary characteristics, and dental history. Specific information regarding hereditary traits such as bone fragility and oral manifestations was assessed and pedigrees were constructed for each family. We formally diagnosed DGI based on either genetic testing or clinical (major and minor criteria). To be classified as affected an individual must have met either 2 major criteria, or 1 major and 2 minor criteria to be diagnosed as having DGI. The major criteria included classic opalescent tooth discoloration, pulp canal obliteration (PCO), cervical constriction, and pronounced attrition. The

minor criteria included increased pulp chamber size, short or slender roots, unprovoked enamel fractures, and multiple teeth with pulp stones or thistle-shaped pulp anatomy.

Genetic analysis for patients recruited through the UNC Hospitals and School of Dentistry were provided by authors KL and TW when available. Referring providers of recruits with unconfirmed diagnosis were contacted to inquire about genetic analysis or previous testing. If the genotype was not known, it was tested by direct sequencing of known DGI and OI candidate genes when possible.

Each participant received a clinical examination. Intraoral and extraoral photographs were taken. Radiographic examination included bitewings and panoramic were completed. All examinations were conducted by two calibrated investigators (TW and JS). Both investigators were calibrated using ten randomly selected patients that underwent a complete re-examination to establish the inter- and intra-examiner reliability and validity of the instruments.

Radiographic Technique and Analysis

All eligible individuals had bilateral digital bitewing radiographs using the long cone technique with a film holding device (DENTSPLY, York, PA). The same type of dental film (Gendex, Des Plaines, IL), exposure settings, and development technique (Gendex, Des Plaines, IL) were used for all evaluations. Digital bitewing radiographs of an additional 290 routine clinic patients with no DGI were randomly selected from patient records. The bitewings were calibrated to account for distortions and mesiodistal crown dimensions of the primary second molar, permanent second premolar and/or the permanent first molar were measured at the height of contour (HOC) and cementoenamel junction (CEJ) (ImageJ Program, NIH). Cervical constriction (CC) was defined as the ratio between HOC/CEJ. Measurements of HOC and CEJ and the CC ratio of the control and DGI groups were compared.

Each participant was evaluated for the number of teeth with PCO. The pulp canal and chambers of each tooth present in the mouth at the time of examination was compared to unaffected patients of similar age and for gross and apparent deviation in size. A PCO Index was formed as a

ratio of all PCO affected teeth divided by all teeth present. Teeth with pulp chambers which were not readily visible were excluded (i.e. teeth with PFM crowns).

Each participant was evaluated for number of posterior teeth with cervical constriction. The bulbous nature of the crown for each molar and premolar in the mouth of the patient at the time of examination was compared to unaffected patients of similar age for gross and apparent deviations in form. A CC Index was formed as the ratio of posterior teeth present with cervical constriction divided by all posterior teeth present. Teeth with full coverage restorations or any other alterations which may have changed the radiographically visible crown contour were excluded. The examiner remained unaware of affected status, race or gender of the radiographs being examined.

Dentofacial Morphology and Morbidity

Dentoalveolar phenotypes including occlusion classification, overjet, overbite, open bite, crossbite, and degree of crowding were compared between different groups. Crowding was assessed in the maxillary and mandibular canine-canines segment. In permanent dentition, if more than half of the contact points were mal-aligned more than 1mm as described by the Little Index of Irregularity[67], in either maxilla or mandible, the subject was classified as crowded. Those in mixed dentition were evaluated by the same criteria. In primary dentition, if the patient lacked interdental spacing of anterior teeth for either arch they were classified as crowded. Any subjects with deflections in eruption path (misangulation of erupting permanent tooth) and none third molar impactions were identified. Agenesis and presence of supernumerary teeth were documented. Degree of dental morbidity including enamel fracturing, enamel defects and attrition was classified as either none, localized, or general. All teeth present were evaluated for enamel fracturing (unrelated to trauma or caries), enamel defect (smooth surface discoloration and enamel hypoplasia), and attrition (apparent exposure of dentin with opposing wear faucets). If any tooth exhibited such findings, the patient was categorized as “localized”, if more than one third of the teeth present exhibited the particular morbidity, the patient was classified as “generalized”. Any patient with at least one arch without a natural tooth was classified as edentulous. Participants (and/or their guardian) were also asked to

report their current degree of dental sensitivity as mild, moderate, or severe. Severity of bone fragility in those with OI was evaluated using self-reported number of fractures; subjects were categorized as having either more or fewer than 10 fractures for statistical analysis.

Statistical Methods

Means and standard deviations were computed for size, and cervical constriction for all teeth combined. Clustering of teeth within subjects was accounted for by using large sample Taylor series based empirical standard errors obtained with the SURVEYMEANS procedure in SAS v. 9.2. Means were also computed by subgroups based on contralateral teeth and standard deviations were computed in the usual way using SAS PROC MEANS. For analysis, we constructed a variable "tooth type" based on pairs of contralateral teeth. Maximum likelihood estimation of linear mixed models was used to compare the groups for size overall and by tooth type. In particular, the first model specified fixed categorical effects of group and tooth type, and random effects for subject. If overall differences among diagnostic groups were indicated adjusting for tooth type, then a series of subgroup analyses by tooth type were performed where the model specified fixed categorical effects of group and random effects for subject. The purpose of the subgroup analyses was to identify the set of contralateral teeth that were contributing to the global differences across diagnostic groups. In each model, statistical significance of an overall test of any differences among the five groups was defined as $p<0.05$. If the overall test was significant, then pairwise comparisons (of all ten possible group pairs) were conducted with Bonferroni adjustment for multiple testing with a p-value below $0.05/6 = 0.0083$ declared significant. We conducted similar descriptive and linear mixed model analyses for cervical constriction as a ratio of HOC/CEJ.

Validation and Considerations in Radiographic Technique

Measurement reliability for HOC and CEJ (defined as the correlation between paired measurements) was calculated using data from 40 random subjects by fitting a multivariate linear model with fixed tooth effects. Figure 1 shows the raw data for all measurements, where each point represents a measurement pair. Measurements agree exactly when on the line, and are in

disagreement when deviating from the line. The within-patient correlation for this model was defined with a Kronecker-product structure, so that paired observations on the same tooth had an exchangeable correlation, and the correlation of within-patient observations on different teeth also had an exchangeable structure. For analysis purposes, each measure was assessed separately. The estimated reliability for each outcome (HOC and CEJ) is on a scale from zero to one, where one corresponds to perfect agreement and zero corresponds to no linear relationship between paired outcomes. Table 7 summarized findings. In both cases, the estimated reliability is above 0.95. Approximate 95% confidence intervals for reliability are also shown.

To compare our tooth size data with existing literature (direct or cast-derived techniques), we will need to consider magnification and distortion in the radiographic technique. We calculate a 7.7 percent magnification by using the known dimension from an aluminum wedge placed in the same sagittal plane as the teeth. The following equation was used to determine this value:
[(Measured Wedge Size - Actual Wedge Size)/Actual Wedge Size] x 100 = Percent magnification

With regards to distortion, non-spherical objects such as teeth will be distorted if the x-ray beam deviated at all from penetrating the tooth from any angle that is not perfectly parallel to the sagittal plane of the tooth. An effort was made to minimize this distortion through proper technique, but we do not have the means to account for this error in the data.

RESULTS

We consented 143 eligible participants. Sixty four were diagnosed with a dentinal disorder. We had 1 with dentinal dysplasia type I (DD-I). Twenty one had DGI-I, 26 had DGI-II, and 16 had dentinal dysplasia type II (DD-II). Thirty two had OI without DGI and 39 were unaffected family members. The remaining 8 could not be diagnosed based on our defined criteria and were dropped from analysis. These individuals were either too young to have full examination or declined to have all examinations necessary for diagnosis. Categorizing based on underlying genetic defect, the subjects were divided into five groups of 1) DGI-I, 2) DGI/DD-II, 3) OI (no DGI), and 4) unaffected

family, 5) healthy unrelated controls. The latter group was selected randomly from existing dental records and only had radiographs available for evaluation. Excluding group 5, there were 83 females, 52 males, with a median age of 12 years (ranging 1-57). There were 105 Caucasians, 13 African Americans, and 17 others. Twenty one of the 53 OI patients (39.6%) had DGI (see Table 1).

Tooth Size

There were statistically significant differences among the five groups with respect to HOC and CEJ (see Table 5). For both measures of size the observed means were larger in OI, unaffected, and healthy groups as compared to DGI affected persons (DD/DGI-II or DGI-I persons). Application of Bonferroni adjustment for pairwise testing reveals HOC for unaffected and healthy subjects is significantly larger than HOC for DD/DGI-II subjects; the remaining eight pairwise group comparisons are not statistically significant (e.g., DGI-I is not significantly different compared with OI, unaffected or healthy).

Application of Bonferroni adjustment for pairwise testing of CEJ reveals that, as in the case of HOC, DD/DGI-II and DGI-I are not statistically different from each other. Further, OI, unaffected, and healthy are not statistically different from each other with respect to CEJ. The remaining six pairwise comparisons are statistically different showing that teeth affected by DD/DGI-II or DGI-I have a significantly smaller CEJ dimension compared with OI, unaffected, and healthy persons.

As overall differences among groups were indicated for size, further statistical analyses for subgroups of tooth type were conducted. Table 5a shows that HOC differed among the groups for primary teeth, but not for permanent teeth. In particular, mean HOC appears larger for the unaffected and healthy groups than for the two DGI groups (a similar but none significant trend tended to be observed for permanent teeth).

In the case of CEJ, differences among groups were seen for all six tooth type groups (Table 5b), including both permanent and primary teeth. For every tooth type, the mean CEJ dimension was larger for the OI, unaffected, and healthy groups compared with the DGI groups.

Cervical Constriction Ratio

There were significant differences among the five groups with respect to the CC ratio (Table 6). Observed mean ratios for OI, unaffected, and healthy subjects appear smaller than for DGI affected persons (DD/DGI-II or DGI-I persons). Application of Bonferroni adjustment for pairwise testing reveals CC for OI, unaffected, and healthy subjects was statistically significantly smaller than CC for DD/DGI-II and DGI-I subjects; degree of CC was not different between DD/DGI-II subjects and those with DGI-I . Table 6a shows that there are significant differences among the five groups with respect to CC for every tooth type.

Genotype and Prevalence of DGI and Bone fragility in OI Subjects

Table 2 reports genetic defect and illustrates prevalence of bone fractures in the DGI-I and OI cohorts. Of those with identified mutations, we found a majority (84.6%) of DGI-I patients had a *COL1A2* mutation, where as nearly all (92.3%) OI patients who did not have DGI had a *COL1A1* mutation. Sixty fiver percent of those with DGI-I had over 10 fractures, as compared to 28.1 percent in the OI (no DGI) group.

Dentofacial Morphology and Morbidity

Table 3 summarizes our findings. Class I molar occlusion was most common in all groups except group 1 with 12 of 17 patients (70.5%) having Class III occlusion. Moderate anterior crowding was highest in the DGI-I group (35%) followed by the DD/DGI-II group (22%). The prevalence of posterior cross bite was 66% in the DGI-I group in comparison to 16% in the OI group and 10% in controls. Similarly the DGI-I group had the highest prevalence of anterior cross bite (60%), compared to 7.4%, and 0% in the DGI-II and unaffected groups respectively. Thirty three percent of DGI-I patients also had an open bite, compared to only 7.7% of patient with OI and no DGI. The DGI-I and OI group had a significant difference in mean overjet and overbite with DGI-I group deviating much more from unaffected controls as compared with OI group subjects.

The DD/DGI-II group had the highest prevalence of enamel defects (23.5%) with most being localized. Enamel fracturing was seen in more DGI-I patients (62.5%) than DD/DGI-II (50.0%), but

among them, DGI-II patients had more severe, generalized presentation. The highest prevalence of self reported sensitivity was found in the DD/DGI-II group with most severe forms reported by DGI-II patients. The prevalence of attrition was 71.4% in DD/DGI-II, and 62.5% in DGI-I group as compared to 20.0% and 6.7% in the OI and unaffected subjects respectively. Of those with attrition, the DGI-I group had more severe generalized attrition (50.0%) as compared to DD/DGI-II group (33.0%). It appears that the DD/DGI-II cohort has the highest overall dental morbidity, especially if we consider the higher edentulism in the group skews the data on enamel defects/fracture, attrition, and reported sensitivity (summarized in table 4).

Supernumerary teeth were noted in 2 of 16 (12.5%) DGI-I patients. No subject in the DGI-I or OI groups had impactions compared to 4 of 33 DD/DGI-II patients (12.1%). Deflections in path of eruption were seen in 18.2%, 6.2%, 20.0%, and 4.3% of DD/DGI-II, DGI-I, OI, and unaffected groups respectively.

DISCUSSION

This is the first prospective study to objectively assess multiple phenotypic features in a large population having DGI-I and DGI-II. We recognize that current diagnosis and subjective phenotype-based classification systems of hereditary conditions such as DGI are imperfect. Objective phenotyping and ultimately genetic-derived diagnosis and classification is the direction we aim to pursue. Several important observations can be derived from this study including the feasibility of diagnosing DGI based on well defined major and minor criteria. We also report the prevalence and range of severity of a variety of dental phenotypic features in these conditions. The findings of this study clearly demonstrate the critical role that the DSPP/collagen complex is critical in development of normal tooth morphology and size. Interestingly, the overproduction of dentin frequently caused by mutations in either of these genes (evidenced by pulp canal obliteration) results in a diminished size and altered morphology of teeth. This provides further support that the interactions between the odontogenic epithelium in the crown and root and the underlying dental mesenchyme are critical for establishing normal tooth morphology. Whether the effect on tooth size and morphology as observed

in this study results from signaling changes between the odontogenic epithelium and mesenchyme or due to alterations in dentin formation itself is not known.

The reported prevalence of DGI in OI populations varies depending on the population and subtype ranging from as low as 8% to as high as 82%[50, 68] . One study suggests the greatest prevalence occurs with OI Type III as compared to I and IV[48]. The prevalence of DGI in our cohort was 39.6%. Forty three of our 53 patients with OI were classified as milder forms of OI (they were Type I, Type IV or indistinguishable Type I/IV). Furthermore, OI patients with dentinal defects had a higher occurrence of fractures (defined as 10 or more fractures) as compared to those without (Table 2). This is in agreement with findings by Paterson who demonstrated that, within their OI population (comprised of OI type I and IV), patients with associated DGI-I had a more severe expression of OI anomalies, a greater fracture rate and increased likelihood of growth impairment, when compared to those without DGI[52, 53]. We now report (Table 2) genetic findings of this population, and find that most DGI-I participants had a mutation in *COL1A2*; whereas, most OI study participants without DGI had mutations in *COL1A1*. This is the first prospective study to report diagnosis of DGI-I and correlate the presence of DGI with specific genes involved. There is only one other study and they failed to find a correlation between DGI and underlying genetic mutation[69]. This study was retrospective and they did not provide any explanation of their criteria for DGI diagnosis as data collection was conducted by medical chart reviews and no mention of involvement of a dentist in the data collection process. The reported prevalence of DGI (around 70%) in their cohort seems unusually high as compared to other studies of similar (mostly type I and IV) OI populations. Whiel the present study's trend is worthwhile noting, given the limited number of subjects with mutation analysis, the reported trend of DGI prevalence and bone fragility should be objectively studied in a larger population to establish detailed cause and effect relations on the cDNA and protein levels

The radiographic technique for measuring tooth related dimensions provides advantages over conventional direct *in vivo* methods and cast measurement. Radiographs allow visualization of the tooth and its different components at transverse planes not accessible with other techniques. The only

study that we are aware of reporting mesiodistal tooth size taken from radiographs evaluated first permanent molars[64]. We are the first study to our knowledge reporting these dimensions in primary teeth.

There are a number of studies that compare the mesiodistal dimension of teeth at the HOC measured indirectly from a cast. Some have been used to establish ethnic norms. Keeping in mind the limitations of comparing radiographic and cast-derived measurements, frequently referenced first molar dimensions in European Americans reported by Moorrees[70] et al are 10.81 in males, 10.52 in female for the maxilla and 11.18 and 10.74 respectively in the mandible. Generally, it's believed that males have larger teeth than females. Looking at inter-racial size comparisons, generally Caucasians have smaller teeth than Asians, who in turn have smaller teeth than African Americans, Arabs, and Australian Aboriginals[70-74]. Considering the race and gender distribution of our sample, and accounting for the approximate radiographic magnification, our control group's dimensions are in agreement with the standard tooth dimensions derived from measuring dental casts.

Objective illustration of smaller teeth with more bulbous crown forms raises the question of the role of *DSPP*, *COL1A1*, and *COL1A2* in morphodifferentiation and cytodifferentiation stages of odontogenesis. All three genes are associated with the same phenotype with regards to tooth size and shape. However, not all, mutations involving the genes for type I collage affect tooth morphology. This observation could be a function of the complex genetic interactions and redundancies in dental genesis, or may be suggestive of epigenetic mechanism at play.

Morphologic variations in DGI patients also have clinical implications relevant to orthodontists and restorative dentists. Tooth size prediction tables and other established dentoalveolar and cephalometric standards may need to be used with caution in DGI patients. Also, clinicians should be certain to use the smallest, custom crimped stainless steel crowns which will minimize over hangs that may lead to impaction of adjacent teeth and or periodontal problems. The quantification of the cervical constriction offered in the present study can add another objective tool for diagnosticians of DGI, which may serve particularly useful in diagnosing those with milder variations of the disease.

We have also noted the similarity in tooth size and cervical constriction between those diagnosed as unaffected family members as compared to the random healthy controls, supporting the use of the major and minor diagnostic criteria we have defined to diagnosis even mild forms of DGI.

The incidence of Class III malocclusion has been reported to range from 61-100% of DGI-I patients depending on subtype of OI[33]. The greatest prevalence was reported in the most severe none-lethal OI subtype (OI-III). However, no study has reported the angle occlusal classification or incidence of crowding in the DGI-II population. Over half of our DGI-II group had Class I occlusion, but we still found a very high prevalence of Class III dental relation (21.4%) as compared to unaffected family members (4.5%). Mutation in *DSPP* had not previously been associated with alternation in craniofacial development and our report calls for further investigation. The DGI-I cohort had a large majority (70.6%) Class III with the rest Class I. The high prevalence of Class III in the DGI-I group contrasts with only 14.8% of OI patients without DGI exhibiting this malocclusion. The high prevalence of dental malocclusion in this population is likely a function of dental and skeletal aberrations. Waltimo-Siren et al. looked at the skeletal changes and reported smaller than normal linear measurements on cephalometric analysis of OI-I subjects. Those with OI types III and IV, had profound craniofacial deformity and impaired growth as a function of differential growth deficiency, bending of the cranial base, and vertical underdevelopment of condylar process[75]. Such literature and the apparent difference between all 5 groups in our study highlights the need for further studies of the role of *COL1A1, COL1A2*, and *DSPP* in craniofacial development.

Previous reports of increased incidence of impacted teeth in patients with DGI-I have been attributed to either a posteriorly positioned maxilla or the bulbous shape of the teeth[33, 46]. However, OI patients without DGI and with a large apical base can also have impactions. Also, these studies had not considered the path of eruption of the impacted teeth to rule out possible deviations and mis-angulation of teeth as the cause of impaction. We noted that DGI-I patients had larger mesiodistal dimension at level of HOC as compared to DGI-II and both groups had more cervical constriction than controls. Larger teeth, and more bulbous crowns could possibility explain increased incidence of

impactions. However, prevalence of impactions remained low in our cohort and no obvious correlation can be suggested (data not shown). The deflection of path of eruption is higher in DD/DGI-II (18.2%) and OI (20.0%) compared to DGI-I (6.2%) patients (data not shown). Missing teeth have been reported in patients with OI and DGI. Malmgren et al (2002) reported 22% of OI patients with at least one missing tooth. Interestingly, those with DGI and OI seem to have a decrease prevalence of agenesis[33]. The prevalence of supernumerary teeth has not been documented in DGI and or OI population. Our cohort had few congenitally missing teeth ranging from 0 to 9.1%. We did have 2 out of 16 DGI-I patients with supernumerary teeth (data not shown).

Further studies with larger sample size may allow comparison of the mentioned clinical and radiographic features on the basis of detailed mutational analysis. Family-based association of the phenotypes can allow evaluation of differences in the spectrum of clinical and radiographic dental features to identify potential associations with mutations in specific genes. Specifically, mutations in different genes can be clustered in groups for analysis of phenotype-genotype relationships as well as mutations clustering in specific domains of the genes of major effect.

Table 1: Demographics

Variable	DD/DGI-II	DI-I	OI	Unaffected	Total
Gender					
F (%)	26(62.0)	11(52.4)	18(56.3)	28(71.8)	83(61.9)
M (%)	16(38.0)	10(47.6)	14(73.7)	11(28.2)	51(38.1)
Total (families)	42(15)	21(16)	32(22)	39(18)	134*(64)
Race					
White (%)	39(92.9)	14(66.7)	19(59.4)	33(84.6)	105(78.4)
Other (%)	3(7.1)	7(33.3)	13(40.6)	6(15.4)	29(21.6)
Age					
Median	14.0	10.0	11.0	26.0	12.0
Mean (SD)	18.9 (14.8)	16.9 (15.7)	15.4 (14.0)	23.3 (14.5)	18.6 (14.9)
Range	3-57	1-56	2-56	3-50	1-57

Note: Prevalence of DGI in our OI cohort was 39.6% of individuals (42.1% of families)
* 9 additional consentees could not be categorized using our criteria due to insufficient data and were dropped from analysis

Table 2: Genotype and prevalence of DGI and fractures in OI cohort

	COLIA1 mutation*	**COLIA2 mutation***	**Under 10 fractures**	**Over 10 fractures**
DGI-I	2 (15.4)	**11(84.6)**	7(35.0)	**13(65.0)**
OI (%)	**12 (92.3)**	1(7.7)	**23(71.9)**	9(28.1)

*Genotype was unavailable for 8 DI-I and 13 OI subjects.

Table 3: Dentofacial morphology

Variable	DD/DGI-II	DGI-I	OI	Unaffected
Occlusion				
Class I(%)	15(53.6)	5(29.4)	13(48.1)	17(77.3)
Class II(%)	7(25.0)	0	10(37.0)	4(18.2)
Class III(%)	**6(21.4)**	**12(70.6)**	4(14.8)	1(4.5)
Mean OJ in mm (SD)	1.87(1.71)	0.73(2.31)	2.65(1.60)	2.73(1.35)
Mean OB in mm (SD)	1.93(2.38)	1.10(2.75)	3.33(2.31)	2.77(2.07)
Open bite (%)	3(10.3)	5(33.3)	2(12.5)	1(4.5)
Cross bite				
Anterior (%)	6(19.3)	9(60.0)	2(7.4)	0
Posterior (%)	10(33.3)	10(66.7)	4(16.0)	2(9.1)
Ant. Crowding				
Crowding(%)	**7(18.4)**	**6(35.3)**	**7(25.0)**	1(4.8)
Normal (%)	31(81.6)	11(64.7)	21(75.0)	20(95.2)

Table 4: Dental morbidity

Variable	DD/DGI-II	DGI-I	OI	Unaffected
Enamel Fracture				
Localized(%)	11(30.6)	9(56.2)	4(13.3)	1(4.3)
Generalized(%)	**7(19.4)**	1(6.3)	0	0
None(%)	18(50.0)	6(37.5)	26(86.7)	22(95.7)
Enamel Defect				
Localized(%)	2(5.9)	0	0	0
Generalized(%)	**6(17.6)**	1(6.7)	5(16.7)	5(22.7)
None(%)	26(76.5)	14(93.3)	25(83.3)	17(77.3)
Attrition				
Localized(%)	10(47.6)	5(23.7)	6(20.0)	1(6.7)
Generalized(%)	**5(23.8)**	**5(23.7)**	0	0
None(%)	6(28.6)	6(28.6)	24(80.0)	14(93.3)
Edentulous				
Yes(%)	**4(10)**	1(5.3)	0	1(3.1)
No(%)	36(90)	18(94.7)	32(100)	32(96.9)
Sensitivity				
Mild-Mod(%)	8(36.7)	4(28.6)	6(20.0)	4(17.4)
Severe(%)	**2(7.1)**	0	0	0
None(%)	18(64.3)	10(71.4)	24(80.0)	19(79.2)

Table 5: Mean (standard error*) in the size of teeth (averaged over all teeth)

	DD/DGI-II (n=22)	DGI-I (n=15)	OI (n=23)	Unaffected (n=15)	Healthy (n=273)	p-value+
HOC						**<.001**
No. teeth	124	59	135	70	1247	
Mean	9.50	9.63	10.12	9.99	9.92	
Range	4.68-13.45	6.20-14.66	6.32-13.34	5.93-13.40	4.68-14.19	
SE	0.22	0.34	0.17	0.29	0.07	
CEJ						**<.001**
No. teeth	124	59	135	70	1247	
Mean	7.07	7.12	7.94	7.91	7.77	
Minimum	3.52-12.04	3.92-12.00	4.79-10.99	4.61-11.61	3.72-12.04	
SE	0.17	0.28	0.12	0.29	0.06	

*large sample Taylor series based empirical standard errors adjust for clustering of teeth within subject. + overall test of any difference among the four groups, based on Wald F-test from linear mixed model specifying subject as a random effect and group and tooth type as fixed effects.

Table 5a: Mean (standard deviation) in the size (HOC) of teeth by tooth type

Tooth	DD/DI-II (n=22)	DI-I (n=15)	OI (n=23)	Unaffected (n=15)	Healthy (n=273)	p-value+
4 or 13						0.670
No. teeth	19	9	20	14	197	
Mean	7.08	7.17	7.39	7.31	7.32	
SD	0.72	0.67	0.57	0.80	0.82	
Range	5.37-8.23	6.20-8.28	6.32-8.42	5.93-8.59	4.96-11.86	
20 or 29						0.253
No. teeth	21	8	24	12	210	
Mean	7.42	7.60	7.96	7.82	7.82	
SD	0.75	0.88	0.66	0.93	0.88	
Range	5.75-8.83	6.52-8.74	7.00-9.40	6.29-8.96	4.68-11.43	
3 or 14						**0.047**
No. teeth	30	10	28	13	240	
Mean	10.88	10.98	11.52	11.72	11.37	
SD	0.90	0.71	0.87	0.72	0.95	
Range	8.87-13.09	9.95-12.04	9.57-13.26	10.40-12.65	7.05-13.19	
19 or 30						**0.051**
No. teeth	28	11	32	12	246	
Mean	11.14	12.00	12.11	12.38	11.83	
SD	1.12	1.00	0.75	0.83	0.98	
Range	9.36-13.45	10.96-14.66	10.51-13.34	10.28-13.40	6.51-14.20	
A or J						0.079
No. teeth	12	10	15	7	183	
Mean	9.28	9.14	9.78	9.74	9.79	
SD	0.65	1.00	0.77	0.38	0.66	
Range	8.15-10.34	7.86-10.80	8.74-11.25	8.94-10.13	7.88-11.65	
K or T						**<0.001**
No. teeth	14	11	17	12	171	
Mean	9.84	9.99	10.47	11.14	10.85	
SD	0.78	1.08	0.72	0.66	0.60	
Range	8.71-10.85	8.30-11.30	9.61-12.03	10.07-12.07	8.98-12.19	

+ overall test of any difference among the four groups, based on Wald F-test from linear mixed model specifying subject as a random effect and group as fixed effect. Controlled for age and sex.

Table 5b: Mean (standard deviation) in the size (CEJ) of teeth by tooth type

Tooth	DD/DGI-II (n=22)	DI-I (n=15)	OI (n=23)	Unaffected (n=15)	Healthy (n=273)	p-value+
4 or 13						0.075
No. teeth	19	9	19	14	197	
Mean	5.11	5.09	5.54	5.58	5.53	
SD	0.71	0.92	0.45	0.69	0.73	
Range	3.52-610	3.92-6.84	4.79-6.47	4.61-6.70	3.72-10.01	
20 or 29						**0.007**
No. teeth	21	8	24	12	210	
Mean	5.45	5.91	6.05	5.94	6.14	
SD	0.68	1.32	0.50	0.71	0.82	
Range	3.83-6.60	4.38-7.52	5.11-7.17	5.07-7.40	4.12-9.69	
3 or 14						**<0.001**
No. teeth	30	10	28	13	240	
Mean	7.97	7.69	9.03	9.21	8.88	
SD	0.81	0.62	0.64	0.80	0.84	
Range	6.00-9.56	6.67-8.69	7.68-10.13	8.18-10.55	5.48-10.78	
19 or 30						**<0.001**
No. teeth	28	11	32	12	246	
Mean	8.66	9.39	9.92	12.38	9.84	
SD	0.91	0.94	0.57	0.83	0.87	
Range	7.10-10.87	8.74-12.00	8.51-10.99	10.28-13.40	5.41-12.04	
A or J						**<0.001**
No. teeth	12	10	15	7	183	
Mean	6.47	6.21	7.28	7.34	6.98	
SD	0.55	0.88	0.55	0.73	0.57	
Range	5.23-7.22	5.36-7.69	6.41-8.32	6.54-8.81	5.60-8.73	
K or T						**<0.001**
No. teeth	14	11	17	12	171	
Mean	7.54	7.73	8.36	9.12	8.65	
SD	0.72	0.99	0.47	0.44	0.50	
Range	5.83-8.36	5.99-9.19	7.60-9.51	8.64-9.74	7.61-9.84	

+ overall test of any difference among the four groups, based on Wald F-test from linear mixed model specifying subject as a random effect and group as fixed effect. Controlled for age and sex.

Table 6: Mean (standard error*) in HOC/CEJ ratio (averaged over all teeth)

	DD/DGI-II (n=22)	**DGI-I (n=15)**	**OI (n=23)**	**Unaffected (n=15)**	**Healthy (n=273)**	**p-value+**
HOC/CEJ						**<0.001**
No. teeth	124	59	135	70	1247	
Mean	1.3551	1.3704	1.2841	1.2751	1.2888	
SE	0.0135	0.0195	0.0108	0.0201	0.0043	

*large sample Taylor series based empirical standard errors adjust for clustering of teeth within subject. + overall test of any difference among the four groups, based on Wald F-test from linear mixed model specifying subject as a random effect and group and tooth type as fixed effects. Controlled for tooth size, age, and sex.

Table 6a: Mean (standard deviation) in cervical constriction by tooth type

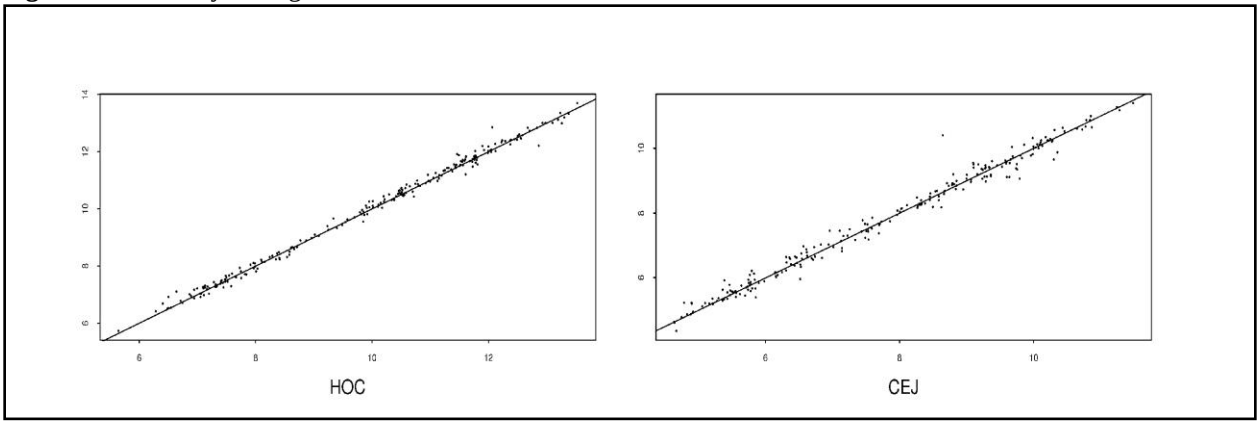
Tooth	DD/DGI-II (n=22)	DI-I (n=15)	OI (n=23)	Unaffected (n=15)	Healthy (n=273)	p-value+
4 or 13						**0.010**
No. teeth	19	9	19	14	197	
Mean	1.3969	1.4277	1.3381	1.3120	1.3296	
SD	0.1234	0.1206	0.0881	0.0853	0.0888	
20 or 29						**0.006**
No. teeth	21	8	24	12	210	
Mean	1.3688	1.3159	1.3186	1.3183	1.2786	
SD	0.0973	0.1633	0.0804	0.0756	0.0957	
3 or 14						**<0.001**
No. teeth	30	10	28	13	240	
Mean	1.3694	1.4327	1.2772	1.2775	1.2839	
SD	0.0723	0.0845	0.0577	0.0807	0.0731	
19 or 30						**<0.001**
No. teeth	28	11	32	12	246	
Mean	1.2879	1.2802	1.2211	1.2031	1.2047	
SD	0.0557	0.0478	0.0519	0.0587	0.0583	
A or J						**0.020**
No. teeth	12	10	15	7	183	
Mean	1.4398	1.4808	1.3445	1.3355	1.4069	
SD	0.1234	0.1093	0.0775	0.1206	0.0959	
K or T						**0.011**
No. teeth	14	11	17	12	171	
Mean	1.3089	1.2965	1.2518	1.2227	1.2559	
SD	0.0831	0.0723	0.0371	0.0692	0.0575	

+ overall test of any difference among the four groups, based on Wald F-test from linear mixed model specifying subject as a random effect and group as fixed effect. Controlled for age and sex.

Table 7: Estimated reliability and associated confidence intervals.

Outcome	N teeth	N patients	Estimated Reliability	95% Confidence Interval for Reliability
HOC	228	40	0.959	(0.948, 0.971)
CEJ	228	40	0.927	(0.907, 0.946)

Figure 1: Reliability testing raw data



Paired height of contour (HOC) and cemento-enamel junction (CEJ) measurements in millimeters. Each point represents a paired measurement, that is in exact agreement when on the line, and in disagreement when deviating from the line.

Table 8: Combined Sillence and Shields classifications of DGI and OI

Type (OMIM #)	Salient Features and Incidence	Inheritance	Gene (OMIM #)
DGI-I (#166240)	Occurs in association with OI. Dental discoloration, pulp chamber and canal obliteration, cervical constriction, short/blunt roots, frequent enamel fractures, excessive attrition.	AD	COL1A1 (120150), COL1A2 (120160)
OI-I (#166200)	Mildest of 8 OI types. Mild to moderate bone fragility with near normal stature. Hearing loss (50%). Blue sclera. If DGI present primary teeth more affected than permanents. 8% reported as type A(with DGI)[50].	AD	COL1A1, COL1A2
OI-II (#166210)	Most severe of OI types. Lethal in the perinatal period. Incidence of type A unknown and may be +/- DGI[42].	AD	COL1A1, COL1A2 (IIA) CRTAP (IIB)
OI-III (#259420)	Most severe non-lethal form. Multiple bone deformities as result of fractures. Short Stature. Failure to thrive. Hearing loss. Incidence of type A reported as 36% permanent, 82% Primary[46].	AD/AR	COL1A1, COL1A2
OI-IV (#166220)	Moderate bone fragility. May fracture at birth, but decreases with age. No blue sclera after adolescence. Less hearing loss that OI-I. Incidence of type B reported as 37-65% permanents and 83% primary[46].	AD	COL1A1, COL1A2
OI-V (%610967)	Moderate-severe bone fragility and short stature. No blue sclera or DGI. Dislocation of radial head. Mineralized intraosseous membrane.	AD	Unknown
OI-VI (%610968)	Moderate to severe bone fragility and short stature. No blue sclera of DGI. Scoliosis. Accumulation of osteoid in bone tissue. Fish-scale pattern of bone lamellation.	Unknown	Unknown
OI-VII (#610682)	Recurrent fractures decrease after puberty. Mild short stature. No blue sclera or DGI.	AR	CRTAP
OI-VIII (#610915)	Extreme bone fragility. No blue sclera. DGI unknown.	AR	LEPRE1
DGI-II (#125490)	Same as DGI-I except no OI present. Often similar severity in primary and permanent teeth. High penetrance and variable expressivity.	AD	DSPP (125485)
DGI-III (#125500)	Reported in Brandywine people. Same as DGI-II but earlier/more extensive wear of enamel. Young primaries with enlarged pulp chamber. Not a unique entity but a milder version (allelic variation) of DGI-II.	AD	DSPP

Table 9a: Non syndromic dentinal anomalies [13]

Genomic DNA	Protein	Dentin and hearing phenotypes	References
g.16T>G	p.Y6D	DD-II	Rajpar et al. 2002
g.44C>T	p.A15V	DGI-II	Malmgren et al. 2004
g.49C>A	p.P17T	DGI-II+ DFNA 39	Xiao et al. 2001
g.49C>T	p.P17S	DGI-II	Zhang et al. 2007
g.1188C>G	IVS2-3C	DGI-II + DFNA 39	Kim et al. 2004
g.1194C>A	IVS2-3	DGI-II	Holappa et al. 2006
g.1197G>T	p.V18F	DGI-II+ DFNA 39 DGI-II and III	Xiao et al. 2001, Kim et al. 2005, Song and al 2006
g.1272C>T	p.Q45X	DGI-II DGI-III	Zhang et al. 2001 Song and al. 2006
g.1275G>A	IVS2-3	DGI-II	Xiao et al. 2001
g.1474A>T	p.R68W	DGI-II	Malmgren et al. 2004
g.3599-3634del36, 3715-3716ins18	p.del1160-1171 and p.ins1198-1199	DGI-III	Dong et al. 2005

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Table 9b: Syndromic Dentinal Anomalies [14]

Syndromes	OMIM N°	Modes	Genes	Dentin phenotype	References / Number of cases
OI type IB	166240	AD	*COL1A1* and *COL1A2*	DGI-I	Levin *et al.*, 1980, 16 patients ; Falk *et al.*, 1986, 10 families; Paterson *et al.*, 1983, 48 patients; Sykes *et al.*, 1990, 38 patients
OI type II	259440	AR	Non *COL1A1* and non *COL1A2*	DGI-I	Shapiro *et al.*, 1982, (1 patient in temporary teeth); *heterogeneity in findings*
OI type IIIB	259420	AD or AR	*COL1A1* and *COL1A2*	DGI-I	O'Connell and Marini, 1999, 40 patients; Lund *et al.*, 1998, 28 patients
OI type IVB	166220	AD	*COL1A1* and *COL1A2*	DGI-I	O'Connell and Marini, 1999, 40 patients; Falk *et al.*, 1986, 10 families; Paterson *et al.*, 1983, 48 patients; Sykes *et al.*, 1990, 38 patients
OI + Ehlers-Danlos syndrome type VII	120160		*COL1A2*	DG-I	Raff, *et al.*, 2000, 1 patient
Goldblatt syndrome	184260 120140	AR	*COL2A1*	DD-I	Goldblatt *et al.*, 1991, 1 patient; Bonnaventure *et al.*, 1992, 2 patients

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Table 9c: Syndromes with non-collagen gene or unknown gene and DGI[14]

Syndromes	OMIM N°	Modes	Chromosome location	Genes	References / Number of cases
Schimke immuno-osseous dysplasia	242900	AR	2q34q36	*SMARCAL 1*	Da Fonseca 2000, 1 patient; (Ludman *et al.*, 1993, 1 patient with microdontia without DGI)
OI with blue sclera and wormian bones, but without fractures	166230	AD			Beighton, 1981, 20 patients
Cortical defects, Wormian bones without osteopenia	604922	AR			Moog *et al.*, 1999, 1 family
OI unclassified with radiopaque-radiolucent lesions surrounding apices in maxilla and mandible		AD			Levin *et al.*, 1985, 13 patients
Generalized connective tissue defect					Komorowska *et al.*, 1989, 44 patients
Short stature, MR, hearing loss	-	AR			Cauwels *et al.*, 2005, 2 patients
Cole-Carpenter syndrome	112240				Macdermot *et al.*, 1995 1 patient

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Table 9d: Syndromes associated with dentin dysplasia type I[14]

Syndromes	OMIM N°	Modes	Chromosome location	Genes	References / Number of cases
Tumoral Calcinosis HFTC ADHR	211900/601756 605380/193100	AR	2q24.q31 12p13.3	*GALTNT 3* *FGF23*	Specktor *et al.,* 2006, 1 patient ; Ichikawa *et al.,* 2005, 15 patients Chefetz *et al.,* 2005, 1 patient
Skeletal dysplasia with opalescent and rootless teeth					Kantaputra 2001b, 1 patient
Skeletal anomalies, sclerotic bones	125440	AD			Morris and Augsburger, 1977, 2 patients
Singleton-Merten syndrome	182250	AD			Singleton and Merten 1973, 2 patients ; Gay and Kuhn 1976, 2 patients. Feigenbaum *et al.,* 1988, 1 family
Ehlers-Danlos syndrome (type undefined)					Barabas, 1969, 6 patients ; Hoff, 1977, 1 patient
Ehlers-Danlos syndrome type I					Pope *et al.,* 1992, 2 patients

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REFERENCES

1. MacDougall M, Dong J, Acevedo AC. Molecular basis of human dentin diseases. Am J Med Genet A 2006;140(23):2536-46.

2. Simon S, Smith AJ, Lumley PJ, Berdal A, Smith G, Finney S, et al. Molecular characterization of young and mature odontoblasts. Bone 2009.

3. Rajpar MH, Koch MJ, Davies RM, Mellody KT, Kielty CM, Dixon MJ. Mutation of the signal peptide region of the bicistronic gene DSPP affects translocation to the endoplasmic reticulum and results in defective dentine biomineralization. Hum Mol Genet 2002;11(21):2559-65.

4. Linde A, Goldberg M. Dentinogenesis. Crit Rev Oral Biol Med 1993;4(5):679-728.

5. Avery JK. Dentine. 8 ed. London: Mosby; 1976.

6. Nanci A, editor. Dentin-pulp complex. 6 ed. St. Louis: Mosby; 2003. Nanci A, editor. Ten Cate's oral histology development, structure, and function.

7. Hu JC, Simmer JP. Developmental biology and genetics of dental malformations. Orthod Craniofac Res 2007;10(2):45-52.

8. Linde A, Lussi A, Crenshaw MA. Mineral induction by immobilized polyanionic proteins. Calcif Tissue Int 1989;44(4):286-95.

9. Hart PS, Hart TC. Disorders of human dentin. Cells Tissues Organs 2007;186(1):70-7.

10. MacDougall M, Simmons D, Luan X, Nydegger J, Feng J, Gu TT. Dentin phosphoprotein and dentin sialoprotein are cleavage products expressed from a single transcript coded by a gene on human chromosome 4. Dentin phosphoprotein DNA sequence determination. J Biol Chem 1997;272(2):835-42.

11. George A, Bannon L, Sabsay B, Dillon JW, Malone J, Veis A, et al. The carboxyl-terminal domain of phosphophoryn contains unique extended triplet amino acid repeat sequences forming ordered carboxyl-phosphate interaction ridges that may be essential in the biomineralization process. J Biol Chem 1996;271(51):32869-73.

12. Yamakoshi Y, Hu JC, Fukae M, Zhang H, Simmer JP. Dentin glycoprotein: the protein in the middle of the dentin sialophosphoprotein chimera. J Biol Chem 2005;280(17):17472-9.

13. Bailleul-Forestier I, Molla M, Verloes A, Berdal A. The genetic basis of inherited anomalies of the teeth. Part 1: clinical and molecular aspects of non-syndromic dental disorders. Eur J Med Genet 2008;51(4):273-91.

14. Bailleul-Forestier I, Berdal A, Vinckier F, de Ravel T, Fryns JP, Verloes A. The genetic basis of inherited anomalies of the teeth. Part 2: syndromes with significant dental involvement. Eur J Med Genet 2008;51(5):383-408.

15. Levin LS, Leaf SH, Jelmini RJ, Rose JJ, Rosenbaum KN. Dentinogenesis imperfecta in the Brandywine isolate (DI type III): clinical, radiologic, and scanning electron microscopic studies of the dentition. Oral Surg Oral Med Oral Pathol 1983;56(3):267-74.

16. Skinner HC, Bartz Z, Ladenbauer-Bellis I, Pooley A, Albright JA. Scanning electron microscopy of osteogenesis imperfecta and normal deciduous human dentin. J Dent Res 1978;57(2):418-9.

17. Kinney JH, Pople JA, Driessen CH, Breunig TM, Marshall GW, Marshall SJ. Intrafibrillar mineral may be absent in dentinogenesis imperfecta type II (DI-II). J Dent Res 2001;80(6):1555-9.

18. Shields ED, Bixler D, el-Kafrawy AM. A proposed classification for heritable human dentine defects with a description of a new entity. Arch Oral Biol 1973;18(4):543-53.

19. Witkop CJ, Jr. Amelogenesis imperfecta, dentinogenesis imperfecta and dentin dysplasia revisited: problems in classification. J Oral Pathol 1988;17(9-10):547-53.

20. Bixler D. Heritable disorders affecting dentin. Oral Facial Genetics 1976:227-61.

21. Beattie ML, Kim JW, Gong SG, Murdoch-Kinch CA, Simmer JP, Hu JC. Phenotypic variation in dentinogenesis imperfecta/dentin dysplasia linked to 4q21. J Dent Res 2006;85(4):329-33.

22. MacDougall M. Dental structural diseases mapping to human chromosome 4q21. Connect Tissue Res 2003;44 Suppl 1:285-91.

23. McKnight DA, Suzanne Hart P, Hart TC, Hartsfield JK, Wilson A, Wright JT, et al. A comprehensive analysis of normal variation and disease-causing mutations in the human DSPP gene. Hum Mutat 2008;29(12):1392-404.

24. Martin E, Shapiro JR. Osteogenesis imperfecta:epidemiology and pathophysiology. Curr Osteoporos Rep 2007;5(3):91-7.

25. Bai H, Agula H, Wu Q, Zhou W, Sun Y, Qi Y, et al. A novel DSPP mutation causes dentinogenesis imperfecta type II in a large Mongolian family. BMC Med Genet;11:23.

26. Waltimo J, Ranta H, Lukinmaa PL. Ultrastructure of dentin matrix in heritable dentin defects. Scanning Microsc 1995;9(1):185-97; discussion 97-8.

27. Waltimo J, Ojanotko-Harri A, Lukinmaa PL. Mild forms of dentinogenesis imperfecta in association with osteogenesis imperfecta as characterized by light and transmission electron microscopy. J Oral Pathol Med 1996;25(5):256-64.

28. Xiao S, Yu C, Chou X, Yuan W, Wang Y, Bu L, et al. Dentinogenesis imperfecta 1 with or without progressive hearing loss is associated with distinct mutations in DSPP. Nat Genet 2001;27(2):201-4.

29. Butler WT. Matrix macromolecules of bone and dentin. Coll Relat Res 1984;4(4):297-307.

30. Specktor P, Cooper JG, Indelman M, Sprecher E. Hyperphosphatemic familial tumoral calcinosis caused by a mutation in GALNT3 in a European kindred. J Hum Genet 2006;51(5):487-90.

31. Chefetz I, Heller R, Galli-Tsinopoulou A, Richard G, Wollnik B, Indelman M, et al. A novel homozygous missense mutation in FGF23 causes Familial Tumoral Calcinosis associated with disseminated visceral calcification. Hum Genet 2005;118(2):261-6.

32. Ludman MD, Cole DE, Crocker JF, Cohen MM, Jr. Schimke immuno-osseous dysplasia: case report and review. Am J Med Genet 1993;47(5):793-6.

33. Malmgren B, Norgren S. Dental aberrations in children and adolescents with osteogenesis imperfecta. Acta Odontol Scand 2002;60(2):65-71.

34. Lukinmaa PL, Ranta H, Ranta K, Kaitila I, Hietanen J. Dental findings in osteogenesis imperfecta: II. Dysplastic and other developmental defects. J Craniofac Genet Dev Biol 1987;7(2):127-35.

35. Hodge HC, Finn SB, Robinson HBG, Manly RS, Lefevre Manly M, Van Huysen G, et al. Hereditary Opalescent Dentin: III. Histological, Chemical and Physical Studies'. Journal of Dental Research 1940;19(6):521-36.

36. Kerebel B, Daculsi G, Menanteau J, Kerebel LM. The inorganic phase in dentinogenesis imperfecta. J Dent Res 1981;60(9):1655-60.

37. Wright JT, Gantt DG. The ultrastructure of the dental tissues in dentinogenesis imperfecta in man. Arch Oral Biol 1985;30(2):201-6.

38. Papagerakis P, Berdal A, Mesbah M, Peuchmaur M, Malaval L, Nydegger J, et al. Investigation of osteocalcin, osteonectin, and dentin sialophosphoprotein in developing human teeth. Bone 2002;30(2):377-85.

39. Lindau B, Dietz W, Lundgren T, Storhaug K, Noren JG. Discrimination of morphological findings in dentine from osteogenesis imperfecta patients using combinations of polarized light microscopy, microradiography and scanning electron microscopy. Int J Paediatr Dent 1999;9(4):253-61.

40. Sunderland EP, Smith CJ. The teeth in osteogenesis and dentinogenesis imperfecta. Br Dent J 1980;149(10):287-9.

41. Siar CH. Quantitative histological analysis of the human coronal dentine in dentinogenesis imperfecta types I and II. Arch Oral Biol 1986;31(6):387-90.

42. L. Stefan Levin, John M. Brady, Michael Melnick, John M. Optiz. Scanning electron microscopy of teeth in dominant osteogenesis imperfecta: Support for genetic heterogeneity. American Journal of Medical Genetics 1980;5(2):189-99.

43. Lygidakis NA, Smith R, Oulis CJ. Scanning electron microscopy of teeth in osteogenesis imperfecta type I. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1996;81(5):567-72.

44. Lindau BM, Dietz W, Hoyer I, Lundgren T, Storhaug K, Noren JG. Morphology of dental enamel and dentine-enamel junction in osteogenesis imperfecta. Int J Paediatr Dent 1999;9(1):13-21.

45. Neville BW. Oral & Maxillofacial Pathology. 2 ed: Saunders; 2001.

46. O'Connell AC, Marini JC. Evaluation of oral problems in an osteogenesis imperfecta population. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1999;87(2):189-96.

47. Petersen K, Wetzel WE. Recent findings in classification of osteogenesis imperfecta by means of existing dental symptoms. ASDC J Dent Child 1998;65(5):305-9, 54.

48. Malmgren B, Lindskog S, Elgadi A, Norgren S. Clinical, histopathologic, and genetic investigation in two large families with dentinogenesis imperfecta type II. Hum Genet 2004;114(5):491-8.

49. Schwartz S, Tsipouras P. Oral findings in osteogenesis imperfecta. Oral Surg Oral Med Oral Pathol 1984;57(2):161-7.

50. Lund AM, Jensen BL, Nielsen LA, Skovby F. Dental manifestations of osteogenesis imperfecta and abnormalities of collagen I metabolism. J Craniofac Genet Dev Biol 1998;18(1):30-7.

51. Malmgren B, Lindskog S. Assessment of dysplastic dentin in osteogenesis imperfecta and dentinogenesis imperfecta. Acta Odontol Scand 2003;61(2):72-80.

52. Paterson CR, McAllion S, Miller R. Osteogenesis imperfecta with dominant inheritance and normal sclerae. J Bone Joint Surg Br 1983;65(1):35-9.

53. Paterson CR, McAllion S, Miller R. Heterogeneity of osteogenesis imperfecta type I. J Med Genet 1983;20(3):203-5.

54. Teixeira CS, Santos Felippe MC, Tadeu Felippe W, Silva-Sousa YT, Sousa-Neto MD. The role of dentists in diagnosing osteogenesis imperfecta in patients with dentinogenesis imperfecta. J Am Dent Assoc 2008;139(7):906-14; quiz 94.

55. Cauwels RG, De Coster PJ, Mortier GR, Marks LA, Martens LC. Dentinogenesis imperfecta associated with short stature, hearing loss and mental retardation: a new syndrome with autosomal recessive inheritance? J Oral Pathol Med 2005;34(7):444-6.

56. Rauch F, Glorieux FH. Osteogenesis imperfecta. Lancet 2004;363(9418):1377-85.

57. Andersen PE, Jr., Hauge M. Osteogenesis imperfecta: a genetic, radiological, and epidemiological study. Clin Genet 1989;36(4):250-5.

58. Pedersen U. Osteogenesis imperfecta clinical features, hearing loss and stapedectomy. Biochemical, osteodensitometric, corneometric and histological aspects in comparison with otosclerosis. Acta Otolaryngol Suppl 1985;415:1-36.

59. Witkop CJ. Hereditary defects in enamel and dentin. Acta Genet Stat Med 1957;7(1):236-9.

60. Guideline on oral heath care/dental management of heritable dental development anomalies. Pediatr Dent 2008;30(7 Suppl):196-201.

61. Pettiette MT, Wright JT, Trope M. Dentinogenesis imperfecta: endodontic implications. Case report. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1998;86(6):733-7.

62. Arana-Chavez VE, Massa LF. Odontoblasts: the cells forming and maintaining dentine. Int J Biochem Cell Biol 2004;36(8):1367-73.

63. Kieser JA. Human adult odontometrics. Cambridge: Cambridge University Press; 1990.

64. Purton DG, Ng BP, Chandler NP, Monteith BD. The bitewing radiograph as an assessment tool in fixed prosthodontics. J Oral Rehabil 2004;31(6):562-7.

65. Tanaka T, Murakami T. Radiological features of hereditary opalescent dentin. Dentomaxillofac Radiol 1998;27(4):251-3.

66. Sillence DO, Rimoin DL. Classification of osteogenesis imperfect. Lancet 1978;1(8072):1041-2.

67. Little RM. The irregularity index: a quantitative score of mandibular anterior alignment. Am J Orthod 1975;68(5):554-63.

68. Santili C, Akkari M, Waisberg G, Bastos Junior JO, Ferreira WM. [Clinical, radiographic and laboratory evaluation of patients with osteogenesis imperfecta]. Rev Assoc Med Bras 2005;51(4):214-20.

69. Rauch F, Lalic L, Roughley P, Glorieux FH. Genotype-phenotype correlations in nonlethal osteogenesis imperfecta caused by mutations in the helical domain of collagen type I. Eur J Hum Genet.

70. Jensen E, Kai-Jen Yen P, Moorrees CF, Thomsen SO. Mesiodistal crown diameters of the deciduous and permanent teeth in individuals. J Dent Res 1957;36(1):39-47.

71. Yuen KK, Tang EL, So LL. Relations between the mesiodistal crown diameters of the primary and permanent teeth of Hong Kong Chinese. Arch Oral Biol 1996;41(1):1-7.

72. Yuen KK, So LL, Tang EL. Mesiodistal crown diameters of the primary and permanent teeth in southern Chinese--a longitudinal study. Eur J Orthod 1997;19(6):721-31.

73. Brown T, Margetts B, Townsend GC. Comparison of mesiodistal crown diameters of the deciduous and permanent teeth in Australian aboriginals. Aust Dent J 1980;25(1):28-33.

74. Hattab FN, al-Khateeb S, Sultan I. Mesiodistal crown diameters of permanent teeth in Jordanians. Arch Oral Biol 1996;41(7):641-5.

75. Waltimo-Siren J, Kolkka M, Pynnonen S, Kuurila K, Kaitila I, Kovero O. Craniofacial features in osteogenesis imperfecta: a cephalometric study. Am J Med Genet A 2005;133A(2):142-50.