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The effect of hereditary disorders on tooth components: a radiographic morphometric study of two syndromes

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Summary *Objective:* The purpose of this study was to compare tooth components (enamel and dentin) in Familial Dysautonomia (FD) and Down syndrome (DS) in order to assess the extent to which each was affected. *Design:* The design was cross-sectional. The sample consisted of 20 FD patients and 45 DS patients. The control group comprised 250 healthy subjects. Mesio-distal crown width (CW), enamel and dentin thickness and pulp chamber dimensions were measured on standardized bitewing radiographs of mandibular second primary and first permanent molars. Statistical analyses were performed between groups using SAS programs. *Results:* CW was reduced in both hereditary disorders. In the DS group enamel height (EH) and dentin thickness were reduced. In FD enamel thickness in the primary and permanent molars as well as dentin height (DH) in permanent molars was increased. *Conclusions:* In both syndromes the reduction in CW suggests reduced proliferation during tooth germ formation. However, the differences in enamel and dentin thickness suggest that ameloblasts and odontoblasts were affected differently in the later phases of cell function. In FD cell function is stimulated resulting in thicker enamel and dentin. In DS cell function is reduced resulting in thin enamel and dentin.
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Introduction

Tooth formation starts at an early stage of embryonic differentiation and is of limited duration. It proceeds sequentially along the tooth row and so provides data on different developmental stages within an individual.[1—3] The final size of a tooth represents the result of the combined effect of genetic and environmental factors in the course of dental development. However, the relatively early age of tooth development means that they are less affected than any other organ in the human body by environmental factors and their morphology is mostly a reflection of genetic factors.[4—8]

Both oral ectoderm and neural crest influenced ecto-mesenchyme are involved in tooth formation. Initiation and morphogenesis appear to be primarily determined by the ectodermally derived enamel organ, which also gives rise to the ameloblasts that form the enamel matrix. The ecto-mesenchyme

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forms the dental sac and dentin—pulp complex. Crown size is determined by two phases: the early stage, the determination of the volume of the dentin—pulp complex, is characterized by completion of dentin—enamel junction (DEJ) in the crown and the dentin cementum junction in the root. The thickness of enamel and dentin is determined by the amount of tissue laid down by the ameloblasts and odontoblasts, while cementum proliferation is associated with the recruitment of additional cells throughout life.[9] Enamel thickness is defined early in life, whereas dentin thickness increases throughout life by secondary (physiological) and tertiary (reactive) apposition, and pulp size is inversely affected by this phenomenon since dentin apposition reduces the size of the pulp.[10]

Individuals with hereditary disorders such as Down syndrome (DS) and Familial Dysautonomia (FD) have remarkably small teeth. This phenomenon has been related to an overall impairment of normal development.[11—14] However, while FD primarily affects the nervous system, DS (Trisomy 21), exhibits general growth retardation. In view of our understanding of the contribution of various tissues on tooth development, each of these conditions may affect a different phase of tooth development.

FD, also known as Riley Day syndrome (MIM #223900) is an autosomal recessive disorder that mainly affects children of Jewish Ashkenazi origin, with an incidence of 1 in 3700 live births, which corresponds to a carrier frequency of 1 in 32 among Ashkenazi Jews.[15,16] It is classified as a hereditary sensory and autonomic neuropathy type III[17] and is the most common and widely recognized of the congenital sensory neuropathies.[18,19] FD affects the development and survival of sensory, sympathetic and parasympathetic neurons. It is a devastating and debilitating disease, present from birth, with a variety of symptoms, including gastrointestinal dysfunction, vomiting crisis, recurrent pneumonias, altered sensitivity to pain and temperature, and cardiovascular instability.[18,20] There is a progressive neuronal degeneration throughout life, and survival statistics indicate that the probability of reaching 30 years of age is only 50%.[21] One of the most distinctive features of the disease is impaired pain perception due to the progressive neuropathy. The diagnosis of FD is based on: absence of fungiform papillae on the tongue, absence of axon flares after injection of intradermal histamine, decrease or absent deep tendon reflexes, absence of overflow emotional tears and Ashkenazi Jewish ancestry.[19] The clinical features of FD are due to a striking progressive depletion of unmyelinated sensory and autonomic neurons.[22] The FD gene coded ''DYS'' was mapped to chromosome 9q31-q33.[23] In FD patients,

neural crest dysfunction is believed to be responsible for the wide range of dysautonomic changes.[8] Tooth components in FD patients have been shown to differ from those of normal healthy controls.[14]

DS (MIM #190685) is caused by trisomy of the 21st chromosome. It is a relatively common anomaly (one in every 600—700 live births), and is the best known example of the severe growth and development abnormalities associated with an extra chromosome. The disorder affects, among other features, general body size, mental and systemic development.[24] The dental characteristics associated with DS include antero-posterior shortened palate,[25] microdontia of permanent dentition, while some primary teeth are larger,[12,13] altered crown morphology[26] and shape,[27] taurodontism[28] and hypodontia.[29] Other body features that may affect the oro-facial complex in DS are the altered development and morphology of bone. Rib growth cartilage in DS fetuses showed an increase in the hypertrophic portion with a concomitant decrease in the proliferating and resting zone.[30] This abnormality may represent an early manifestation of an abnormal cartilage maturation pattern, which appears postnatally in long bones, leading to diminished growth rates. Bone mineral density in DS is reduced compared to healthy population,[31—34] due, probably, to their muscular hypotonia.[32,33]

The purpose of this study was to compare the severity and pattern of change in tooth components of FD individuals with those of children with a different genetic disorder that is also characterized by reduced tooth size. In order to determine the effect of these conditions at different stages of tooth development on the two tissues involved, we measured crown size, enamel and dentin thickness in the early developing mandibular second primary molars and later developing mandibular first permanent molars. Our basic hypothesis was that crown size can be partitioned into two phases: an early proliferative stage represented by the volume of the tooth at the DEJ and a latter stage of cell function represented by enamel and dentin thickness.

Materials and methods

Routine diagnostic bitewing radiographs of 20 patients diagnosed with FD and 45 patients with cytogenetically determined DS were studied. The control group consisted of 250 healthy children and adolescents and comprised 132 males and 118 females aged 3—16 years. The FD group included 14 males and 6 females aged 5—22 years and the DS group consisted of 20 females and 25 males aged 3—27 years.
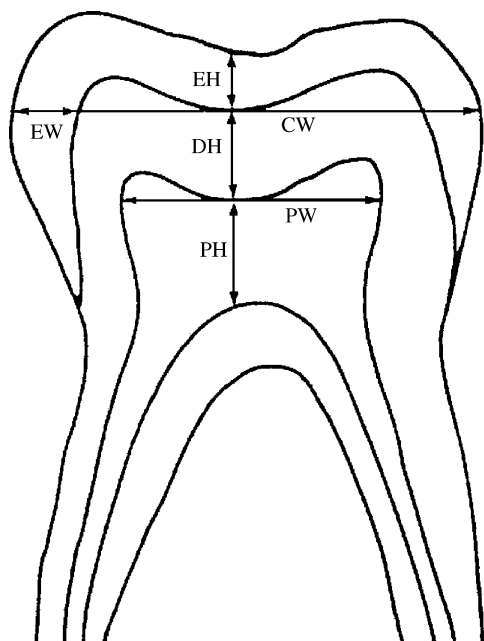
Figure 1 The measurement of crown components. EH: enamel height, DH: dentin height, PH: pulp height, EW: enamel width, PW: pulp width, CW: crown width.

Only radiographs with minimal or no distortion and minimal or no overlapping between the proximal surfaces were selected for the study.

All radiographs were coded, and the examiner was unaware as to which group the radiographs belonged to. The measured teeth were the mandibular second primary and first permanent molars. Only intact teeth without restorations or marked attrition were measured. The measurements were performed using a digital caliper on a light table with the use of a magnifying glass, as was previously described.[35] The results were in mm. Six measurements were taken on each tooth crown (Fig. 1). Enamel height (EH), dentin height (DH) and pulp height (PH) were measured parallel to the long axis of the tooth, one millimeter mesial to the central fossa on the same line. This line was chosen for reproducibility reason and for the fact that attrition on enamel affects mainly tooth cusps and not the central fossa. Pulp width (PW) was measured perpendicular to the long axis of the tooth on the widest part of the pulp. Mesial enamel width (EW) and maximal crown width (CW) were measured on the widest mesio-distal length of the tooth on a line perpendicular to the long axis of the tooth. Each measurement was performed separately. All measurements were also expressed as a ratio of mesio-distal CW, in order to minimize the effect of external tooth size and possible errors due to magnification on each variable. The same examiner performed all measurements.

Measurement accuracy and reliability

When taken by experienced personnel dental radiographs provide an easily reproducible two-dimensional representation of the maximum diameters of the crown, enamel, dentine and pulp cavity, whose accuracy can be easily verified by repeated radiographs.[35] Enamel as measured from radiographs represents its maximal thickness, given that all the levels of enamel from buccal to lingual are projected on two dimensions.[36] The mesial EW is in fact the maximal enamel thickness measured from tooth slices.[35] The occlusal enamel measured from radiographs is in the same range of dimensions obtained from tooth slices and naturally broken teeth.[37] Pulp chamber dimensions measured on radiographs show the maximal size.[38]

In order to test the reliability of measurements taken from radiographs, 10 randomly chosen teeth were re-measured. The range of variation found between measurements taken on different accepted radiographs was 2—5% and averaged 3.2%. Variation was least for CW measurements and greatest for EH.

Statistical analysis

All data were transferred to a computer and statistically analyzed using the SAS package.[39] In cases where both right and left teeth were measured the mean value was used. A previous study on FD and controls showed that sexual dimorphism in CW and tooth components was insignificant in comparison to the differences between groups.[14] Therefore, in this study, males and females were grouped together for analysis.

Univariate ANOVA was performed, using each variable as dependent variable and health status as independent variable, in order to determine the influence of FD or DS on tooth components. The procedure used was the post-hoc, multiple comparisons for means, with $\alpha = 5\%$. Multifactorial discriminant analysis was performed to determine the distance between the controls and the two syndromes. The procedure used was the biplot-multivariate analysis of variance.[40]

Results

A total of 175 mandibular second primary and 160 mandibular permanent first molars were computed in the control group. They were compared to the FD group (11 second primary and 9 first permanent molars) and DS group (29 second primary and 41 first permanent molars). One way analysis of variance (ANOVA, REGWR procedure. Post-hoc, $\alpha = 5\%$)

Table 1 Measurements of tooth components.

		Second primary molars			First permanent molars		
		Controls	FD	DS	Controls	FD	DS
EH	Mean	0.96	1.16	0.92	1.66	1.74	1.42
	N	175	10	28	160	9	41
	S.D.	0.15	0.18	0.16	0.40	0.17	0.18
	Minimum	0.64	0.94	0.73	0.64	1.45	0.87
	Maximum	1.64	1.42	1.53	2.78	2.00	1.72
DH	Mean	2.83	2.58	2.27	3.30	3.64	2.85
	N	175	11	28	160	9	39
	S.D.	0.38	0.47	0.46	0.52	0.48	0.33
	Minimum	2.08	1.72	1.33	2.03	2.91	2.36
	Maximum	3.92	3.14	2.91	4.92	4.17	3.57
PH	Mean	1.10	0.97	1.52	1.69	0.99	1.81
	N	175	11	28	160	9	39
	S.D.	0.40	0.34	0.59	0.60	0.63	0.87
	Minimum	0.33	0.50	0.69	0.47	0.38	0.64
	Maximum	2.28	1.51	2.55	3.55	2.16	3.89
EW	Mean	0.83	0.76	0.88	1.32	1.29	1.22
	N	175	11	26	160	9	41
	S.D.	0.10	0.10	0.13	0.29	0.11	0.18
	Minimum	0.59	0.60	0.75	0.62	1.18	0.76
	Maximum	1.16	0.90	1.37	2.12	1.50	1.53
PW	Mean	5.00	4.78	4.54	4.57	4.08	4.38
	N	175	11	28	160	9	39
	S.D.	0.41	0.43	0.35	0.49	0.43	0.51
	Minimum	3.93	4.14	4.03	3.35	3.30	3.39
	Maximum	5.87	5.38	5.42	6.02	4.59	5.32
CW	Mean	10.25	9.96	9.77	11.39	11.05	11.11
	N	175	10	28	160	9	41
	S.D.	0.49	0.59	0.37	0.73	0.51	0.74
	Minimum	9.07	8.87	8.79	9.61	10.30	9.70
	Maximum	11.44	10.75	10.60	12.96	11.73	12.27
EH1	Mean	0.09	0.12	0.09	0.14	0.16	0.13
	N	175	9	28	160	9	41
	S.D.	0.01	0.02	0.02	0.03	0.01	0.01
DH1	Mean	0.28	0.27	0.23	0.29	0.33	0.26
	N	175	10	28	160	9	39
	S.D.	0.04	0.03	0.05	0.04	0.04	0.03
PH1	Mean	0.11	0.10	0.15	0.15	0.09	0.16
	N	175	10	28	160	9	39
	S.D.	0.04	0.03	0.06	0.05	0.06	0.08
EW1	Mean	0.08	0.08	0.09	0.12	0.12	0.11
	N	175	10	26	160	9	41
	S.D.	0.01	0.01	0.02	0.02	0.01	0.02
PW1	Mean	0.49	0.48	0.46	0.40	0.37	0.39
	N	175	10	28	160	9	39
	S.D.	0.03	0.03	0.03	0.05	0.03	0.04

The data for the FD was taken from a previous report.[14] *Note*: EH, enamel height; DH, dentin height; PH, pulp height; EW: enamel width; PW, pulp width; CW, crown width; EH1, EH/CW; DH1, DH/CW; PH1, PH/CW; EW1, EW/CW; PW1, PW/CW.

for each tooth component showed significant differences between FD, DS and control groups (Table 1).

Mesio-distal crown diameter (CW) of second primary and first permanent molars in children with both syndromes was smaller than that of the control group (Table 1). In FD EH and dentin thickness was significantly greater than that of the controls in the permanent molars and PH was reduced. In the primary molars, EH was also increased relative to the other groups, and PH decreased, although DH showed no significant difference. The ratio of EH to CW was greater in both primary and permanent molars of individuals with FD than in controls or DS.

In DS permanent molars EH and width and dentin thickness were reduced, absolutely and relatively. In DS primary molars dentin thickness was reduced and PH was greater, both absolutely and relatively than that of the controls and FD groups.

If these changes were related to age or function, then a similar trend would be expected in all tooth components. The results demonstrate that this is not the case. This is exemplified by Figs. 2 and 3 that show biplot representations of the multivariate

analysis of variances for second primary and first permanent molars. The biplot is a graphic device for approximate display of various features of a matrix of multivariate means for several samples. It is especially revealing in principal component analysis, where the biplot can show inter-unit distances and indicate clustering of units as well as display variances and correlations of the variables. Each group can be graphically determined as a confidence circle for samples. The center is the mean of the analyzed group and the radius is the simultaneous distance from the mean. The level of significance chosen was $\alpha = 2.5\%$. The size of each circle depends on the group size—the smaller the group, the larger the circle. Overlapping circles mean that the distances between groups are not significant statistically. The vectors represent variables. Vectors perpendicular to the line connecting two circles centers represent variables that make the difference between groups. Vectors parallel to the line connecting between two centers represent variables that are similar in both groups. From the biplot one may learn what sort of linear
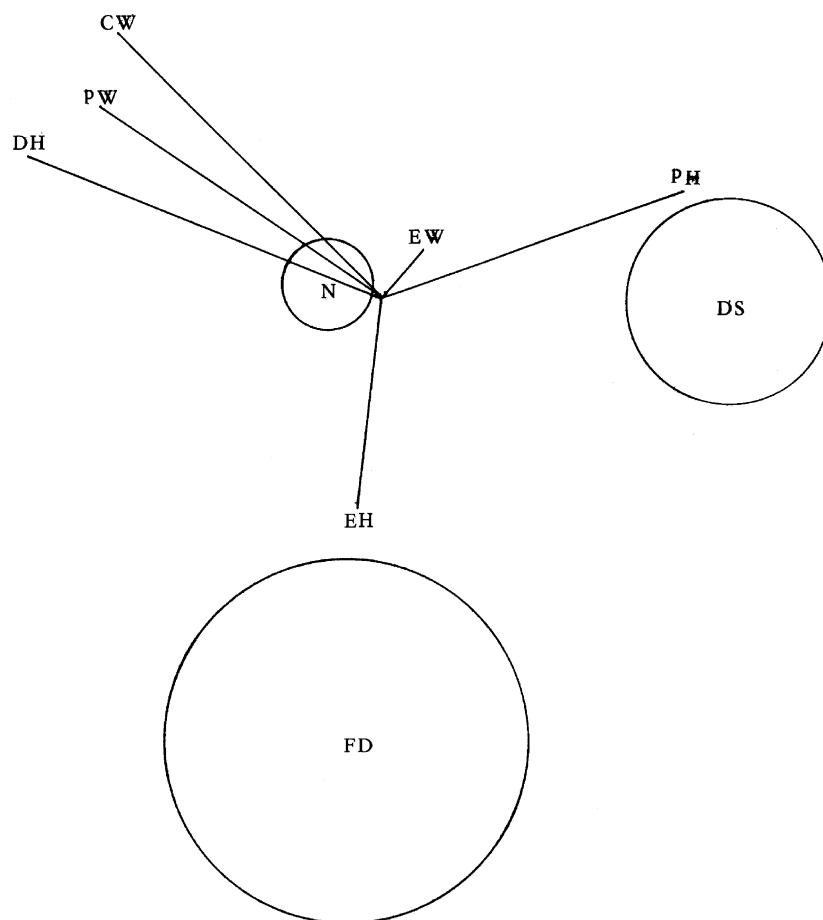

Figure 2 Biplot of second primary molars. N: control group, FD: Familial Disautonomia, DS: Down syndrome. Vectors abbreviations similar to Table 1.
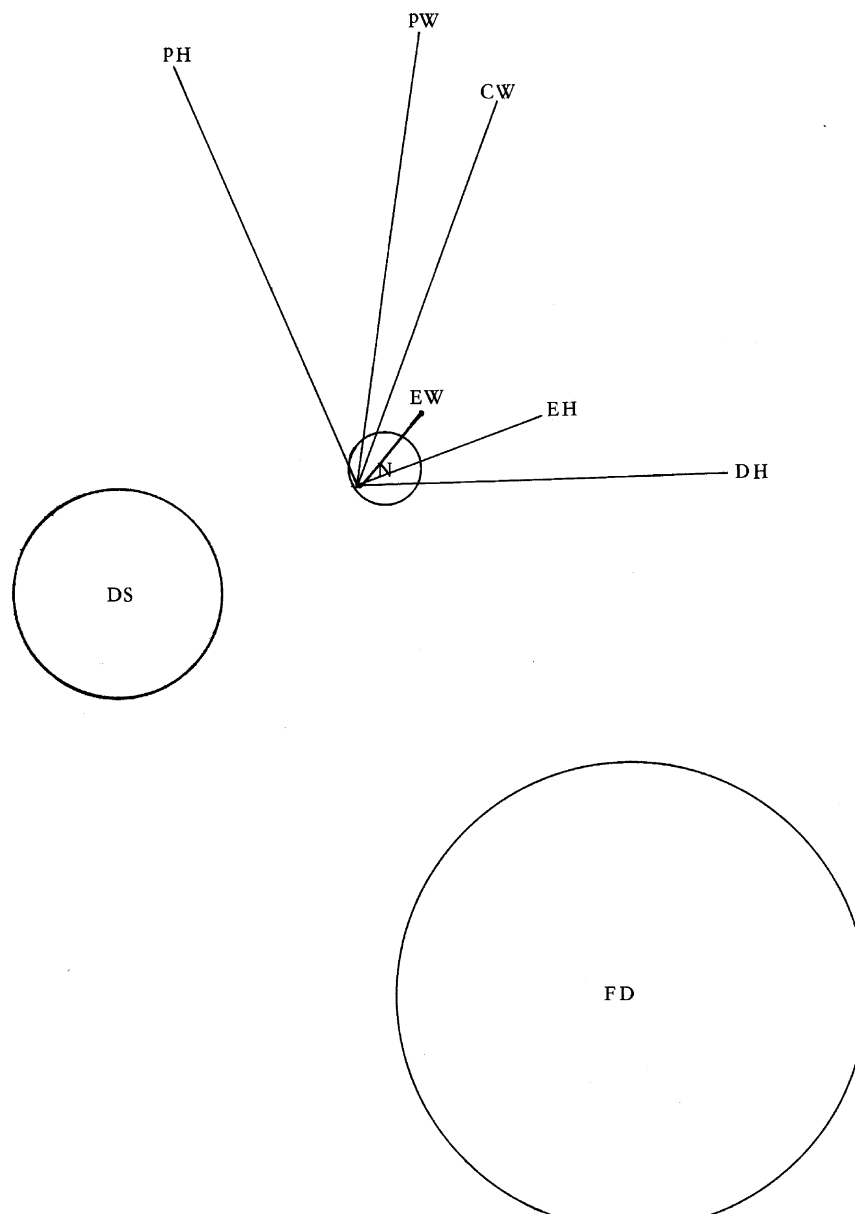
Figure 3 Biplot of permanent first molar. FD: Familial Disautonomia, DS: Down syndrome. Vectors abbreviations similar to Table 1.

combination of the given variables will show up the relatively large sample differences, among all samples or between some given pair of samples.

As observed in Figs. 2 and 3, FD and DS groups differ significantly from the control group (N). The main variables contributing to the difference between DS and N groups in primary molars are enamel dimensions and CW and in permanent molars pulp dimensions and CW. Between FD and N groups pulp dimensions and DH in primary molars and enamel dimensions and DH in permanent molars showed the greatest differences. DS differ from FD in DH, pulp and CW in primary molars and in enamel thickness, DH, and pulp and CW in permanent molars.

Discussion

The growth pattern of the molar teeth in the human embryo is characterized by a rapid increase in dimensions of the tooth germ, from the beginning of the cap stage to the initiation of calcification on the mesio-buccal cusp. Thereafter, there is a gradual deceleration in growth as cell differentiation proceeds at the interphase between the enamel organ and dental sac until the outline of the DEJ is completed. Once this is achieved any further increase in crown size is dependant on the amount of enamel laid down by the ameloblasts, and not by mitotic activity.[2]

Regulation of tooth size has long been known to be polygenic, and influenced by genes on both the X and Y chromosomes[6,41—44] and their absence or duplication grossly affects tooth size and enamel thickness. The X chromosome has a role in the determination of tooth shape and enamel apposition and the Y chromosome has been related to larger tooth size in males and especially in the canine tooth.[45—51] The X amelogenin gene has been mapped to the short arm and is now considered to be the source of the genomic defect for X-linked Amelogenesis Imperfecta[52] which may also affect other ectodermal derived tissues.

Our understanding of the processes involved in the regulation of tooth development is rapidly increasing and many hundreds of genes appear to be involved.[5,8,53—55]

The limited duration and ''irreversible'' sequence of events leading to development of the teeth, means that examination of the different components of teeth in the living can provide information on the timing and extent of abnormalities in early stages of development since the form, size and even number of teeth is affected in many different syndromes.[7,11] In this paper, we have attempted to distinguish between the different stages of tooth development defined by the DEJ and enamel and dentin thickness implicated in FD and DS, both of which show reduced crown size.

A number of studies have shown that the later developing teeth in DS are the most severely affected, in keeping with the general deceleration in growth and development.[26] Townsend[12] reported that in the primary dentition only the second molars showed any reduction in size although all permanent teeth are smaller. This has been attributed to a transitory acceleration in mitotic activity in early life prior to the characteristic retardation in growth that has been reported in the second trimester[56] and is expressed in the reduced size of the primary second molars and all permanent teeth.[26,27]

The biplot analysis highlights the differences found between the three groups, demonstrating the distinct behavior of the tooth components in the two syndromes, as summarized in Table 2. Reduction in enamel thickness of DS patients has

previously been described by Zilberman[57] and by Bell et al.[58] on extracted mandibular permanent incisors. The increased severity of the defect observed on the permanent molars may be due to their later timing and rate of development. The duration of enamel apposition in the primary second molar is shorter than that of the first permanent molar and starts earlier. In the primary second molar enamel formation begins at 6 months in utero and crown formation is complete at 12 months (a total of 15 months), while in the first permanent molar enamel formation begins at birth and the crown is completed at 2.5—3 years (a total of 30—36 months). The rate of recruitment and enamel apposition of the ameloblasts in the primary teeth is faster than that of the permanent molars. In the permanent molars both recruitment and apposition, determined by the cross-striation interval in enamel varies. It is small close to the DEJ, 2—3 μm per day, and increases toward the surface to 5—6 μm per day, in the final stages of amelogenesis.[59]

In the DS sample, the reduction in crown size in primary second molars is mainly due to the reduction in proliferation shown by the reduced dimensions of the dentin and pulp, while in permanent molars both proliferation and apposition of enamel and dentin are affected. The reduction in enamel and dentin thickness of the permanent first molars takes place within the context of the overall deceleration of body growth observed in DS patients after the age of 2 years.[23]

In FD patients despite the reduction observed in crown size both enamel and dentin were thicker than in the DS group, suggesting hyperfunction of both ameloblasts and odontoblasts. Since enamel is laid down only over a restricted period of time, this suggests that ameloblastic activity was either accelerated or prolonged. We hope to be able to clarify this in the future through examination of the ultrastructure of exfoliated primary teeth.

In conclusion, the results showed that these two hereditary disorders differ significantly in their effect on tooth formation. DS was associated with reduced activity of the ameloblasts and odontoblasts, while FD was associated with increased activity of both components. This study showed

Table 2 Summary of the effects of DS and FD on tooth components.

Tissue	Tooth component	Cell division (proliferation)		Function (apposition)	
		DS	FD	DS	FD
Ectodermal	Enamel	Reduced	Reduced	Reduced	Increased
Ecto-mesenchymal	Dentin—pulp complex height	Not affected		Reduced	Increased in M₁

that the use of standard roentgenographs can provide valuable information on the contribution of different phases in tooth formation to the external expression of growth insults in the dentition.

Acknowledgements

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