ORAL DISEASES

Oral Diseases (2010) 16, 292–298. doi:10.1111/j.1601-0825.2009.01641.x

http://www.blackwellmunksgaard.com

ORIGINAL ARTICLE

# Quantification of taurodontism: interests in the early diagnosis of hypohidrotic ectodermal dysplasia

**C-I Gros[1,2,5,6], F Clauss[1,3,5,6], F Obry[1,3,5], MC Manière[1,3,5], M Schmittbuhl[1,4,5,6]**

[1]*Faculty of Dentistry, University of Strasbourg, France;* [2]*Department of Oral Medicine and Oral Surgery, Oral and dental care unit, University Hospital, Strasbourg, France;* [3]*Department of Pediatric Dentistry, Oral and dental care unit, University Hospital, Strasbourg, France;* [4]*Department of Radiology, Oral and dental care unit, University Hospital, Strasbourg, France;* [5]*National French Reference Center for Dental Manifestations of Rare Diseases, University Hospital, Strasbourg, France;* [6]*Team research UMR INSERM 977, Faculty of Dentistry, University of Strasbourg, France*

**OBJECTIVE: The aim of this study was to provide a quantification of taurodontism in Hypohidrotic Ectodermal Dysplasia (HED) and to report its occurrence in a cohort of HED patients to assess phenotypic–genotypic correlations.**

**PATIENTS AND METHODS: Of 68 HED patients retrospectively reviewed, 16 patients aged 7–51 years were selected and compared with a control sample (*n* = 351). The pulp surface index of the first lower permanent molar was calculated from the panoramic radiograph of each individual, and statistical comparisons between the HED patients and the control sample were performed.**

**RESULTS: Whatever the genetic disorder, 81.25% of the HED patients exhibited a relative enlargement (≥1 s.d.) of the pulp. Major deviations (>5 s.d.) were respectively related to men affected by large deletion of the *EDA* gene or missense mutation. The autosomal recessive form was linked to a relative moderate pulp enlargement (3.44 s.d.). In *NEMO* forms, the increase of pulp size in men appeared to be less marked than in *EDA* mutations.**

**CONCLUSION: This study provides for the first time an objective assessment of pulp enlargement in HED patients, and the various degrees of taurodontism depicted could be interesting dental phenotypic markers of HED forms.**

*Oral Diseases* (2010) **16**, 292–298

**Keywords:** taurodontism; hypohidrotic ectodermal dysplasia; *EDA*; *EDAR*; *NEMO*

Correspondence: Catherine-Isabelle Gros, DDS, Assistant, Faculty of Dentistry, 1 place de l'Hôpital, F-67000 Strasbourg, France. Tel: +33 90 24 39 24, Fax: + 33 90 24 39 10, E-mail: catherine-isabelle.gros@chru-strasbourg.fr
Received 5 October 2009; accepted 13 October 2009

## Introduction

Hypohidrotic ectodermal dysplasia belongs to a large and heterogeneous nosological group of polymalformative syndromes characterized by abnormal development of ectodermal structures, including teeth, nails, hairs and eccrine glands (Pinheiro and Freire-Maia, 1994; Mikkola and Thesleff, 2003). This syndrome is caused by mutations of the genes involved in the Ectodysplasin (EDA)-NF-$\kappa$B pathway. X-linked hypohidrotic ectodermal dysplasia (XLHED) corresponds to mutations of the *EDA* gene encoding for the functional EDA-A1 isoform interacting with EDA Receptor (EDAR) (Kere *et al*, 1996; Schneider *et al*, 2001; RamaDevi *et al*, 2008). The autosomal forms (recessive and dominant) involve mutations of both EDA Receptor and EDA Receptor-associated Death-domain genes (*EDAR* and *EDARADD*) (Chassaing *et al*, 2006; Bal *et al*, 2007; Van der Hout *et al*, 2008). Syndromic HED with immunodeficiency (HED-ID) is caused by mutations in NF-$\kappa$B Essential Modulator gene (*NEMO*) (Zonana *et al*, 2000; Smahi *et al*, 2002).

Phenotypic signs consist of hypotrichosis, hypohidrosis and craniofacial and dental abnormalities (Montonen *et al*, 1998; Ruhin *et al*, 2001; Johnson *et al*, 2002; Lamartine, 2003; Clauss *et al*, 2008). Dental phenotype consists of various degrees of oligodontia with other dental abnormalities (microdontia, cone-shaped teeth). These abnormalities could correspond to interesting telltale signs in the early diagnosis of this syndrome and for the genetic counselling of female carriers.

Taurodontism is one of the dental abnormalities frequently encountered in HED syndrome, as reported in the literature (Jorgenson, 1982; Crawford *et al*, 1991; Glavina *et al*, 2001; Lexner *et al*, 2007). This abnormality is characterized by a vertical elongation of the pulp chamber associated with an apical displacement of the root bifurcation or trifurcation (Keith, 1913; Jaspers and Witkop, 1980; Jafarzadeh *et al*, 2008). Diagnosis of taurodontism is usually based on a subjective evaluation

of the radiographic apical displacement of the pulp chamber floor (Shaw, 1928). Some attempts for quantification of taurodontism have, however, been proposed (Keene, 1966; Blumberg *et al*, 1971; Shifman and Chanannel, 1978), but these approaches remain relatively confidential and surprisingly, they do not account for a well-known biological phenomenon, i.e. the age-related reduction of the pulp chamber size (Ketterl, 1983; Morse, 1991).

In HED patients, taurodontism seems to affect preferentially primary molars and permanent molars with a relatively high prevalence (Crawford *et al*, 1991; Lexner *et al*, 2007). To date, no attempt of taurodontism quantification has been proposed in the context of this syndrome (Jafarzadeh *et al*, 2008).

The aim of the study was (i) to characterize the taurodontism by calculating the pulp surface index from digital panoramic radiographs, (ii) to report the occurrence of this abnormality in the present cohort of HED patients. Furthermore, the precise quantification of the degree of taurodontism could provide the opportunity to assess phenotypic–genotypic correspondence, and could therefore lead to an interesting clinical predictor of HED variants.

## Patients and methods

The patients with clinically diagnosed HED were recruited from the National French Reference Center for Dental Manifestations of Rare Diseases (Oral and Dental Care Unit, University Hospital, Strasbourg, France) and the National French Reference Center for Genodermatosis (Department of Dermatology, Necker-Enfants Malades Hospital, AP-HP, Paris). A total of 68 patients were retrospectively reviewed and 42 of them presented mutations identified either in *EDA, EDAR* and *NEMO* genes. The genotyping was performed by the Department of Medical Genetics (Purpan University Hospital, Toulouse, France) and the Laboratory of Genetics (Necker-Enfants Malades Hospital, AP-HP, Paris) using previously published protocols (Vincent *et al*, 2001; Chassaing *et al*, 2006; Bal *et al*, 2007). A control sample of 351 individuals (205 men and 146 women) was also included in the study. The age of the subjects ranged from 5 to 76 years, with an average age of 37.33 years.

For this retrospective study, the majority of panoramic radiographs were collected from patients who were referred to the Department of Radiology (Oral Health Care Department, University Hospital, Strasbourg, France) between 2007 and 2008. All the imaging examinations were performed by the same operator using a ProMax Digital Panoramic device (Planmeca, Helsinki, Finland). For some HED patients, radiographs were provided by their dentist. The panoramic radiograph of each individual was examined. The selection criteria for inclusion of panoramic radiographs in the study were: equality of the mandibular ramus width, upper concavity of the occlusion plane and minimal tooth proximal superimpositions.

Only the first permanent mandibular molars were retained for the purpose of this study. Patients with carious lesions, apical infectious pathologies, endodontic treatments and crown restorations were excluded from the study. The dental maturity of the selected teeth corresponded at least to Dermirjian F stage (Demirjian and Levesque, 1980). As no significant differences between controlateral teeth of the same type exist (Solheim, 1992; Kvaal *et al*, 1995; Bosmans *et al*, 2005), left and right teeth were pooled for the analysis.

After filtering out HED patients according to the previous selection criteria, only 16 patients aged 7–51 years were selected. Among these patients, 11 presented mutations identified in *EDA*, *EDAR* or *NEMO* genes.

Pulp size and crown size were estimated using an original procedure of image processing and analysis. Outlines of the first molar and of the pulp were obtained using the Adobe Illustrator® CS3 software (Adobe, San Jose, CA, USA) by fitting a Bezier curve to the corresponding margins of the tooth and the pulp from each panoramic radiograph. The pulp crown surface was defined as the part of the pulp located above a parallel line to the cement–enamel junction and tangent to the highest point of the pulp floor (Figure 1). The crown surface corresponded to the tooth portion located above this line. Quantification of the pulp and of the crown areas was automatically performed using a routine developed with Visilog 5.4® image analysis software (Noesis, France).

The Pulp Surface Index, corresponding to the ratio between the pulp crown surface (PCS) and the crown surface (CS), was calculated for each first molar as follows:

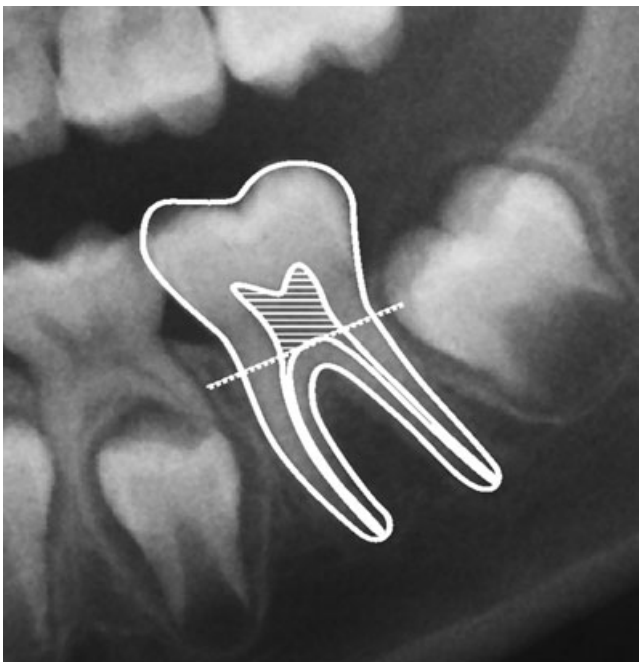

**Figure 1** Pulp surface index (PSI) corresponding to the ratio between the pulp crown surface (hatched area) and the crown surface (area located above the dashed line)

$$\mathrm{PSI} = \frac{\mathrm{PCS}}{\mathrm{CS}} \times 100$$

Statistical analyses were performed using Statistica 7.0® software (Statsoft, Tulsa, OK, USA). To test the inter-observer variation of the PSI, a random subset of 18 radiographs, selected from the control sample, were reassessed by a second examiner. Differences between the measurements of the two examiners were analysed using a *t*-test. A Pearson correlation coefficient was calculated between the PSI values of the two examiners to evaluate inter-observer variation.

Descriptive statistics (mean, standard deviation) of the PSI were calculated according to age first in the gender-pooled control sample and then in each gender group. Non-parametric Mann–Whitney tests were used to examine gender differences of PSI values in each age group. The relationship between age and PSI was evaluated by calculating the corresponding correlation coefficients.

For the HED group of patients, the deviation ($D$) of PSI from the control sample was determined as follows:

$$D = \frac{\mathrm{PSI}_{\mathrm{i.DEH}} - \mathrm{PSI}_{\mathrm{age.gender}}}{\mathrm{s.d.}_{\mathrm{age.gender}}} \quad (1)$$

with $\mathrm{PSI}_{\mathrm{i.DEH}}$ corresponding to the PSI value of the DEH patient studied; $\mathrm{PSI}_{\mathrm{age.gender}}$ corresponding to the PSI mean value of the age-matched and gender control group, and $\mathrm{s.d.}_{\mathrm{age.gender}}$ corresponding to the standard deviation of the corresponding age and gender control group.

## Results

No significant inter-observer differences of PSI values were observed as indicated by the *t*-test comparison between the two examiners ($t = 0.83$, $P = 0.42$). Furthermore, a high inter-observer concordance in PSI was demonstrated as suggested by the high value of the correlation coefficient between the measurements of the two observers ($r = 0.95$, $P = 0.01$).

Data from the control sample demonstrated a physiological age-related reduction of the pulp surface, as indicated by the descriptive statistics of the PSI in the combined sample and in each gender group (Table 1, Figure 2). Furthermore, the existence of significant negative correlations between pulp size and chronological age confirmed this biological trend (combined sample: $r = -0.597$; men: $r = -0.613$; women: $r = -0.626$) (Figure 2).

In the HED group, molecular data were available for 11 individuals (Table 2); the X-linked mode of inheritance was encountered in 10 patients and an autosomal recessive form was detected in one patient. *EDA* gene mutations were identified in seven patients and *NEMO* gene mutations were found in three individuals. *EDA* gene defects corresponded principally to missense mutations involving respectively exon 2, exon 6 and exon 8, and for two patients (one mother P4 and her son P5), mutations were large deletions encompassing exons 3–8 (Table 2). The *EDAR* gene mutation corresponded to a missense mutation located in exon 12 encoding the death domain of the receptor protein.

In the HED group, 81.25% of patients exhibited a relative enlargement of the pulp, as suggested by the deviations of PSI values equal or superior to 1 s.d. (Table 2). Deviations of the pulp size were more marked in men affected with X-linked HED than that in heterozygous female carriers of the mutation. Major deviations ($>5$ s.d.) were respectively related to a large deletion encompassing exons 3–8 (P4) and a missense mutation of exon 8 (P1; Table 2, Figure 2). Interestingly, their mothers, carriers of the same mutations (P5, P7), exhibited normal or moderate enlargement of pulp size. Minor deviations ($<2.5$ s.d.) were observed in women respectively affected by missense mutation of *EDA* gene exon 8 or by *NEMO* gene defect. The autosomal recessive form was linked to a relative moderate pulp enlargement, as suggested by the deviation slightly $>3$ s.d. (Table 2, Figure 2).

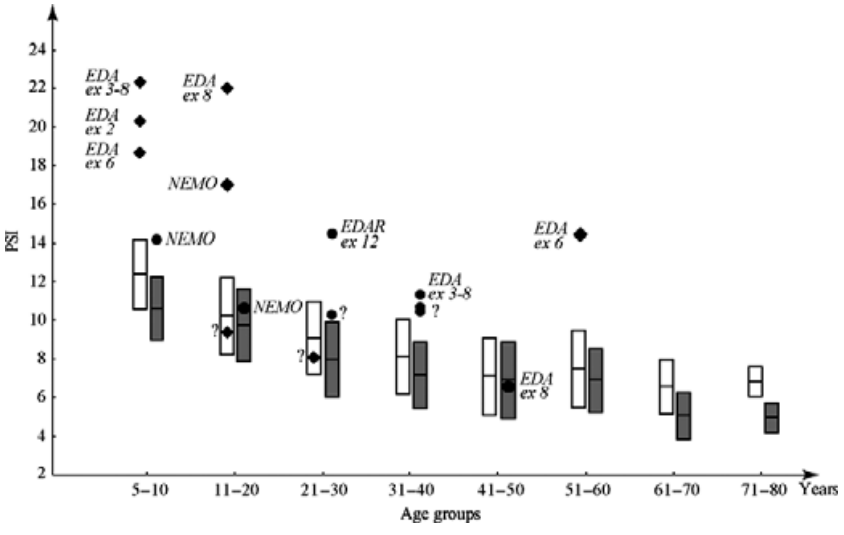


**Figure 2** Variations of the pulp size in HED patients compared with those of the control sample. Cohort of HED patients: round symbols (●) denote women, diamond symbols (♦), men; *EDA*, *EDA* gene mutation; *NEMO*, *NEMO* gene mutation; *EDAR*, *EDAR* gene mutation; ex, exon; ?, unknown molecular diagnosis. For the control sample, PSI variations according to chronological age are given; for each age group, boxes correspond to the mean ± 1 s.d.; white boxes, men; grey boxes, women

**Table 1** Pulp surface index (PSI): descriptive statistics (mean, standard deviation) in the gender-pooled control sample and in each gender group; results of the between-gender comparisons (Mann–Whitney test)

| | *Male + Female* | | | *Male* | | | *Female* | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| *Age group* | *n* | *Mean* | *s.d.* | *n* | *Mean* | *s.d.* | *n* | *Mean* | *s.d.* | *M–W* |
| 5–10 | 34 | 11.58 | 1.93 | 20 | 12.32 | 1.80 | 14 | 10.53 | 1.63 | ** |
| 11–20 | 36 | 9.90 | 1.90 | 18 | 10.14 | 1.96 | 18 | 9.67 | 1.87 | n.s. |
| 21–30 | 59 | 8.44 | 1.96 | 30 | 8.99 | 1.88 | 29 | 7.88 | 1.92 | * |
| 31–40 | 71 | 7.67 | 1.89 | 43 | 8.04 | 1.93 | 28 | 7.08 | 1.72 | * |
| 41–50 | 55 | 6.91 | 1.97 | 32 | 7.00 | 1.99 | 23 | 6.80 | 1.98 | n.s. |
| 51–60 | 60 | 7.17 | 1.87 | 37 | 7.38 | 1.99 | 23 | 6.84 | 1.64 | n.s. |
| 61–70 | 27 | 6.02 | 1.49 | 19 | 6.47 | 1.40 | 8 | 4.97 | 1.16 | * |
| 71–80 | 9 | 6.12 | 1.17 | 6 | 6.75 | 0.75 | 3 | 4.88 | 0.74 | * |
| Age group combined | 351 | 8.04 | 2.41 | 205 | 8.31 | 2.48 | 146 | 7.65 | 2.27 | * |

*n*, sample size; s.d., standard deviation; Min, minimum; Max, maximum; M–W, Mann–Whitney test; n.s., non-significant.
$^{**}P < 0.01$; $^{*}P < 0.05$.

**Table 2** Deviation of pulp size in HED patients *vs* control sample

| *Patient* | *Age* | *Gender* | *Gene involved* | *Gene location* | *Mode of inheritance* | *PSI value* | *Deviation of HED vs* Control* |
|---|---|---|---|---|---|---|---|
| P6 | 8 | M | *EDA* | Exon 6 | X-linked | 18.62 | +3.50 |
| P9 | 8 | M | *EDA* | Exon 2 | X-linked | 20.27 | +4.42 |
| P4 | 9 | M | *EDA* | Exons 3–8 | X-linked | 22.25 | +5.52 |
| P1 | 14 | M | *EDA* | Exon 8 | X-linked | 21.89 | +5.99 |
| P2 | 51 | M | *EDA* | Exon 6 | X-linked | 14.38 | +3.53 |
| P5 | 40 | F | *EDA* | Exons 3–8[a] | X-linked | 11.27 | +2.44 |
| P7 | 41 | F | *EDA* | Exon 8[b] | X-linked | 6.49 | −0.16 |
| P12 | 25 | F | *EDAR* | Exon 12 | AR (htz.) | 14.40 | +3.44 |
| P3 | 11 | M | *NEMO* | n.d. | X-linked | 16.88 | +3.44 |
| P14 | 7 | F | *NEMO* | n.d. | X-linked | 14.07 | +2.17 |
| P15 | 18 | F | *NEMO* | n.d. | X-linked | 10.61 | +0.50 |
| P10 | 17 | M | n.d. | n.d. | n.d. | 9.30 | −0.43 |
| P8 | 24 | M | n.d. | n.d. | n.d. | 8.06 | −0.50 |
| P16 | 22 | F | n.d. | n.d. | n.d. | 10.24 | +1.23 |
| P11 | 31 | F | n.d. | n.d. | n.d. | 10.43 | +1.95 |
| P13 | 39 | F | n.d. | n.d. | n.d. | 10.56 | +2.53 |

M, male; F, female; AR (htz.), heterozygous autosomal recessive form in a heterozygous female patient; n.d., mutation not defined.
*For each HED patient, deviation of the PSI value from mean PSI value of the corresponding age and gender control group is given.
Superscript alphabets indicate mothers of patients P4 and P1.

In *NEMO* mutations, the increase of pulp size in men appeared to be less marked than that in *EDA* mutations as suggested by the corresponding deviations (Table 2). In women, pulp size is comparable with that linked with *EDA* mutations (Figure 2).

## Discussion

This study provides, for the first time, an objective assessment of pulp enlargement in HED patients. Various approaches for pulp size description have been proposed, essentially in the context of forensic age estimation (Kvaal and Solheim, 1994; Drusini *et al*, 1997; Paewinsky, 2005; Cameriere *et al*, 2007), but no surface characterization in syndromes, especially in HED, seems to be reported in the literature.

The diagnosis of taurodontism corresponds generally to a subjective estimation of pulp enlargement in molars. From these examinations, the varying degrees of expression of this abnormality were classified in increasing order of severity as hypotaurodontism, mesotaurodontism and hypertaurodontism (Shaw, 1928; Keene, 1966; Shifman and Chanannel, 1978). According to Seow and Lai (1989), a crown (C) and body height (B) to root length (R) ratio (CB:R) of slightly $<1$ is considered to be the normal limit for the diagnosis of taurodontism. In our study, the definition of taurodont tooth relies on statistical distribution parameters, i.e. a PSI value larger than 1 standard deviation; moreover, age-related physiological reduction of pulp size chamber and gender differences are taking into account for pulp enlargement estimation as indicated by the mode of calculation of PSI deviation (1). Thus, the present quantitative approach avoids the inherent bias resulting from observer subjectivity, improves reliability and allows statistical determination of taurodontism.

Dental pulp reduction, which corresponds to a continuous deposition of dentin throughout life after crown formation, has been extensively studied (Ketterl,

1983; Morse, 1991) and this feature is mainly used for age estimation (Solheim, 1992; Paewinsky *et al*, 2005; Meinl *et al*, 2007). In the context of syndromes, the physiological pulp reduction was sometimes taken into consideration to characterize taurodontism (Chaussain-Miller *et al*, 2003), but never in HED. Sexual dimorphism is another biological aspect that might be considered in pulp size quantification. Although conflicting results have been demonstrated in the literature (Demirjian and Levesque, 1980; Drusini *et al*, 1997; Chandler *et al*, 2003; Cameriere *et al*, 2007), some significant gender differences are outlined in our study. Thus, these differences need to be considered and led us to include gender as a factor in the determination of the PSI deviation (1).

Area assessment of the pulp size seems to be a more sensitive approach than conventional measurements for depiction of pulp enlargement. Indeed, area corresponds to a square function maximizing much more subtle size deviations than a linear measurement ratio would characterize. This aspect may be of interest in the detection of pulp changes in HED syndromes.

Panoramic radiographs were used in our approach for estimating pulp enlargement. This examination provides a large view in which all the teeth are recorded in a single image. The assessment of the first permanent mandibular molars was preferred as these teeth were better defined than the permanent maxillary molars, and their pulp chambers were fully visible. According to Seow and Lai (1989), orthopantomograms may be associated with distortions, but the authors demonstrated that there were no statistically significant differences between measurements from periapical radiographs and measurements from panoramic radiographs for the evaluation of taurodontism in the first mandibular molar. In addition, the methodology used in this study seems to be reliable for the investigations of large samples as panoramic view is the exploration of choice for initial examination of patients (FDA, 1987; White and Pharoah, 2009).

The majority of the identified mutations corresponded to *EDA* mutations (7/11). The other genetic defects were identified as one *EDAR* and three *NEMO* mutations. Ectodermal dysplasia caused by *NEMO* gene mutations remains scarce; the incidence of *NEMO* mutations resulting in HED-ID is $<1$ in 250 000 live male births (Orange *et al*, 2004). Surprisingly, our cohort of HED patients includes an exceptional number of individuals affected by *NEMO* mutations. This may be as a result of the inclusion protocol of the National French Reference Center for Genodermatosis (Department of Dermatology, Necker-Enfants Malades Hospital, AP-HP, Paris) that gathers information on patients with this type of syndrome from all over the French territory.

Of the *EDA* mutations identified, the majority of them were missense mutations (5/7). These results parallel the corresponding frequencies reported in the literature, underlying the high frequency of missense mutations among *EDA* gene defects (Pääkkönen *et al*, 2001; Vincent *et al*, 2001; Conte *et al*, 2008).

The large deletion affecting exons 3–8 is unique. Generally, most of the corresponding mutations encompass between 1 and 3 exons, as indicated in earlier reports (Kere *et al*, 1996; Bayés *et al*, 1998; Conte *et al*, 2008). Vincent *et al* (2001) described also a large deletion but involving all the *EDA* gene exons. Large *EDA* gene deletions are associated with severe molecular consequences (Vincent *et al*, 2001; Conte *et al*, 2008). However, these genetic defects remain rare, as suggested by the corresponding low prevalencies, i.e. 5.5% and 5.9% as respectively described by Conte *et al* (2008) and Vincent *et al* (2001).

Compared with the reported prevalence of pulp enlargement in normal populations, ranging from 0.1% to 48.0% (Pindborg, 1970; Sarr *et al*, 2000), a higher frequency seems to characterize our cohort of HED patients irrespective of the molecular defects. Indeed, 81.25% of the patients exhibited PSI values equal or superior to 1 s.d. (Table 2). Moreover, the prevalence of pulp enlargement is higher in our HED sample than the 66.15% and 70.21% reported respectively by Crawford *et al* (1991) and Lexner *et al* (2007). As these previous approaches relied generally on a subjective evaluation, it might be hypothesized that the degree of taurodontism might have been slightly underestimated by these authors.

The pulp enlargement in men affected by *EDA* mutations corresponds to a marked taurodontism, as underlined by the large deviations of PSI values (Table 2, Figure 2). This clinical sign may provide an interesting dental phenotypic marker of XLHED. Moreover, the degree of taurodontism in patients bearing *EDA* gene abnormalities seems to be more severe than that detected in *NEMO* mutation (Figure 2). Even if further phenotypic analyses with larger HED samples need to be conducted, our observation gives for the first time a distinctive characterization of pulp enlargement between XLHED and HED-ID forms, which might be useful for their respective diagnosis.

The most important pulp enlargement seems to be associated with the large deletion encompassing exons 3–8 (patient P4). In this genetic disorder, exon 4 encoding the $(Gly\text{-}X\text{-}Y)_{19}$ domain is affected, leading to severe anomalies of the extracellular trimerization, EDA–EDAR binding and EDA-A1 functions (Visinoni *et al*, 2003) likely to induce dental developmental alterations and thus pulp disorders. Marked pulp enlargement is also associated with missense mutations in exons 2 and 8, encoding respectively for proteolytic cleavage site for a furin protease (Pääkkönen *et al*, 2001; Schneider *et al*, 2001) and for the extracellular TNF homology sub-domain necessary for receptor binding (Tariq *et al*, 2007; RamaDevi *et al*, 2008). Moreover, the resulting severe molecular anomalies seem to be associated with marked dental phenotypic manifestations (Clauss *et al*, 2008), emphasizing thus the major role of these different coding regions in odontogenesis.

Moderate pulp enlargements are encountered in patients (P2, P6) affected by exon 6 mutations (Figure 2); this exon being involved in the encoding of the TNF homology sub-domain necessary for receptor

binding. In these patients, the moderate pulp enlargement is associated with a relatively mild-to-moderate dental phenotype resulting in oligodontia and non-systematic dental dysmorphologies (Clauss *et al*, 2008). Such a moderate phenotype suggests the existence of a more conservative mutation on the EDA-A1 quaternary molecular structure, allowing for the partial EDA–EDAR binding.

Mild pulp enlargement or normal pulp sizes (Table 2, Figure 2) are found in heterozygous female carriers of *EDA* mutation (exons 3–8 large deletion and exon 8 missense mutation observed respectively in patients P5 and P7) or *NEMO* mutations (patients P14 and P15). For female carriers of *EDA* gene mutations, our cohort included their sons. The subnormal pulp size encountered in women affected by *EDA*, compared with the marked pulp enlargement in the affected sons, emphasizes thus the existence of lyonization phenomenon consisting in the accidental inactivation of an X-chromosome in HED syndromes (Lyon, 1961; Glavina *et al*, 2001). This phenomenon is probably responsible for the reduced pulp enlargement in *NEMO* women.

Interestingly, the change in the *EDAR* gene involving exon 12 (patient P12), which encodes the Receptor death domain, seems to be linked to a relatively marked pulp enlargement (+3.44 s.d.). Besides, the patient exhibited a mild dental phenotype: the agenesis of the two maxillary permanent lateral incisors (data not shown). Therefore, these observations suggest that the size of the pulp could provide a tell-tale sign in the diagnosis of autosomal recessive forms. This is all the more interesting as phenotypic differences between the autosomal recessive and dominant forms remain difficult to interpret (Chassaing *et al*, 2006).

In conclusion, this study provides, for the first time, an objective assessment of the pulp enlargement in HED patients. Neither surface characterization of the pulp nor any physiological pulp reduction in HED patients seem to have been reported to date in the literature. As area assessments seem to be more sensitive than conventional measurements to quantify pulp enlargement, the approach employed in this study could be of interest to detect subtle size changes in HED. Irrespective of the genetic disorder, the prevalence of pulp enlargement seems to be higher in our sample than that in normal population. The higher prevalence, compared with those reported in other HED studies, suggests a possible underestimation of the degree of taurodontism in previous attempts of pulp size characterization. Even if further phenotypic analyses with larger HED samples need to be conducted, the different degrees of pulp enlargement seem to provide interesting dental phenotypic markers of the HED forms. The degree of taurodontism associated with *EDA* defects seems to be more severe than that detected in *NEMO* mutations, leading for the first time to a distinctive characterization of pulp enlargement between XLHED and HED-ID forms. Moreover, the relatively marked pulp enlargement associated with mild dental phenotypes, as encountered in *EDAR* mutations, may suggest that the pulp size could be of interest in the depiction of autosomal recessive forms.

## References

Bal E, Baala L, Cluzeau C *et al* (2007). Autosomal dominant anhidrotic ectodermal dysplasias at the *EDARADD* locus. *Hum Mutat* **28:** 703–709.

Bayés M, Hartung AJ, Ezer S *et al* (1998). The anhidrotic ectodermal dysplasia gene (*EDA*) undergoes alternative splicing and encodes ectodysplasin-A with deletion mutations in collagenous repeats. *Hum Mol Genet* **7:** 1661–1669.

Blumberg JE, Hylander WL, Goepp RA (1971). Taurodontism: a biometric study. *Am J Phys Anthropol* **34:** 243–255.

Bosmans N, Ann P, Aly M, Willems G (2005). The application of Kvaal's dental age calculation technique on panoramic dental radiographs. *Forensic Sci Int* **153:** 208–212.

Cameriere R, Ferrante L, Belcastro MB, Bonfiglioli B, Rastelli E, Congolani M (2007). Age estimation by pulp/tooth ratio in canines by peri-apical x-rays. *J Forensic Sci* **52:** 166–170.

Chandler NP, Pitt Ford TR, Monteith BD (2003). Coronal pulp size in molars: a study of bitewing radiographs. *Int Endod J* **36:** 757–763.

Chassaing N, Bourthoumieu S, Cossee M, Calvas P, Vincent MC (2006). Mutations in *EDAR* account for one-quarter of non-*ED1*-related hypohidrotic ectodermal dysplasia. *Hum Mutat* **27:** 255–259.

Chaussain-Miller C, Sinding C, Wolikow M, Lasfargues JJ, Godeau G, Garabédian M (2003). Dental abnormalities in patients with familial hypophosphatemic vitamin D-resistant rickets: prevention by early treatment with 1-Hydrovitamin D. *J Pediatr* **142:** 324–331.

Clauss F, Manière MC, Obry F *et al* (2008). Dento-craniofacial phenotypes and underlying molecular mechanisms in hypohidrotic ectodermal dysplasia (HED): a review. *J Dent Res* **87:** 1089–1099.

Conte C, Gambardella S, Bulli C *et al* (2008). Screening of *EDA1* gene in X-linked anhidrotic ectodermal dysplasia using DHPLC: identification of 14 novel mutations in Italian patients. *Genet Test* **12:** 437–442.

Crawford PJM, Aldred MJ, Clarke A (1991). Clinical and radiographic dental findings in X linked hypohidrotic ectodermal dysplasia. *J Med Genet* **28:** 181–185.

Demirjian A, Levesque GY (1980). Sexual differences in dental development and prediction of emergence. *J Dent Res* **59:** 1110–1122.

Drusini AG, Toso O, Ranzato C (1997). The coronal pulp cavity index: a biomarker for age determination in human adults. *Am J Phys Anthropol* **103:** 353–363.

Food and Drug Administration (1987). *The selection of patients for X-ray examinations: dental radiographic examinations*. FDA editions: Rockville.

Glavina D, Majstorović M, Lulić-Dukić O, Jurić H (2001). hypohidrotic ectodermal dysplasia; dental features and carriers detection. *Coll Antropol* **25:** 303–310.

Jafarzadeh H, Azarpazhooh A, Mayhall JT (2008). Taurodontism: a review of the condition and endodontic treatment challenges. *Int Endod J* **41:** 375–388.

Jaspers MT, Witkop CJ (1980). Taurodontism, an isolated trait associated with syndromes and X-chromosomal aneuploidy. *Am J Hum Genet* **32:** 396–413.

Johnson EL, Roberts MW, Guckes AD, Bailey LJ, Phillips CL, Wright JT (2002). Analysis of craniofacial development in children with hypohidrotic ectodermal dysplasia. *Am J Med Genet* **112:** 327–334.

Jorgenson RJ (1982). The conditions manifesting taurodontism. *Am J Med Genet* **11:** 435–442.

Keene HJ (1966). A morphologic and biometric study of taurodontism in a contemporary population. *Am J Phys Anthropol* **25:** 208–209.

Keith A (1913). Problems relating to the teeth of the earlier forms of prehistoric men. *Proc R Soc Med* **6** (Odontol Sect)**:** 103–124.

Kere J, Srivastava AK, Montonen O *et al* (1996). X-linked anhidrotic (hypohidrotic) ectodermal dysplasia is caused by mutation in a novel transmembrane protein. *Nat Genet* **13:** 409–416.

Ketterl W (1983). Age-induced changes in the teeth and their attachment apparatus. *Int Dent J* **33:** 262–271.

Kvaal S, Solheim T (1994). A non-destructive dental method for age estimation. *J Forensic Odontostomatol* **12:** 6–11.

Kvaal SI, Kolltveit KM, Thomsen IO, Solheim T (1995). Age estimation of adults from dental radiographs. *Forensic Sci Int* **74:** 175–185.

Lamartine J (2003). Towards a new classification of ectodermal dysplasias. *Clin Exp Dermatol* **28:** 351–355.

Lexner MO, Bardow A, Bjorn-Jorgensen J, Hertz JM, Almer L, Kreiborg S (2007). Anthropometric and cephalometric measurements in X-linked hypohidrotic ectodermal dysplasia. *Orthod Craniofac Res* **10:** 203–215.

Lyon MF (1961). Gene action in the X chromosome of the mouse (*Mus musculis* L.). *Nature* **190:** 372–373.

Meinl A, Tangl S, Pernicka E, Fenes C, Watzek G (2007). On the applicability of the secondary dentin formation to radiological age estimation in young adults. *J Forensic Sci*, **52:** 438–441.

Mikkola ML, Thesleff I (2003). Ectodysplasin signaling in development. *Cytokine Growth Factor Rev* **14:** 211–224.

Montonen O, Ezer S, Saarialho-Kere UK *et al* (1998). The gene defective in anhidrotic ectodermal dysplasia is expressed in the developing epithelium, neurectoderm, thymus, and bone. *J Histochem Cytochem* **46:** 281–289.

Morse DR (1991). Age-related changes of the dental pulp complex and their relationship to systemic aging. *Oral Surg Oral Med Oral Pathol* **72:** 721–745.

Orange JS, Jain A, Ballas ZK, Schneider LC, Geha RS, Bonilla FA (2004). The presentation and natural history of immunodeficiency caused by nuclear factor kappaB essential modulator mutation. *J Allergy Clin Immunol* **113:** 725–733.

Pääkkönen K, Cambiaghi S, Novelli G *et al* (2001). The mutation spectrum of the *EDA* gene in X-linked anhidrotic ectodermal dysplasia. *Hum Mutat* **17:** 349.

Paewinsky E, Pfeiffer H, Brinkmann B (2005). Quantification of secondary dentine formation from orthopantomograms–a contribution to forensic age estimation methods in adults. *Int J Legal Med* **119:** 27–30.

Pindborg JJ (1970). *Pathology of the dental hard tissues*. Munksgaard: Copenhagen.

Pinheiro M, Freire-Maia N (1994). Ectodermal dysplasias: a clinical classification and a causal review. *Am J Med Genet* **53:** 153–162.

RamaDevi AR, Reddy EC, Ranjan S, Bashyam MD (2008). Molecular genetic analysis of patients from India with hypohidrotic ectodermal dysplasia reveals novel mutations in the *EDA* and *EDAR* genes. *Br J Dermatol* **158:** 163–167.

Ruhin B, Martinot V, Lafforgue P, Catteau B, Manouvrier-Hanu S, Ferri J (2001). Pure ectodermal dysplasia: retrospective study of 16 cases and literature review. *Cleft Palate Craniofac J* **38:** 504–518.

Sarr M, Toure B, Kane AW, Fall F, Wone MM (2000). Taurodontism and the pyramidal tooth at the level of the molar. Prevalence in the Senegalese population 15 to 19 years of age. *Odontostomatol Trop* **23:** 31–34.

Schneider P, Street SL, Gaide O *et al* (2001). Mutations leading to X-linked hypohidrotic ectodermal dysplasia affect three major functional domains in the tumor necrosis factor family member ectodysplasin-A. *J Biol Chem* **276:** 18819–18827.

Seow WK, Lai PY (1989). Association of taurodontism with hypodontia: a controlled study. *Pediatr Dent* **11:** 214–219.

Shaw JCM (1928). Taurodont teeth in South African races. *J Anat* **62:** 476–498.

Shifman A, Chanannel I (1978). Prevalence of taurodontism found in radiographic dental examination of 1200 young adult Israeli patients. *Community Dent Oral Epidemiol* **6:** 200–203.

Smahi A, Courtois G, HadjRabia S *et al* (2002). The NF-$\kappa$B signaling pathway in human diseases: from incontinentia pigmenti to ectodermal dysplasias and immune-deficiency syndromes. *Hum Mol Genet* **11:** 2371–2375.

Solheim T (1992). Amount of secondary dentin as an indicator of age. *Scand J Dent Res* **100:** 193–199.

Tariq M, Wasif N, Ayub M, Ahmad W (2007). A novel 4-bp insertion in *EDA1* gene in a Pakistani family with X-linked hypohidrotic ectodermal dysplasia. *Eur J Dermatol* **17:** 209–212.

Van der Hout AH, Oudesluijs GG, Venema A *et al* (2008). Mutation screening of the Ectodysplasin-A receptor gene *EDAR* in hypohidrotic ectodermal dysplasia. *Eur J Hum Genet* **16:** 673–679.

Vincent MC, Biancalana V, Ginisty D, Mandel JL, Calvas P (2001). Mutational spectrum of the ED1 in X-linked hypohidrotic ectodermal dysplasia. *Eur J Hum Genet* **9:** 355–363.

Visinoni AF, de Souza RL, Freire-Maia N, Gollop TR, Chautard-Freire-Maia EA (2003). X-linked hypohidrotic ectodermal dysplasia mutations in Brazilian families. *Am J Med Genet* **22:** 51–55.

White SC, Pharoah MJ (2009). *Oral radiology – Principles and interpretation*. 6th edition. Mosby Elsevier: St Louis.

Zonana J, Elder ME, Schneider LC *et al* (2000). A navel X-linked disorder of immune deficiency and hypohidrotic ectodermal dysplasia is allelic to incontinentia pigmenti and due to mutations in *IKK-gamma* (*NEMO*). *Am J Hum Genet* **67:** 1555–1562.