Tooth Dimensions in hypodontia with a known PAX9 mutation

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Abstract

Aims: Congenital absence of teeth is a complex condition affecting several parameters of oral development. This is the first study to measure tooth crown dimensions using image analysis in a family with hypodontia in whom the mutation has been identified, and compare them with a control group.

Methods and Results: Study models were obtained from ten family members from three generations affected by severe hypodontia with a missense mutation in PAX9 and ten unaffected, unrelated controls. Using established image analysis techniques all teeth up to and including the first permanent molars were digitally imaged by two operators from the occlusal (O) and buccal (B) aspects three times and an average made for the mesio-distal (MDO and MDB) bucco-lingual (BL), area (A) and perimeter (P) measurements. Intra-Class Correlation Coefficients (ICCC) were calculated to assess intra- and inter-operator reliability. Two-sample t-tests were then used to compare these dimensions with those of the controls. Reliability of the technique was high (mean $r>0.95$). The majority of tooth types throughout the dentition were significantly smaller in the family members with hypodontia than in the control group for all parameters measured. The levels of significance were very high for upper lateral incisors ($p<0.0001$) whilst the canines and first molars were less different. The greatest number of significant differences were found in BL and P, closely followed by MD and A measurements.

Conclusions: the significantly smaller tooth crown dimensions recorded in the affected family members show that the effect of the PAX9 mutation is seen not only in the congenitally missing teeth but also in smaller crown size throughout the dentition.

Key words:
Hypodontia
PAX9
Reliability
Image analysis

Introduction

Hypodontia, the congenital absence of teeth ranges in severity from mild, involving the absence of one or two teeth, to severe, when many of the teeth are missing. It is a complex condition associated clinically with anomalies in the size and shape of the teeth formed, deficient alveolar bone growth and delayed eruption. The aetiology of hypodontia is multifactorial with major genetic and environmental factors [1]. The reported prevalence of hypodontia in different populations has varied between 2.3% and 10.1% in the permanent dentition with 3rd molars, 2nd pre-molars and upper lateral incisors the most frequently missing teeth. Females are more often affected than males. Occasionally hypodontia occurs in conjunction with certain syndromes such as ectodermal dysplasia, cleft lip and/ or palate [2] and Down's [1,2].

The World Health Organisation classifies this condition under the heading of 'Handicapping Dentofacial Anomalies' describing the presentation as 'an anomaly which causes disfigurement or which impedes function and which requires treatment if the disfigurement or functional defect is, or is likely to be, an obstacle to the patient's physical or emotional well being [3].

Various genes including Pax9, MSX1 and AXIN2 [4-9] have been implicated in the aetiology of hypodontia. The PAX9 gene on chromosome 14 is a controlling factor during embryonic development with particular effect on dental development and mutations are strongly related to missing teeth. Several different mutations have been found since Stockton et al recorded the first in 2000 [4-16].

Following linkage analysis three families were identified with different mutations within the PAX9 gene and demonstrating severe hypodontia of the primary and permanent dentitions [12].

In several studies of individuals with severe hypodontia small tooth size has been found [1,16-19]. Most previous studies investigating crown size in hypodontia have been carried out using manual measurements of erupted teeth on study models. A more accurate and versatile approach is to use an image analysis technique which permits both more extensive investigation and has a high reliability [18,20,21]. A study using this technique showed that patients with mild, moderate and severe hypodontia all had tooth crown dimensions that were smaller than controls[18]. However the degree of difference was greater the more severe the hypodontia[18].

The aim of this study was to measure the crown dimensions on study models using image analysis in a family with severe hypodontia with an identified missense mutation in the human PAX9 gene[12] and compare the findings with a control group to determine whether tooth size was different in the hypodontia patients from the controls and whether any differences affected the whole dentition.

Materials and Methods

The DEN 9 family.

Three novel mutations were identified in PAX9 associated with hypodontia in 3 families (DEN3, DEN8 and DEN9)[12]. In the DEN9 family members affected by hypodontia clinical phenotype information, the designation of teeth present/absent, was cross referenced with a T62C transition mutation causing a coding amino acid exchange of leucine for praline within the PAX9 protein at position 21, leading to the removal of the MspA1 site in exon 2. This was the first time a hypodontia phenotype had been linked directly with that genotype. All affected DEN9 family members having this genetic mutation demonstrated hypodontia. This study involved the DEN9 family only.

Sample collection

Following ethical approval by the South Sheffield Research Ethics Committee, consent was obtained from family members to the study and their dentists contacted. The various dentists of the family members took the required upper and lower dental impressions in alginate. For the control group, matched for sex, age and ethnicity impressions were taken from ten unaffected, unrelated individuals. The impressions were cast in yellow stone (Kaffir D, British Gypsum, Newark, UK).

Imaging and measurement

Study models were mounted on an adjustable stand (Figure 1) and both buccal and occlusal surfaces of all sound, fully erupted, permanent teeth only up to and including the first permanent molars were imaged, using a 32-bit digital camera (Kodak Nikon DCS410 Digital Camera (CCD Dynamic Random Access Memory (DRAM) imager, giving an ISO of 100, providing 1.5 mega pixel resolution in an array of 1012 x 1524 pixels, producing 4.6MB TIF files), with a 90mm high quality Elicar macro lens.

The camera was attached to a gridded-base copy stand (Kaiser, Germany). The stand supported two white strip lights (RB 5000, with Phillips Fluorescent daylight bulbs, Kaiser, Germany) to provide standardisation of illumination and incorporated adjustments for recordable positioning (Figure 1). The camera was connected to a Viglen CX1 Dual processor, Viglen Ltd, UK) via an Adaptec 2940 SCSI card (KJP Ltd, UK).

A scale was included in each image for calibration purposes. Each tooth was imaged 3 times from the occlusal and buccal aspects independently by 2 examiners.

From the occlusal aspect the mean mesio-distal (MDO), bucco-lingual (BL), projected two dimensional area (A) measured within the boundary of the maximum perimeter possible from a particular image of the tooth (P) were calculated using Image Pro Plus software (V5, Media Cybernetics, USA; Figure 2). The measurements from the buccal view were the mean mesio-distal (MDB). Variables such as the occluso-gingival, area and perimeter were not measured from the buccal aspect as they depend on the position of the gingival margin, which can vary due to local pathology, making them unreliable (Figure 3).

Statistical analysis

Fleiss' Coefficient of Reliability [22] was used to assess intra-operator repeatability and inter-operator reproducibility for all occlusal and buccal measurements including the re-imaging procedure. The results were categorised according to Donner and Eliasziw's (1987) classification[23]. Percentage differences are given between the DEN9 family data and those of the control group. Two-sample t-tests were used to check for between antimere teeth for asymmetry determination and to evaluate the differences between test and control groups. A binomial test evaluated any significance patterns of differences per tooth type and measurement, whilst correcting for multiple nesting [25]. To further test significance levels of findings, Meta-analysis [26] was applied to the p values from the control/test comparisons. In this test, which is more stringent than the binomial, the original p values are transformed to give a truncated product and this is then used to assess the probability of the original values being true.

Results

All inter- and intra-operator measurements fell into Donner and Eliasziw's category of excellence for the Coefficient of Reliability (Table 1).

In the individuals with hypodontia, differences were apparent for most of the tooth types being significantly smaller for all variables than the control group (Tables 2, 3 and 4).

From the occlusal aspect (Tables 3 and 4), the majority of the teeth, from all types were significantly smaller in the family members with hypodontia. The perimeter and buccal-lingual dimensions showed the most significant differences closely followed by the mesio-distal. The area shows greatest difference but this variable is a squared function of the perimeter. However, the area differences were greatest in the first molars.

From the buccal view, considering the mesio-distal dimension, those affected by hypodontia had smaller MDB compared to the controls with the exception of the upper left second premolar where the measurements are the same in both test and control groups (Tables 2 and 4).

Table 4 shows that the meta-analysis has confirmed the significance of the p values calculated.

No significant asymmetry seen between antimere tooth measurements.

Discussion

The results in Table 1 clearly show that the image analysis technique is highly reliable with all intra- and inter- examiner measurements for all variables falling into Donner and Eliasziw’s category of excellence; this is better than those reported for manual measurement. These data therefore show this technique is valid for the measurement of MD, BL, P and A from the occlusal view and MD from the buccal view using image analysis to measure controls and hypodontia cases from study models.

In agreement with Khalif et al [27] there was no significant asymmetry seen in either the control or hypodontia groups. Results from the occlusal view for crown size, showed a reduction for each variable in the hypodontia cases when compared with the controls (Tables 3 and 4). This is the first time that this difference has been published in the literature for the parameters of perimeter and area. The effect was seen to the whole dentition with the canines and first molars least effected. The MD from the buccal view confirmed the result from the occlusal view that this dimension was smaller in the hypodontia patients (Table 2).

The pattern of congenitally missing teeth in affected family members was that the molar regions were particularly affected but that some premolars and incisors were also missing[2]. This study shows that the crown size of the whole dentition is affected, including the anterior segments. The varying degrees of effect on the different variables e.g. BL more affected than MD is worth investigating further in future studies.

This study has provided further evidence, from the effects of a PAX9 mutation, confirming the findings of Brook [1], Al-Sharood [2] , McKeown et al [26] and Brook et al [28] that there is an association between hypodontia and smaller crown dimensions in the remaining dentition and so supporting the aetiological model proposed by Brook [1]. This would incorporate major influences such as single genes of major effect (e.g. PAX 9), mutations, chromosomal anomalies and major environmental insults within a background of polygenic and general environmental factors such as nutrition.

Conclusions

The image analysis method proved highly reliable for the measurement of variables from two views when assessing tooth dimensional changes from study models. The method permits reliable, accurate, increasingly flexible approaches to analysing tooth shape and size, producing a database of standardised images for future study. There was no evidence of left / right asymmetry in tooth dimensions.

All measurements from the occlusal view and the MD from the buccal view showed a reduction, often highly significant, in the hypodontia cases when compared to controls.

Thus the study demonstrated that hypodontia includes not only reduced tooth number but also smaller than average tooth size throughout the dentition.

References

1. Brook AH. A unifying aetiological explanation for anomalies of human tooth number and size. *Arch Oral Biol* 1984;**29**:373-378.
2. Al-Sharood MH. Measurement of tooth size and shape in subjects with hypodontia and a control group using a new image analysis technique. PhD thesis, University of Sheffield, United Kingdom. 2000.
3. World Health Organisation. Standardisation of Reporting of Dental Diseases and conditions. 6. The Assessment of Handicapping Dentofacial Anomalies. Technical report series. No. 242, Geneva, WHO. 1962.
4. Vastardis H, Karimbux N, Guthua SW, Seidman JG, Seidman CE. A Human MSX1 homeodomain missense mutation causes selective tooth agenesis. *Nat Genet* 1996;**13**:417–421.
5. Stockton DW, Das P, Goldenberg M, D'Souza RN, Patel PI. Mutation of PAX9 is associated with oligodontia. *Nat Genet* 2000;**24**:18–19.
6. Lammi S, Arte S, Somer M, Järvinen H, Lahermo P, Thesleff I. Mutations in AXIN2 cause familial tooth agenesis and predispose to colorectal cancer. *Am J Hum Genet* 2004;**74**:1043–1050.
7. De Muynck S, Schollen E, Matthijs G, Verdonck A, Devriendt K, Carels C. A novel MSX1 mutation in hypodontia. Am J Med Genet 2004;**128A**:401–403.
8. Kim JW, Simmer JP, Lin BP, Hu JC. Novel MSX1 Frameshift causes autosomal-dominant oligodontia. *J Dent Res* 2006;**85**:267–271.
9. Mostowska A, Kobielak A, Biedziak B, Trzeciak WH. A novel mutation in the paired box sequence of PAX9 gene in a sporadic form of oligodontia. *Eur J Oral Sci* 2003;**111**:371–376.

10. Nieminen P, Arte S, Tanner D, Paulin L, Alaluusua S, Thesleff I, *et al*. Identification of a nonsense mutation in the PAX9 gene in molar oligodontia. *Eur J Hum Genet* 2001;**9**:743–746.
11. Das P, Stockton DW, Bauer C, Shaffer LG, D'Souza RN, Wright TJ. Haploinsufficiency of PAX9 is associated with autosomal dominant hypodontia. *Hum Genet* 2002;**110**:371–376.
12. Das P, Hai M, Elcock C, Leal SM, Brown DT, Brook AH, *et al*. Novel missence mutations and a 288-bp exonic insertion in PAX9 families with autosomal dominant hypodontia. *Am J Med Genet* 2003;**118**:35–42.
13. Jumlongras D, Lin JY, Chappa A, Seidman CE, Seidman JG, Maas RL. A novel missence mutation in the paired domain of PAX9 causes non - syndromic oligodontia. *Hum Genet* 2004;**114**:242–249.
14. Kapadia H, Frazier-Bowers S, Ogawa T, D'Souza RN. Molecular characterization of a novel PAX9 missense mutation causing posterior tooth agenesis. *Eur J Hum Genet* 2006;**14**:403–409.
15. Gerits A, Nieminen P, De Muynck S, Carels S. Exclusion of coding region mutations in MSX1, PAX9 and AXIN2 in eight patients with severe oligodontia phenotype. *Orthod Craniofac Res* 2006;**9**:129–136.
16. Tallón-Walton V, Manzanares-Céspedes MC, Arte S, Carvalho-Labato P, Valdivia-Gandhur I, Garcia-Susperregui A, *et al*. Identification of a novel mutation in the PAX9 gene in a family affected by oligodontia and other dental anomalies. *Eur J Oral Sci* 2007;**115**:427-432
17. Baccetti T. A controlled study of associated dental anomalies. *Angle Orthod* 1998;**68**:267–274.

18. Brook AH, Elcock C, Al-Sharood MH, McKeown HF, Khalaf K, Smith RN. Further studies of a model for the etiology of anomalies of tooth number and size in Humans. *Con Tissue Res* 2002;**4**:289-295.
19. Lammi L, Halonen K, Pirinen S, Thesleff I, Arte S, Nieminen P. A novel missence mutation in PAX9 in a family with distinct phenotype of oligodontia. *Eur J Hum Genet* 2003;**11**:866–871.
20. Brook AH, Smith RN, Elcock C, Al-Sharood MH, Shah AA, Karmo M. The measurement of tooth morphology: development and validation of a new image analysis system. In: Mayhall JT, Heikinnen T, eds. Dental Morphology 1998. Oulu: Oulu University Press, 1999. p. 380-387.
21. Brook AH, Smith RN, Elcock C, Al-Sharood MH, Shah AA, Karmo M, *et al*. Accurate tooth morphology measurement using a new analysis system. *J Dent Res* 1999;**78**:336.
22. Fleiss JL. Reliability of measurement, The design and analysis of clinical experiments. Chichester, NY: Wiley, 1986. p. 4-32.
23. Donner A, Eliasziw M. Sample sizes for reliability studies. *Statistics in Medicine* 1987;**6**:441-448.
24. Neuhäuser M. Testing whether any of the significant tests within a table are indeed significant. *OIKOS* 106 2004;**2**:409-410.
25. Whitehead A. Meta analysis of controlled clinical trials. Chichester NY: Wiley, 2002. p. 237-240.
26. McKeown HF, Robinson DL, Elcock C, Al-Sharood MH, Brook AH. Tooth dimensions in hypodontia patients, their unaffected relatives and a control group measured by a new image analysis system. *Eur J Ortho* 2002;**24**:131-141.

27. Khalaf K, Elcock C, Smith RN and Brook AH. Fluctuating Dental Asymmetry of Multiple Crown Variables Measured by an Image Analysis. *Arch Oral Biol* 2005;**50**:249-253

28. Brook AH, Griffin RC, Smith RN, Townsend GC, Kaur G, Davis GR *et al.* Tooth Size patterns in Patients with Hypodontia and Supernumerary teeth. *Arch Oral Biol* 2008; (In Press).

Table 1. Intra-operator repeatability and inter-operator reproducibility: Fleiss' Coefficient of Reliability (R; range plus mean in brackets) for all buccal and occlusal measurements. (R of 0.81-1.00 = excellent reliability).

	Intra-operator		**Inter-operator**
	Operator 1	**Operator 2**	
Buccal aspect			
Test group	0.987-0.999 (0.995)	0.986-0.999 (0.995)	0.877-0.966 (0.962)
Control group	0.986-0.998 (0.993)	0.987-0.999 (0.994)	0.890-0.995 (0.954)
Occlusal aspect			
Test group	0.980-0.999 (0.996)	0.988-0.999 (0.997)	0.887-0.996 (0.974)
Control group	0.998-0.999 (0.994)	0.988-0.999 (0.994)	0.904-0.999 (0.963)

Table 2. Tooth dimensions for tooth types 1 to 6 in all four quadrants from the buccal aspect. Percentage differences for each variable are provided.

Tooth type	N (Test)	Test Data MDB	N (Control)	Control MDB	% Diff’
UL1	10	7.24	9	8.83	18.01
UL2	9	4.19	9	4.97	15.69
UL3	10	6.40	8	7.91	19.09
UL4	7	5.37	9	7.09	24.26
UL5	7	6.97	9	6.97	0.00
UL6	5	9.41	8	10.62	11.39
UR1	9	7.08	9	8.69	18.53
UR2	9	3.61	9	5.21	30.71
UR3	7	5.84	8	7.96	26.63
UR4	8	6.41	9	6.93	7.50
UR5	7	6.76	9	7.16	5.59
UR6	5	9.01	8	10.61	15.08
LL1	6	5.03	10	5.39	6.68
LL2	10	4.82	10	5.21	7.49
LL3	10	5.75	10	6.60	12.88
LL4	9	6.01	10	6.39	5.95
LL5	4	6.92	10	7.01	1.28
LL6	5	9.57	9	11.30	15.31
LR1	5	4.54	10	5.23	13.19
LR2	10	4.61	10	5.19	11.18
LR3	10	5.47	10	7.32	25.27
LR4	8	6.24	10	7.40	15.68
LR5	5	6.14	10	7.46	17.70
LR6	7	10.47	10	11.48	8.80

N = number of teeth (test and control listed / tooth type).

Table 3. Tooth dimensions for all four quadrants from the occlusal aspect. Percentage differences for each variable are provided.

Tooth Type	N (test)	Test	N (control)	Control	% Diff'	Test	Control	% Diff'	Test	Control	% Diff'	Test	Control	% Diff'
		MDO		MDO		BL	BL		A	A		P	P	
UL1	10	7.23	9	9.35	22.67	6.76	8.15	17.06	37.80	56.27	32.82	22.77	28.77	20.86
UL2	9	5.53	9	7.45	25.77	5.25	7.33	28.38	23.41	42.19	44.51	17.44	23.87	26.94
UL3	10	7.07	8	8.39	15.73	6.99	6.81	-2.64	39.04	41.37	5.63	22.92	24.45	6.26
UL4	7	6.04	9	7.11	15.05	6.60	10.31	35.98	32.18	60.86	47.12	20.27	28.59	29.10
UL5	7	6.15	9	6.79	9.43	7.20	10.20	29.41	34.98	58.47	40.17	21.30	28.41	25.03
UL6	5	9.02	8	10.8	16.48	7.95	12.15	34.57	56.53	115.40	51.01	27.27	39.69	31.29
UR1	9	7.14	9	9.27	22.98	6.44	8.14	20.88	37.00	55.67	33.54	22.33	28.23	20.90
UR2	9	5.48	9	7.27	24.62	5.24	6.94	24.50	22.17	39.94	44.49	16.91	23.09	26.76
UR3	7	6.02	8	8.58	29.84	7.12	7.35	3.13	34.90	47.94	27.20	21.44	24.81	13.58
UR4	8	6.03	9	7.07	14.71	7.56	10.05	24.78	37.03	59.49	37.75	21.99	28.28	22.24
UR5	7	6.08	9	6.83	10.98	7.23	10.43	30.68	36.08	58.27	38.08	21.72	28.18	22.92
UR6	5	8.55	8	10.78	20.69	8.25	11.91	30.73	54.40	110.66	50.84	26.76	38.83	31.08
LL1	6	5.40	10	5.65	4.42	4.93	7.01	29.67	21.35	28.65	25.48	16.68	20.95	20.38
LL2	10	5.17	10	6.39	19.09	5.43	7.35	26.12	22.08	33.63	34.34	17.38	21.92	20.71
LL3	10	5.99	10	7.50	20.13	6.17	7.32	15.71	29.99	41.60	27.91	21.18	24.20	12.48
LL4	9	6.38	10	7.35	13.20	6.88	8.71	21.01	35.94	51.23	29.85	21.72	25.78	15.75
LL5	4	6.57	10	7.54	12.86	7.29	9.18	20.59	39.78	56.52	29.62	23.01	27.31	15.75
LL6	5	9.79	9	11.12	11.96	7.82	11.53	32.18	62.45	111.41	43.95	28.53	38.28	25.47
LR1	5	4.62	10	5.78	20.07	5.09	6.54	22.17	19.30	26.92	28.31	15.74	20.25	22.27
LR2	10	5.05	10	6.17	18.15	5.65	7.07	20.08	21.42	31.82	32.68	17.17	21.40	19.77
LR3	10	6.22	10	7.47	16.73	6.71	7.04	4.69	32.36	39.52	18.12	20.96	23.58	11.11
LR4	8	6.51	10	7.42	12.26	7.04	8.49	17.08	37.40	50.62	26.12	21.91	25.84	15.21
LR5	5	5.86	10	7.48	21.66	6.86	9.26	25.92	33.88	56.95	40.51	21.48	27.45	21.75
LR6	7	10.39	10	11.22	7.40	8.70	11.42	23.82	73.94	111.72	33.82	31.04	38.42	19.21

Table 4. Meta analysis p values for the 24 tooth types and the 24 teeth combined for each of the 7 aspects.

Tooth type	Meta analysis p values		Tooth aspect	Meta analysis p values
UL1	1.426E-09		Perimeter (occlusal)	4.392E-30
UL2	7.402E-10		Area (occlusal)	3.341E-28
UL3	1.374E-03		Bucco-lingual	8.144E-30
UL4	2.730E-06		MD (occlusal)	1.469E-28
UL5	1.259E-11		MD (buccal)	3.956E-11
UL6	2.627E-05			
UR1	2.021E-12			
UR2	3.294E-09			
UR3	9.689E-04			
UR4	1.436E-06			
UR5	8.631E-08			
UR6	3.341E-03			
LL1	2.965E-07			
LL2	5.548E-11			
LL3	8.459E-05			
LL4	4.746E-08			
LL5	2.839E-07			
LL6	1.805E-04			
LR1	1.228E-05			
LR2	5.035E-08			
LR3	1.437E-05			
LR4	2.824E-06			
LR5	2.693E-09			
LR6	5.944E-05			

Figures

Figure 1. The imaging station.

Figure 2. Occlusal view of a study model showing the area (AO), perimeter (PO,), mesio-distal (MDO), and) buccal lingual (BL) measurements.

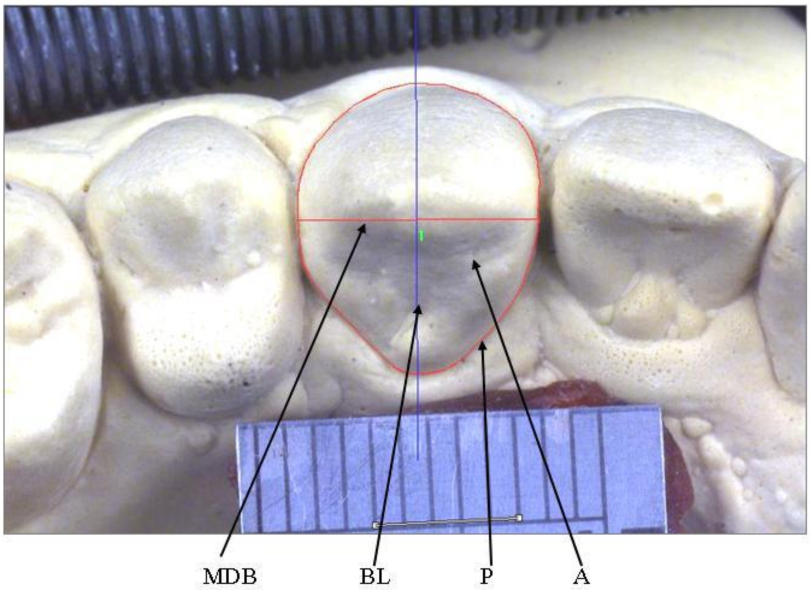

Figure 3. Buccal view of a study model showing the mesio-distal (MDB) dimension.