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ORIGINAL ARTICLE

Three-dimensional analysis of tooth dimensions in the *MSX1*-missense mutation

Marijn Créton · Marie-José van den Boogaard · Thomas Maal · Luc Verhamme · Willem Fennis · Carine Carels · Anne Marie Kuijpers-Jagtman · Marco Cune

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Abstract
Objectives A novel, 3D technique to measure the differences in tooth crown morphology between the *MSX1* cases and non-affected controls was designed to get a better understanding of dental phenotype-genotype associations.
Materials and methods Eight Dutch subjects from a single family with tooth agenesis, all with an established nonsense mutation c.332 C > A, p. Ser 111 Stop in exon 1 of *MSX1*, were compared with unaffected controls regarding several aspects of tooth crown morphology of incisor and molar teeth. A novel method of quantitative three-dimensional analysis was used to detect differences.
Results Statistically significant shape differences were observed for the maxillary incisor in the *MSX1* family compared with the controls on the following parameters: surface area, buccolingual dimension, squareness, and crown volume ($P \leq 0.002$). Molar crown shape was unaffected.
Conclusions A better understanding of dental phenotype-genotype associations may contribute to earlier diagnosis of some multiple-anomaly congenital syndromes involving dental anomalies.
Clinical relevance A "shape database" that includes associated gene mutations resulting from developmental syndromes may facilitate the genetic identification of hypodontia cases.

Keywords Hypodontia · Congenitally missing teeth · MSX1 · Crown dimensions · 3D analysis

M. Créton (✉) · W. Fennis
Department of Oral and Maxillofacial Surgery, Prosthodontics and Special Dental Care, University Medical Centre Utrecht,
P. O. Box 85.500, 3508 GA Utrecht, The Netherlands
e-mail: mcreton@umcutrecht.nl

M.-J. van den Boogaard
Department of Medical Genetics,
University Medical Center Utrecht,
Utrecht, The Netherlands

T. Maal · L. Verhamme
Department of Oral and Maxillofacial Surgery,
Radboud University Nijmegen Medical Centre,
Nijmegen, The Netherlands

C. Carels · A. M. Kuijpers-Jagtman
Department of Orthodontics and Craniofacial Biology,
Radboud University Nijmegen Medical Centre,
Nijmegen, The Netherlands

M. Cune
Department of Oral-Maxillofacial Surgery,
Prosthodontics and Special Dental Care, St. Antonius Hospital,
Nieuwegein, The Netherlands

M. Cune
Department of Fixed and Removable Prosthodontics, Centre for Dentistry and Oral Hygiene, University Medical Center Groningen, The University of Groningen,
Groningen, The Netherlands

Introduction

Hypodontia, the congenital absence of one or more permanent teeth, is the most common developmental anomaly in humans, with a prevalence of 5.5 % in Europeans and a 1.37:1 preference for women compared with men. Hypodontia appears as part of a syndrome or as a non-syndromic trait [9, 10, 29, 32, 33] and has a heterogeneous dental and dento-facial presentation.

There is an increasing understanding with respect to the genetic background of tooth development and, more specifically, to the molecular mechanisms during cell and tissue interactions. The etiology of disturbances of tooth development is considered multifactorial and genetic, epigenetic, and environmental, and their interaction factors play a role [2, 6, 11, 39]. Over 300 genes have been associated with tooth development to date, at least in mouse embryos. Most

of these genes seem to have a function in preserved signaling pathways during repetitive and reciprocal cellular communication between epithelial and mesenchymal tissues [6, 20, 27, 39, 40].

When tooth development is disrupted, a wide spectrum of clinical phenotypes can be expected, including typical patterns of tooth agenesis and variations in tooth morphology and size. For example, in familial non-syndromic oligodontia, it has been suggested that the defects in *PAX9* predispose for agenesis of maxillary and mandibular second molars, while in *MSX1*-associated oligodontia, agenesis of bicuspids is typically observed.

More recently, some variations in the *EDA* gene have been demonstrated to cause (X-linked) non-syndromic oligodontia as well. The patterns of missing teeth associated with *EDA* mutations seem to differ distinctly from those observed with *PAX9* and *MSX1*. There appears to be a tendency towards agenesis of maxillary and mandibular central incisors, lateral incisors, and canines in the presence of an *EDA* mutation. In case of *PAX9* or *MSX1* mutations, maxillary and mandibular first preliminary molars frequently persist [14, 38]. In the case of mutations in the *SHH* gene, fusion of the central incisor tooth buds may result in a single central incisor [15]. In addition, subjects with a single missing central upper incisor can be heterozygous carriers for holoprosencephaly [12], a potentially more serious syndromic condition affecting the midline development of the brain and face.

Some examples of aberrations in tooth shape and dimensions that are associated with genetic disturbances are provided below. Mutations in the EDA pathway (*EDA*, *EDAR*, *EDARADD*, and *NEMO*) result in hypohydrotic ectodermal dysplasia, commonly with hypodontia and conical, peg-shaped teeth when they do develop [25, 26]. In addition, it has been established that a common variation in *EDAR* (EDAR 1540C) in a Japanese population is strongly associated with the degree to which marginal ridges on the lingual surfaces of upper incisors (tooth shoveling) are developed [24]. A typical combination of morphological tooth features is observed in association with a *DLX3* mutation (amelogenesis imperfecta with taurodontism) [13]. In cases of hypodontia, teeth that are formed are generally smaller (microdontia) than those encountered in subjects without tooth agenesis [7, 8, 25, 34], while subjects with supernumerary teeth (hyperdontia) generally have larger teeth (macrodontia) than the controls [8, 22]. Recently, mutations in the PCNT gene have been shown to be associated with very small teeth, possibly the smallest ever reported [19]. Taurodontia is frequently observed in subjects with hypodontia [35, 36], but not those with hyperdontia [18]. Therefore, a better understanding of dental phenotype–genotype associations may contribute to earlier diagnosis of some multiple congenital anomaly syndromes involving tooth anomalies; additionally, precise measuring tools for shape analysis are desirable [1].

Morphological tooth traits, parameters of tooth dimension, and agenesis patterns may also serve as biomarkers for a dental phenotype, enabling early diagnosis of syndromes or specific genetic disorders [25]. The National Institutes of Health defines a biomarker as a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention. Biomarkers may be anatomic, physiologic, biochemical, or molecular measures that reflect the presence or severity of specific disease states.

Quantitative morphological analysis of the teeth poses a fundamental problem because teeth are multi-dimensional, irregular objects that are difficult to measure and quantify [31]. Continuous measures are preferred over descriptive ones, such as the presence or absence of Carabelli's trait or hypocone reduction. In odontometric analyses, linear measurements such as the mesiodistal and buccolingual tooth dimensions are traditionally performed on dental casts by means of analogue or digital calipers. This type of measurement can be obtained with a high degree of inter- and intra-observer reliabilities [8]. More recently, two-dimensional (2D) image analysis systems became available, and non-linear measurements, such as surface areas and perimeters, could be reliably determined [21]. Because both mesiodistal and buccolingual dimensions are generally smaller in subjects affected by hypodontia [8], tooth volume is expected to be an even more discriminative three-dimensional (3D) parameter which distinguishes small differences in tooth dimension between subgroups of patients.

For this purpose, we have developed a technique to geometrically evaluate the morphological parameters of teeth in three dimensions. This technique was applied to compare a series of patients with a known *MSX1* mutation with healthy controls under the null hypothesis that they are similar. Observed differences in tooth crown morphology between the *MSX1* cases and non-affected controls will be discussed in light of the present understanding of the biological regulation regarding some features of tooth crown morphogenesis.

Materials and methods

This study was designed as a case–control study.

Cases

Eight Dutch subjects from one family (four males and four females) with tooth agenesis and cleft palate or cleft lip and palate participated in the study. Subjects' ages varied from 9 to 68 years (mean age, 39 years). All subjects had an established nonsense mutation c.332 C > A, p. Ser 111 Stop in exon 1 of

MSX1. This particular population has been previously described in detail by van den Boogaard et al., and this mutation was known as p.Ser105Stop. The nomenclature has been changed in accord with the guidelines of the Human Genome Variation Society with reference sequence NM_002448.3 [42]. Absent teeth are presented in Table 1.

Selection of target teeth

Like other families with an *MSX1* mutation, the majority of the family members in the present study lack the premolars, predominantly the second premolars (Table 1) [22]. Therefore, we selected two tooth types that were present in all family members and, in general, are present in even the most severe hypodontia cases: the right maxillary first molar and the central incisor.

Controls

Healthy Caucasian subjects without hypodontia served as controls (21 males and 21 females). They were selected from the database of the Department of Orthodontics and Craniofacial Biology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands. To be included, all permanent teeth had to be present, including the third molars. The right maxillary central incisor and maxillary first molar should be non-restored and fully erupted. Subjects were excluded when the target teeth were damaged, showed excessive tooth wear, or with severe crowding.

3D measurements of tooth dimensions

Conventional gypsum models were processed into digital dental models, and their raw geometric data were obtained for all cases and controls (Digimodel, OrthoProof B.V., Doorn, The Netherlands). Target teeth were virtually cut from the models using commercially available software (Maxilim, Medicim B.V., Mechelen, Belgium). Subsequently, the teeth were loaded into a computer program that enables the mathematical analysis of three-dimensional shapes (Matlab 2007b, the Mathworks, Natick, MA, USA; Fig. 1).

The teeth must be positioned reproducibly in the geometric model. For this purpose, a reference plane was defined for both the molar and the incisor on the basis of pre-defined reference points, as described below. All geometric measurements were performed from this reference plane.

Maxillary first molar

For the first molar, a line was drawn between the mesial buccal and lingual cusp tips. A second line was drawn

Table 1 Congenitally absent teeth in the *MSX1* cases

	Maxilla Mandible	18 48	17 47	16 46	15 45	14 44	13 43	12 42	11 41	21 31	22 32	23 33	24 34	25 35	26 36	27 37	28 38
Male																	
1	Maxilla	X			X	X							X	X			X
	Mandible	X			X									X			X
2	Maxilla	X	X		X	X							X	X			X
	Mandible	X			X									X		X	X
3	Maxilla	X			X	X							X	X			X
	Mandible	X			X									X		X	X
4	Maxilla	X			X	X							X	X			X
	Mandible	X	X	X	X									X	X	X	X
Female																	
1	Maxilla	X			X	X							X	X			X
	Mandible	X			X									X			X
2	Maxilla				X	X							X	X			
	Mandible	X			X									X			X
3	Maxilla	X			X	X							X	X			X
	Mandible	X	X	X	X									X	X	X	X
4	Maxilla	X	X		X	X							X	X		X	X
	Mandible	X	X	X	X				X	X				X	X	X	X

X congenitally missing

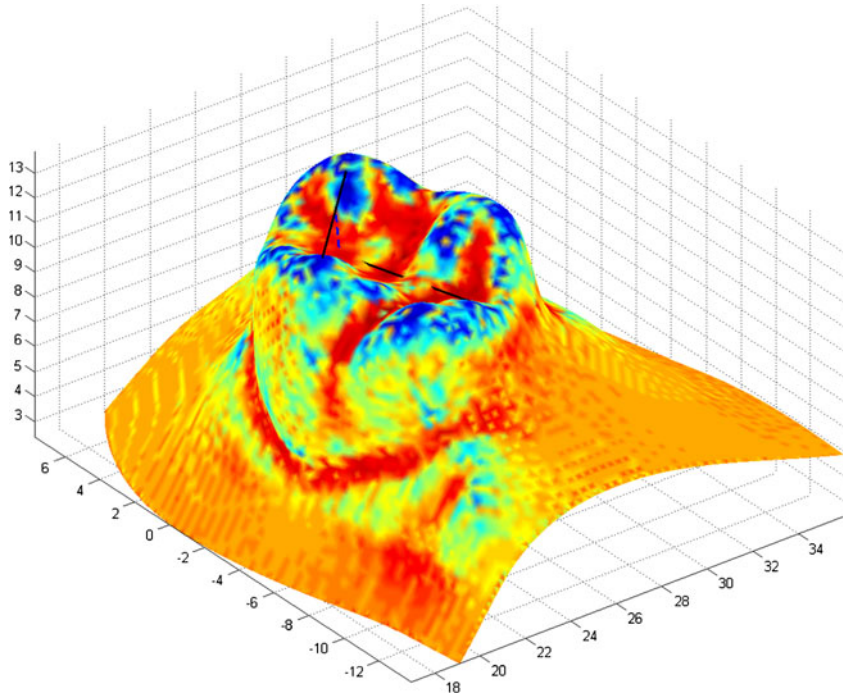

Fig. 1 The teeth were loaded into a computer program that enables mathematical analysis of three-dimensional shapes. For the first molar, a line is drawn between the two mesial cusp tips. A second line is drawn from the mesial to the distal margin. These two lines are then projected onto each other to form a plane parallel to the occlusal plane

from the mesial to the distal margin (Fig. 1). These two lines were projected onto each other to form a plane parallel to the occlusal plane. The molar was then rotated automatically until the constructed plane was parallel to the *X*–*Y* plane (Fig. 2).

The initial idea was to lower the plane until the largest perimeter of the molar was reached. Unfortunately, this often presents sub-gingivally, where no data regarding tooth dimensions are available. Therefore, the plane was lowered 1.2 mm below the deepest point in the fissure (which was determined automatically). At this depth, all models could be included in the study. The position of the reference plane had now been established.

Maxillary central incisor

For the incisor, a line was drawn through the incisal edge. Subsequently, the most prominent point on the buccal surface and the margin of the cingulum on the palatal surface were determined, and lines were drawn through these two points (perpendicular to the incisal edge line). To create a plane, the model was rotated along the incisal edge line until these two newly constructed lines were equal in length (Fig. 3). Finally, the reference plane was determined 2.3 mm beneath the incisal edge line. This position was chosen because it ensured that the reference plane would be above the gingival margin in all cases.

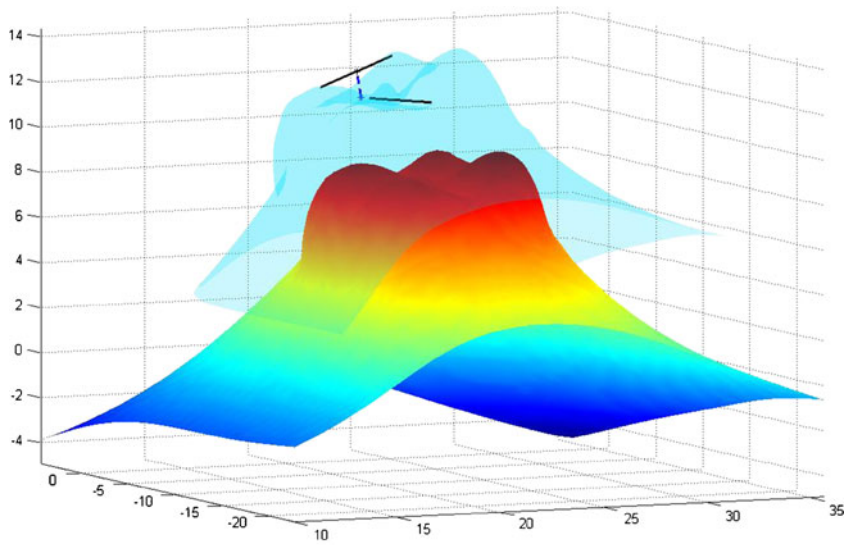

Fig. 2 The molar is rotated automatically until the constructed plane is parallel to the *X*–*Y* plane

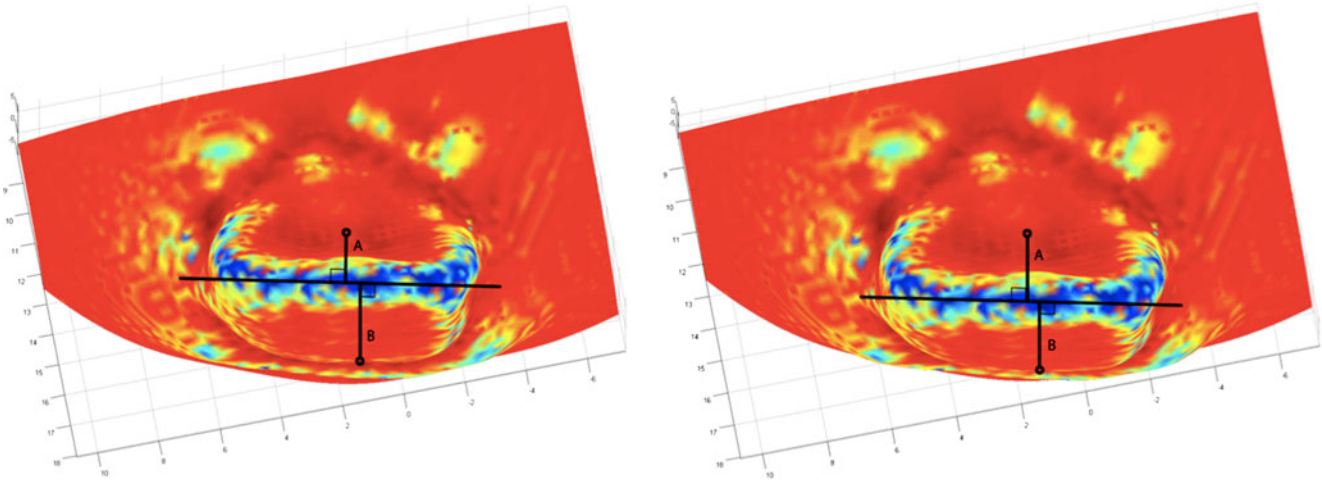

Fig. 3 To create a plane, the model is rotated along the incisal edge line until the two newly constructed lines (*A* and *B*) are equal in length. Line A is drawn perpendicular to the incisal edge line, through the center of the cingulum. Line B is also drawn perpendicular to the incisal edge line, through the most prominent buccal point

Morphological quantitative parameters

Tooth crown shape was quantified by means of six parameters that were thought to be representative, all of which were calculated at the level of the reference plane. These parameters were defined as follows:

- perimeter: the perimeter of the crown at the level of the reference plane (mm);
- surface area: the surface of the plane at the level of the reference plane (mm^2);
- buccolingual distance: the maximal distance between the buccal surface and the lingual surface of the crown (mm);
- mesiodistal distance: the maximum distance between the mesial and distal proximal surfaces (mm);
- squareness: indicates to what degree the tooth crown shape was square and was the ratio between the mesiodistal and buccolingual distances (mm/mm); and
- volume: the volume of the crown was calculated from the reference plane to the incisal edge and cusps (mm^3).

Repeatability

Twenty randomly selected incisors and 20 randomly selected molars were measured and remeasured by the same observer, and 20 molars and 20 incisors were measured by another observer to assess intra- and inter-observer repeatabilities. Repeatability of the measurements was expressed as the coefficient of repeatability (CR) in accordance with Bland and Altman [4].

Statistical analysis

Two-way analysis of variance with the geometric parameters as dependent variables and group and gender as fixed factors was applied for the measurements on both the molar and the incisor. Tooth dimensions are likely to be correlated, and multiple-testing correction to overcome the increase in type I error was advisable; hence, Bonferroni correction was performed. Consequently, α was set at 0.01. This is considered to be a conservative approach.

Results

The CRs for both intra- and inter-observer repeatabilities are presented in Table 2. The CR was interpreted in accordance with the guidelines of the British Standards Institution, which states that 95 % of the difference between the first and second measurements is expected to be within two standard deviations of the mean difference [5]. This was the case for the inter-observer repeatability of all measurements on both molars and incisors and was considered satisfactory. Regarding the intra-observer repeatability of the mesiodistal distance, area, and volume, 90 % of the differences fell within two standard deviations of the mean difference, while all other parameters were at 95 % (Table 2).

The mean values and standard deviations for *MSX1* cases and controls, as well as the statistical comparisons, are presented in Table 3. For the first maxillary molar, no statistically significant differences were noted for any of the six parameters nor were interaction effects noticeable

Table 2 Intra- and inter-observer repeatabilities for incisors (n=20) and molars (n=20)

	Coefficient of repeatability [4]	
	Intra-observer repeatability	Inter-observer repeatability
Incisors		
Perimeter (mm)	0.8	1.1
Mesiodistal distance (mm)	0.3	0.5[a]
Buccolingual distance (mm)	0.3	0.3
Area (mm^2)	1.9	1.4[a]
Volume (mm^3)	4.5	2.0[a]
Molars		
Perimeter (mm)	1.2	3.2
Mesiodistal distance (mm)	1.0	1.4
Buccolingual distance (mm)	0.3	0.6
Area (mm^2)	3.1	6.6
Volume (mm^3)	12.2	12.3

[a]A total of 90 % of differences fall within two standard deviations of the mean difference. With all other coefficients, 95 % of differences fall within two standard deviations of the mean difference

between gender and group (*MSX1* case or control). Therefore, the null hypothesis that there are no differences in dimensions of the maxillary right first molar between *MSX1* cases and non-affected controls could not be rejected.

Regarding the central incisor, the *MSX1* cases have significantly higher values for the area of the reference plane as well as its buccolingual distance, squareness, and volume. Again, there were no interaction effects between gender and group. For the central incisor, the null hypothesis that there are no differences in dimensions between *MSX1* cases and controls can be rejected. Digital models of a typical *MSX1* case and a control are seen in Fig. 4a, b respectively.

Discussion

We suggest that quantification of tooth crown shapes may contribute to the early diagnosis of congenital anomaly syndromes involving teeth. A "shape database" that includes associated gene mutations resulting from developmental syndromes may facilitate the genetic identification of hypodontia cases. So far, the authors have not found an existing technique that enables volumetric measurements in isolated teeth for this purpose; only 2D techniques have been described in the literature.

Teeth are multi-dimensional, irregular objects and are, therefore, difficult to measure in 2D. To measure 2D

Table 3 Descriptive statistics for geometric parameters of crown morphology for molars and incisors in *MSX1* cases and controls

	Control (n=42) Mean (SD)	Case *MSX1* (n=8) Mean (SD)	Gender P value	Group P value	Gender × group P value
Molar					
Perimeter (mm)	35.9 (2.4)	34.7 (4.6)	0.962	0.264	0.199
Area (mm^2)	93.2 (10.7)	89.8 (23.0)	0.733	0.513	0.191
Buccolingual distance (mm)	9.7 (0.5)	9.3 (1.3)	0.673	0.114	0.699
Mesiodistal distance (mm)	10.7 (0.8)	10.2 (1.2)	0.767	0.193	0.962
Squareness (mm/mm)	1.1 (0.1)	1.1 (0.1)	0.688	0.336	0.848
Volume (mm^3)	151.6 (24.5)	152.1 (57.9)	0.708	0.958	0.159
Incisor					
Perimeter (mm)	21.2 (1.7)	22.1 (1.6)	0.096	0.250	0.598
Area (mm^2)	24.3 (3.7)	31.3 (7.5)	0.079	0.000[a]	0.484
Buccolingual distance (mm)	3.0 (0.3)	4.2 (0.7)	0.051	0.000[a]	0.912
Mesiodistal distance (mm)	8.6 (0.6)	8.7 (0.9)	0.127	0.693	0.732
Squareness (mm/mm)	2.8 (0.3)	1.9 (0.3)	0.380	0.000[a]	0.965
Volume (mm^3)	35.8 (5.6)	45.6 (13.1)	0.301	0.002[a]	0.986

Mean values and standard deviations (in parentheses). Univariate analysis of variance with geometric parameters as dependent variables and gender and group as fixed factors, P values given

[a] Statistically significant values below α=0.01 (Bonferroni correction)

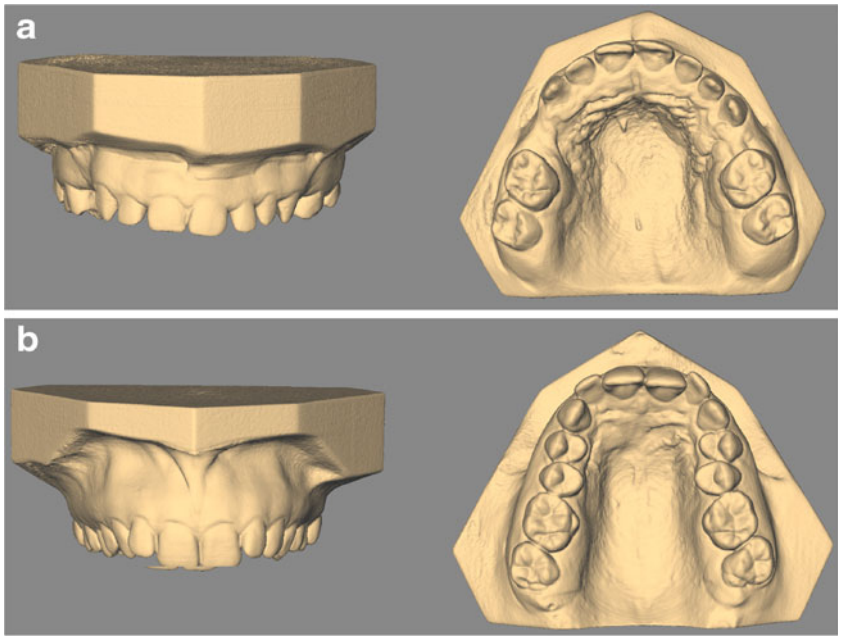

Fig. 4 **a** Digital model of the frontal (*left*) and occlusal (*right*) views of a typical *MSX1* case (female). It is noticeable that the teeth of the *MSX1* female are distinctive in shape and size compared to a control (i.e., **b**). In particular, the central incisor has a distinctive "square" appearance. **b** Digital model of the frontal (*left*) and occlusal (*right*) views of a healthy control (female)

parameters such as the perimeter correctly, the 2D picture must be perpendicular to the occlusal plane [8]. However, each tooth has a specific angulation within the jaw. The analysis of multiple teeth would require a separate picture for each individual tooth. Also, additional phenotypes of tooth morphology and dimensions that involve volumetric aberrations can only be obtained from 3D measurements. Because both mesiodistal and buccolingual dimensions in subjects affected with hypodontia are generally smaller, tooth volume is expected to be an even more discriminative parameter.

Because of scattering and related issues, computed tomography (CT) cannot produce 3D data in sufficient detail to allow detailed geometric measurements of the teeth. Other disadvantages of CT are that it would require exposure of a subject to radiation, and CT equipment is not yet generally available. A laser scanner-based image analysis system that can acquire 3D data of small objects, such as tooth crowns in dental casts, has been described in the literature. The system was considered reliable when comparing the same parameters in 2D and 3D; however, it has not yet been applied to obtain 3D data, such as tooth volume, and would require an experimental setup, which is not very practical [37]. The drawbacks of other techniques have led to the development of the currently described method of 3D analysis to quantify tooth morphology. The method applied is relatively simple, minimally invasive, and inexpensive compared to other methods and conventional gene tests. The only required input is a dental impression of the upper jaw, which is transferred to a 3D digital model. Direct intra-oral scanning of tooth crowns to obtain a 3D digital dataset, without requiring an impression, is also a realistic option. Presently, the described method is rather time-consuming, although this concern could be resolved considerably by automating the process, which would probably enhance measurement precision as well.

In the present *MSX1* family, a specific pattern of tooth agenesis was observed; however, aberrations in tooth morphology were also noted. The central incisor and first molar were chosen because they present in the mouth at an early age (6 years of age) and because they are almost never congenitally absent, even in cases of severe hypodontia [10]. Future studies may involve other tooth types as well. This may help diagnose other genotype–phenotype correlations. Affected teeth may be different among diseases. To ensure accurate and reproducible measurements, the reference points were chosen on the hard tissues and in non-abrasive zones. Reference points in the vicinity of the gingival margin were avoided; they were deemed unreliable because of the soft nature of the gingiva and variation due to gingivitis. Furthermore, only the supragingival area of a tooth is available for evaluation, and the size of the area is dependent upon the eruption phase.

Striking features of the central incisor are significantly larger area of the reference plane and a larger buccolingual distance and volume. We did not take the body size into account because this information was not available for the controls. This may have helped in interpreting these findings. *MSX1* cases also present with incisors that have a more square appearance (outcome variable: "squareness"). These findings are in contrast with the finding that all teeth that develop in hypodontia are generally smaller than in control groups [7, 28]. A possible explanation for the enlarged incisors in the studied family may arise from proximal–distal patterning during tooth development. Tucker showed that the developing oral epithelium can be divided into two domains, one distal and one proximal [41]. The epithelium

of the presumptive incisor domain expresses *BMP4*, which positively regulates the expression of *MSX1* and *MSX2* in the underlying neural crest-derived mesenchyme. Meanwhile, *FGF8* is expressed in the epithelial presumptive molar region and regulates the expression of *Barx1*. *BMP4* and *FGF8* negatively regulate each other, thereby restricting *Barx1* expression to the presumptive molar region. The boundary between *MSX1* and *Barx1* demarcates the presumptive incisor- and molar-forming regions. The crown shape could be changed by manipulating the expression of these signaling factors. When beads with noggin protein, which antagonizes BMP signaling, are placed in the distal mesenchyme, and the expression of *MSX1* is lost, a molar tooth is formed in the presumptive incisor region.

Interestingly, in K14-noggin mice, in which overexpression of noggin blocks BMP signaling, the incisors were thick, wide, and blunt-ended [30]. They stated that subtle differences in the level, distribution, and timing of signaling molecules could have morpho-regulatory consequences [30]. Modulation of *BMP4* signaling can transform a conically shaped tooth into a tooth with a more complex morphology. Because *MSX1* plays an important role in BMP signaling, one can hypothesize that the incisors in this family have a more posterior, molar-like appearance as a result of decreased *MSX1* expression.

No statistically significant differences were observed for any of the six parameters in the first molar. Clinically, we did observe a deviating morphology in the cusps. The results have a tendency towards a smaller volume and more squared appearance. We expect that these small differences in morphology would also be expressed statistically if the case group was larger. In one case, there was a small extra cusp present. The shape of the tooth crown results from morphogenesis of the epithelium during the cap and bell stages of tooth development, through differential growth and folding of the epithelium [3]. The enamel knots express growth stimulatory signals. It has been demonstrated that apoptosis in the enamel knot plays an important role in regulating tooth size and shape, and the expression of *BMP4* in the enamel knot is associated with apoptosis [3, 17].

A model has been presented in which the pattern of tooth cusps is regulated by *FGF4*, which functions as an activator promoting cusp initiation and growth. Inhibitors, such as *BMP4*, control the distance between the enamel knots and negatively regulate cusp growth. It has also been reported that using z-VAD-fmk treatment to block apoptosis results in morphological anomalies. The morphology of mouse molars was similar to that observed in human macrodontia [23].

MSX1 and *BMP4* are closely associated during tooth development. *BMP4* and *MSX1* regulate one another in a positive feedback loop [3]. Jernvall et al. suggest that *MSX1* is needed for *BMP4* expression, which in turn induces the expression of p21, which is responsible for apoptosis [16]. Thus, apoptosis in the enamel knot is necessary for the proper formation of molar teeth, including appropriate shape and size. It is tempting to speculate that *MSX1*, by inducing *BMP4*, is involved in this stage of tooth morphogenesis as well. Such speculation could explain the deviating morphology of the molar cusps presented in the studied family. Reduced *MSX1* expression could result in reduced expression of *BMP4* and *p21*, which in turn would inhibit apoptosis.

Conclusions

We introduced a novel, 3D technique to measure the aspects of tooth morphology to get a better understanding of dental phenotype–genotype associations. This measurement technique may contribute to earlier diagnosis of some multiple-anomaly congenital syndromes involving teeth anomalies. These findings suggest that *MSX1* may play a role not only in tooth patterning but also in tooth morphogenesis, as expressed by distinct shape differences, particularly in maxillary incisors, between the *MSX1* family members and controls regarding the parameters of area, buccolingual distance, squareness, and volume.

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Conflict of interest The authors declare that they have no conflict of interest.

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