MSX1 and PAX9 Genetic Alteration in Malaysian Families with Hypodontia

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Abstract

Hypodontia is characterized by the absence of one to six teeth. Malaysia has a high prevalence of hypodontia (2.8%). This study aimed to investigate the MSX1 mutation with clinical variability in Malaysian hypodontia families and to correlate the findings a previous study of the PAX9 mutation. Materials and methods: We re-examined seven individuals from two families of the previous PAX9 study. Orthophantomogram (OPG) and intraoral photos were re-assessed. Saliva was collected for genetic analysis. Direct sequencing was done on exons 1 and 2 of MSX1 and exons 2 and 3 of PAX9. Results: In family 1, three out of five members are affected. The mother has posterior hypodontia, while the daughters have anterior hypodontia. Point mutations on exon 1 of MSX1 (c.599C>T, c.732G>A) and on exon 3 of PAX9 (c.477delG, c.480delT) were identified on 1B (mother) and 1D (second daughter). Her carrier-son (1E) exhibited c.597C>T, c.730G>A on exon 1 of MSX1 and c.273T>G on exon3 of PAX9. In family 2, the daughter has a missing lower premolar with a point mutation on exon 1 of MSX1 (c.730G>A). A similar point mutation in her non-hypodontia father on exon 2 of PAX9 (c.628C>T) was observed. Conclusion: Mutation of MSX1 is observed in familial hypodontia; both genes MSX1 and PAX9 are needed to manifest hypodontia whereby PAX9 is the predominant gene mutation.

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Introduction

Tooth agenesis is one of the most common craniofacial anomalies. It is the congenital absence of one or more teeth. This anomaly is classified clinically based on the number of missing teeth; hypodontia, oligodontia, and anodontia. Hypodontia is defined as the absence of one to six teeth, excluding the third molar. It is reported that hypodontia affects 0.3–11.3% of the population, with the third molars excluded.[1] Tooth agenesis can either be non-syndromic (isolated condition) or syndromic (associated with congenital anomalies).[2] One of the syndromic conditions associated with hypodontia is Down Syndrome. Non-syndromic hypodontia can either be sporadic or familial. It is most common in secondary dentition. The third molar is the most common missing tooth, occurring in 20% of population.[3]

The majority of hypodontia cases are reported to have a genetic basis. Familial hypodontia is reported to be inherited in autosomal dominant, autosomal recessive, and x-linked manners.[1,4]

Studies on tooth development carried out in knockout mice have revealed that more than 200 genes are involved in odontogenesis with MSX1 and PAX9 being the genes most associated with tooth agenesis. Both genes play a crucial role in tooth development as they encode transcription factors involved in activating the expression of signaling factors for epithelial mesenchyme interaction.[2]

Studies on knockout mice reveal that mutations on both genes causes the tooth development to be arrested at the bud stage. Thus, MSX1 and PAX9 show correlations that both are essential for odontogenesis.

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In this study, we assess the relationship between MSX1 mutation and non-syndromic hypodontia. There is a high prevalence of hypodontia in Malaysia—about 2.8% as reported by Hussein.[5] Our previous study suggested that PAX9 on either exon 2 or 3 is responsible for the hypodontia phenotype in these families. However, no studies linking the MSX1 mutation to hypodontia have been reported in Malaysia.

This study also aims to correlate the findings obtained from previous studies on MSX1 and PAX9 mutations, as evidence suggests that both MSX1 and PAX9 genes interact during odontogenesis at gene and protein levels. While much progress has been made in understanding the developmental basis of tooth formation, knowledge of the aetiological basis of congenital tooth loss remains poor, especially in Malaysian families with hypodontia.

Materials and Methods

Study design and ethical approval

This study was designed as a quantitative experimental study conducted in the International Islamic University Malaysia (IIUM) Dental Polyclinic and Molecular Laboratory of Integrated Centre for Research Animal Care and Use (ICRACU). Ethical approval was obtained from the Research Ethic Committee of IIUM with IREC ID 554.

Clinical assessment

Three selected probands and their respective families from the previous PAX9 study were selected for this study.

All the participants were briefed on the study and written consent was obtained. Medical, birth defect, and family history were re-assessed to identify the associated anomalies and to differentiate syndromic from non-syndromic conditions. Reassessment of dental charting and orthophantomograms (OPG) were done to locate missing teeth. The non-affected family members were included as a control group.

Genetic assessment

The methods used were previously described by Xuan et al.[6] with slight modifications. A non-invasive method was used, in which 2 ml of saliva was collected from each participant to obtain deoxyribonucleic acid (DNA) samples. The saliva sample was kept at -80ºC in a refrigerator until used. Genomic DNA was extracted from the saliva using the QIAamp sample DNA Minikit (Qiagen, Germany).

To obtain the DNA template, Thermofisher Nanodrop equipment was used to calculate the extracted DNA concentration. Then each sample was run with primers for exon 1 and exon 2, as described by the previous study.[7]

The DNA samples were amplified using polymerase chain reaction (PCR) for each exon of the MSX1 gene. The samples underwent denaturation, annealing, and extension in order to obtain the expected base pair size.

The primers and PCR conditions are listed in Table 1 and Table 2.

Table 1. List of MSX1 primers for exon 1 and exon 2 (8).

Exon	Type	Primers	Expected size (bp)
Exon 1	Forward	5’-CTG GCC TCG CCT TAT TAG C-3’	766
	Reverse	5’-GCC TGG GTT CTG GCT ACT C-3’	
Exon 2	Forward	5’-ACT TGG CGG CAC TCA ATA TC-3’	698
	Reverse	5’-CAG GGA GCA AAG AGG TGA AA-3’	

Table 2. List of PAX9 primers for exon 2 and exon 3.

Exon	Type	Primers	Expected size (bp)
Exon 2	Forward	5'-CCA GCC TTC GGG GAG GTG AA -3'	640
	Reverse	5'-CAC GAA GGA TCT GGC TCG T -3'	
Exon 3	Forward	5'-GTG GGT CAG AGA ATT TGG AA -3'	589
	Reverse	5'-CAC GAA GGA TCT GGC TCG T -3'	

Table 3. Specific conditions for polymerase chain reaction for MSX1 both exons.

PCR steps	Temperature	Time
Initial denaturation	95°C	5 min
Denaturation	95°C	1 min
Annealing	57°C	30 sec
Extension	72°C	2 min
Final extension	72°C	5 min

Table 4. Specific conditions for polymerase chain reaction for PAX9 both exons.

PCR steps	Temperature	Time
Initial denaturation	95°C	5 min
Denaturation	95°C	1 min
Annealing	58°C (exon 2) 59°C (exon 3)	30 sec
Extension	72°C	2 min
Final extension	72°C	5 min

The amplified DNA was run through gel electrophoresis. For the gel electrophoresis, 1% of agarose gel was prepared and loaded with samples (8 µl) and indicator ladder (5 µl). An electric current (75–100 kV) was applied across the gel for 50 minutes and the resulting bands were visualized using Biorad Chemidoc. Each primer for the different exons was expected to be on a specific band under the gel electrophoresis. DNA purification was done using Gene aid kit. The DNA fragment was cut under UV light and transferred into a micro centrifuge tube. After purification, both DNA strands were sent for sequencing in order to identify the mutations.

Data Analysis

The DNA sequences were analyzed and compared against DNA references using Basic Local Alignment Search Tool (BLAST); meanwhile chromatograms were also viewed and analyzed using Sequence Scanner software. The results from all three families were collected and organized in a database with complete dental descriptions. History taking was also done and OPG recorded. The clinical data was compared against sequencing results. The results from the MSX1 mutation were also compared with PAX9 mutation findings from our previous study.

Results

In family 1, all the female members are affected with hypodontia, suggesting that the condition is inherited in an autosomal dominant pattern, according to the pedigree constructed in Figure 1. Clinical examination revealed that the mother (1B) has a missing posterior tooth, while both daughters have missing anterior teeth; the first daughter is missing 23 and the second daughter is missing 32. Similar point mutations were observed in patient 1B and 1D on exon 1 of MSX1 (1B, 1D; c.599C>T, c.732G>A), while on exon 3 of PAX9 point mutations were found on c.465delG and c.480delT, respectively. No mutation of MSX1 was observed in patient 1C; however, the PAX9 point mutation was observed on exon 2 (c.629G>T, c.632insA). The carrier-son (1E) exhibited c.597C>T, c.730G>A on exon 1 of MSX1 and c.273T>G on exon 3 of PAX9.

Figures 2 to 4 show the clinical, radiologic, and genetic results of each affected family members.

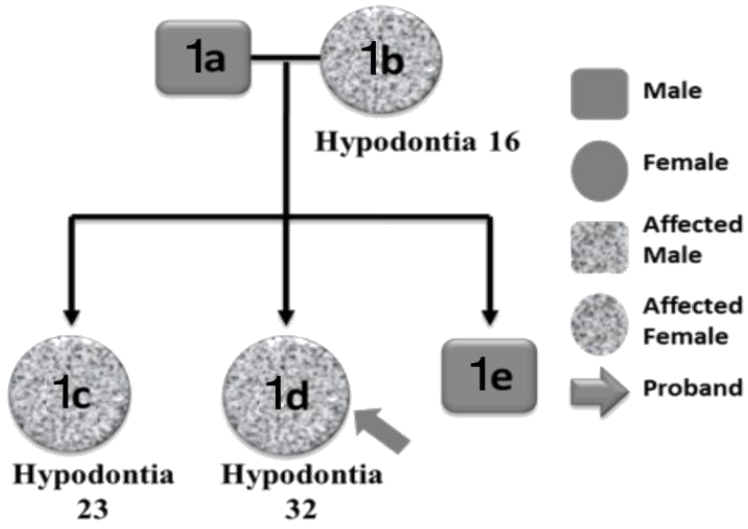

Figure 1 show the pedigree constructed for family 1 where the mother and the first and second daughters are affected, indicated by the shaded figures. Missing were 16, 23, and 32, respectively. The arrow represents the proband (second daughter).

For family 2, the pedigree constructed in Figure 5 shows that only the daughter (2C) is affected with hypodontia with missing 35. This condition suggests that the condition is inherited in an autosomal recessive manner, whereby the condition may be inherited from the patient's grandparents.

Figure 6 shows a point mutation was observed in patient 2C on exon 2 of MSX1 (c.730G>A). An almost identical point mutation was observed in patient (2C) and her carrier-father (2A) on exon 2 of PAX9 (c.627T>G, c.628T>G).

The overall summary of the phenotypes and genotypes of each patient are listed in the table 5, compared against the PAX9 mutation.

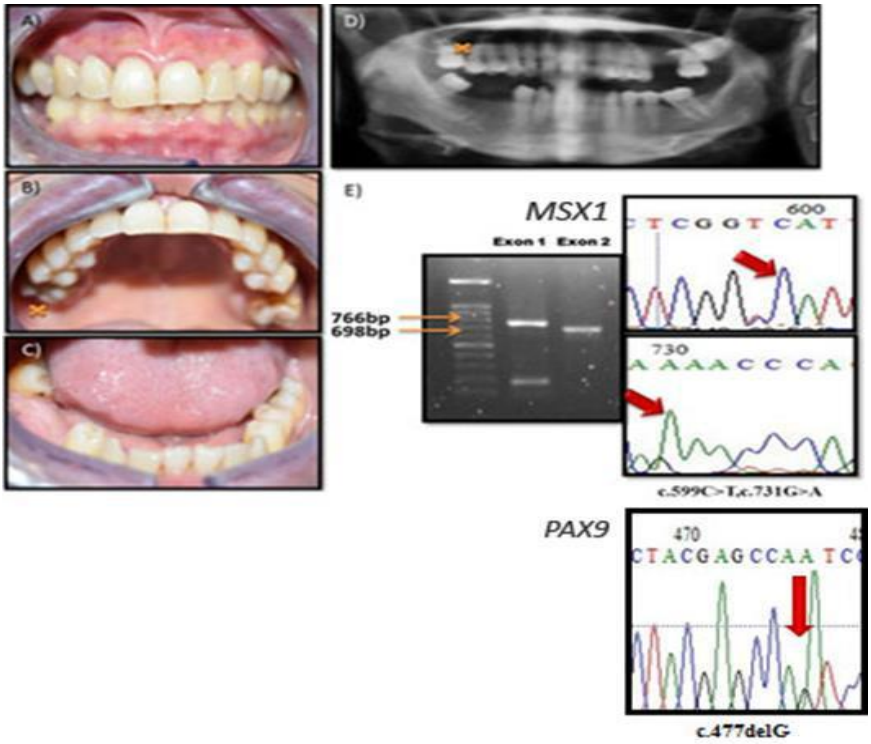

Figure 2 shows sample 1B (mother) of the family clinical findings (A, B, C) showing missing radiographic findings (D) of the respective missing tooth are indicated using 'X'; and genetic findings (E) of electrophoresis and chromatogram show point mutations on exon 2 of MSX1 c.599C>T and c.731G>A and on exon 3 of PAX9 c.477delG.

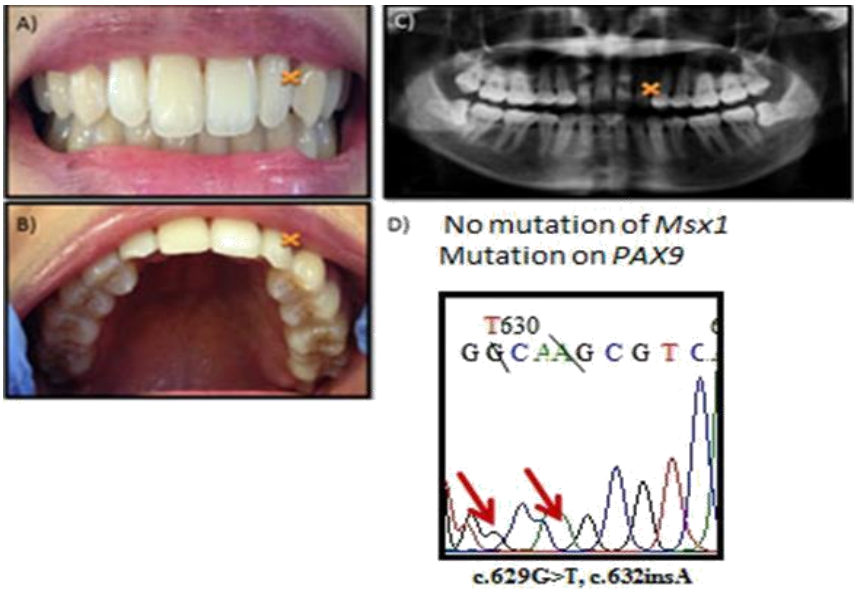

Figure 3 shows sample 1C (first daughter) of the family clinical findings (A, B) showing missing 23; radiographic findings (C) of the respective missing tooth are indicated using 'X'; and genetic findings (D) showing no mutation in MSX1, but point mutations were found on PAX9 exon 2 c.629G>T, c.632insA

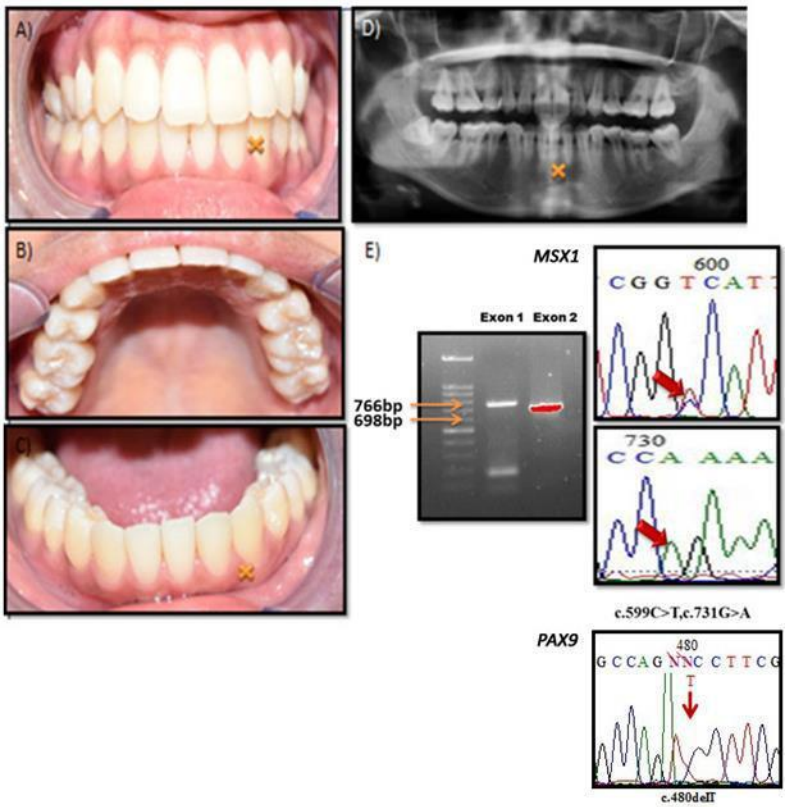

Figure 4 shows sample 1D (second daughter, proband) of the family clinical findings (A, B, showing missing 32; radiographic findings (D) of the respective missing tooth are indicated with 'X'; and genetic findings (E) of electrophoresis and chromatogram show point mutations on exon 1 of MSX1 c.599C>T and c.731G>A and exon 3 of PAX9 c.480delT

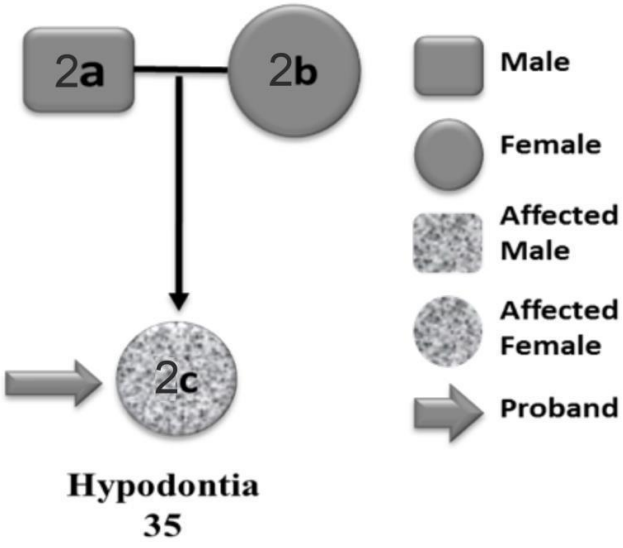

Figure 5 shows the pedigree constructed for family 2, where only the daughter is affected with missing 35 shown as the shaded figure, while the father and mother are unaffected. The arrow represents the proband (daughter).

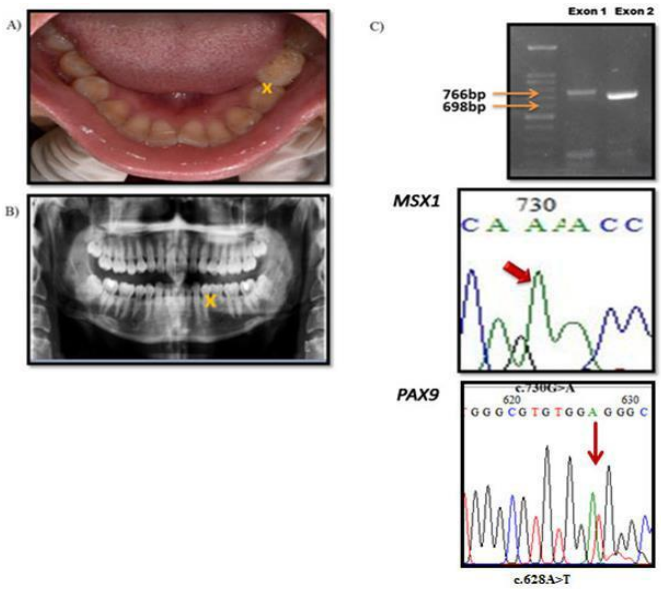

Figure 6 shows sample 2C (daughter, proband) of the family clinical findings (A) showing missing 35 and the retained deciduous molar; radiographic findings (B) of the respective missing tooth are indicated using 'X'; and genetic findings (C) of electrophoresis and chromatogram show a point mutation on exon 1 of MSX1 c.730G>A and on exon 2 of PAX9 c.628A>T.

Table 5. Summary of two Malaysian families with hypodontia, affected teeth, involvement of environmental factors and type of MSX1 and PAX9 mutations.

ID number	Gender	Missing teeth	Environmental involvement	MSX1 mutation		PAX9 mutation	
				Exon1	Exon2	Exon2	Exon3
1A	Male	None	Extraction of 15, 24, 26, 27, 46, 47	No mutation	No mutation	No mutation	No mutation
1B	Female	16	Extraction of 25, 37, 45, 46	c.599C>T c.732G>A	No mutation	No mutation	c.477delG
1C	Female	23	Extraction of 13	No mutation	No mutation	c.629G>T c.632insA	No mutation
1D	Female	32	Extraction of 15 & 25	c.599C>T c.732G>A	No mutation	No mutation	c.480delT
1E	Male	None	None	c.597C>T c.730G>A	No mutation	No mutation	No mutation
2A	Male	None	None	No mutation	No mutation	c.627C>T	No mutation
2B	Female	None	None	No mutation	No mutation	No mutation	No mutation
2C	Female	35	None	c.730G>A	No mutation	c.628A>T	No mutation

Discussion

This research was carried out to identify the role of MSX1 mutation in familial hypodontia, and to correlate findings to our previous study of PAX9 mutation. Both MSX1 and PAX9 are important in tooth development; PAX9 mutation prevents MSX1 activation, which is involved in the transition of teeth from the bud to the cap stage.[8] Detailed clinical and radiographic re-assessment was done. No remarkable extra oral features that suggested an underlying syndrome were observed in any patient.

Previous studies concluded that familial tooth agenesis can be inherited in an autosomal dominant, autosomal recessive, or X-linked manner.[1] In this study, autosomal dominant inheritance was observed in family 1. In this family, the mother was affected with hypodontia and passed the condition down to her daughters. For family 2, the daughters were affected with hypodontia which was inherited from the mother. Conversely, inheritance through an autosomal recessive pattern can be observed in family 2, where only the daughter is affected and probably inherited the condition from her grandparents, while the father is only a carrier.

Both MSX1 and PAX9 are involved in tooth agenesis, whether hypodontia or oligodontia.[5] In this research, MSX1 mutation was present in all affected patients except patient 1C. However, a mutation of PAX9 either on exon 2 or exon 3 can be observed in all patients affected by hypodontia. The non-hypodontia family members were not affected with mutations of MSX1 and PAX9. These results suggest that the MSX1 gene is less significant in hypodontia, as it exerts a larger role in oligodontia.[2] A previous study by Cudney and Vieira[9] also states that PAX9 is the predominant factor contributing to hypodontia.

In common hypodontia, upper lateral incisors or lower second premolars are usually the most affected teeth.[2] In this research, missing lateral incisors can be seen in patient 1D, while second premolars were missing in patient 2C. However, one patient (patient 2C) was missing canines, which is uncommon. Prevalence studies state that missing canines are rare in the Caucasian population, but may be relatively more common in Asian groups.[10] However, one patient in family 1 (patient 1D) had posterior hypodontia. This is in line with the report by Wang et al.[11] stating that PAX9 is associated with agenesis of the permanent molars, mostly including the maxillary first molars, maxillary second molars, and mandibular second molars.

To plan the best possible treatment for the developing dentition, the screening of children with a family history of segregating tooth agenesis is crucial at six to seven years of age, by clinical and radiographic means.[12] In this case, if hypodontia patients can be diagnosed early, treatment can be carried out early thus reducing the cost of treatment in the future. The successful management of hypodontia requires a multidisciplinary approach comprising pediatric dentistry, conservative dentistry, orthodontics, and oral surgery besides diagnostic set-ups.[13] Overall, both MSX1 and PAX9 contribute to hypodontia. In this case, MSX1 may be mutated in familial hypodontia; however, the mutation of MSX1 alone may not cause phenotype change to the patient. PAX9 mutation is the more predominant factor contributing to hypodontia compared to MSX1.

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