

Identification of MSX1 Mutation in Malaysian Hypodontia Family

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Abstract–Hypodontia is defined as the absence of one to six teeth. There is high prevalence of hypodontia recorded in Malaysia (2.8%). This study aimed to identify any mutation of MSX1 in Malaysian family with hypodontia and its clinical finding. We re-examined 4 individuals from a family 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. 2 out of 4 members (1A and 1D) in the family have anterior hypodontia. Point mutation on exon1 of MSX1 (c.731G>A) was observed in 1A (father) with missing 13 and 23 and 1C (carrier-son). c.732G>A was found on exon1 of MSX1 of his daughter (1D) with missing 32. MSX1 mutation is involved in the occurrence of hypodontia in patient.

Keyword–MSX1, mutation, hypodontia

I. INTRODUCTION

Tooth agenesis is the congenitally missing of one or more teeth and is being one of the most craniofacial anomalies encountered. It ranges about 2-10% excluding 3rd molar. It is classified into hypodontia, oligodontia and anodontia. Hypodontia involved missing of one to six teeth, excluding the third molar. Tooth agenesis can either be non-syndromic (isolated condition) or syndromic (associated with congenital anomalies [1]). Tooth agenesis is caused by the genetic and environmental factors. Environmental factors include jaw fracture, surgical procedure or malnutrition. MSX1 and PAX9 are the genes responsible for tooth agenesis with tooth development are found to be arrested at the bud stage in MSX1 and PAX9 knockout mice [2,3]. Hypodontia can occur as syndromic or non-syndromic with the non-syndromic hypodontia can either be in sporadic or familial fashion. Non-syndromic hypodontia is more common in tooth agenesis with variable number of teeth involved. Teeth most commonly involved are maxillary lateral incisor and mandibular second premolar with prevalence of 2.2%

and 3.4% respectively [3,4]. Maxillary permanent central incisors, canines, and first molar are rarely seen in tooth agenesis.

MSX1 (muscle segment homeobox) is a member of a distinct sub family of homeobox genes that is expressed in spatially restricted regions of the head during early development and is localized to regions of condensing embryonic connective tissue or ectomesenchyme in tooth germ. Thus, MSX1 is essential for odontogenesis. The gene is found to be associated with tooth agenesis, orofacial cleft and nail dysplasia. Analysis conducted on family affected with oligodontia revealed a causative locus on chromosome 4p, the place where MSX1 gene resides. Missense mutation was detected within a critical region of MSX1 protein in all affected family members when sequence analysis was done. MSX1 was found to be inactive in vivo and haploinsufficiency [2].

II. MATERIALS AND METHODS

A. Study design & ethical approval

This study is a quantitative experimental study conducted in IIUM. For this study, ethical approval was obtained from the Research Ethic Committee of International Islamic University Malaysia (IIUM) with IREC ID 554.

B. Clinical assessment

Four individuals from a family of the previous PAX9 study (data not shown) were selected for this study. All the participants were explained on the study that will be conducted and written consents were obtained. A thorough history taking was done. Reassessment of dental charting and orthopantomograms (OPG) were performed to locate the missing teeth. The non-affected family members were included as control group.

C. Genetic assessment

Methods used were previously described by Xuan et al. (2008) with slight modification. 2 ml of saliva was collected from each participant to get their deoxyribonucleic acid (DNA) sample and was then kept in -80°C refrigerator for storage. Genomic DNA was extracted from the saliva using the QIAamp sample DNA Minikit (Qiagen, Germany).

The DNA samples were amplified using polymerase chain reaction (PCR) for each exon of MSX1 gene and involved the steps of initial denaturation, denaturation, annealing, extension and final extension. The primers are listed in Table I.

TABLE I. LIST OF MSX1 PRIMERS FOR EXON 1 AND EXON 2 [5]

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 CCG CAC TCA ATA TC-3'	698
	Reverse	5'-CAG GGA GCA AAG AGG TGA AA-3'	

Then, the amplified DNA was run through gel electrophoresis. DNA purification was done using the Geneaid kit. Both DNA strands were sent for sequencing in order to identify the mutations after the purification.

D. Data analysis

The DNA sequences were analyzed and compared with DNA reference using Basic Local Alignment Search Tool (BLAST); meanwhile chromatograms were also viewed and analyzed using Sequence Scanner software. The results was collected and organized in a database with complete dental description. OPG was recorded. The clinical data was compared against the sequencing results.

III. RESULTS

The father (1A) and the daughter (1D) are affected with hypodontia, suggesting that the condition is inherited in autosomal dominant pattern as shown in Figure 1. Clinical examination of family 1 revealed that both father and daughter had missing anterior teeth, where the father had missing of 13 and 23, while the daughter with the missing of 32. Point mutation was observed on exon 1 of MSX1 (1A;c.731G>A) and (1D;c.732G>A). Figures 2 and 3 showed the clinical, radiological, and genetic results on each affected family members.

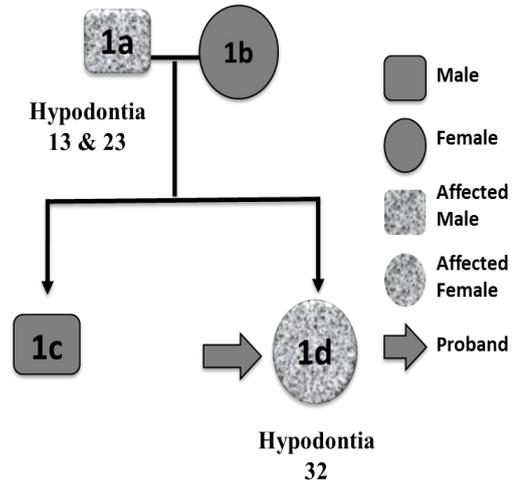


Figure 1. The pedigree constructed for Family 1; the shaded parts represent the affected samples (father and daughter of the family) with the missing of teeth shown above. Arrow represents the proband.

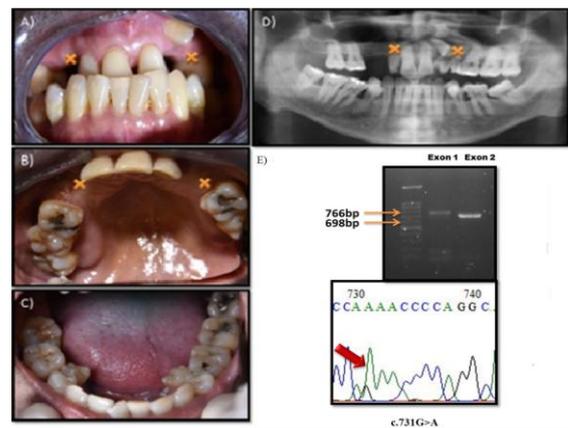


Figure 2. Sample 1A (Father) of the family consisting the clinical findings (A,B,C) which shows the missing of 13 and 23, radiographic findings (D) shown in 'X' marks of the respected missing teeth, and genetic findings (E) of electrophoresis and chromatogram which show point mutation on exon 1 of MSX1 c.731G>A.

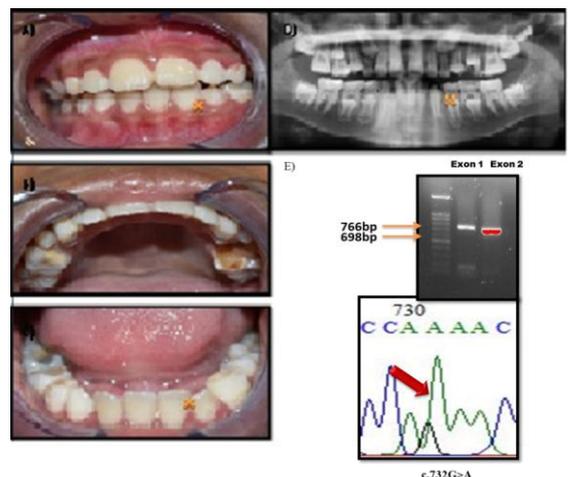


Figure 3. Sample 1D (daughter, proband) of the family consisting the clinical findings (A,B,C) which show the missing of 32, radiographic findings (D) shown in 'X' marks of the respected missing tooth, and genetic findings (E) of electrophoresis and chromatogram which show point mutation on exon 1 of MSX1 c.732G>A.

TABLE I. SUMMARY CONSISTS OF PHENOTYPE AND GENOTYPE OF EACH OF THE PARTICIPANT

ID Number	Gender	Missing teeth	Environmental involvement	MSX1 mutation	
				Exon1	Exon2
1A	Male	13 & 23	Extraction 14,15,25, 45	c.731G>A	No mutation
1B	Female	None	Extraction of 41	No mutation	No mutation
1C	Male	None	None	c.731G>A	No mutation
1D	Female	32	None	c.732G>A	No mutation

IV. DISCUSSION

This research was done to identify the involvement of MSX1 mutation in familial hypodontia. MSX1 is important in tooth development. A family from the previous PAX9 study was selected for this study in which a thorough re-assessment in term of clinical and radiographic was done.

Autosomal dominant, autosomal recessive or X-linked manner can be inherited in tooth agenesis based on previous study [6]. The father has hypodontia and is passed down to the daughter, while the mother is not affected and the son is being a carrier. Thus, hypodontia is inherited through autosomal dominance in this family. Both MSX1 and PAX9 are involved in tooth agenesis, either hypodontia or oligodontia. In this study, MSX1 mutation is found in all affected family members.

Upper lateral incisors or lower second premolars are the most teeth commonly involved in hypodontia [1,4]. In this research, missing of lateral incisors can be seen in patient 1D. However, patient 1A has missing both upper canines, which is uncommon. It is best to plan the treatment as early as six to seven years of age for those with family history of hypodontia through clinical and radiographical examination [7].

All in all, MSX1 mutation has been linked with non syndromic hypodontia. MSX1 gene plays a crucial role in tooth development during the epithelial mesenchymal interaction.

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REFERENCES

- [1] B.R. Boeira Junior, S. Echeverrigaray, "Polymorphism in the MSX1 gene in a family with upper lateral incisor agenesis," *Arch. Oral Biol.* vol. 57(10), pp. 1423-1428, 2012.
- [2] M. Cobourne, "Familial human hypodontia--is it all in the genes?" *Br. Dent. J.*, vol. 203, pp. 203-208, 2007.
- [3] T. Shimizu, T. Maeda, "Prevalence and genetic basis of tooth agenesis," *Jpn. Dent. Sci. Rev.*, vol. 45(1), pp. 52-58, 2009.
- [4] V.R. Paixao-Cortes, T. Braga, F.M. Salzano, K. Mundstock, C.A. Mundstock, M.C.C. Bortolini, et al., "PAX9 and MSX1 transcription factor genes in non-syndromic dental agenesis," *Arch. Oral Biol.*, vol. 56(4), pp. 337-344, April 2011.
- [5] K. Xuan, F. Jin, Y.L. Liu, L.T. Yuan, L.Y. Wen, F.S. Yang, et al., "Identification of a novel missense mutation of MSX1 gene in Chinese family with autosomal-dominant oligodontia," *Arch. Oral Biol.*, vol. 53, pp.773-779, 2008.
- [6] X. Zhu Yang, C. Zhang, L. Ge, S. Zheng, "A novel nonsense mutation in PAX9 is associated with sporadic hypodontia," *Mutagenesis*, pp. 1-5, 2011.
- [7] I.N. Farahiyah, R.N. Syahira, I.N. Irfanita, Y.D. Ardini, J.I. Solachuddin, W. Lestari, "PAX9 Mutation of Non-syndromic Hypodontia in a Malaysian Family," In: *UI Proceeding on Health and Medicine*, 2016.