PAX9 Mutation of Non-Syndromic Hypodontia in a Malaysian Family

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Abstract

Objective: Hypodontia is portrayed by the missing of one to six numbers of teeth. PAX9 is one of the genes that caused non-syndromic hypodontia. We aimed to investigate the PAX9 mutation of non-syndromic hypodontia with clinical variability in a Malaysian hypodontia family.

Methods: Clinical examinations for all participants whilst orthopantomogram (OPG) was taken for hypodontia patient only. Saliva was collected for genetic analysis. Direct sequencing was performed by using exon 2 and 3 of PAX9 gene.

Results: 3 out of 5 family members are affected with hypodontia. The mother has missing posterior tooth and her daughters have missing anterior teeth. The point mutation was identified on exon 2 on patient 1C; c.620G>T and on exon 3 on patients 1B; c.465delG, 1C; c.273T>G, 1D; c.462delT.

Conclusions: Our findings suggested those identified points mutations of PAX9 either on exon 2 or exon 3 is responsible for the hypodontia phenotype in this family.

Keywords: hypodontia, mutation, PAX9

Introduction

Hypodontia is one of the most common dental anomalies, characterized by missing one to six teeth in the mouth. This anomaly can be caused by the environmental factors or genetic. It has been identified as both non-syndromic and syndromic. The non-syndromic form of hypodontia can be sporadic or familial.1 Familial tooth agenesis can be inherited in an autosomal dominant, autosomal recessive or X-linked manner.

To date it has been demonstrated MSX! And PAX9 are responsible for familial and sporadic hypodontia. Previously, in animal based studies have revealed that MSX1 and PAX9 knockout mice induced an arrest in tooth formation at the bud stages. Among them, PAX9 was more intensively studied. PAX9 is a transcription factor that is express in dental mesenchyme at initiation, bud, cap and bell stages of odontogenesis.3

The prevalence of hypodontia in Malaysian was (2.8%) and it is considered as high.4 While much progress has been made in understanding the developmental basis of the tooth formation, knowledge of the aetiological basis of inherited tooth loss remain poor especially in Malaysian hypodontia families.

Materials and Methods

Research ethic approval
Ethical approval was obtained for this study from Research Ethic Committee of the International Islamic University Malaysia (IREC-342).

Family selection and pedigree construction
The proband whom was selected randomly attending Dental Polyclinic International Islamic University Malaysia, Kuantan, Pahang, Malaysia. All participants are Malaysian descent. The proband and the family members including siblings and parents (first degree relatives only) were invited to participate in this study. All of medical information related to hypodontia such as birth defect, trauma and radiations were gathered. Full dental charting was done to locate the missing tooth. Tooth agenesis was characterized by orthopantomogram (OPG) and careful examination of their clinical charts. Those non hypodontia family members were included as our control group.

DNA collection, screening and mutational analysis
Methods that have been used are described by Paixao-Cortes et al with slightly modifications. A non-invasive method was used to collect patient’s deoxyribonucleotides (DNA) where by a 2ml of saliva was collected from each of participants. Then, genomic DNA will be extracted from saliva using the QIAamp sample DNA MiniKit
(Qiagen). PAX9 exons 2 (640 bp) and exon 3 (589 bp) were amplified using primers and conditions described in Pereira et al.\cite{9} PCR products were purified using Gel/PCR DNA fragment extraction kit (Geneaid). Both DNA strands were sent for sequencing.

**Data analysis**

Result of all participants were collected and organized in database with complete dental description. History taking include patient gender, missing tooth (third molars, molars, premolars, canines and incisors) and other dental categories (left and right quadrant; upper and lower arches). OPG was recorded each hypodontia patient. All these clinical data were compared with sequencing results.

**Results**

<table>
<thead>
<tr>
<th>ID NUMBER</th>
<th>SEX</th>
<th>MISSING TOOTH</th>
<th>ENVIRONMENTAL INVOLVEMENT</th>
<th>PAX9 MUTATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>M</td>
<td>none</td>
<td>Extraction of 15,24,26,27,46,47</td>
<td>None</td>
</tr>
<tr>
<td>1b</td>
<td>F</td>
<td>16</td>
<td>Extraction of 26, 37, 35,36</td>
<td>c.465delG</td>
</tr>
<tr>
<td>1c</td>
<td>F</td>
<td>23</td>
<td>Extraction of 13</td>
<td>c.620G&gt;T, c.273T&gt;G</td>
</tr>
<tr>
<td>1d</td>
<td>F</td>
<td>32</td>
<td>Extraction of 15 &amp; 24</td>
<td>c.462delT</td>
</tr>
<tr>
<td>1e</td>
<td>M</td>
<td>None</td>
<td>none</td>
<td>None</td>
</tr>
</tbody>
</table>

Only patient 1b, 1c and 1d are affected with hypodontia.

**Clinical diagnosis**

![Pedigree of family 1](image)

Figure 1. A constructed pedigree of family 1 which result the mother and both of her daughter are affected with hypodontia with respective numbers of missing teeth. The arrow shows the selected proband.

**Patient 1b**

![Image of Patient 1b](image)

Figure 2. Missing of 16 was identified in this patient, 52 year old lady (B, D). However, 45 and 46 were missing due to extraction (C). Chromatogram shows the point mutations c.465delG.

**Patient 1c**

![Image of Patient 1c](image)

Figure 3. Missing of 23 was identified in this patient, 27 year old lady (B, D). However, 13 was extracted due to orthodontic purposes (C). Chromatogram shows the point mutations c.620G>T, c.624_625insA, c.273T>C.

**Patient 1d**

![Image of Patient 1d](image)
Figure 4. Missing of 32 was identified in this patient, 25 year old lady (B, D). However, 15 and 25 were extracted due to orthodontic purposes (C). Chromatogram shows the point mutation c.462delT.

Discussion

This research attempted to identify the involvement PAX9 mutation in hypodontia patients. A proband and her family members were recruited and they underwent clinical and radiological examinations. Panoramic radiographs and examination of dental chartings characterized tooth agenesis. A complete family history was taken to exclude the possibilities of previous medical problem associated anomalies and to ensure that the families suffered from true non-syndromic hypodontia. In addition, there were no remarkable extra oral features that suggested an underlying syndrome in any subject.

In this family, the mother and 2 daughters are affected with the hypodontia, with different distribution of missing teeth for each of them which suggest that combination of genes, epigenetic and environmental factors play roles in expression of hypodontia. According to the pedigree constructed, the type inherit pattern is autosomal recessive. The mother has missing unilateral first maxillary molar. The prevalence of missing unilateral first maxillary molar is considered low compared to missing bilaterally. Meanwhile, for the first and second daughter have missing anterior teeth. Both patients underwent orthodontics treatment at early 20s. The first daughter has missing left maxillary canine which is consider as rare case. The contra lateral maxillary canine was extracted prior to orthodontic treatment and both upper 4’s has been camouflaged to imitate the morphology of the upper canine to restore the patient’s aesthetic. Lastly, the second daughter, she has missing lower left lateral incisor, which is quite common for lower lateral to be missing in dentition. This patient underwent orthodontic treatment due to crooked anterior teeth and palatally displaced left maxillary second premolar.

Genetics are very important and obviously associated with this dental anomaly, it is required that a family history of hypodontia/oligodontia as well as dental anomalies is observed in clinical paediatric dentistry. Screening children of families with segregating tooth agenesis by clinical and radiographic examinations should be carried out at 6 to 7 years of age to plan the best possible treatment for the developing dentition.

There are some benefits to the patient throughout this study. First, the can get consultation regarding the possibilities of hypodontia in future generation. Secondly, successful management of hypodontia required a multidisciplinary approach with input from paediatric dentistry, restorative dentistry, orthodontic and oral surgery aided by diagnostic set-up. Furthermore, further clarification of mutational analysis need to carried out.

Conclusions

From this study, we can conclude that there is correlation between PAX9 mutations with all hypodontia patients. For the non-hypodontia patients which are our control samples, they showed no PAX9 mutation. Currently, study regarding mutation of MSX1 in hypodontia patients is still in progress for further confirmation. In this 20th century the prevalence of hypodontia increasing, and with today’s modern molecular genetic analysis more families with hypodontia can be identified. To achieve this, dental professional needs to collaborate with human genetics so that hypodontia can be diagnosed early thus patient will received early treatment and reducing cost in the future.

Acknowledgment

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References