The Application of Clinical Genetics

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ORIGINAL RESEARCH

The role of *PAX9* promoter gene polymorphisms in causing hypodontia: a study in the Jordanian population

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Background: The congenital absence of one or few teeth, hypodontia, is considered one of the utmost dental ageneses in human beings. Several genes have been shown to be involved in the development of hypodontia such as paired box gene 9 (*PAX9*). The expression of *PAX9* is controlled by several polymorphic elements in the promoter region of the gene on 14q13.3 locus. The aim of this study was to find any association between *PAX9* c.-912T>C (rs2073247) and c.-1031G>A (rs2073244) promoter polymorphisms and the development of hypodontia among the Jordanian population.

Methods: Genotyping of the polymorphisms in 72 unrelated subjects with hypodontia was performed using PCR-restriction fragment length polymorphism (RFLP) technique and compared with that of 72 normal healthy unrelated control individuals.

Results: The hypodontia group had a significantly higher -1031GG genotype ($P<0.01$) and a significantly lower -912TC genotype ($P<0.01$) compared with the control group. The results suggest that the transcriptional activity of *PAX9* gene is affected by polymorphisms in the promoter region of this gene and is associated with hypodontia phenotype.

Conclusion: The rs2073247) and rs2073244 promoter polymorphisms of PAX9 might play a role in the development of hypodontia in the Jordanian population.

Keywords: *PAX9*, hypodontia, Jordan, polymorphism, promoter

Introduction

Hypodontia, the congenital absence of less than six permanent teeth, is one of the most prevalent craniofacial congenital malformations in human beings,[1] with an overall incidence of 2.0%–10.0%, depending on the population studied excluding the absence of third molars.[2] Hypodontia is common for the lower second premolars and the upper lateral incisors.[3,4] Hypodontia can occur as a result of both environmental and genetic factors. For example, trauma of the dental region, chemotherapy, or radiotherapy has been shown to be associated with hypodontia. Genetic factors such as variations in *MSX1* and paired box gene 9 (*PAX9*) have also been shown to be related to non-syndromic hypodontia.[5,6]

The *PAX9* gene is a member of the paired box (PAX) family of transcriptional factors. The gene is localized on the long arm of chromosome 14 (14q21) and has been shown to play an important role in dental development especially in the bud stage.[3,7,8] Mutations in *PAX9* gene have been shown to cause familial tooth agenesis.[9–12] Moreover, several polymorphisms in *PAX9* gene have been shown to increase the risk factor for the development of hypodontia. The c.-1031G>A and c.-912T>C single-nucleotide polymorphisms (SNPs) in *PAX9* gene have been shown to be associated with hypodontia in

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the Caucasian population.[13] Similarly, Bianchi et al[14] showed that the C allele of c.-915G>C SNP of *PAX9* gene is related to hypodontia. Therefore, polymorphic variants of *PAX9* gene in addition to mutations might be a risk factor for tooth agenesis.

This study aimed to investigate the association between *PAX9* c.-1031G>A and c.-912T>C promoter polymorphisms and the development of hypodontia among the Jordanian population.

Subjects and methods

In this study, 72 unrelated healthy subjects diagnosed with non-syndromic hypodontia and a similar number of healthy controls of unrelated individuals with the same mean age of 20 years were enrolled.

Sample collection was done in the Dental Teaching Center, Jordan University of Science and Technology (JUST), Jordan. Approval from the institutional review board (IRB) at the university was obtained. Both the IRB at JUST committees utilized the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments. The procedures and possible discomforts, risks, and benefits were explained to the patients involved, and their written informed consents were obtained.

The selection criteria were as follows: healthy, unrelated individuals with agenesis of one to five permanent teeth, excluding third molars, and the lack of any other craniofacial malformations. Control subjects were all healthy, unrelated, and had normal primary and permanent dentition and did not show any craniofacial abnormalities. The diagnosis was confirmed by the same dentist after obtaining thorough history and making orthopantomographs for all subjects.

The DNA for the genotyping of examined SNPs was isolated from the blood collected in EDTA sterile tubes using DNA extraction kit obtained from Promega Corporation (Fitchburg, WI, USA) according to the manufacturer's instructions. DNA concentration and quality were assessed spectrophotometrically, and DNA was stored at –20°C until used.

Polymorphisms of *PAX9* c.-1031G>A and c.-912T>C were typed by restriction fragment length polymorphism (RFLP) according to the criteria specified by Peres et al[13] using *HaeIII* and *MseI* restriction enzymes (Fermentas, Glen Burnie, MD, USA) respectively. For both polymorphisms, the used primers were as follows: forward: 5'-AGC CTG AAT CCT GTG TGC AC-3' and reverse: 5'-CTA ATC TAA AGT GTA CCG TAT GC-3'. Amplification was performed in PCR tubes containing 100 ng of DNA, 1 μM of each primer and ready-to-use PCR master mix obtained from Promega Corporation. An Eppendorf thermocycler (Eppendorf, Hamburg, Germany) were used, and the cycling conditions were as follows: 95°C for 5 minutes and 35 cycles at 95°C for 60 seconds, 58°C for 30 seconds, and 72°C for 60 seconds, followed by a final extension of 5 minutes at 72°C. The amplified fragment (202 bp) was detected using 2% agarose gel electrophoresis prior to restriction digestion, which was carried out for each polymorphism separately in 20 μL final volume reaction using 10 μL of PCR product and 1 unit of *HaeIII* for c.-1031G>A or 2.0 units of *MseI* for c.-912T>C. The reactions were incubated at 37°C for 5 hours. Amplified fragment for -1031G and -912T alleles stayed uncut, whereas the -1031A allele cut with *HaeIII* to give 176 bp and 26 bp fragments (Figure 1), and -912C allele cut with *MseI* to give 144 bp and 58 bp fragments (Figure 2).

Hardy–Weinberg equation was used to test whether the examined SNPs were in equilibrium. The SPSS (version 21; IBM Corporation, Armonk, NY, USA) program was used for all statistical evaluations. *P*-value of <0.05 was considered significant.

Results

Male-to-female ratio was 1:1.18 in the hypodontia group and 1:1.25 in the control group ($P>0.05$; Table 1). About 16.7% of hypodontia subjects had three or more missing teeth, while

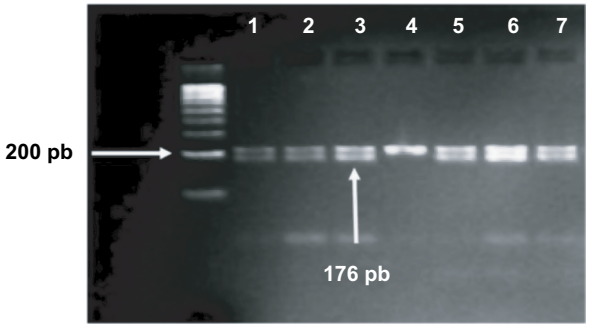


Figure 1 Gel electrophoresis of c.-1031G>A polymorphism.
Notes: PCR fragments (202 bp) were restricted with *HaeIII* enzyme. Samples in lanes 1–3 and 5–7 represents heterozygous GA genotype. Sample in lane 4 represents homozygous AA genotype.

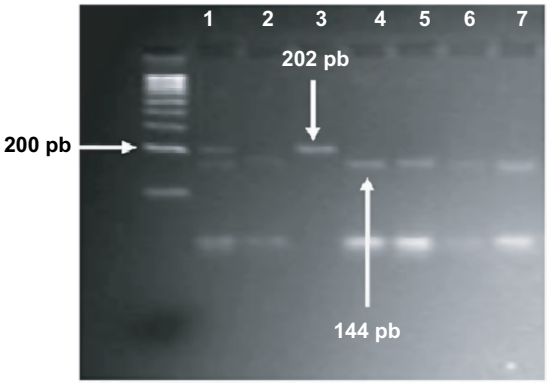


Figure 2 Gel electrophoresis of c.-912T>C polymorphism.
Notes: PCR fragments (202bp) were restricted with *MseI* enzyme. Sample in lane 1 represents heterozygous CT genotype. Samples in lane 2, 4–7 represent homozygous TT genotype. Sample in lane 3 represents CC genotype.

Table 1 Aspects of population studied

Family characteristics	N (%)
Gender	
Male	33 (45.8)
Female	39 (54.2)
Average age (years)	20.88
Number of teeth missing	
1	28 (38.9)
2	32 (44.4)
3 or more	12 (16.7)
Type of teeth more often missing (total teeth missing =164)	
Central incisor	30 (18.3)
Lateral incisor	74 (45.1)
Canines	10 (6.1)
First premolar	4 (2.4)
Second premolar	36 (22)
First molar	4 (2.4)
Second molar	6 (3.7)

the percentage of subjects with one and two missing teeth was 83.3% (Table 1). The most prevalent missing teeth in the sample were incisors and the premolars (Table 1).

Table 2 summarizes the genotype distributions and allele frequencies of c.-1031G>A *PAX9* SNPs in different groups. Significant differences in the distribution of genotypes of the c.-1031G>A SNPs were detected (Fisher's exact test, $P<0.001$) with the GG homozygous genotype being more frequent among hypodontia subjects. Accordingly, the distribution of G allele to A allele was different between hypodontia subjects and controls (chi-squared test, $P<0.001$). Therefore, the presence of the -1031 GG homozygous genotype increases the risk of hypodontia in the studied population.

The distribution of genotypes and the frequencies of alleles at *PAX9* c.-912T>C promoter SNPs in the two groups are summarized in Table 3. Similar to c.-1031G>A SNPs, the distribution of *PAX9* c.-912T>C SNP genotypes (Fisher's exact test, $P<0.001$) and T to C allele (chi-squared test, $P<0.001$) was significantly different between the hypodontia group and the control group. Among the hypodontia group, only one subject (<1% of the sample) was CT heterozygous compared with 24 subjects (33% of the sample) in the control group. Therefore, the presence of the -912TC heterozygous genotype might be associated with low risk of hypodontia in the Jordanian population.

Table 2 Genotype and allele frequencies of *PAX* c.-1031G>A polymorphism in the Jordanian population

	Control subjects		Hypodontia subjects		*P*-value
	n	%	n	%	
AA	43	59.7	27	37.5	<0.001[a]
GA	28	38.9	35	48.6	
GG	1	1.4	10	13.9	
Allele A	114	79.2	89	61.8	
Allele G	30	20.8	55	38.2	

Notes: [a]Fisher's exact test. [b]Chi-squared test.

Table 3 Genotype and allele frequencies of *PAX9* c.-912T>C polymorphism in the Jordanian population

	Control subjects		Hypodontia subjects		*P*-value
	n	%	n	%	
CC	3	4.2	3	4.2	<0.01[a]
CT	24	33.3	1	1.4	
TT	45	62.5	68	94.4	
Allele C	30	20.8	7	4.9	<0.01[b]
Allele T	114	79.2	137	95.1	

Notes: [a]Fisher's exact test. [b]Chi-squared test.

Discussion

In this study, we found that *PAX9* c.-1031G>A and c.-912T>C gene promoter polymorphisms were strongly associated with the development of non-syndromic hypodontia in the Jordanian population. The results showed that the presence of the -1031 GG and -912TC genotypes might be associated with hypodontia in the Jordanian population.

The role of *PAX9* in tooth development is well established. *PAX9* is a member of gene family that encodes for transcriptional factors which is important during embryogenesis.[15,16] *PAX9* plays a significant role during odontogenesis in the progressive and reciprocal signal transduction pathways that normally occur in epithelial–mesenchymal cells.[6] The gene product of *PAX9* appears early in the mesenchyme before ectodermal thickening and reaches its higher expression level during the initiation of bud and cap stages.[17] In the dental mesenchyme, PAX9 protein interacts with MSX1 homeoprotein to regulate BMP4, which has an implication in downstream signaling cascade that is essential for enamel knot formation.[16,18,19] Therefore, proper regulation of PAX9 signaling pathway is essential for odontogenic potential of mesenchyme and normal tooth development.

The results of this study agree with the finding in the Caucasian population (12). About 13.8% of hypodontia patients had GG genotype at -1031 position, while it was present in one subject from the control group (1.4%). Similar finding was reported in the Caucasian population in Brazil where the GG genotype at -1031 (9.8%) in hypodontia subjects compared with 2.9% in controls.[13] Regarding the c.-912T>C polymorphism, the frequency of the TT genotype was 94.4% in the hypodontia group compared with 62.5% in the control

Table 4 Mutations and polymorphisms in *PAX9* gene that has been shown to cause tooth agenesis

Mutation/ polymorphism	Type of mutation	Amino acid change	Reference
219 Ins G	Frame shift	G73fsX316	12
c.340A>T	Nonsense	K114X	29
c.793insC	Frame shift	A264fsX315	22
Deletion of gene	Del	L21P	10
c.62T>C	Missense	R26W	9
c.76C>T	Missense	G51S	26
c.151G>A	Missense	A58fsX177	21
c.176ins288nt	Frame shift	K91E	9
c.271A>G	Missense	R28P	9
c.83G>C	Missense	I37fsX41	23
c.109insG	Frame shift	R47W	27
c.139C>T	Missense	G73fsX316	27
c.219insG	Frame shift	M1V	27
c.1A>G	Missense	I87F	24
c.259A>T	Missense	I207fsX211	16
c.619–621delATCins24nt	Frame shift	Q145X	21
c.433C>T	Nonsense	R59X	28
c.175C>T	Nonsense		30
c.-915G>C	SNP		14
c.-912T>Cc.-1031G>A	SNPSNP		13, 20, this study

group. In the Caucasian population, Peres et al[13] showed that TT genotype was strongly associated with hypodontia. In their study, TT genotype was 12.7% in hypodontia subjects compared with 2.8% in controls. However, in the present study, the frequency of the T/C genotype was higher in the control group (33.3%) compared with the hypodontia subjects (1.4%) which indicated that the T/C genotype was associated with low risk of hypodontia. In contrast, Peres et al[13] reported similar frequencies of the T/C genotype in hypodontia individuals and the control group. On the other hand, a study from Turkey showed that AC haplotype of c.-1031G>A and c.-912T>C SNPs carries less risk in having hypodontia.[20] The discrepancy between the different studies could be due to variations in the genetic background and in the frequency of the T/C alleles in the different populations. Despite such disparity, results of the current study and those of the study by Peres et al,[13] provide strong evidence for the involvement of the *PAX9* c.-912T>C promoter polymorphism in causing hypodontia phenotype in the two populations. In addition, the association between gene polymorphisms and hypodontia might have population specificity being affected by certain environmental factors or/and population genetic background.

Several mutations and polymorphisms in *PAX9* gene were reported to cause hypodontia in other populations (Table 4). For example, the deletion of the *PAX9* gene in American and Polish families was described to cause autosomal dominant agenesis which involves several primary and permanent molars.[10,21] In the American population, frame shift mutations in exon 2 of *PAX9* were reported in individuals suffer from hypodontia.[9,22] In addition, missense mutations in *PAX9* gene were also reported to cause hypodontia in several populations.[4,9,11,16,21,23–27] Furthermore, nonsense mutations in *PAX9* that produce truncated proteins were identified to cause familial hypodontia and oligodontia phenotypes in subjects from Denmark, Finland, Spain, and USA.[22,28–30] In the Caucasian population in Brazil, the G-915C SNP in the promoter region of *PAX9* gene was found to be associated with hypodontia.[14] Mitsui et al[31] reported two novel mutations (c.73_75delATC and c.146C>T) in the paired domain of PAX9 in patients with non-syndromic tooth agenesis from Japan. In a study from Saudi Arabia, a new mutation p.N40I in PAX9 was detected in two patients with non-syndromic tooth agenesis.[32] A new initiation codon mutation (changes the ATG initiation codon to AGG) related to oligodontia was identified in the Chinese population.[33] Thus, the abovementioned literature indicates a significant role for *PAX9* in tooth development in human beings.

Conclusion

Findings of this study indicated that c.-1031G>A and c.-912T>C promoter polymorphisms in *PAX9* gene might be associated with hypodontia in the Jordanian population. The analysis of genes that involved in the development of hypodontia is important for understanding the genetic basis of hypodontia and might help in the management of this condition.

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Disclosure

The authors report no conflicts of interest in this work.

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