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Screening for MSX1 and PAX9 gene variants among hypodontia patients in the Jordanian population

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Abstract

This study aimed to sequence the PAX9 and MSX1 genes in a group of Jordanian patients diagnosed with non-syndromic hypodontia. The study also examined whether the identified genetic variants in these key odontogenic genes could be used to assess susceptibility to hypodontia. Fifty Jordanian individuals diagnosed with non-syndromic hypodontia were enrolled in this study. Genomic DNA was extracted, and all exons of PAX9 (Exons 1,2, 3, and 4) and MSX1 (Exons 1 and 2) were amplified using PCR. The Sanger sequencing technique was used to detect genetic variants, which were analyzed for their clinical significance and evolutionary conservation. Several previously reported genetic variants were detected. In MSX1, two missense variants, c. 77G>C; p. (Gly26Ala)(rs567549350) and c. 119C>G; p. (Ala40Gly)(rs36059701), and a regulatory variant, c. *6C > T(rs8670), were identified. In PAX9, six genetic variants were found: c. 5-109G>C (rs12883298), c. 5-54A>G (rs1288923), c. 5-41A>G(rs12883049), c. 631+41G>A(rs2236007), c. 717C>T; p. (His239His) (rs12881240) and c. 718G>C; p. (Ala240Pro)(rs4904210). Additionally, the c. *6C > T (rs8670) variant in MSX1 was associated with upper lateral incisor agenesis. The c. -18G>A (rs186861426) variant in MSX1 was evolutionarily conserved across multiple species, suggesting a potential regulatory role in odontogenesis. This study confirmed the presence of several previously reported PAX9 and MSX1 variants among Jordanian individuals with hypodontia. The findings emphasize the importance of genetic screening for hypodontia but suggest that the identified variants are likely benign polymorphisms rather than causative variants. Further studies with larger sample sizes and functional analyses are needed to determine the regulatory impact of non-coding variants and their role in tooth agenesis.

Keywords: Hypodontia, PAX9 gene, MSX1 gene, Gene variants, Tooth agenesis, Jordan.

1. Introduction

Tooth agenesis is a condition in which one or more of the usual 20 primary or 32 permanent teeth fail to develop due to genetic or environmental factors [1]. It can occur as an independent disorder or as part of a syndrome [2]. Hypodontia refers to the absence of one to five teeth, while oligodontia describes the absence of six or more teeth [3]. The prevalence of hypodontia varies across different populations, ranging from 1. 6% to 36. 5%, with evidence suggesting a possible increase over time [4]. In the Jordanian population, hypodontia affects approximately 5. 5% of adults and 8. 83% of children [5]. Globally, the prevalence is highest in Europe (males: 4. 6%, females: 6. 3%) and Australia (males: 5. 5%, females: 7. 6%) compared to North American Caucasians (males: 3. 2%, females: 4. 6%) [6].

The most frequently missing teeth in non-syndromic hypodontia are the maxillary lateral incisors and mandibular second premolars [6, 7]. The genetic basis of tooth development disorders, including agenesis, supernumerary teeth, and morphological abnormalities, has been extensively studied [8-10]. Several candidate genes have been identified, including *MSX1, PAX9, AXIN2, WNT10A, EDA, EDAR, BMP4, FGF8, IRF6,* and *PITX2* [11-21]. Among these, *MSX1* and *PAX9* have been particularly associated with non-syndromic familial and sporadic hypodontia [22-31]. *MSX1* and *PAX9* encode transcription factors that are expressed in the dental mesenchyme during early tooth development, specifically in the dental follicle and dental papilla [32, 33].

The *PAX9* gene, located on chromosome 14, encodes a protein with 341 amino acid residues, playing a crucial role in multiple stages of tooth formation [34]. The *MSX1* gene is located on chromosome 4 and contributes to the regulation of odontogenesis [34]. Variants, rather than mutations, in these genes can lead to disruptions in protein function, which may contribute to dental anomalies. Missense and nonsense variants may lead to protein loss but

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do not result in complete gene loss [35,36]. *PAX9* plays a role in early bud-stage tooth development, as demonstrated in *PAX9*-knockout mouse models, where tooth formation is disrupted [34].

A thorough examination of the *PAX9* gene reveals genetic alterations in both its coding and non-coding regions, mainly within exons 1 to 4. The paired domain, a highly conserved region encoded by exon 2, is known for its high mutation rate [37]. A variant (c. 1057G>A) in *PAX9* has been reported to alter its functional activity, affecting *BMP4* gene expression and contributing to hypodontia [38]. This emphasizes the critical role of *PAX9* in dental development, as its variants have been implicated in delayed tooth formation at the bud stage [38]. Studies conducted on Portuguese populations have linked *PAX9* polymorphisms to maxillary lateral incisor agenesis, reinforcing its potential role in hypodontia [38].

Further studies using animal models lacking *PAX9* have provided insight into the molecular pathways influencing dental anomalies, demonstrating that the severity of hypodontia correlates with specific genetic alterations [37]. Similarly, sequencing analysis of *MSX1* has identified a key nonsense variant (c. 610G>T) associated with tooth agenesis, which leads to a premature stop codon and reduced *MSX1* protein function [39]. Another study reported a frameshift mutation in the *MSX1* homeodomain, suggesting that *MSX1* alterations affect dental development through mechanisms such as pulp stem cell regulation via the ERK signaling pathway [40,41].

Understanding the genetic and environmental factors contributing to hypodontia is essential, given the complexity of treatment and the potential need for extensive orthodontic intervention with variable outcomes. Acquiring this fundamental knowledge is key to developing new preventive and therapeutic strategies [38,42].

The objective of this study is to sequence the *PAX9* and *MSX1* genes in Jordanian patients diagnosed with hypodontia to identify genetic variants and assess their potential relevance. This study aims to contribute valuable genetic data that can help in early identification of individuals at risk and enhance genetic counseling services for affected families.

2. Materials and methods

2. 1. Ethical approval and sample collection

This study was conducted in full accordance with the ethical principles of the Declaration of Helsinki and was approved by the Institutional Review Board (IRB) of King Abdullah University Hospital (KAUH) and Jordan University of Science and Technology (JUST), Irbid, Jordan, IRB number 50/128/2019. Blood samples were collected from 50 Jordanian individuals diagnosed with non-syndromic hypodontia who were undergoing treatment at an orthodontic clinic. Written informed consent was obtained from all participants or their guardians.

2. 2. Genomic DNA extraction and quantification

Genomic DNA was extracted from whole blood samples using the QIAamp DNA Blood Mini Kit (Qiagen, Germany), following the manufacturer's protocol. The DNA concentration and purity were assessed using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, USA). DNA samples were stored at -20°C for short-term use and -80°C for long-term storage.

2. 3. Targeted Gene Sequencing: PCR amplification and sanger sequencing

PCR amplification was performed to amplify exons 1 and 2 of the MSX1 gene and exons 1, 2, 3, and 4 of the PAX9 gene. Primers were designed using Primer3 software based on sequences obtained from the Ensembl Genome Browser. The sequences of the primers used are shown in Tables 1 and 2.

2. 4. PCR conditions and amplification

PCR amplification was performed to amplify exons 1 and 2 of the MSX1 gene and exons 1, 2, 3, and 4 of the PAX9 gene. Primers were designed using Primer3 software based on sequences obtained from the Ensembl Genome Browser. The sequences of the primers used are shown in Tables 1 and 2.

Polymerase chain reactions were performed using 1 µL genomic DNA (40 ng/µL), 1 µL of each primer (5 pmol) and 12. 5 µL of 2X Taq Master Mix (Thermo Fisher Scientific, USA) in a 25 µL volume. The PCR program was initiated with a 95ºC denaturation for 7 min, followed by 35 cycles of 95ºC for 45s, 60ºC for 45s, 72ºC for45s; and a final extension of 72ºC for 7 minutes. Negative control (without DNA template) was included in all reactions to check for contamination.

2. 5. Agarose gel electrophoresis and PCR product purification

PCR products were visualized using 2% agarose gel electrophoresis with GelRed™ nucleic acid stain under a UV trans illuminator. A 100 bp DNA ladder (Thermo Fisher Scientific, USA) was used as a reference for fragment size confirmation. Successfully amplified products were purified before sequencing.

2. 6. Sanger sequencing and variant identification

Sanger sequencing was performed on purified PCR products using Macrogen Corporation (Seoul, South Korea). Bidirectional sequencing was carried out to confirm detected variants. Sequence chromatograms were analyzed using CHROMAS Pro software (Technolysium Ltd.), and sequences were aligned with reference sequences from GenBank.

2. 7. Criteria for variant classification

Variants were classified as benign, likely benign, or pathogenic based on:

Table 1. Primer sequences used for PAX9 gene amplification.

Exon	Forward Primer (5'-3')	Reverse Primer (5'-3')	Size (bp)
1	TTTCATCGGGGCACAGACTT	CTGACCCTTAGCGTGTTCCT	353
2	TTCGGCTATGTTCAGGGACC	TCAGGTGGTGGGAAAGACAG	895
3	TTTGGGTCCCGTCTCAAGAG	GAAGCTCAGAAAGGGACCCA	302

Table 2. Primer sequences used for MSX1 gene amplification.

Exon	Forward Primer (5'-3')	Reverse Primer (5'-3')	Size (bp)
1	GAGTGCTCCCGGGAACTC	CCTGGGTTCTGGCTACTCAC	708
2	GGGCTGATCATGCTCCAATG	CAGGGCTGGAGGAATCGG	554

- ACMG guidelines for variant classification
- Minor allele frequency (MAF) in population databases
- Functional predictions from bioinformatics tools

2. 8. Statistical analysis

All statistical analyses were performed using SPSS software (version 26. 0; IBM Corp., Armonk, NY, USA). Descriptive statistics were used to summarize the demographic and clinical characteristics of the study cohort, as well as the frequencies of genotypes and alleles for each identified variant. Genotype and allele frequencies were calculated by direct counting. The Hardy-Weinberg equilibrium (HWE) for each variant was assessed using a Chi-square (χ^2) goodness-of-fit test.

Associations between genetic variants (in MSX1 and PAX9) and the presence/type of tooth agenesis were evaluated. For categorical phenotypes (e. g., agenesis of a specific tooth type: present/absent), Chi-square (χ^2) or Fisher's exact tests were employed, as appropriate. Odds ratios (ORs) along with their 95% confidence intervals (CIs) were calculated to estimate the strength of associations between specific genotypes and hypodontia phenotypes.

A p-value of less than 0. 05 was considered statistically significant for all tests. Given the exploratory nature of this study and the multiple comparisons performed, the findings should be interpreted with caution, and replication in larger cohorts is warranted.

3. Results

The present study aimed to identify genetic variants in both the *PAX9* and *MSX1*genes among Jordanian patients diagnosed with non-syndromic hypodontia. DNA sequencing of *MSX1* exons 1 and 2 and *PAX9* exons1, 2, 3, and 4 was conducted in a cohort of 50 individuals. The results identified several missense, synonymous, and regulatory variants, none of which were classified as pathogenic. All detected variants have been previously documented in genomic databases as benign polymorphisms.

3. 1. Phenotypic patterns of missing teeth

The prevalence and distribution of missing teeth were analyzed across the study population. The most frequently absent teeth were maxillary lateral incisors and mandibular second premolars, consistent with previous findings in hypodontia patients. In some cases, multiple teeth were missing, with a few individuals exhibiting oligodontia-like patterns summarized in Table 3, which shows the frequency of agenesis for each tooth type.

Table 3. Phenotypes of patients with missing teeth.

Tooth Type	Frequency of agenesis (%)
Central Incisors	0. 12%
Lateral Incisors	0. 38%
Canines	0. 16%
First Premolars	0. 24%
Second Premolars	0. 16%
First Molars	0. 12%
Second Molars	0. 2%

3. 2. PAX9 gene variants

The sequencing analysis identified six variants in PAX9, including one missense mutation and five polymorphisms located in intronic and coding regions. The c. 718G>C; p. (Ala240Pro) (rs4904210) missense variant in exon 3 was observed in 32 patients, with 23 in heterozygous and 9 in homozygous. This variant has been previously documented in dbSNP and ClinVar as benign, suggesting that while it is common in hypodontia cases, it may not be causative. The synonymous variant c.717C>T; p.(His239His) (rs12881240) was observed in 32 patients, with 30 heterozygous and 2 homozygous cases. Both rs4904210 and rs12881240 were detected in the heterozygous state in one patient (Figure 1E). Further investigation is required to determine whether these variants are located on the same allele.

Additional intronic variants were detected, including c. 5-54A>G (rs12882923) and c. 5-41A>G (rs12883049), both located in intron 2. The c. 5-54A>G variant was found in 42 patients, with 22 homozygous and 20 heterozygous cases. The c. 5-41A>G variant was present in 17 individuals, with 5 homozygous and 12 heterozygous cases. These variants, although not protein-altering, may influence splicing mechanisms or gene expression. The c. 5-109G>C (rs12883298) variant was found in 15 patients, with 10 homozygous and 5 heterozygous. The c. 631+41G>A (rs2236007) variant, a splice-region polymorphism, was detected in 13 patients and warrants further investigation regarding its potential regulatory effects.

DNA sequencing chromatograms show mutations and polymorphisms seen in the PAX9 gene are presented in Fig. 1.

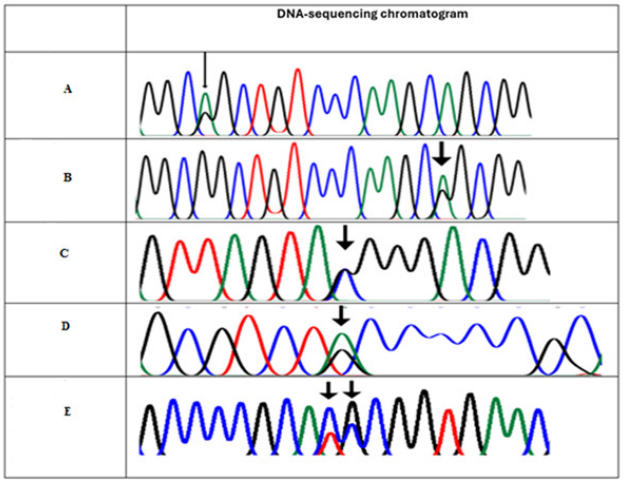


Fig. 1. DNA sequencing chromatograms show mutations and polymorphisms seen in the PAX9 gene (A) c. 5-54A>G (rs12882923) (B) c. 5-41A>G (rs12883049) (C) c. 5-109G>C (rs12883298) (D) c. 631+41G>A (rs2236007) (E) c. 717C>T (rs12881240) and c. 718 G>C (rs4904210).

3. 3. MSX1 gene variants

The MSX1 gene sequencing revealed five variants, including two missense variants, one synonymous variant, and two regulatory region variants. The c. 77G>C; p. (Gly26Ala) missense variant in exon 1 was identified in two individuals, with one homozygous and one heterozygous case. Similarly, the c. 119C>G; p. (Ala40Gly) missense variant was found in 10 patients, with 8 heterozygous and 2 homozygous cases. While these variants alter amino acid sequences, they have been previously classified as benign in genetic databases and may not be directly associated with hypodontia.

A synonymous variant, c. 348C>T; p. (Gly116Gly) was detected in five heterozygous patients. This variant, though not altering protein structure, could affect mRNA stability or splicing efficiency. Its presence in affected individuals suggests that synonymous mutations should not be overlooked when studying genetic contributions to hypodontia.

In addition to coding region variants, a 3' untranslated region (UTR) variant, c. *6C >T (rs8670) was detected in four patients in a homozygous state. Given that UTR variants can influence mRNA stability, translation efficiency, and gene expression, this variant may play a regulatory role in MSX1 gene function. Another regulatory variant, c. -18G>A (rs186861426) in the 5' UTR was identified in 11 patients.

3. 4. Genotype-phenotype correlation

To assess whether specific genetic variants correlated with missing teeth phenotypes, a comparison was conducted between variant carriers and their dental findings. Patients carrying c. 718G>C; p. (Ala240Pro) in PAX9 frequently exhibited missing lateral incisors and second premolars, consistent with previous reports associating PAX9 variants with molar and premolar agenesis.

Patients with MSX1 c. *6C > T (3' UTR) variants exhibited missing lateral incisors and second molars, reinforcing the hypothesis that MSX1 regulatory elements play a role in dental development. Notably, patients carrying the c. 119C>G; p. (Ala40Gly)variant showed a tendency toward central incisor agenesis, though no statistically significant association was established due to the limited sample size results summarized in Table 4.

3. 5. Patients with variants in both *PAX9* and *MSX1*

Table 5 summarizes the findings for patients carrying variants in both *MSX1* and *PAX9*, highlighting potential genetic interactions.

4. Discussion

The results emphasize that while genetic variants in MSX1 and PAX9 are common in hypodontia patients, none of the identified variants demonstrate a clear pathogenic role. Further functional studies are needed to evaluate the biological impact of these variants on gene expression and tooth morphogenesis.

Research on non-syndromic hypodontia has emphasized the importance of studying PAX9 and MSX1, as these genes play a critical role in tooth development. This is supported by both current literature and the findings of this study. Prior research has identified a genetic basis for dental anomalies in one-third of Saudi Arabian patients by screening for variants in these genes [42]. However, limitations such as small sample sizes may obscure potential genotype-phenotype correlations.

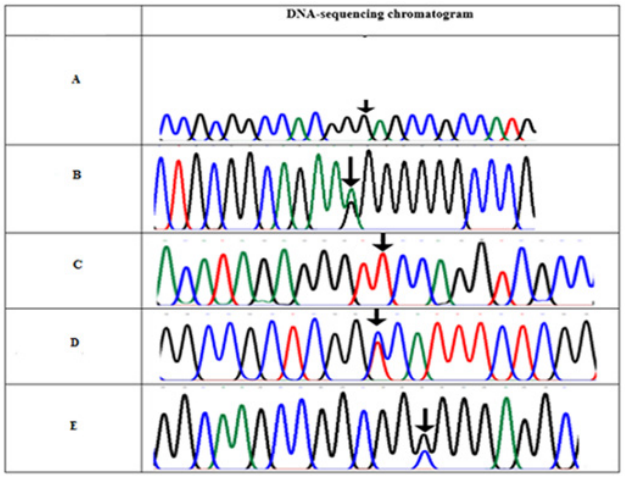


Fig. 2. DNA sequencing chromatograms show mutations and polymorphisms seen in the *MSX1* gene (A) c. 119C>G; p. (Ala40Gly) (rs36059701) (B) c. -18G>A (rs186861426) (C) c. *6C >T (rs8670) (D) c. 348C>T; p. (Gly116Gly) (rs34165410) (E)(rs567549350).

This study examined MSX1 (exons 1 and 2) and PAX9 (exons1, 2, 3, and 4) in 50 individuals with hypodontia. The results align with prior investigations [43], confirming the presence of previously reported polymorphisms but no pathogenic mutations in the DNA-binding domains of either gene. However, this study expands on previous data by reporting the presence of rs186861426 (c. -18G>A) in MSX1, which was found to be highly conserved across multiple species, including chimpanzees, cattle, mice, and rats. The conservation of this variant suggests a potential regulatory role rather than a direct coding sequence alteration, warranting further investigation into its impact on MSX1 expression and tooth development.

The interaction between PAX9 and MSX1 is critical for tooth development, particularly through the regulation of BMP4, which influences enamel knot formation [37]. MSX1 variants have been primarily linked to second premolar agenesis, while PAX9 variants more commonly affect molar development [44]. Studies suggest that PAX9 and MSX1 proteins interact to form a regulatory network governing early odontogenesis [45]. Notably, heterozygous loss-of-function variants in these genes can significantly affect the development of multi-cusped teeth, such as molars [34].

The role of PAX9 extends beyond dental development, as it encodes a transcription factor necessary for various embryonic processes [46]. It is particularly crucial during the bud and cap stages of tooth formation, where epithelial-mesenchymal interactions drive tooth morphogenesis [47].

In this study, 14 single nucleotide polymorphisms (SNPs) were identified, seven of which were polymorphic. Two of these SNPs have previously been associated with peg-shaped teeth in Jordanian families, highlighting a potential genetic predisposition to this anomaly [48]. While no variants were detected in PAX9 exons 2 and 4, a missense variant in Exon 3 was identified, which aligns with previous family studies suggesting a recessive inheritance pattern [43].

The presence of the c. 718G>C; p. (Ala240Pro) variant

Table 4. MSX1 and PAX9 gene variants and their clinical findings.

sample number	*MSX1*	*PAX9*	Number of Missing teeth	Clinical details
8	c. 77G>C C\C	Wild-type	1	Lower/Right/Second Premolar
7	c. 119C>G G/G c. *6C > T T/T	Wild-type	2	Lower/Right/Central incisor Lower/Left/Central incisor
35	c. *6C > T T/T	c. 718G>C G/C	5	Lower/Left/Second Molar Lower/Right/Second Molar Upper/Left/Second Molar Upper/Right/Second Molar Upper/Left/lateral Incisor
37	c. 119C>G G\G c. *6C > T T/T	c. 718G>C G/C	1	Lower/Right/Second Molar
46	c. *6C > T T/T c. 119C>G C/G	c. 718G>C G/C	2	Upper/Right//lateral Incisor Upper/Left/lateral Incisor
20	Wild-type	c. 718G>C C/C	3	Upper/Left/lateral Incisor Lower/Right/Second Premolar Lower/Left/Second premolar
23	c. 119C>G C\G	c. 718G>C C/C	1	Upper/Right/lateral Incisor
24	c. 119C>G C\G	c. 718G>C C/C	1	Lower/Right/Central Incisor
28	Wild-type	c. 718G>C C/C	5	Lower/Right/Central Incisor Lower/Left/Central Incisor Lower/Right/First Molar Lower/Left/First Molar Lower/Left/Second Molar
30	Wild-type	c. 718G>C C/C	1	Lower/Right/Second premolar
34	Wild-type	c. 718G>C C/C	2	Upper/Right//lateral Incisor Upper/Left/lateral Incisor
42	Wild-type	c. 718G>C C/C	1	Upper/Left/lateral Incisor
43	c. 119C>G C/G	c. 718G>C C/C	1	Lower/Right/Second Premolar

Table 5. Patients with variants in both *MSX1* and *PAX9* genes.

		PAX9	MSX1	MSX1	
sample number	**Number of Missing teeth**	**c. 718G>C p. Ala240Pro**	**c. 119C>G p. Ala40 Gly**	**c. *6C > T**	**Clinical details**
19	1	G/C	C/G	C/C	Upper/Right/lateral Incisor
22	1	G/C	C/G	C/C	Lower/Right/Second Premolar
23	1	C/C	C/G	C/C	Upper/Right/lateral Incisor
24	1	C/C	C/G	C/C	Lower/Right/Central Incisor
37	1	G/C	G/G	T/T	Lower/Right/Second Molar
42	1	C/C	C/G	C/C	Upper/Left/lateral Incisor
46	2	G/C	C/G	T/T	Upper/Right/lateral Incisor Upper/Left/lateral Incisor

in PAX9 exon 3 raises questions about its functional significance, particularly because exon 3 encodes a DNA-binding domain [49, 50]. Similar studies in Pakistani populations have linked MSX1 variants to dental abnormalities, reinforcing its role in tooth agenesis [51]. However, some studies have debated the pathogenicity of the identified variants, with certain PAX9 polymorphisms showing no association with non-syndromic hypodontia in specific populations [44]. Emerging research suggests that non-DNA-binding mutations might still alter PAX9 protein conformation or mRNA stability, thereby contributing to tooth agenesis [37].

This study also found that the rs8670 variant c. *6C > T in MSX1, located in the 3' untranslated region (UTR), was present in four individuals in a homozygous state. Given its location in a regulatory region, this variant may play a role in gene expression regulation, potentially influencing protein translation and stability. This aligns with previous findings linking rs8670 to non-syndromic tooth agenesis [43, 44, 58, 59].

In addition, the c. 348C>T; p. (Gly116Gly) variant (rs34165410) in MSX1 was identified in five heterozygous individuals. While this is a synonymous variant and does not change the amino acid sequence, it might affect mRNA stability or splicing efficiency, impacting gene function.

Previous meta-analyses and genome-wide association studies (GWAS) have reported inconclusive findings regarding the role of specific PAX9 SNPs in hypodontia, indicating the need for larger population-based studies [52-54]. The involvement of MSX1 in craniofacial development is well-documented, as MSX1 mutations have been associated with various congenital conditions affecting tooth formation [55-57].

This study employed Sanger sequencing, which remains the gold standard for detecting point mutations and polymorphisms, ensuring high genetic accuracy. The study also provides valuable insight into the Jordanian population, contributing to the global understanding of hypodontia genetics. However, several limitations must be considered when interpreting these results. First, the relatively small sample size may have constrained statistical power, potentially resulting in the oversight of rarer variants. Second, as the cohort was exclusively Jordanian, the findings may lack generalizability to other ethnic groups. Third, the study was restricted to *PAX9* and *MSX1*, meaning other relevant genes—such as *WNT10A*, *AXIN2*, and *EDA*—were not screened. Finally, the absence of functional validation prevents confirmation of the biological impact of the detected variants, and the influence of gene-environment interactions was not assessed. Despite these limitations, this study enhances our understanding of *PAX9* and *MSX1* in hypodontia and provides a foundation for future genetic research.

Although this study did not identify any novel pathogenic variants, it confirmed the presence of several previously reported polymorphisms in *MSX1* and *PAX9* among Jordanian individuals with hypodontia. Key findings included two missense variants (c. 77G>C; p. Gly26Ala and c. 119C>G; p. Ala40Gly) and one regulatory variant (c. *6C>T) in *MSX1*, with the latter potentially possessing implications for gene expression regulation. Furthermore, multiple variants were detected in *PAX9*—specifically c. 5-109G>C, c. 5-54A>G, c. 5-41A>G, c. 631+41G>A, c. 717C>T, and c. 718G>C—all of which are currently documented as benign polymorphisms in dbSNP and ClinVar. While these specific variants are unlikely to be causative mutations, these findings reinforce the value of genetic profiling in this population. Consequently, future research should prioritize functional validation, larger sample sizes, and broader genetic screening to further elucidate the complex etiology of hypodontia.

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Conflict of interests
Author’s has no conflicts with any step of the article preparation.

Consent for publications
The author read and approved the final manuscript for publication.

Ethics approval and consent to participate
King Abdullah University Hospital (KAUH), Jordan University of Science and Technology, Irbid, Jordan, was the Institutional Review Board (IRB) that approved this research.

Informed consent
The authors declare that written informed consent was obtained from all participants or their guardians.

Availability of data and material
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' contributions
Areej Quran: Research concept and design; writing the article; Ahmed Maslat: Supervision and critical revision of the article; Saied Jaradat: Data analysis and interpretation; Eman Hammouri: Critical revision of the article and final approval of the article; Abeer Quran: Performed all laboratory procedures and final approval of the article.

Use of artificial intelligence tools
Some sentences in this manuscript were revised with the assistance of artificial intelligence–based language models to improve clarity and readability. All intellectual content, interpretations, and conclusions are solely the responsibility of the authors.

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