Family and case–control genetic study of MSX1 polymorphisms in peg-shaped teeth Jordanian population

Rami Alkhatib1,2*, Razan Hawamdeh1, Laith Al-Eitan1,2, Nour Abdo3, Fadi Obeidat4, Mohamed Al-Bataineh4 and Hatem Aman2

Abstract

Background: This study aimed to investigate the genetic association of specific Single Nucleotide Polymorphisms (SNPs) within the muscle segment homeobox gene 1 (MSX1) with susceptibility to the peg-shaped teeth in 36 Jordanian Arab families and case–control samples in the Jordanian Arab population.

Methods: This cohort involved 108 individuals (36 trios families), which were used for family-based genetic study. Additionally, 56 patients and 57 controls were used for case–control study. Genomic DNA samples from both families and case–control were extracted according to distinguished processes. Then, polymerase chain reaction technique (PCR) was conducted using specific primers for the axons of MSX1. Moreover, DNA sequencing genotyping method analysis of SNPs was used to detect specified SNPs in the MSX1 linked with peg-shaped teeth. Hardy–Weinberg Equilibrium and Chi-square were used to evaluate the data quality and the presence of any genotypic error. In addition, Transmission Disequilibrium Test (TDT) was used to identify family-based association in which trios of parents and proband are used.

Results: The results of this study showed fourteen polymorphic sites in this gene, eight of them (rs121913129, rs104893852, rs104893853, rs121913130, rs1095, rs3775261, and rs1042484) were none-polymorphic. Meanwhile, the minor allele frequencies of the rest of the SNPs were polymorphic (rs8670, rs12532, rs3821949, rs4464513, rs1907998, and rs6446693). However, none of these SNPs were associated with peg-shaped teeth. Moreover, the haplotype genetic analysis revealed that there was no genetic association with peg-shaped teeth disorder susceptibility (P > 0.05) in the Jordanian families of Arab descent.

Conclusions: The present findings can be used in estimation of prevalence of peg-shaped teeth in the Jordanian population. However, our findings revealed that there is no evidence that the MSX1 polymorphisms had a crucial role in the peg-shaped teeth phenomenon, emphasizing that other genes might have this role. These findings are beneficial for clinicians to comprehensively understand the molecular aspects of teeth abnormalities.

Keywords: MSX1, Allele frequency, SNPs, Exons, Primers

Background

Tooth agenesis is among the well-recognized morphological anomalies in humans. Peg-shaped teeth are a hereditary dental disorder called microdontia, a condition where one or more teeth appear smaller than average (microdontia) [1]. In general, the most common
teeth affected are the upper lateral incisors or sometimes third molars. Moreover, it might be seen on both sides in most instances, and they have shorter roots than usual teeth [2]. Peg-shaped teeth are characterized by autosomal dominant inheritance that might cause defects during teeth development. As a result, there is an insufficient development compared to normal development. The prevalence of agenesis in the permanent dentition, excluding the third molar, ranges between 0.15 and 16.2% [3] with a higher prevalence in females than males [4–6]. Hua et al. [7] reported that women were 1.35 times more likely than men to have peg-shaped maxillary permanent upper lateral incisors. In general, the prevalence of peg-shaped teeth is about 1.8% [7]. The prevalence of tooth agenesis in permanent teeth in both genders varies among different populations. For example, in Europe the prevalence is 4.6% in males and 6.3% in females; in North American Caucasians is 3.2% in males and 4.6% in females. Meanwhile, the prevalence was the highest in Australia (males 5.5% and females 7.6%) [8].

Odentogenesis undergoes restricted genetic and morphological manipulation depending on cell–cell interactions resulting in the initiation and generation of tooth induced by morphological signaling pathway [9, 10]. Thus, any defect in the germ tooth can lead to dental anomalies either in shape, number, structure, or size of the teeth which exhibit morphological anomalies in humans [11]. Numerous genes and signaling pathways participate in tooth formation and cell differentiation at specific stages of odentogenesis [12] which may cause mutation in tooth agenesis [13]. Dental anomalies could be a combination between genetic, epigenetic and environmental factors during the process of dental development [14]. There are many genes involved in peg-shaped teeth. More than 350 genes have been associated with teeth development such as PAX9, MSX1, AXIN2, EDA, EDAR and WNT10a [15]. However, few genetic studies reported how genes are related to this disorder. In Jordanian population, the effect of MSX1 variation on the peg-shaped teeth is still not clear. The rate of persons with peg-shaped teeth disorder has been increased with a lack of information about the causes of this disorder and occurrence. According to several studies on this disorder, various environmental factors have been investigated in details with very restricted attention has been paved to the genetic component factor [1]. Therefore, a multi genetic factorial epidemiology study has been actually focusing on the genetic aspects including several genes such as MSXI [16].

Expression assays and transgenic mouse phenotypes revealed that MSXI has a critical role in craniofacial development [17]. The MSXI is expressed in the mesenchyme of developing tooth germ especially at the bud and cap stages as a response to epithelial signals [18]. Muscle segment homebox is a non-clustered home box protein, which is located in the small arm of chromosome 4 (chromosome 4p16.2) [19]. It contains two exons which are separated by an intron. The MSXI is a member of the mammalian MSX gene family, which consists of three physically unlinked members (MSXI, MSX2, and MSX3). MSX genes are essential for normal craniofacial, limb and ectodermal organ morphogenesis, and are also essential to survival in mice [20]. The MSX1 is a protein that in humans is encoded by MSXI [21]. The encoded protein acts as transcription factors repressor during morphogenesis through interaction with components of the core transcription component complex and other home proteins. Phenomena caused by the lack of MSXI protein may depend on the localization of the mutations and their effect on the protein structure and function. Moreover, a polymorphism in the MSXI leads to hypodontia and most of the affected teeth were second premolar and third molar. In addition, the upper lateral incisor, upper first molar, lower central incisor, and lower first molar could be absent [17, 22]. For example, mice with a homozygous deletion of MSXI exhibit a complete cleft palate and failure of tooth development [23].

The objective of this study was to investigate the genetic association of the MSXI and its susceptibility to the peg-shaped teeth in 36 families and case–control samples Jordanian Arab population.

Methods

Participants

Ethical approval for this study was in compliance with the Institutional Review Board (IRB) Guidelines at Jordan University of Science and Technology (IRB# 19/85/2015). All clinical investigations were conducted according to the principles expressed in the Declaration of Helsinki consent. Participants in this study consisted of 36 Jordanian Arab families with peg-shaped teeth cases and their biological parents; 137 samples were involved in this study (58 males [42.3%] and 79 females [57.7%]). The 137 samples consisted of 36 trios (108 individuals) and additional 29 unrelated individual cases. Of the latter 29 cases, nine cases failed genotyping and were excluded in the final case–control analyses. A total of 57 controls were added to the case–control study (Fig. S1). Peg-shaped teeth were diagnosed by a specialized dentist based on the morphology of the tooth only (cone shape). All cases of peg-shaped teeth used in this study were permanent upper lateral incisors only. Clinical and demographic data from each participant were collected using a semi-structured interview, which was built on a standard protocol. Demographic data including gender and age
were collected (Table 1). Written informed consent was provided by all subjects in this study.

The candidate genes and the selected SNPs
In this study, National Centre for Biotechnology Information (NCBI), SNPs Database (dbSNP), and HapMap project databases were used to select the SNPs, which were selected based on: Map position, minor allele frequency, and previous SNPs studies. The MSX1 and its SNPs and positions, and genotyping data based on the whole cohort (137 Subject) is shown in (Table 2). The rational for selecting these SNPs was: SNPs with significant functional relevance, SNPs that can cover the genes of interest as widely as possible, and SNPs that had been already genotyped in other studies. Moreover, these SNPs had a higher ability to be implicated in several tooth disorders and may have a major effect on peg-shaped teeth cases. Based on that, fourteen SNPs in chromosome 4 were selected.

DNA collection and analysis
DNA was extracted using commercially available kit (Promega, USA) according to the manufacturer’s instructions. Then, the DNA samples were diluted making the DNA concentration to be 20 ng/μl. DNA quantity and purity were verified using Nano-Drop ND-1000 UV-V Spectrophotometer (Bio-Drop Spectrophotometer, Cambridge, UK).

DNA genotyping
DNA samples were genotyped using the Sequenom Mass ARRAY® system (iPLEX GOLD) (Sequenom, San Diego, CA, USA) in the Australian Genome Research Facility [AGRF] (Melbourne Node, Australia).

Statistical and haplotype analyses
Hardy–Weinberg Equilibrium (HWE), Mendelian inheritance of the genotypes, and haplotype test were used in this study. Hardy–Weinberg Equilibrium and Chi-square were used to evaluate the data quality and the presence of any genotypic error. The genetic differences in allele frequencies and genotype distribution of each

Table 1 Characteristics of 36 Peg‑shaped teeth families of Jordanian descent in this study

| Characteristics of patients | N (%) |
|-----------------------------|-------|
| Age (years)                 |       |
| 9–29                        | 6 (48.2) |
| 30–50                       | 50 (36.5) |
| 51–71                       | 18 (13.1) |
| 72–92                       | 3 (2.2) |
| Gender                      |       |
| Male                        | 58 (42.3) |
| Female                      | 79 (57.7) |
| Position of peg‑shaped tooth|       |
| Bilateral                   | 26 (19.0) |
| Upper right                 | 14 (10.2) |
| Upper left                  | 12 (8.8) |
| Other                       | 85 (62.0) |
| Other anomalies             |       |
| Hyperodontia (> 32 teeth in the mouth) | 1 (0.7) |
| Hypodontia (1–6 teeth excluding third molar) | 12 (8.8) |
| Macroodontia (1 or 2 teeth larger than normal) | 3 (2.2) |
| Microdontia (1 or 2 teeth smaller than normal) | 5 (3.6) |

Table 2 The basic information of the selected SNPs in this study

| SNP ID     | Position | SNP | SNP location                        |
|------------|----------|-----|-------------------------------------|
| rs121913129| 4862836  | C>G | MISSENSE                            |
| rs104893852| 4860231  | A>C | STOP GAIND                          |
| rs104893853| 4862854  | A>C | STOP GAIND                          |
| rs121913130| 4860099  | A>T | MISSENSE                            |
| rs104893850| 4862808  | T>C | STOP GAIND                          |
| rs8670     | 4863149  | C>T | UTR variant 3 prime                 |
| rs1095     | 4863211  | C>T | UTR variant 3 prime                 |
| rs12532    | 4863419  | A>G | UTR variant 3 prime                 |
| rs3821949  | 4858675  | A>G | Upstream variant 2 KB               |
| rs4464513  | 4865595  | G>T | By 1000G, by 2hit 2allele, by cluster, by frequency |
| rs3775261  | 4862018  | A>G | Intron variant                      |
| rs1042484  | 4862654  | C>T | Intron variant                      |
| rs1907998  | 4854852  | A>G | By 1000G, by 2hit 2allele, by cluster, by frequency, by HapMap, by submitte |
| rs6446693  | 4853353  | C>T | By 1000G, by 2hit 2allele, by cluster, by frequency, by HapMap |

* Chromosome positions are based on NCBI Human Genome Assembly Build 4p16.2
polymorphism of interest in the family study and case–control study were compared using the Chi-square test. Moreover, odd ratio was calculated with 95% confidence interval (CI). In addition, haplotype test was used for Linkage Disequilibrium (LD) for each individual polymorphism using Haploview Software (Version 4.2). Transmission Disequilibrium Test (TDT) was used identify family-based association in which trios of parents and proband are used. Stratified genotype analysis for each SNP was calculated using PC SAS (v. 9.2; SAS Institute, Cary, NC, USA).

Results
Hardy–Weinberg equilibrium test (HWE)
All the genotyped polymorphisms met the HWE and Mendelian errors tests for the case–control and family groups. The Sequenom MassARRAY® system data was very precise with an average success rate of 99%. No Mendelian errors were observed. The 14 SNPs in \textit{MSX1} gene (Chromosome 4q16.2) were used; eight of them (rs121913129, rs104893852, rs104893853, rs121913130, rs104893850, rs1095, rs3775261, and rs1042484) were none-polymorphic. Meanwhile, the minor allele frequency of the other six SNPs was polymorphic (rs8670, rs12532, rs3821949, rs4464513, rs1907998, and rs6446693). The minor alleles of the studied SNPs and their frequencies for both families and case–control are shown in (Table 3).

Association of SNPs candidate genes with peg-shaped teeth
The distribution of alleles and genotypes of the genotyped SNPs in the peg-shaped teeth patients and their parents, brothers, and sisters were recorded. In addition, the association of these SNPs was tested using the TDT. The TDT test was carried out on 36 trios (male and female probands) and exhibited no significant preferential transmission in any of the six SNPs studies (Table 4). The observed value of D, a representation of allelic frequencies of SNPs, was not equal to 1, suggesting that the SNPs were in perfect LD. The genetic haplotype block of rs8670, rs12532, and rs4464513 within the \textit{MSXI} was shown in (Fig. 1). The haplotype program revealed that one SNP (rs6446693) out of the six SNPs from Sequenom Massarray data was not associated with peg-shaped teeth.

The genetic association analysis of all six SNPs in peg-shaped teeth cases and controls using different genetic models are exhibited (Table S1). The statistical analysis for the heterozygous versus both common homozygous and rare homozygous or even testing the latter two together was performed (Table S2). Moreover, stratified genotype analysis by gender for each SNP (adjusted by age) was conducted (Table 5). For case and control samples, all genotype SNPs were checked for HWE and Mendelian errors. The minor allele frequency for all six SNPs was within normal distribution in both studies ($P = 0.05$). The genotype frequencies of all six SNPs (rs12532, rs1907998, rs3821949, rs4464513, rs6446693, and rs8670) within the \textit{MSXI} displayed no association with peg-shaped teeth in both studies. Schematic structure of the human \textit{MSX1}, and the positions of the fourteen SNPs genotyped and their db SNP IDs displayed in Fig. 2.

Discussion
To the best of our knowledge, this is the first study that has analyzed the association of \textit{MSX1} in peg-shaped teeth syndrome in the Jordanian population of Arab descent. In addition, these individuals were characterized by typical symptoms such as tooth phenotypic structure that look like tapered maxillary lateral incisor which is commonly called morphological anomalies conical shape (The emergence of sharp teeth clearly like a screw) [24].

In this study, the sample set included 36 families with at least one affected child with peg-shaped teeth syndrome. The results of the current study revealed no

| Gene | SNP ID | (36) families | Controls n = (57) | Cases n = (56) |
|------|--------|---------------|------------------|---------------|
|      |        | MA | MAF | HWE-value | MA | MAF | HWE-value | MA | MAF | HWE-value |
| MSX1 | rs12532 | G  | 0.28 | 0.077 | G  | 0.31 | 0.270 | G  | 0.04 | 0.000 |
|      | rs1907998 | G  | 0.35 | 0.721 | G  | 0.24 | 0.741 | G  | 0.36 | 0.434 |
|      | rs3821949 | A  | 0.23 | 0.934 | A  | 0.27 | 0.040 | A  | 0.22 | 0.688 |
|      | rs4464513 | T  | 0.44 | 0.942 | T  | 0.36 | 0.934 | T  | 0.39 | 0.926 |
|      | rs6446693 | C  | 0.47 | 0.001 | C  | 0.46 | 0.742 | C  | 0.45 | 0.112 |
|      | rs8670 | T  | 0.23 | 0.643 | T  | 0.27 | 0.536 | T  | 0.29 | 0.309 |

\textit{MA} minor allele, \textit{MAF} minor allele frequency, \textit{HWE} Hardy–Weinberg equilibrium
significant association of the fourteen SNPs with the peg-shaped teeth in all samples in both genders. Even though, it has been reported that inactivation of MSX1 and PAX9 in mice showed that these genes are relevant to dental and craniofacial development [23, 25]. Interestingly, Alkhatib et al. (2020) reported that two SNPs in the paired box gene 9 (PAX9) were significantly associated with peg-shaped teeth in the same subjects used for this current study [26]. In this study, the 36 families were separated according to the gender of patients (male-only (MO) comparing with female-containing (FC) families) and analyzed based on a specific gender. However, the results showed that there was no significant association of these SNPs with the peg-shaped teeth in families with affected males only or in families with affected females only in trios [27].

Numerous former researches indicated that there is a genetic linkage in the small arm of chromosome 4 (4p16.2). Moreover, there is strong evidence that MSX1 is expressed in the developing craniofacial region [19, 23]. Based on their findings, there are no investigated results or evidences that confirm a significant genetic association between the MSX1 and peg-shaped teeth, which is similar to the presented results in this study. Alvesalo and Portin reported that agenesis and peg-shaped teeth could be autosomal dominant genes with reduced penetrance [28]. This reinforces our conclusion in the present study that there is an ethnic marker due to a genetic heterogeneity of the studied population in different ethnicities. The reason for these conflicting results might be firstly due to the ethnic differences between populations which contribute to genetic variation. Secondly, this study included only the Jordanian population of Arab ethnicity, which is considered as an isolated population. Thirdly, the genetic heterogeneity of peg-shaped teeth disorder and the possibility of the involvement of other genes or environmental factors such as gene–gene interaction or gene-environmental interaction must be considered for further research. This is also the limitation of this study.

### Table 4: The Transmission Disequilibrium Test (TDT) analysis for allelic association in 36 Peg-shaped tooth trios in Jordanian Population

| SNP ID  | Allele | T<sup>a</sup> | NT<sup>b</sup> | Ratio (T/NT) | X²  | P-value<sup>c</sup> |
|---------|--------|--------------|--------------|-------------|-----|------------------|
| rs12532 | A      | G            | A            | 12.70       | 1.316 | 0.251            |
|         | G      |              |              |             |      |                  |
| rs1907998 | A     | A            | G            | 14.12       | 0.154 | 0.695            |
|         | G      |              |              |             |      |                  |
| rs3821949 | A     | A            | G            | 12.11       | 0.043 | 0.835            |
|         | G      |              |              |             |      |                  |
| rs4464513 | T     | G            | T            | 14.60       | 3.200 | 0.074            |
|         | G      |              |              |             |      |                  |
| rs6446693 | T     | T            | C            | 20.16       | 0.444 | 0.505            |
|         | C      |              |              |             |      |                  |
| rs8670  | T      | T            | C            | 12.90       | 0.429 | 0.513            |
|         | C      |              |              |             |      |                  |

<sup>a</sup> Transmitted allele
<sup>b</sup> None transmitted allele
<sup>c</sup> P-value is significant when P < 0.05
Finally, there was a lack of information about environmental risk factors. Also, the promoter of this gene which has a direct effect on the regulation of the MSX1 expression was not investigated in this study.

**Conclusion**
This is the first genetic project of the MSX1 in the Jordanian peg-shaped teeth patients. No prior studies have been carried out investigating the MSX1 and its association with the peg-shaped teeth in the Arab population. These results revealed that there was no evidence that the MSX1 polymorphisms play a crucial role in peg-shaped teeth phenomenon. More genetic studies on Jordanian peg-shaped teeth in Arab population should be explored in the future.

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**Table 5** Stratified genotype analysis for each SNP (by gender and adjusted by age)

| SNP      | Genotype | Gender | Cases | Controls | Odds ratio (95% CI) | P-value |
|----------|----------|--------|-------|----------|---------------------|---------|
| rs2073244| A/A      | Female | 18    | 5        | 1.00                | 0.039   |
|          |          | Male   | 2     | 4        | 12.14 (1.13–130.56) |         |
|          | G/A      | Female | 14    | 12       | 1.00                | 0.887   |
|          |          | Male   | 7     | 5        | 0.89 (0.20–3.92)    |         |
|          | G/G      | Female | 6     | 2        | 1.00                | 0.897   |
|          |          | Male   | 4     | 1        | 1.24 (0.06–25.14)   |         |
| rs2073246| C/C      | Female | 18    | 5        | 1.00                | 0.039   |
|          |          | Male   | 2     | 4        | 11.16 (1.12–121.43) |         |
|          | C/T      | Female | 15    | 12       | 1.00                | 0.802   |
|          |          | Male   | 7     | 4        | 0.81 (0.17–3.76)    |         |
|          | T/T      | Female | 5     | 2        | 1.00                | 0.946   |
|          |          | Male   | 4     | 1        | 0.89 (0.04–18.48)   |         |
| rs2295222| C/C      | Female | 22    | 7        | 1                   | 0.157   |
|          |          | Male   | 5     | 4        | 3.59 (0.61–21.04)   |         |
|          | C/A      | Female | 15    | 12       | 1.00                | 0.982   |
|          |          | Male   | 7     | 4        | 0.98 (0.20–4.73)    |         |
|          | A/A      | Female | 3     | 0        | 1.00                |         |
|          |          | Male   | 1     | 1        | –                   |         |
| rs4904155| C/C      | Female | 16    | 5        | 1.00                | 0.059   |
|          |          | Male   | 2     | 4        | 9.82 (0.91–105.47)  |         |
|          | G/C      | Female | 14    | 12       | 1.00                | 0.664   |
|          |          | Male   | 7     | 4        | 0.70 (0.15–3.30)    |         |
|          | G/G      | Female | 8     | 2        | 1.00                | 0.703   |
|          |          | Male   | 4     | 1        | 1.84 (0.09–35.81)   |         |
| rs4904210| G/G      | Female | 16    | 4        | 1.00                | 0.085   |
|          |          | Male   | 2     | 3        | 8.70 (0.74–102.10)  |         |
|          | G/C      | Female | 16    | 12       | 1.00                | 0.787   |
|          |          | Male   | 6     | 6        | 1.23 (0.30–5.10)    |         |
|          | C/C      | Female | 6     | 2        | 1.00                | 0.871   |
|          |          | Male   | 4     | 1        | 1.30 (0.07–25.13)   |         |

CI confidence interval
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Authors’ contributions

RA: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing—original draft, Writing—review and editing. RH: Data curation, Formal analysis, Investigation, Methodology, Resources, Writing—original draft, Writing—review and editing. LA: Data curation, Methodology, Resources, Validation, Writing—review and editing. NA: Data curation, Methodology, Resources, Validation, Writing—review and editing. FO and MA: Investigation, Methodology, Resources, Writing—review and editing. HA: Methodology, Resources, Validation, Writing—review and editing. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analysed during this study are included in this published article. All relevant raw data will be freely available to any researcher wishing to use them for non-commercial purposes, without breaching participant confidentiality using the following link below: (https://mega.nz/ file/ bHoSn).

Declarations

Ethics approval and consent to participate

The study was approved by the ethics committee of Jordan University of Science and Technology (IRB# 19/85/2015). All patients consented to the use of their teeth data and signed an informed consent form for this purpose.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests in relation to the present study.
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