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· 基础研究 ·

2个非综合征型先天缺牙家系的MSX1基因突变检测与分析

丁婷婷[1]，　刘浩辰[2]
1. 国家儿童医学中心 首都医科大学附属北京儿童医院口腔科，北京（100045）；　2. 北京大学口腔医学院·口腔医院修复科，国家口腔医学中心，国家口腔疾病临床医学研究中心，口腔生物材料和数字诊疗装备国家工程研究中心，北京（100081）

【摘要】 **目的**　对2个非综合征型先天缺牙家系进行突变筛查及分析，为先天缺牙的诊断和治疗提供依据。**方法**　本研究已通过单位医学伦理委员会审查批准，并获得患者知情同意。收集了2个非综合征型先天缺牙核心家系的信息及血液样本，另外收集了100例正常对照的血液样本。通过外显子测序及Sanger测序来探索致病基因突变。使用预测软件Polyphen-2、CADD及fammth分析所发现的突变的致病性。使用Mupro、DUET和I-Mutant软件预测突变对蛋白质稳定性的影响。使用保守性分析及蛋白质二维/三维结构分析来预测突变对蛋白质功能的影响。使用DeepLoc 2.1软件预测突变蛋白对亚细胞定位的影响。**结果**　在本研究2个家系中发现了肌节同源异性盒基因1（muscle segment homeobox 1，MSX1）的2个新突变：c.547C > A（p.Gln183Lys）和c.854 T > C（p.Val285Ala）。Polyphen-2、CADD及fammth预测这2个突变存在致病性，ACMG分类这2个突变可能致病。保守性分析显示这2个突变位点（Gln183和Val285）位于进化过程中高度保守区域。蛋白质稳定性预测这2个突变对蛋白质稳定性造成影响。蛋白质二维结构分析显示这2个突变会对蛋白质二维结构造成影响。三维结构分析显示这2个突变造成了三维结构的改变。软件预测这2个突变对蛋白质的亚细胞定位没有影响。**结论**　本研究首次报道了MSX1基因的2个突变（c.547C > A和c.854 T > C）可以导致先天缺牙，为先天缺牙诊断和治疗提供了依据。

【关键词】 先天缺牙；　基因突变；　肌节同源异性盒基因1；　遗传病；　发育异常；　突变筛查；　外显子测序；　遗传咨询

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Detection and analysis of MSX1 gene mutations in two families with non-syndromic tooth agenesis

DING Tingting[1], LIU Haochen[2].　1. Department of Stomatology, Beijing Children's Hospital, Capital Medical University, National Center for Children's Health, Beijing 100045, China;　2. Department of Prosthodontics, Peking University School and Hospital of Stomatology & National Center for Stomatology & National Clinical Research Center for Oral Diseases & National Engineering Research Center of Oral Biomaterials and Digital Medical Devices & Central Laboratory, Beijing 100081, China
Corresponding author: LIU Haochen, Email: lhc@bjmu.edu.cn, Tel: 86-10-82195393

【Abstract】 **Objective**　To screen and analyze mutations in two families with non-syndromic tooth agenesis, providing a theoretical basis for the diagnosis and treatment of tooth agenesis. **Methods**　This study was reviewed and approved by the Medical Ethics Committee, and informed consent was obtained from patients. Information and blood samples from two core families with non-syndromic congenital tooth agenesis were collected, along with blood samples from

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【作者简介】丁婷婷，主治医师，博士，Email：dingtingting@bch.com.cn
【通信作者】刘浩辰，副主任医师，博士，Email：lhc@bjmu.edu.cn，Tel：86-10-82195393

100 normal controls. Pathogenic gene mutations were explored through whole exome sequencing and Sanger sequencing. The pathogenicity of the identified mutations was analyzed using prediction software Polyphen-2, CADD, and FAMMTH. The impact of the mutations on protein stability was predicted using Mupro, DUET, and I-Mutant software. Conservation analysis and protein 2D/3D structure analysis were used to predict the impact of mutations on protein function. The impact of the mutant proteins on subcellular localization was predicted using DeepLoc 2.1 software. **Results**　We identified two novel mutations in the muscle segment homeobox 1 (MSX1) gene: c.547C>A (p.Gln183Lys) and c.854T>C (p.Val285Ala) in the two families. Polyphen-2, CADD, and FATHMM predicted these mutations to be pathogenic, and ACMG classified these mutations as likely pathogenic. Conservation analysis showed that the two mutation sites (Gln183 and Val285) are located in highly conserved regions during evolution. Protein stability predictions indicated that these mutations influence protein stability. Protein 2D structure analysis indicated that these two mutations affect the 2D structure of the protein. 3D structure analysis showed that these two mutations can cause changes in the 3D structure. Software predictions indicated that these mutations do not affect the subcellular localization of the protein. **Conclusion** This study is the first to report two novel mutations in the MSX1 gene (c.547C>A and c.854T>C) associated with tooth agenesis, providing a basis for clinical diagnosis and treatment of congenital tooth loss.

【Key words】　tooth agenesis; gene mutation; muscle segment homeobox 1; genetic disorder; developmental anomaly; mutation screening; exome sequencing; genetic counseling

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【Competing interests】　The authors declare no competing interests.

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先天缺牙是一种口腔临床最常见的发育性疾病，在不同种族和人群中的发生率为2.2%～10.1%[1-2]。先天缺牙可以作为一种孤立的/非综合征性情况发生，也可以作为综合征的一部分出现，如外胚层发育不良综合征（ectodermal dysplasia syndrome）、Van der Woude综合征或Axenfeld-Rieger综合征等[3-5]。先天缺牙是一种复杂的多因素疾病。遗传因素和环境因素如创伤、感染、毒素和营养缺乏等，都可能导致牙齿缺失[6-7]。其中，遗传因素被认为是主要的致病因素[8-9]。

先天缺牙的致病基因一直是研究的热点。综合征型先天缺牙的致病基因一般都比较明确，而非综合征型先天缺牙的致病基因比较复杂，单基因或多基因协同作用都有可能导致非综合征型先天缺牙的发生[9-10]。目前，已确定几种基因变异是非综合征性牙齿缺失的原因。这些基因包括肌节同源异性盒基因1（muscle segment homeobox 1，MSX1）、轴抑制蛋白2（axis inhibition protein 2，AXIN2）、配对盒基因1（paired box 9，PAX9）、外异蛋白A基因（ectodysplasin A，EDA）、外异蛋白A受体基因（ectodysplasin A receptor，EDAR）、EDAR相关死亡结构域蛋白基因（EDAR-associated death domain，EDARDD）、无翼型小鼠乳腺肿瘤病毒整合位点家族成员10A（wingless-type mouse mammary tumor virus integration site family member 10A，WNT10A）和无翼型小鼠乳腺肿瘤病毒整合位点家族成员10B（wingless-type mouse mammary tumor virus integration site family member 10B，WNT10B）、低密度脂蛋白受体相关蛋白6（low-density lipoprotein receptor-related protein 6，LRP6）和成对样同源结构域转录因子2（paired-like homeodomain transcription factor 2，PITX2）等[11-17]。然而，目前仍有很多非综合征型先天缺牙患者无法找到其致病基因，需要不断深入研究才能帮助进一步理解非综合征型先天缺牙的病因机制。

本研究收集了2个非综合征型先天缺牙的家系，通过外显子测序检测致病基因突变，并对检测到的致病基因突变进行了验证和分析。进一步扩大了非综合征型先天缺牙的致病基因突变谱，有助于遗传咨询并加强医师对牙齿发育的理解。

1　资料与方法

1.1　研究对象

本研究招募的2个家系先证者为2021年至2024年在北京大学口腔医院的修复科就诊患者。对先证者进行了口腔检查及拍摄曲面断层片来确定缺牙的数目。对家系的其他成员进行了口腔检查或通过远程问诊及复习病例确定是否有先天缺牙的情况。由首都医科大学附属北京儿童医院内科、外科、皮肤科、耳鼻喉科及眼科的医生对先证

者进行全身情况的检查，判断是否存在除牙齿外其他器官的病变。100名牙齿发育正常的志愿者作为正常对照（男、女各50名）。所有参与本研究的人员均签署了知情同意。本研究已获得北京大学口腔医院医学伦理委员会的批准（PKUSSIRB-202162021）。

1.2　研究方法

1.2.1　外显子测序　收集了参与2个家系成员的静脉血。使用全血基因组DNA提取试剂盒（百泰克，北京，中国）提取基因组DNA。将先证者家系成员的DNA送至北京安琪儿基因医学科技有限（北京，中国）使用Illumina-X10平台进行全外显子测序（whole exome sequencing，WES）。在WES结果中，注释并筛选出口腔发育相关基因[18]。然后，根据数据库中最小等位基因频率（minor allele frequency，MAF）≤0.01的标准筛选了所有非同义单核苷酸变异和插入/缺失，使用数据库包括单核苷酸多态性数据库（dbSNP，https: //www.ncbi.nlm.nih.gov/snp/）和基因组聚合数据库（gnomAD，http: //gnomad.broadinstitute.org）。

对于筛选出的突变位点，使用在线预测软件Polyphen-2（http: //genetics.bwh.harvard.edu/pph2/）、CADD（https: //cadd. gs. washington. edu/）及fammth（http: //fathmm.biocompute.org.uk）预测突变对蛋白质功能的影响。

1.2.2　突变基因测序　为了验证WES结果，使用Sanger测序对先证者家系及正常对照的人群的MSX1（NM_002448）基因外显子进行了测序。MSX1基因的外显子和内含子-外显子边界通过聚合酶链反应（polymerase chain reaction，PCR）用特定引物进行扩增（可根据要求提供引物）。PCR产物由北京擎科生物科技有限公司（北京，中国）进行测序。

1.2.3　氨基酸序列保守性分析　从NCBI数据库（https: //www.ncbi.nlm.nih.gov/）获取了不同物种的Msx1的蛋白质序列。使用MEGA（Molecular Evolutionary Genetics Analysis）软件进行多序列对比。使用ClustalW2软件分析突变位点进化过程中的保守性。

1.2.4　蛋白质稳定性预测　为了预测突变蛋白质的稳定性。使用了在线预测软件Mupro（http: //mupro.proteomics.ics.uci.edu/）、DUET（https: //biosig.lab.uq.edu.au/duet/stability；DUTE预测结果包括mCSM方法、SDM方法及总体的预测结果）和I-Mutant（https: //folding.biofold.org/i-mutant/i-mutant2.0.html）预测了突变对蛋白质稳定性造成的影响。

1.2.5　蛋白质二维结构分析　使用了PsiPred 4.0（http: //bioinf.cs.ucl.ac.uk/psipred）在线软件预测了野生型和突变的MSX1蛋白质二维结构，并进行了对比。

1.2.6　蛋白质三维结构分析　使用AlphaFold蛋白质结构数据库（https: //alphafold. ebi. ac. uk/）预测MSX1（NP_002439.2）蛋白质三维结构。使用PyMol v2.1软件分析突变造成的MSX1蛋白质三维结构改变。

1.2.7　突变蛋白亚细胞定位预测　为预测突变会不会影响蛋白的亚细胞定位，使用了DeepLoc 2.1（https: //services. healthtech. dtu. dk/services/DeepLoc-2.1/）预测突变蛋白的亚细胞定位情况。

2　结　果

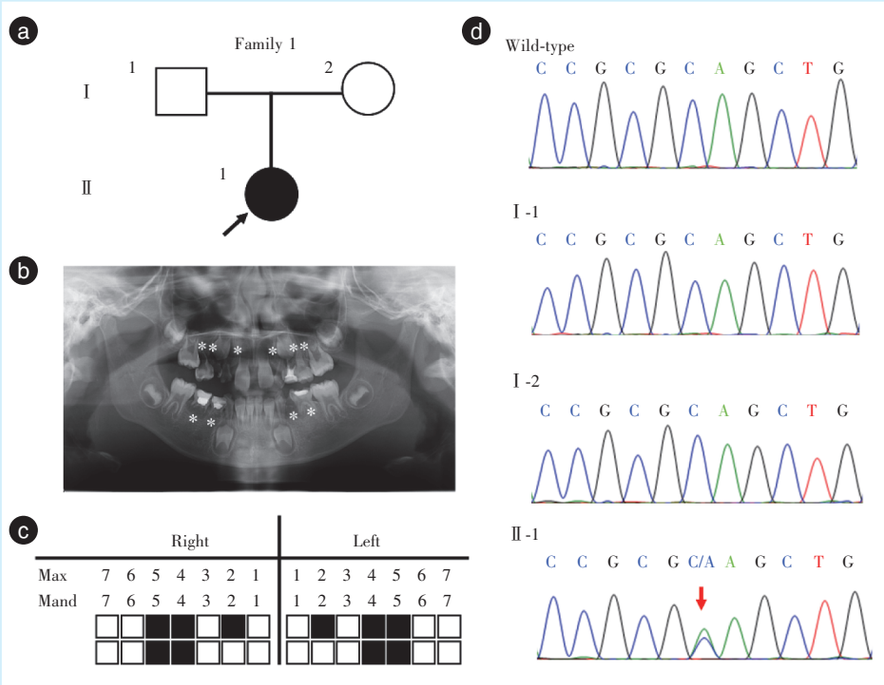
2.1　临床检查及突变基因筛查结果

家系1的先证者是1名8岁的女孩，存在先天恒牙缺失的症状，其父母是正常人，没有先天缺牙的症状（图1a）。经检查发现患者先天恒牙缺失10颗（图1b & 1c），没有发现其他器官的病变，诊断为非综合征型先天缺牙。通过WES和Sanger测序，发现患者携带MSX1基因的杂合错义突变（c.547C > A），而患者的父母的基因型与野生型基因型相同（图1d）。结果符合遗传共分离，患者携带的突变为新发突变。

家系2的先证者是1名8岁的女孩，存在先天恒牙缺失的症状，她的母亲也存在缺牙症状，她的父亲没有缺牙症状（图2a）。经检查发现患者先天恒牙缺失2颗（图2b & 2c），没有发现其他器官的病变，诊断为非综合征型先天缺牙。先证者的母亲恒牙先天缺牙4颗（图2d）。通过WES和Sanger测序，发现先证者和她的母亲存在MSX1基因的杂合错义突变（c.854 T > C），她的父亲的基因型与野生型相同（图2e）。结果符合遗传共分离，先证者的致病基因遗传自她的母亲。

2.2　MSX1突变对功能影响预测分析

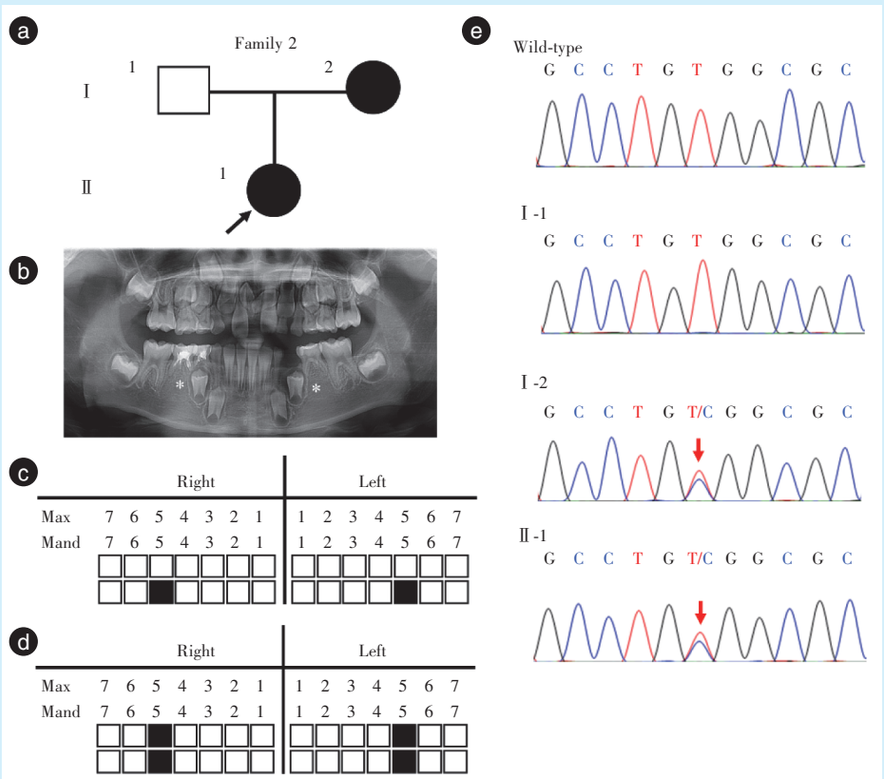
通过数据库检索，发现检出的MSX1突变（c.547C > A和c.854 T > C）在数据库中（gnomAD、dbSNP）均没有检出，提示发现的2个突变是MSX1的新突变。预测软件PolyPhen-2、CADD和fammth均显示这2个突变可能对蛋白质功能产生影响。根据美国医学遗传学与基因组学学会（The Ameri-



a: pedigree chart of the family; b: panoramic radiograph of the proband; c: schematic diagram of the missing teeth positions in the proband; d: Sanger sequencing results showing that the proband carries a heterozygous missense mutation in the MSX1 gene (c.547C > A), with both parents having the wild-type genotype. Asterisks and solid squares indicate congenitally missing teeth; Max: maxillary; Mand: mandibular

Figure 1　Clinical examination results and sequencing diagram of Family 1 with non-syndromic tooth agenesis

图1　非综合征型先天缺牙家系1的临床检查结果及测序图



a: pedigree chart of the family; b: panoramic radiograph of the proband; c: schematic diagram of the missing teeth positions in the proband; d: schematic diagram of the missing teeth positions in the proband's mother; e: Sanger sequencing results showing that the proband and her mother carry a heterozygous missense mutation in the MSX1 gene (c.854 T > C), while her father has the wild-type genotype. Asterisks and solid squares indicate congenitally missing teeth; Max: maxillary; Mand: mandibular

Figure 2　Clinical examination results and sequencing diagram of Family 2 with non-syndromic tooth agenesis

图2　非综合征型先天缺牙家系2的临床检查结果及测序图

can College of Medical Genetics and Genomics, ACMG）的标准分类，这2个变异是可能致病的（likely pathogenic）（表1）。

2.3　突变位点保守性分析结果

所发现的2个突变（c.547C > A和c.854 T > C）造成了MSX1蛋白的氨基酸改变（p.Gln183Lys和

表1　MSX1突变（c.547C > A和c.854 T > C）的功能影响预测

Table 1　Functional impact prediction of MSX1 mutations (c.547C > A and c.854 T > C)

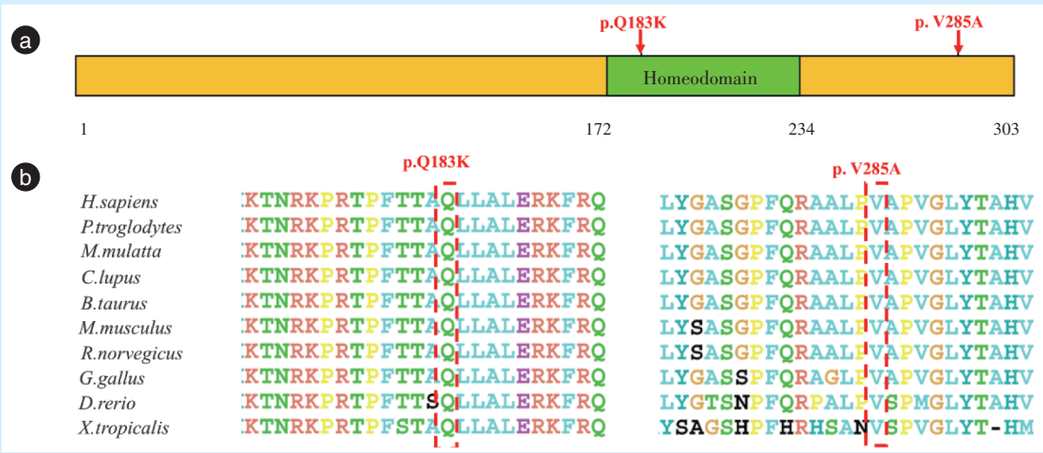
Nucleotide change	Protein change	Exon	Variation type	PolyPhen-2	CADD	Fammth	gnomAD, dbSNP	ACMG classification
c.547C > A	p.Gln183Lys	2	Missense	Probably damaging	Likely deleterious	DAMAGING	Not found	Likely pathogenic
c.854 T > C	p.Val285Ala	2	Missense	Probably damaging	Likely deleterious	DAMAGING	Not found	Likely pathogenic

Online prediction software Polyphen-2 (http://genetics.bwh.Harvard.edu/pph2/), CADD (http://cadd.gs.washington.edu/), and Fammth (http://fathmm.biocompute.org.uk) were used to predict the impact of mutations on protein function. All non-synonymous single nucleotide variations and insertions/deletions were screened using databases including single nucleotide polymorphism database (dbSNP, https://www.ncbi.nlm.nih.gov/snp/) and genome aggregation database (gnomAD, http://gnomad.broinstitute.org). ACMG: The American College of Medical Genetics and Genomics. MSXI: muscle segment hombox 1

p.Val285Ala）。其中p.Gln183Lys位于MSX1蛋白重要的结构域Homeodomain上，而p.Val285Ala位于蛋白质的尾部。保守性分析结果显示Gln183和Val285在进化过程中高度保守（图3）。提示这2个突变对蛋白质功能可能产生影响。

2.4　突变蛋白质稳定性分析结果

使用了不同软件预测突变后蛋白质稳定性的变化。发现对于p.Gln183Lys和p.Val285Ala在5个预测中均有4个预测显示蛋白质稳定性出现降低（表2），提示这2个突变对蛋白质稳定性造成影响。



The protein sequences of MSX1 from different species were obtained from the NCBI database (https://www.ncbi.nlm.nih.gov/). Multiple sequence comparisons were performed using Molecular Evolutionary Genetics Analysis software. ClustalW2 software was used to analyze the conservation during the evolution of mutation sites. a: schematic diagram of the wild-type human MSX1 protein and the distribution of the two mutations identified in this study; b: conservation analysis of the affected amino acids in MSX1 protein. MSXI: muscle segment hombox 1; Q183K: Gln183Lys; V285A: Val285Ala

Figure 3　Location and conservation analyses of MSX1 mutation (p.Gln183Lys and p.Val285Ala)

图3　MSX1蛋白质突变（p.Gln183Lys和p.Val285Ala）的位置和保守性分析

表2　MSX1蛋白质突变（p.Gln183Lys和p.Val285Ala）对蛋白质稳定性影响预测

Table 2　Prediction of the impact of MSX1 protein mutations (p.Gln183Lys and p.Val285Ala) on protein stability

Mutation	Mupro (ΔΔG)	DUET predicted stability change (ΔΔG)	mCSM predicted stability change (ΔΔG)	SDM predicted stability change (ΔΔG)	I-Mutant (ΔΔG)
Gln183Lys	−1.171 037 9 kcal/mol (Decreased stability)	0.183 kcal/mol (Stabilizing)	−0.088 kcal/mol (Destabilizing)	−0.41 kcal/mol (Destabilizing)	−0.61 kcal/mol (Decreased stability)
Val285Ala	−0.899 2306 8 kcal/mol (Decreased stability)	−0.075 kcal/mol (Destabilizing)	−0.405 kcal/mol (Destabilizing)	0.05 kcal/mol (Stabilizing)	−2.9 kcal/mol (Decreased stability)

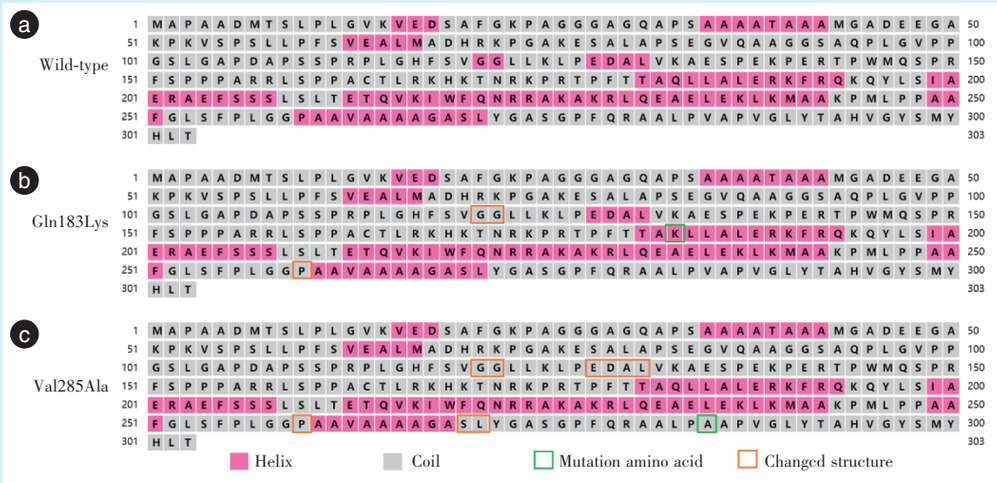
Mupro (http://mupro.proteomics.ics.uci.edu/), I-Mutant (https://folding.biofold.org/i-mutant/i-mutant2.0.html), and DUET (https://biosig.lab.uq.edu.au/duet/stability) were used to predict protein stability. The prediction results from DUET include the mCSM method and SDM method. MSXI: muscle segment hombox 1

2.5　突变蛋白质二维结构分析

二维结构预测分析显示，较野生型 Gln183Lys 和 Val285Ala 的突变蛋白的二维结构出现改变（图 4），提示这 2 个变异会对蛋白质的稳定造成影响。

2.6　突变蛋白质三维结构分析

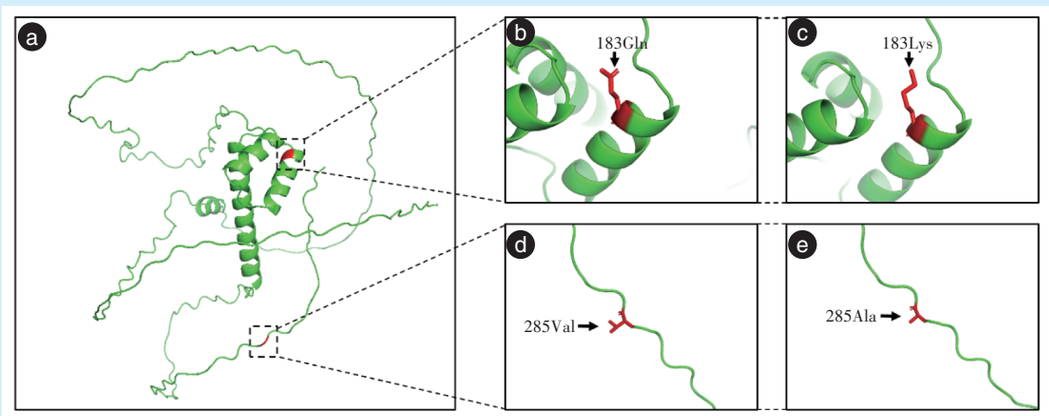
预测出的 MSX1 蛋白三维结构见图 5a。该蛋白质序列中第 183 位的残基是谷氨酰胺（Gln），其侧链为中性（图 5b）。变异残基是赖氨酸（Lys），其侧链带正电荷，使其具有亲水性（图 5c）。该蛋白质序列中第 285 位的残基是缬氨酸（Val），其侧链是脂肪族的，具有疏水性（图 5d）。变异残基是丙氨酸（Ala），其侧链也是脂肪族的，同样具有疏水性（图 5e）。三维分析结果显示 2 个突变（p.Gln183Lys



The two-dimensional structures of wild-type and mutant MSX1 proteins were predicted and compared using the online software PsiPred 4.0 (http://bioinf.cs.ucl.ac.uk/psipred). a: predicted two-dimensional structure of the wild-type MSX1 protein; b: predicted two-dimensional structure of the MSX1 protein with the p.Gln183Lys mutation; c: predicted two-dimensional structure of the MSX1 protein with the p.Val285Ala mutation. MSXI: muscle segment hombox 1

Figure 4　Two-dimensional structure predictions of MSX1 protein mutations (p.Gln183Lys and p.Val285Ala)

图 4　MSX1 蛋白质突变（p.Gln183Lys 和 p.Val285Ala）二维结构预测



The AlphaFold protein structure database (https://alphafold.ebi.ac.uk/) was used to predict the three-dimensional structure of MSX1 (NP_002439.2) protein. PyMol v2.1 software was used to analyze the three-dimensional structural changes of MSX1 protein caused by mutations. a: three-dimensional structure of wild-type MSX1 protein; b: structure of 183Gln in MSX1; c: structure of 183Lys in WNT10A; d: structure of 285Val in MSX1; e: structure of 285Ala in MSX1. MSXI: muscle segment hombox 1

Figure 5　Three-dimensional structural analysis of two MSX1 protein mutations (p.Gln183Lys and p.Val285Ala)

图 5　MSX1 蛋白质两种质突变（p.Gln183Lys 和 p.Val285Ala）的三维结构分析

和p.Val285Ala）对MSX1蛋白质三维构象均有改变，其中p.Gln183Lys对功能影响更大。

2.7　突变蛋白质亚细胞定位预测

DeepLoc 2.1预测结果显示，野生型MSX1蛋白和突变的MSX1蛋白（p.Gln183Lys和p.Val285Ala）亚细胞定位均为细胞核，提示这2个突变对蛋白质的亚细胞定位可能没有影响。

3　讨　论

MSX1基因属于同源框基因家族，位于染色体4p16.2[19]。它编码的蛋白质在Wnt和BMP4信号通路中作为转录抑制因子发挥作用[20-21]。MSX1在胚胎发育过程中在上皮-间充质相互作用中起着关键作用[22]。Msx1基因敲除的小鼠表现出牙齿发育停止、牙槽骨丧失和腭裂等症状[23]。在人类中，MSX1基因的变异与非综合征型牙齿缺失（Online Mendelian Inheritance in Man，OMIM #106600）、非综合征性唇裂伴或不伴腭裂（OMIM #608874）、Witkop综合征（OMIM #189500）和Wolf-Hirschhorn综合征（OMIM#194190）相关[24-27]。

非综合征型牙齿缺失由MSX1变异引起的遗传模式为常染色体显性遗传[17, 28-30]。在本研究中，家系1中的先证者为杂合错义突变（c.547C＞A）。先证者父母的基因型和表型均正常。推测先证者的变异为新发变异，这与常染色体显性遗传模式一致。在家系2中，先证者和先证者的母亲均表现出非综合征性牙齿缺失的表型，基因型为杂合错义变异（c.854T＞C）。先证者的父亲的基因型和表型均正常，推测先证者的突变基因遗传自母亲，这与常染色体显性遗传模式一致。非综合征性牙齿缺失中的MSX1基因变异主要有2种类型，即错义变异和移码变异[28-29]。Zheng等[29]的研究表明，MSX1的错义变异均发生在第二外显子的高度保守区域。本研究中发现的2个新变异位于MSX1基因的第二外显子，编码的氨基酸在进化过程中高度保守。本研究结果与这一现象一致。氨基酸的高度保守区域是结构或功能重要区域，是发现错义变异的热点区域[31]。本研究结果进一步证实了MSX1第二外显子的高度保守区域是错义变异的热点。

MSX1蛋白由多个结构域组成，其中最重要的是同源域（Homeodomain），它是一个高度保守的DNA结合结构域[32]。发生在同源域区域的变异会影响MSX1蛋白的核定位，从而影响细胞功能，对蛋白质功能有较大影响[33]。本研究中发现的一个突变c.547C＞A（p.Gln183Lys）位于同源结构域上，而另一个突变c.854 T＞C（p.Val285Ala）不在这个结构域上。从表型来说家系1的先证者c.547C＞A（p.Gln183Lys）先天缺失10颗恒牙，而家系2的先证者c.547C＞A（p.Gln183Lys）先天缺失2颗恒牙，从表型上来看位于同源结构域上的突变造成的表型更重。进一步证实了同源结构域对MSX1功能影响更大。以往研究显示移码突变会影响MSX1的亚细胞定位，从而影响蛋白质的功能，而错义突变的MSX1蛋白能进入细胞核[28-29]。通过软件预测了这2个突变蛋白（p.Gln183Lys和p.Val285Ala）的亚细胞定位情况，发现这2个突变定位情况与野生型一样，均能进入细胞核。有研究显示突变的MSX1能进入细胞核，但是会影响部分转录激活能力，从而造成疾病的发生[19]。本研究的2个突变可能会影响蛋白质部分功能，这个需要后续的实验进行验证。

MSX1单倍剂量不足也是引起疾病发生的机制之一[19, 34]。突变蛋白的不稳定会影响蛋白质的合成量，从而导致疾病的发生。预测了2个突变蛋白（p.Gln183Lys和p.Val285Ala）的稳定性，发现突变以后蛋白质稳定性受到了影响。二维结构分析来看2个突变（p.Gln183Lys和p.Val285Ala）经预测均能造成蛋白质的改变，可能会影响蛋白质的稳定性。进一步的三维结构分析显示，p.Gln183Lys的突变造成了谷氨酰胺到赖氨酸的改变，侧链由中性变为亲水性；而p.Val285Ala的突变由缬氨酸变为丙氨酸，同样具有疏水性。推测这突变p.Gln183Lys对蛋白质功能影响更大。这些结果说明突变后蛋白质稳定性降低，合成量减少，从而可能导致疾病的发生。由于p.Gln183Lys对蛋白质功能影响更大，其缺牙表型更严重。当然，具体的致病机制尚不明确，需要在未来进一步研究。

多个基因的变异可以导致先天性牙齿缺失。不同基因变异引起的先天性牙齿缺失不仅在缺失牙齿的数量和位置上有所不同，还可能伴有其他系统症状[7, 35-39]。Zheng等[29]的研究显示，MSX1突变造成的先天缺牙患者，最容易缺失的牙齿是上颌第二前磨牙、下颌第二前磨牙及上颌第一前磨牙。Zhao等[28]的研究显示MSX1突变患者最容易缺失第二前磨牙。本研究家系1的先证者缺失了8颗前磨牙及2颗上颌侧切牙，家系2中的先证者缺失了双侧下颌前磨牙，她的母亲缺失了4颗前磨牙。本研究患者的表型符合MSX1突变患者的缺

牙模式。综合以上分析，可以发现，前磨牙缺失是MSX1突变导致的非综合征型先天缺牙的最大特点。在临床诊断中，对于仅有前磨牙缺失的非综合征型先天缺牙患者，首先要怀疑是否是MSX1基因突变。

总的来说，本研究首次报道了MSX1基因的2个突变（c.547C＞A和c.854 T＞C），进一步扩展了该基因的变异谱。这个发现可以帮助进一步理解MSX1基因突变与先天缺牙的关系，并为临床诊断和遗传咨询提供帮助。本研究还存在很多不足，后续需要进行细胞水平及动物水平的实验来进一步验证的结果。

【Author contributions】 Ding TT performed the experiments and wrote the article. Liu HC recruited participants, designed the study and revised the article. All authors read and approved the final manuscript as submitted.

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