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Novel missense mutations in the *AXIN2* gene associated with non-syndromic oligodontia

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ABSTRACT

Objective: Oligodontia, which is the congenital absence of six or more permanent teeth excluding third molars, may contribute to masticatory dysfunction, speech alteration, aesthetic problems and malocclusion. To date, mutations in *EDA*, *AXIN2*, *MSX1*, *PAX9*, *WNT10A*, *EDAR*, *EDARADD*, *NEMO* and *KRT 17* are known to associate with non-syndromic oligodontia. The aim of the study was to search for *AXIN2* mutations in 96 patients with non-syndromic oligodontia.

Design: We performed mutation analysis of 10 exons of the *AXIN2* gene in 96 patients with isolated non-syndromic oligodontia.

Results: We identified two novel missense mutations (Exon 3 c.923C > T and Exon 11 c.2490G > C) in two patients. One mutation (c.923C > T) results in a Thr308Met substitution and the other mutation (c.2490G > C) results in a Met830Ile substitution.

Conclusions: This is the first report indicating that mutations in *AXIN2* are responsible for oligodontia in the Chinese population. Our findings indicate that *AXIN2* can be regarded as a candidate gene for mutation detection in individuals with non-syndromic oligodontia in the Chinese population.

1. Introduction

Oligodontia which is the congenital absence of six or more permanent teeth excluding third molar, has been reported to affect between 0.08% and 0.16% of humans in Scandinavian countries.[1,2] It can contribute to masticatory dysfunction, speech alteration, aesthetic problems and malocclusion.[3] Oligodontia may present as part of a syndrome; however, the isolated, non-syndromic form is more common.

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Abbreviations: *AXIN2*, axis inhibition protein 2; *EDA*, ectodysplasin A; *MSX1*, msh homeobox 1; *PAX9*, paired box 9; *WNT10A*, wingless-type *MMTV* integration site family member 10a; *EDAR*, ectodysplasin A receptor; *EDARADD*, EDAR-associated death domain; *NEMO*, nuclear factor-kappaB essential modulator; *KRT 17*, keratin 17; *Dkk1*, Dickkopf-related protein 1; *Lef1*, lymphoid enhancer-binding factor 1.

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Although several potential and verified environmental factors have been identified in tooth agenesis, genetic defects play a major role in the aetiology.[4] To date, mutations in *EDA*, *AXIN2*, *MSX1*, *PAX9*, *WNT10A*, *EDAR*, *EDARADD*, *NEMO* and *KRT 17*are known to cause isolated oligodontia.[5–14] Reports of the analysis of *AXIN2* mutations in isolated tooth agenesis are rare in comparison with investigations of other causative genes; therefore, in this study, we focused on the role of the *AXIN2* gene in isolated oligodontia.

Axin2 is a feedback inhibitor in the canonical Wnt pathway, involving targeted degradation of free β-catenin.[15] Somatic *AXIN2* mutations have been described in a number of human cancers, including skin, gastrointestinal, liver and ovarian.[16,17]

Germline *AXIN2* mutations were first identified in a Finnish four-generation family presenting with familial oligodontia and a predisposition to colorectal cancer with complete penetrance.[18] Subsequently, *AXIN2* mutations were reported in patients with syndromic or non-syndromic tooth agenesis.[7,19]

Therefore, we selected *AXIN2* as a candidate gene for screening oligodontia patients in the Chinese population. In this study, we identified two novel missense mutations in two subjects with non-syndromic oligodontia, both of which were located in highly conservative region of the encoded protein.

2. Materials and methods

2.1. Participants

Ninety-six non-consanguineous participants diagnosed with isolated oligodontia were recruited from the Department of Prosthodontics, Peking University School and Hospital of Stomatology and the Department of Prosthodontics, Beijing Stomatological Hospital (China). A total of 200 normal volunteers without tooth agenesis (100 males and 100 females) were selected as control individuals. This study was approved by the Institutional Review Board of Peking University School and Hospital of Stomatology (No. IRB0001052-07068). All of participants provided written consent to DNA sequencing analysis and the reproduction of radiographs.

2.2. DNA extraction

Genomic DNA was extracted from peripheral blood lymphocytes and buccal epithelial cells of the oligodontia patients and their relatives using the Biotek DNA minikit (Biotek, Beijing, China) according to manufacturers' instructions. Genomic DNA was extracted from the buccal epithelial cells of normal volunteers using swab DNA mini kit (Tiangen, Beijing, China) according to manufacturers' instructions.

2.3. Mutation detection

The entire coding region and intron–exon junctions of *AXIN2* genes were amplified by polymerase chain reaction (PCR) with Amplitaq Gold 360 Master Mix (Applied Biosystems, Foster City, USA). The PCR products were sent to Sangon Biotech Company (Beijing, China) for direct sequencing using a BigDye terminator v3.1 (Applied Biosystems) and a 3730 DNA sequencer (Applied Biosystems). Primer sequences and PCR conditions are available upon request. SeqMan Pro genetic analysis software (DNASTAR, Madison, USA) was used for sequencing analysis.

2.4. Conservation analysis

Multiple species amino acid sequence alignment analysis of the AXIN2 protein (NP_004646.3) was carried out using ClustalX 2.1 and Jalview. The Axin2 sequences of nine different vertebrate species were obtained from ENSEMBL.

3. Results

3.1. Clinical details

Oral examinations of all participants were performed by prosthodontists to record dentition status, as well as the shapes and sizes of residual teeth. Panoramic radiographs were taken to confirm the diagnosis and the number of missing teeth. Phenotypic characteristics of scalp and body hair, skin, nails, tolerance to heat, and ability to sweat were examined through observation, palpation, and inquiry. All participants reported normal sweating and lachrymal secretions. None of the participants reported dry mouth sensations, intolerance to heat, or susceptibility to respiratory tract

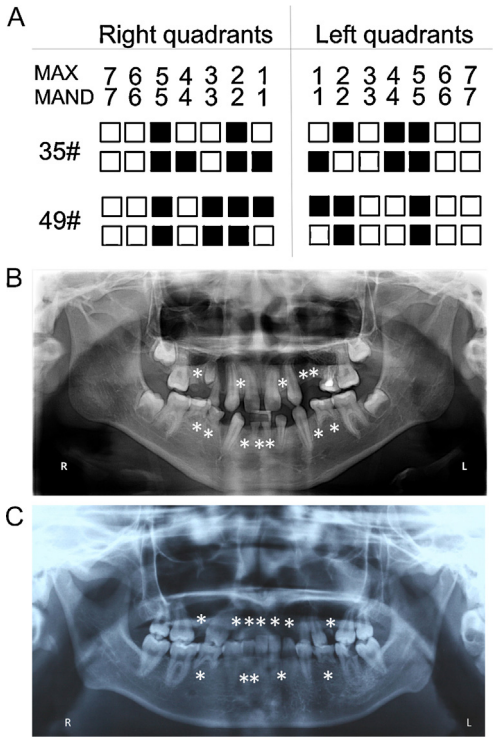

Fig. 1 – Clinical characteristics of non-syndromic oligodontia patients with *AXIN2* mutations. (A) Schematic presentation of congenitally missing teeth in two (35# and 49#) non-syndromic oligodontia patients with *AXIN2* mutations. The missing teeth are represented by a filled square. Max, maxillary; Mand, mandibular. (B) Panoramic radiograph of Patient 35#. (C) Panoramic radiograph of Patient 49#. * indicates the position of missing teeth.

infections. The participants had hair on the body and scalp, and their facial features, skin, and nails were observed to be normal.

Patient 35# was a 12-year-old female in good health. Clinical and radiographic examinations revealed that, in addition to retained deciduous teeth, the patient was missing a total of 12 permanent teeth, including five incisors and seven premolars (Fig. 1A and B). This individual had no other ectodermal abnormalities or systemic disease. There was no history of tooth agenesis or cancer in either of the maternal or paternal ancestries.

Patient 49# was a 22-year-old female with normal appearance. The clinical and radiographic examination of Patient 49# revealed oligodontia with agenesis of 12 teeth, including all the second premolars, as well as all upper incisors and two lower incisors (Fig. 1A and C). All other ectodermal organs appeared normal and the patient reported no family history of tooth agenesis or cancer.

3.2. *Mutation analysis*

In this study, mutation analysis of exons 2–11 of the *AXIN2* gene was conducted in 96 patients with non-syndromic oligodontia. Two novel missense mutations, c.923C > T (p.Thr308Met) and c.2490G > C (p.Met830Ile), were identified in two patients (35# and 49#).

For Patient 35#, the nucleotide sequence showed a heterozygous C to T transition at nucleotide 923 (c.923C > T) of the coding sequence in exon 3 of *AXIN2*, which results in the substitution of Thr at residue 308 to Met (Fig. 2A). Neither parent carried the mutation, suggesting that the mutation was *de novo*.

For Patient 49#, the nucleotide sequence showed a heterozygous G to C transition at nucleotide 2490 (c.2490G > C) of the coding sequence in exon 11 of *AXIN2*, which results in the substitution of Met at residue 830 to Ile (Fig. 2B). The participant's mother did not carry the mutation. The paternal sample was not available although a follow-up interview showed that her father had normal dentition. None of four grandparents was diagnosed with tooth agenesis. Therefore, this mutation is likely to have occurred *de novo*.

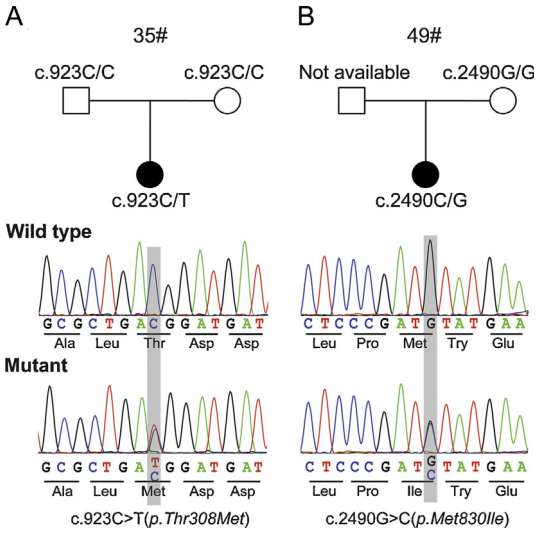

Fig. 2 – Sequence analyses of the *AXIN2* gene in two patients with congenital non-syndromic oligodontia. (A) A *de novo* heterozygous mutation c.923C > T was detected in Patient 35#. (B) A heterozygous mutation c.2490G > C was detected in Patient 49#.

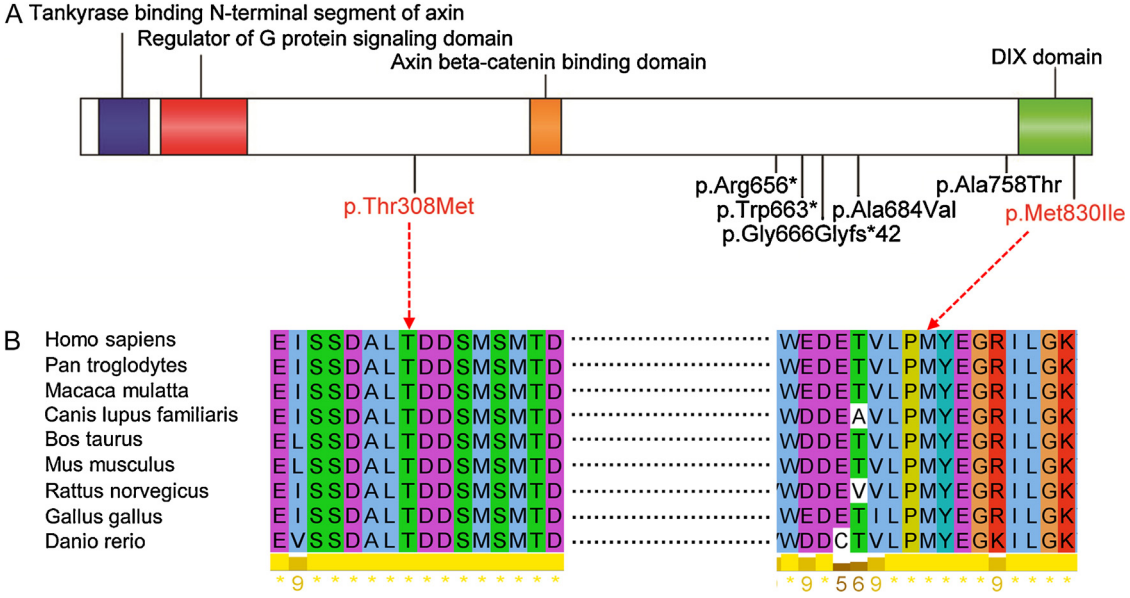

Fig. 3 – Structure and conservation analysis of the *AXIN2* mutations associated with oligodontia. (A) Distribution of *AXIN2* gene mutations identified in oligodontia. Mutations are marked at the corresponding positions of the gene. Red indicates the mutations identified in this study. Black indicates the mutations identified in previous studies. (B) Conservation analysis of nine different vertebrate species indicates Thr308 and Met830 in the AXIN2 protein are highly conserved. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

3.3. *Conservation analysis*

Thr308 is located between the Regulator of G protein signalling domain and the axin beta-catenin binding domain of the AXIN2 protein. Met830 is located at the DIX domain of the AXIN2 protein (Fig. 3A). Multiple species amino acid sequence alignment analysis of the AXIN2 protein showed that Thr308 and Met830 were highly conserved during evolution (Fig. 3B).

4. Discussion

In this study, we found two novel heterozygous *AXIN2* missense mutations (c.923C > T and c.2490G > C) in two Chinese patients with non-syndromic oligodontia. One mutation (c.923C > T) results in a Thr308Met substitution located between the Regulator of G protein signalling domain and the axin beta-catenin binding domain of the AXIN2 protein. The other mutation (c.2490G > C) results in a Met830Ile substitution located in the DIX domain of the AXIN2 protein. Conservation analysis of nine different vertebrate species indicated that Thr308 and Met830 in the AXIN2 protein were highly conserved, suggesting that the mutations identified in this study might affect the function of AXIN2 protein and lead to isolated oligodontia.

Axin2 forms part of the Wnt pathway, which plays a critical and evolutionary conserved role in directing cell fates during craniofacial morphogenesis.[20] Wnt signalling is essential for tooth development. A number of Wnt genes are expressed in the developing tooth and changes in their expression may be one of the factors determining tooth agenesis.[21,22] Ectopic expression of the extracellular Wnt antagonist *Dkk1* in oral epithelium arrests tooth development at the placode stage, whereas null mutants of *Lef1*, a transcription factor mediating Wnt signals, arrest tooth development at the bud stage.[23,24] Ectopic expression of *Lef1* or excess beta-catenin levels in oral epithelium lead to ectopic development of teeth.[25] Loss of function mutations in *adenomatous polyposis coli* (APC) leads to supernumerary teeth in humans.[26] APC and AXINs are components of the intracellular protein complex that antagonizes Wnt signal transduction by promoting degradation of beta-catenin, an intracellular messenger of Wnt signalling. In mouse embryos, *Axin2* is expressed intensively in the dental mesenchyme and enamel knots during odontogenesis, suggesting the involvement of *Axin2* in regulating tooth formation.[18] Thus, AXIN2 plays an important role in tooth development *via* the Wnt pathway and mutations in *AXIN2* may affect tooth development *via* this pathway.

AXIN2 mutations have been linked to cancer and developmental defects including tooth agenesis. In 2004, Lammi et al. identified a nonsense mutation c.1966C > T, resulting in Arg656Stop in the AXIN2 protein in a family with oligodontia and colorectal cancer or precancerous lesions of variable types. In addition, a *de novo* frameshift mutation (1994–1995insG) was identified in *AXIN2* in an unrelated young patient with severe tooth agenesis.[18] In 2009, Marvin et al. identified a family with a novel, inherited *AXIN2* mutation c.1989G > A, resulting in p.Tyr663Stop in the AXIN2 protein. This gene segregates in an autosomal dominant pattern with oligodontia and variable other clinical findings including colonic polyposis, gastric polyps, a mild ectodermal dysplasia phenotype with sparse hair and eyebrows, and early onset colorectal and breast cancers.[19] Bergendal et al. identified two novel *AXIN2* mutations (c.2272G > A and c.2051C > T) in 93 individuals with non-syndromic oligodontia (7–10 teeth absent in each individual); however, there was no history of colorectal cancer in either family.[7] In our study, all patients with *AXIN2* mutations and oligodontia were otherwise healthy. A follow-up interview revealed that none of the participants had a family history of cancer. In the two families diagnosed with oligodontia and cancer history, the mutations identified were nonsense mutation, resulting in truncation of the AXIN2 protein at the last three exons, including the DIX (Dishevelled and AXIN interacting) domain. These nonsense mutations were predicted to destroy the inhibitory action of AXIN2 on Wnt signalling. In other non-syndromic oligodontia cases, most of these mutations were missense mutation.[7,18] Therefore, we hypothesized that when the AXIN2 protein function is severely affected, individuals are likely to have oligodontia and cancer, whereas when AXIN2 protein function is affected mildly, individuals are prone to non-syndromic oligodontia. All of the mutations in *AXIN2* reported in previous studies were heterozygous mutations,[7,18,19] indicating that *AXIN2* mutations are inherited in an autosomal dominant pattern. Both of the two novel missense mutations of *AXIN2* identified in this study are heterozygous, thus confirming this hypothesis.

Regarding the number of missing teeth, we found that all of the individuals with *AXIN2* mutations, including those in previous studies,[7,18,19] were missing six or more permanent teeth, while there are no reports *AXIN2* mutations in individuals with hypodontia (missing less than six permanent teeth). Thus, in combination, these data suggest that *AXIN2* is crucial during tooth development and any change in AXIN2 protein can lead to severe tooth agenesis. Interestingly, in our study, both of the participants with novel heterozygous *AXIN2* missense mutations had four molars and four second molars, while, all subjects in previous studies reported the absence of at least one molar or second molar, thus suggesting that the dentition phenotype varies with different *AXIN2* mutations. The function of AXIN2 in tooth development is clearly complicated; therefore, the mechanism underlying this phenomenon remains to be investigated.

In conclusion, we identified two novel missense mutations of *AXIN2* in two patients diagnosed with non-syndromic oligodontia. This is the first report indicating that *AXIN2* mutation is responsible for oligodontia in the Chinese population. Our findings may provide evidence that *AXIN2* can be regarded as a candidate gene for mutation detection in individuals with non-syndromic oligodontia in the Chinese population. However, the precise role of *AXIN2* in tooth development remains to be elucidated.

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Conflict of interest

All of the authors declare that there are no conflicts of interest.

Ethical approval

This study was approved by the Institutional Review Board of Peking University School and Hospital of Stomatology (No. IRB0001052-07068).

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