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Tooth agenesis patterns and variants in *PAX9*: A systematic review

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ABSTRACT

Mutations in *PAX9* are the most common genetic cause of tooth agenesis (TA). The aim of this study was to systematically review the profiles of the TA and *PAX9* variants and establish their genotype-phenotype correlation. Forty articles were eligible for 178 patients and 61 mutations (26 in frame and 32 null mutations). *PAX9* mutations predominantly affected molars, mostly the second molar, and the mandibular first premolar was the least affected. More missing teeth were found in the maxilla than the mandible, and with null mutations than in-frame mutations. The number of missing teeth was correlated with the locations of the in-frame mutations with the C-terminus mutations demonstrating the fewest missing teeth. The null mutation location did not influence the number of missing teeth. Null mutations in all locations predominantly affected molars. For the in-frame mutations, a missing second molar was commonly associated with mutations in the highly conserved paired DNA-binding domain, particularly the linking peptide (100% prevalence). In contrast, C-terminus mutations were rarely associated with missing second molars and anterior teeth, but were commonly related to an absent second premolar. These finding indicate that the mutation type and position contribute to different degrees of loss of *PAX9* function that further differentially influences the manifestations of TA. This study provides novel information on the correlation of the *PAX9* genotype-phenotype, aiding in the genetic counseling for TA.
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1. Introduction

Tooth agenesis (TA) is the most common developmental anomaly with a prevalence of 2.5–13.4% [1,2]. The absence of less than six teeth is defined as hypodontia, the absence of more than six teeth is termed oligodontia, and the absence of all teeth is called anodontia. TA is determined by genetic and/or environmental factors and occurs as an isolated disease or related to syndromes.

Tooth development is regulated by several genes and signaling pathways [3]. Variants in odontogenic genes, such as the paired box 9 (*PAX9)* [4], axin inhibitor 2 (*AXIN2*) [5], ectodysplasin A (*EDA*) [6], kringle domain-containing transmembrane protein 1 containing the kringle domain (*KREMEN1*) [7], *MSX1* [8], paired-like homeodomain transcription factor 2 paired (*PITX2*) [9], and wingless-type MMTV integration site family member 10 A (*WNT10A*) [10] genes result in arrested tooth development.

PAX9 mutations (OMIM *167416) are the most common causes of non-syndromic TA with phenotypic variability [11]. The *PAX9* gene, a member of the paired-box transcription factor, is located on chromosome 14 (14q13.3) and consists of 5 exons encoding 341 amino acids. The PAX9 protein comprises a highly conserved paired DNA binding domain (N-terminal subdomain [NSD], the linking peptide, C-terminal subdomain [CSD]), and the octapeptide motif. *PAX9* is strongly expressed in the oral mesenchyme and required for the mesenchymal expression of *BMP4, MSX1,* and *LEF1* during tooth development [12]. It is also involved in the formation of the medial nasal process, the palatal shelf, and the maxilla [13]. *PAX9* loss-of-function in mice results in missing teeth, cleft palate, and skeletal abnormalities [14].

Genotype-to-phenotype prediction has been a central problem in human genetics with practical applications in different fields, such as personalized medicine aiming to predict disease risk and treatment outcomes for specific individuals. Furthermore, predicting how an individual phenotype varies is an important challenge in biology. More than 150 variants of the *PAX9* gene with more than 50 different types of mutation have been reported, including missense, frameshift, nonsense, deletion, and insertion mutations [15]. However, the *PAX9* genotype-phenotype correlation has not yet been fully established. Thus, the objective of this study was to systematically review the characteristics of TA associated with *PAX9* mutations, and to establish its genotype-phenotype correlation.

2. Materials and methods

2.1. Bibliographic search

This study was performed according to the PRISMA 2020 guideline for reporting systematic reviews [16]. The systematic search covered from January 2000 to July 2022 and included articles that reported TA associated with *PAX9* variants. The Pubmed and Scopus searches were performed using the terms “oligodontia” or ”hypodontia” or “tooth agenesis” and “PAX9″. After removing the duplicate articles, the English-language articles were screened based on their titles and abstracts. The relevant articles were included if they met the following criteria: 1) the *PAX9* mutation was identified as the TA-causative gene and 2) had an adequate description of the dental phenotype. The articles reporting *PAX9* polymorphisms, association studies, and literature reviews were excluded. The protocol was submitted to PROSPERO (CRD42022352625)(https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42022352625).

2.2. Data extraction and analysis

The data was extracted by three independent reviewers (NI, KaT, and KiT). The types and locations of the *PAX9* mutations and type, number, and location of the missing teeth were recorded for each patient. Oral/dental images and radiographs when available were used to confirm the in-text phenotypic description.

The mutations in *PAX9* (NCBI CCDS9662.1, NM_006194.4, NP_006185.1) were divided into 2 main groups: 1) in-frame mutations (missense mutations and in-frame insertions or deletions) and 2) null mutations (truncated mutations, nonsense mutations, out-of-frame insertions or deletions, and initiation codon mutations). The null mutations were defined as nonsense or frameshift mutations or mutations in a gene leading to its not being transcribed or less transcribed into RNA and/or not translated into a functional protein [15]. The mutation nomenclature was in accordance with the Human Genome Variation Society (HGVS) guideline [17].

The mutations were further classified according to their location on the gene or protein comprising the 5′untranslated region (5′UTR), N-terminus (amino acids 1–6), NSD (7–63), link peptide (64–81), CSD (82–130), C-terminus (132–341) and intron. The analyzed data was presented as the total number, location (maxilla vs. mandible or right vs. left), and type of missing teeth according to the type of mutation. The types of mutations per tooth type were also analyzed. The percentages of each missing tooth type were calculated from the total number of missing teeth analyzed and the total number of affected patients, and classified into three groups according to a previous study [11], consisting of the ‘common’, equal to/more than 50% absent; ‘less common’, 30–49% absent; and “rare,” less than 30% absent. For example, the term ‘common’ was given for the missing upper right second molar that was found in > 50% of the patients with TA. CI stands for central incisor, LI: lateral incisor, Ca: canine, PM1: first premolar, PM2: second premolar, Mo1: first molar, and Mo2: second molar.

2.3. Statistical analysis

The normally distributed data were analyzed by the independent t test, and nonnormally distributed data by the Mann-Whitney test (GraphPad Software, Inc., San Diego, CA, USA). The Chi-square test was used for comparing the prevalence of the missing teeth cases among specific missing teeth, by mutation type, and PAX9 regions (IBM SPSS Statistics for Windows, IBM Corp, NY). A significant difference was defined as $p\ value < 0.05$.

3. Results

Forty articles reporting patients with TA and *PAX9* variants were included for analysis (Fig. 1). Sixty-one *PAX9* mutations were identified, i.e., 29 in-frame and 32 null mutations in 178 patients (Fig. 2A, Supplementary Tables 1 and 2). The mutations were predominantly located in the paired DNA binding domain (PD) (73.7%, n = 45/61). Twenty-nine in-frame mutations were all missense, comprising 27 mutations (93.1%) in the PD and 2 mutations (6.9%) in the C-terminus. Within the PD, the mutations were predominantly present in

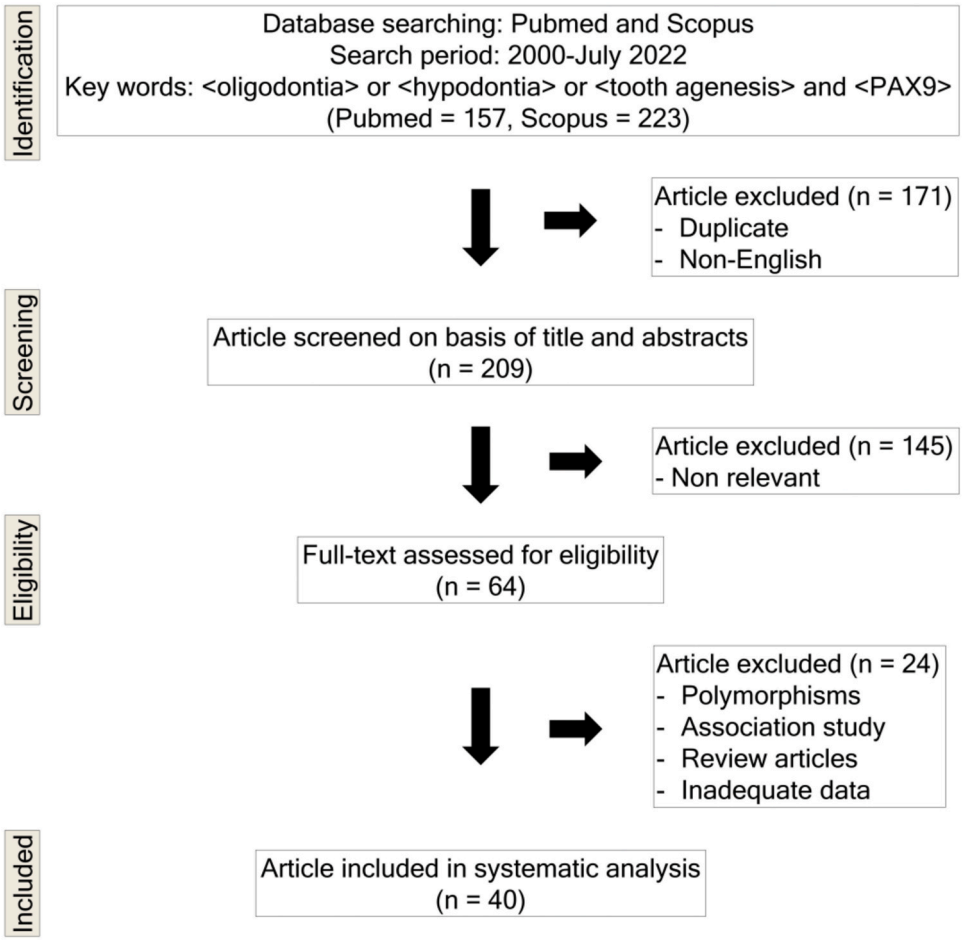


Fig. 1. Flow chart of the literature search and article selection process.

the NSD (63.0%), followed by the CSD (22.2%) and the linking peptide (14.8%). Regarding the null mutations, the PD accounted for 56.2% (n = 18/32) (27.8% NSD, 38.9% linking peptide, and 33.3% CSD), C-terminus for 25% (n = 8/32), N-terminus for 12.5% (n = 4/32), 5′UTR for 3.1% (n = 1 / 32) and intron 2 for 3.1% (n = 1/32) of the mutations.

3.1. The PAX9 mutations and the number of missing teeth

The average number of missing teeth in the *PAX9* patients was 10 teeth. In-frame mutations (n = 8) caused significantly fewer missing teeth compared with the null mutations (n = 11.6) (Fig. 2B). For the in-frame mutations, missing teeth were most commonly found in the link peptide (11.8 teeth), followed by the NSD (8.4 teeth), CSD (7.5 teeth), and C-terminus (4.7 teeth). The number of missing teeth with C-terminus mutations was the lowest and significantly lower than in the other regions (Fig. 2B).

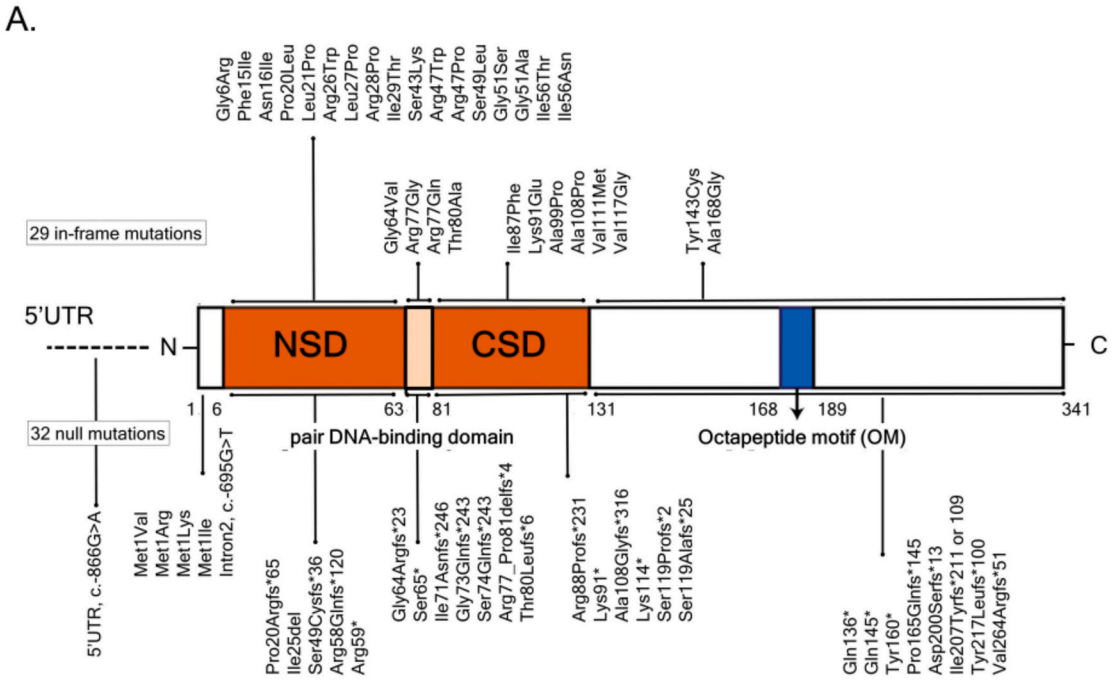
For the null mutations, missing teeth were found more in the N-terminus (12.9 teeth), followed by the CSD (12.2 teeth), C-terminus (12.1 teeth), linking peptide (11.8 teeth), intron 2 (10.7 teeth) NSD (9.7 teeth), and 5′UTR (8.7 teeth). In the CSD and C-terminus, null mutations caused more missing teeth than in-frame mutations (Fig. 2B). Considering the specific type of mutation, nonsense and frameshift mutations caused more missing teeth than missense mutations (Fig. 2C).

When evaluated by the dental arches, the total number of missing teeth in the maxilla (5.6 teeth) was significantly larger than in the mandible (4.4 teeth) (Fig. 3A). The in-frame and the null mutations consistently had more missing maxillary teeth than mandibular teeth. Likewise, when considering the locations and types of mutations, this trend was also observed for in-frame mutations in the NSD and null mutations in the N-terminus, NSD, linking peptide, and intron 2, as well as for missense, nonsense, and frameshift mutations. Within the C-terminus, null mutations caused more missing maxillary teeth than in-frame mutations. Moreover, nonsense and frameshift mutations resulted in more missing maxillary teeth than missense mutations, corresponding to the total missing teeth results (Fig. 3A, B).

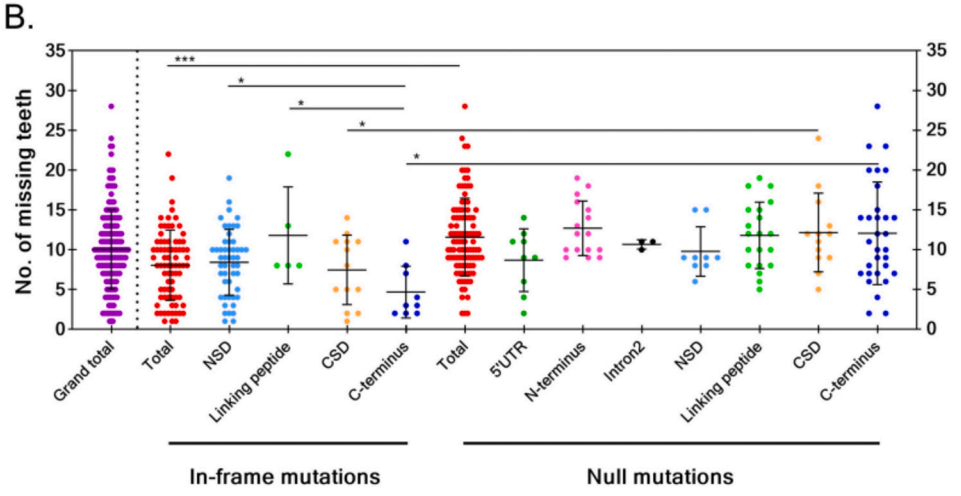
3.2. The PAX9 mutation and the dental agenesis profile

The number of *PAX9* patients and the types of missing teeth are presented in Fig. 4. Absent maxillary and mandibular Mo2 was the most consistent feature in *PAX9* patients with in-frame and null mutations, followed by maxillary Mo1 and maxillary PM2. The absence of posterior teeth was common in *PAX9* patients. A missing lower central incisor was also frequently observed, while a missing PM1, Ca, LI, and maxillary CI was less observed. Interestingly, a missing mandibular Mo1 was commonly seen with null mutations, whereas it was rarely found with in-frame mutations (Fig. 4A).

A pattern of missing teeth was noticeable for in-frame mutations. Within the PD, the mandibular CI was frequently absent with NSD and CSD mutations, but not the linking peptide, while a missing Mo1 (80%) and Mo2 (100%) was more common with linking peptide mutations compared with the NSD and CSD. Uniquely for the in-frame mutations in the C-terminus, a missing PM2 was common, but not Mo1 and Mo2. Furthermore, the absence of Mo1 or any upper anterior teeth was not observed (Fig. 4B).



Pair DNA-binding domain 6-131; N-terminal subdomain (NSD) 7-63; Linking peptide 64-81;
C-terminal subdomain (CSD) 82-130; Octapeptide motif (OM) 168-189



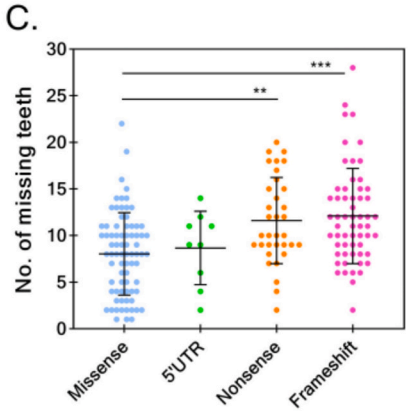


Fig. 2. The PAX9 protein domains, mutations, and number of missing teeth. (A) Mutations previously reported in the PAX9 protein domains. The mutations above the protein diagram were in-frame mutations, whereas the ones below were null mutations. (B, C) The number of missing teeth classified according to the PAX9 regions and types of mutations. The amino acid positions: N-terminus: 1–5, Paired DNA-binding domain: 6–131, N-terminal subdomain (NSD): 7–63, Linking peptide: 64–81, C-terminal subdomain (CSD): 82–130, C-terminus: 132–341, and Octapeptide motif (OM): 168–189. The data are illustrated as mean ± SD. A statistical difference was determined at $p \leq 0.05$. 5′ UTR: 5′ untranslated region.

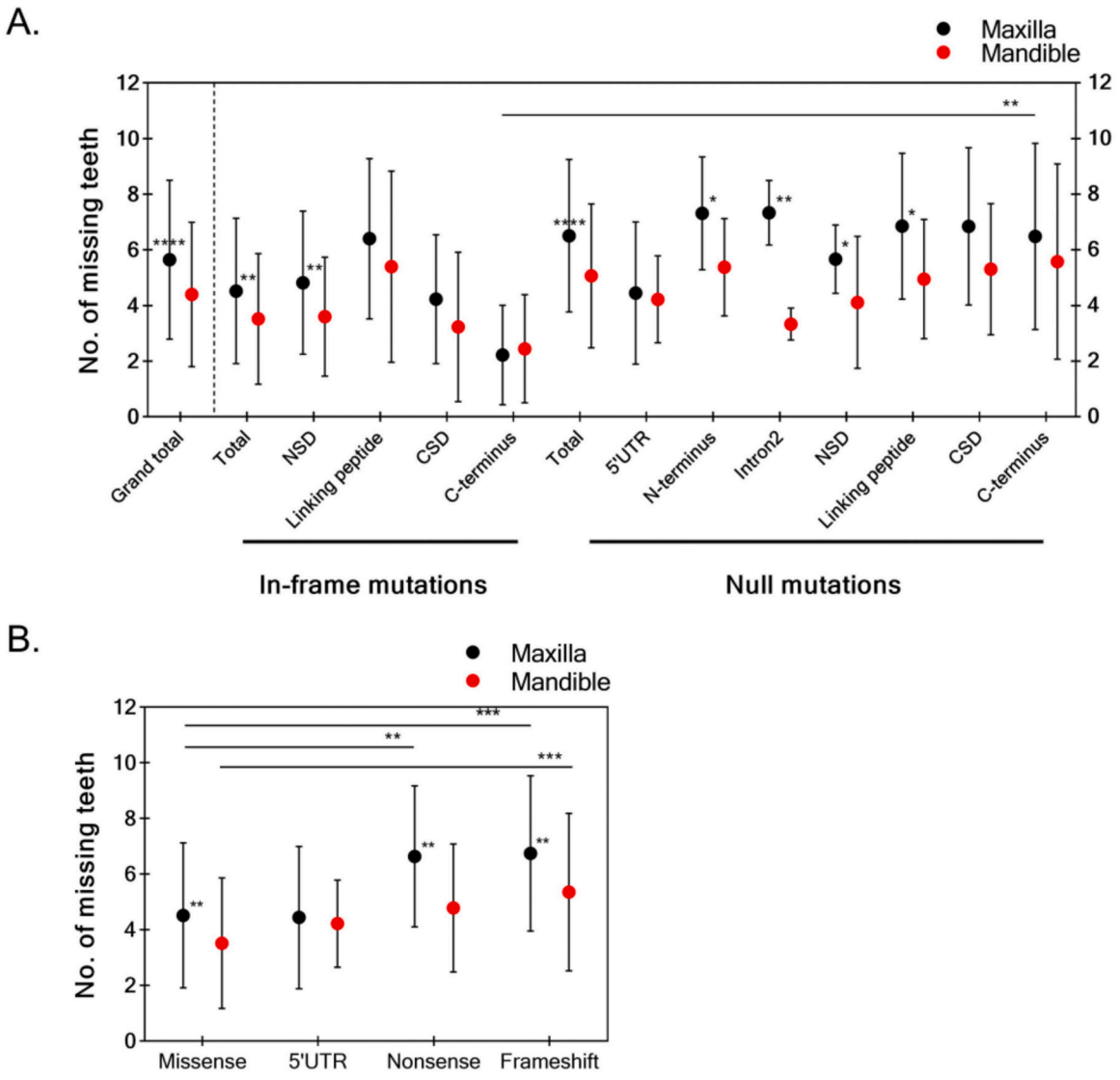


Fig. 3. The number of missing teeth in the maxilla and mandible classified by positions and types of *PAX9* mutations. The data is presented as mean ± SD. A statistical difference was determined at $p \leq 0.05$. $^{*}p \leq 0.05$, $^{**}p < 0.001$, $^{***}p < 0.0001$. NSD: N-terminal subdomain, CSD: C-terminal subdomain, 5′ UTR: 5′ untranslated region.

For the null mutations, the missing tooth pattern was comparable across the PAX9 regions. A missing mandibular CI missing was occasionally found. The absence of Mo2, maxillary Mo1, or maxillary PM2 was a common feature, except for the 5′UTR mutations that rarely caused a missing maxillary PM2. Furthermore, mutations in the 5′UTR resulted in much fewer missing Mo2 (56–89%) compared with the other regions. However, mutations in intron 2 generated a 100% absence of Mo2 (Fig. 4C).

Considering the number of affected individuals, there were more cases with missing maxillary teeth than cases with missing mandibular teeth for all tooth types, except for Mo2 (maxilla=mandible) and CI (mandible > maxilla). In contrast, the cases with missing teeth on the right or left sides were similar (Fig. 5B, Supplement Tables 3–5).

Regarding the types of mutations, significantly fewer patients with missense mutations has a missing upper Mo1 than those with null mutation, and significantly fewer missing lower Mo1 than those with frameshift and nonsense mutations (Fig. 5C and Supplement Table 6). Moreover, an upper Mo2 or PM2 were missing more often in patients with nonsense and frameshift mutations than those with missense and 5′UTR mutations.

Regarding in-frame mutations, linking peptide mutations had a missing Mo2 and mandibular Mo1, while the C-terminus mutations demonstrated a missing mandibular PM1 and PM2 more frequently than the other regions. Interestingly, Mo2, Mo1 and lower CI were missing the least often in the C-terminus (Fig. 5D, Supplement Table 7). A missing upper canine was found more frequently with NSD and linking peptide mutations than the CSD and C-terminus mutations.

For null mutations, a missing maxillary Mo2 and PM2 were found the least among 5′UTR mutations (Fig. 5E, Supplement Table 8). In contrast, the intron 2 mutations caused the larger number of missing maxillary LI and the lowest number of missing mandibular Mo1 compared with the other regions.

4. Discussion

PAX9 mutations are one of the most common genetic causes of TA with more than 50 different mutations reported [18,19]. The present systematic review revealed new insights into the *PAX9* genotype-phenotype correlation.

Genotypically, *PAX9* null mutations were observed throughout the protein with their majority in the PD, and almost all in-frame

A.

Mutation type	Location	Right							Left							No. of patients
		Mo2	Mo1	PM2	PM1	Ca	LI	CI	CI	LI	Ca	PM1	PM2	Mo1	Mo2	
In-frame	Maxilla	61	47	52	21	22	26	4	5	19	19	23	48	47	57	77
	Mandible	57	18	40	6	6	6	32	38	6	8	6	42	23	61	
Null	Maxilla	93	84	70	23	19	23	13	12	24	17	21	70	90	92	101
	Mandible	91	54	41	4	8	10	46	48	10	5	4	42	51	94	
Total	Maxilla	79	68	62	22	20	24	9	9	22	18	22	61	71	77	178
	Mandible	76	39	40	5	7	8	40	43	8	6	5	42	39	80	

Percent of case missing
- Common (≥50%)
- Less common (30-49%)
- Rare (1-29%)
- 0%

B.

In-frame mutations

Region	Location	Right							Left							No. of patients
		Mo2	Mo1	PM2	PM1	Ca	LI	CI	CI	LI	Ca	PM1	PM2	Mo1	Mo2	
NSD	Maxilla	62	48	52	18	30	30	6	6	22	24	22	52	50	60	50
	Mandible	60	14	38	4	8	6	38	46	8	10	6	38	18	66	
Linking peptide	Maxilla	100	80	40	20	20	40	0	20	40	40	20	40	80	100	5
	Mandible	100	80	40	0	20	0	20	20	20	20	0	40	80	100	
CSD	Maxilla	77	62	38	23	8	23	0	0	15	8	23	31	54	62	13
	Mandible	62	23	31	0	0	8	38	38	0	0	0	31	38	54	
C-terminus	Maxilla	11	0	78	33	0	0	0	0	0	0	33	56	0	11	9
	Mandible	11	0	67	33	0	11	0	0	0	0	22	78	0	22	
Total	Maxilla	61	47	52	21	22	26	4	5	19	19	23	48	47	57	77
	Mandible	57	18	40	6	6	6	32	38	6	8	6	42	23	61	

C.

Null mutations

Region	Location	Right							Left							No. of patients
		Mo2	Mo1	PM2	PM1	Ca	LI	CI	CI	LI	Ca	PM1	PM2	Mo1	Mo2	
5'UTR	Maxilla	67	78	22	22	11	22	0	0	22	11	33	11	89	56	9
	Mandible	89	33	33	0	11	11	33	44	11	0	0	33	44	78	
N-terminus	Maxilla	100	69	75	31	38	25	25	25	19	31	19	81	94	100	16
	Mandible	94	75	31	6	6	0	50	50	6	0	6	38	75	100	
Intron2	Maxilla	100	100	100	0	0	67	0	0	67	0	0	100	100	100	3
	Mandible	100	33	33	0	0	0	33	0	0	0	0	33	0	100	
NSD	Maxilla	100	100	56	11	11	11	0	0	0	0	11	67	100	100	9
	Mandible	89	44	11	0	0	11	33	22	0	0	11	33	67	89	
Linking peptide	Maxilla	100	85	75	25	25	20	10	5	30	20	25	80	90	95	20
	Mandible	95	55	35	5	15	5	40	50	5	10	0	40	45	95	
CSD	Maxilla	92	92	77	15	15	31	15	15	38	15	8	77	92	100	13
	Mandible	92	69	46	0	15	8	31	46	8	15	0	38	62	100	
C-terminus	Maxilla	90	84	77	26	13	19	16	16	19	16	26	71	84	90	31
	Mandible	87	48	58	6	3	19	61	58	19	3	6	52	42	94	
Total	Maxilla	93	84	70	23	19	23	13	12	24	17	21	70	90	92	101
	Mandible	91	54	41	4	8	10	46	48	10	5	4	42	51	94	

Fig. 4. Patterns of missing teeth according to the types and locations of the *PAX9* mutations. The prevalence of a missing tooth was defined as follows: "common" (≥50%) as dark blue, "less common" (30–49%) as blue, and "rare" (1–29%) as pale blue. NSD: N-terminal subdomain, CSD: C-terminal subdomain, 5′ UTR: 5′ untranslated region, Mo2: second molar, Mo1: first molar, PM2: second premolar, PM1: first premolar, Ca: canine, LI: lateral incisor, and CI: central incisor.

mutations occurred in the PD. These results indicate that the PD is a mutation hotspot for *PAX9* variants. Phenotypically, the number of missing teeth in the maxilla was larger than that in the mandible, while the number of missing teeth on the right or left side was comparable. These findings are consistent with previous reports of *PAX9* patients [11,15,19].

A correlation was found between the number of missing teeth and the location of the mutations for the in-frame mutations. The number of missing teeth with mutations in the NSD and linking peptide within the PD was large, and that of the C-terminus (outside of the PD) was significantly lower. The PD is a highly conserved domain and vital for DNA binding [20]. Mutations in the PD affect DNA binding activities, protein stabilization, and activation of the *Msx1 and Bmp4* reporter genes, which are odontogenic genes [21]. These findings suggest that the PD plays an important role during tooth development and that its mutations lead to severe TA. In contrast, no significant differences were observed in the number of missing teeth between different locations of the null mutations.

Null *PAX9* mutations correlated with more missing teeth than in-frame mutations, which is consistent with a previous report [19]. Null mutations generally result in rapidly degraded or unstable mRNAs and aberrant truncated proteins that are likely to be degraded by ribosome-associated protein quality control pathways [22]. The severe TA phenotype with null *PAX9* variants is expected to

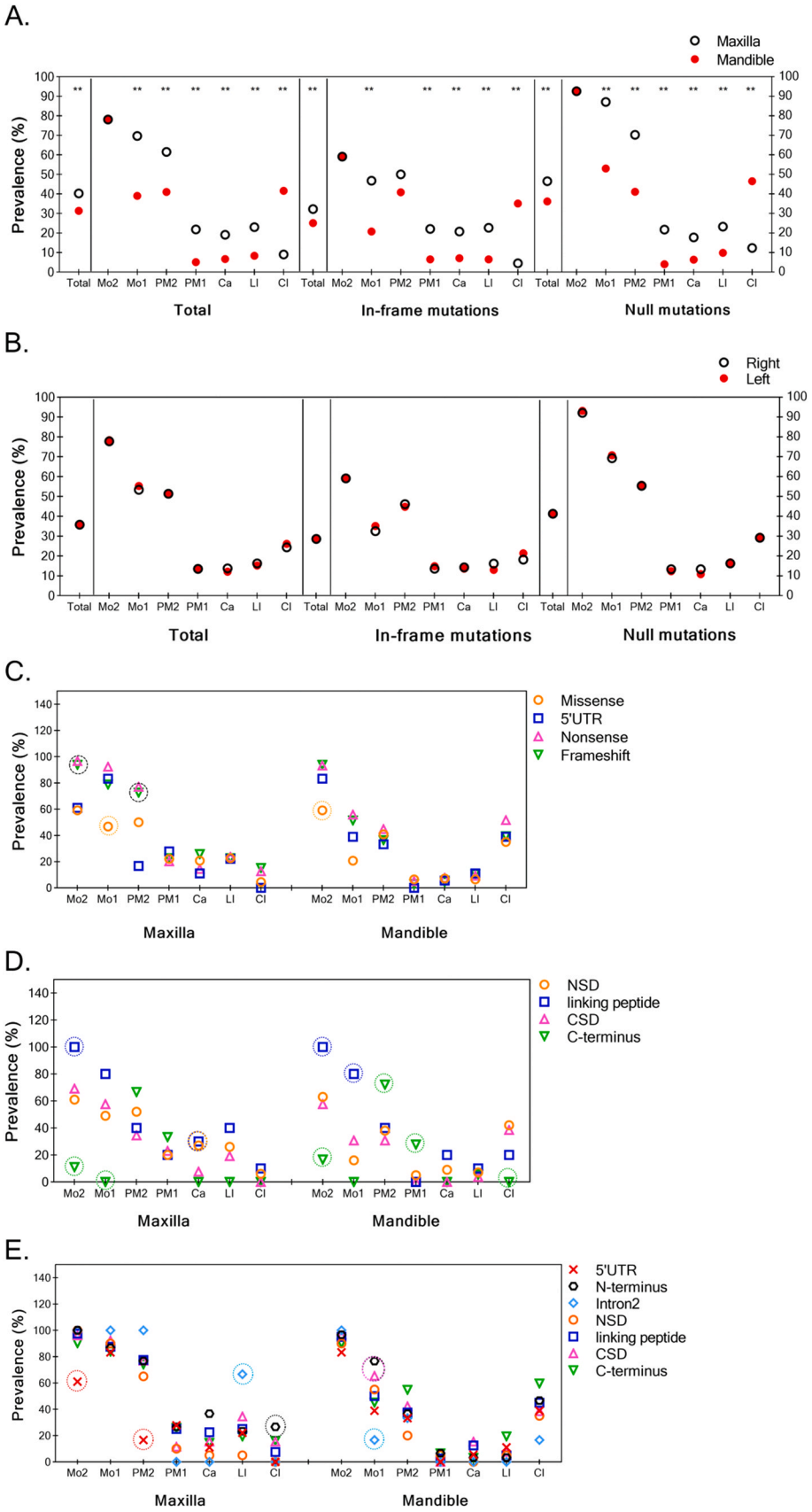


Fig. 5. Tooth agenesis prevalence according to the locations of the missing teeth and types of *PAX9* mutation. The prevalence missing teeth (%) is presented in the maxilla and mandible (A), right and left arches (B), type of mutation (C), and location of the in-frame (D) and null mutations (E). A statistical difference was determined at $p \leq 0.05$. $^{*}p \leq 0.05$, $^{**}p < 0.001$, $^{***}p < 0.0001$. The colored dashed circle in (C), (D), and (E) indicated a statistical difference in the prevalence between the symbol in the circle and the other symbols. NSD: N-terminal subdomain, CSD: C-terminal subdomain, 5′ UTR: 5′ untranslated region, Mo2: second molar, Mo1: first molar, PM2: second premolar, PM1: first premolar, Ca: canine, LI: lateral incisor, and CI: central incisor.

result from the combined defects of transactivation function, protein expression, and/or DNA binding activities of the mutants that lead to *PAX9* haploinsufficiency during odontogenesis [23,24]. In contrast, although in-frame mutations failed to activate the *BMP4* reporter and lost their DNA binding ability, they could express proteins in vitro and had a normal nuclear localization [19,21]. These findings suggest that the *PAX9* in-frame mutations have milder functional consequences.

PAX9 is essential for the development of a variety of organs and skeletal elements. To date, there is no report on lethal *PAX9* mutations in humans. The only evidence of lethality was found in *Pax9*-deficient mice. Homozygous *Pax9* mutant mice died shortly after birth due to a cleft palate and exhibited a wide range of developmental defects involving the thymus, parathyroid glands, and craniofacial and visceral skeleton. Furthermore, all teeth were absent. In contrast, *Pax9* heterozygous mutant mice were viable, fertile, and did not show obvious abnormalities [14]. These findings indicate the pathogenesis of the *PAX9* mutation as haploinsufficiency during embryonic development and odontogenesis.

In terms of TA pattern, *PAX9* mutations predominantly affected molar teeth, which is consistent with clinical studies [19,21,25]. We observed that the second molar was the most commonly missing tooth (78% of *PAX9* patients), while the least common was the mandibular first premolar (4%). Null mutations that occurred in all locations predominantly affected the molars. In mice, the hypomorphic *Pax9* allele resulted in oligodontia and arrested molar tooth development at different stages. Changes in *Pax9* expression levels were found to determine dental patterning [26]. Furthermore, *Pax9* plays a vital role in tooth and palate development [27,28]. Inactivation of *Pax9* using Wnt1-Cre resulted in a cleft secondary palate and missing teeth in mice [28]. Based on the above findings in mutant mice and humans, it is suggested that *PAX9* predominantly regulates the development of molar teeth, especially in the maxilla, and its dosage influences the patterning of the dentition.

PAX9 is involved in the formation of the posterior teeth and mandibular incisors [26,29]. *In vitro* functional studies demonstrated that *PAX9* null mutations reduced mRNA stability and protein production [15,19,30]. The transactivation capacity of PAX9 to the *BMP4* promoter, a direct downstream target, was reduced more in the *Pax9* null mutations compared with the in-frame mutations [15]. The *Pax9* null mice died shortly after birth, likely due to a cleft palate, and their teeth were arrested at the bud stage [14]. Mice that were homozygous for the hypomorphic *Pax9* alleles exhibited hypoplastic or missing lower incisors and third molars, while mice that were compound heterozygote for the hypomorphic and null *Pax9* alleles developed severe forms of oligodontia with variable severity. In hypomorphic mutants, third molar development was arrested prior to the bud stage, and that of the lower second molars was arrested at the bud stage, while the upper molars were less affected [26]. Furthermore, *PAX9* functions in the mandibular incisor development involves the EDA pathway [29]. These findings indicate that reduced *PAX9* gene dosage affects tooth development in multiple stages and the presence of a feedback mechanism and other signaling could affect the phenotypic consequence *Pax9* reduction.

Tooth agenesis is considered rare in the deciduous dentition compared with that in the permanent dentition. A Chinese patient identified with the heterozygous mutation, p.Tyr160*, in *PAX9* was missing 6 primary teeth and 20 permanent teeth [23]. A Finnish patient with the *PAX9* p.Lys114* mutation was missing deciduous second molars [31]. Another study reported that a seven-year-old patient had agenesis of all deciduous and permanent molars associated with deletion of the entire PAX9 gene and the 3′end of the *SLC25A21* gene that encodes the mitochondrial oxodicarboxylate carrier [32]. Furthermore, a reduced tooth size in the deciduous and permanent teeth was reported in a three-generation family with oligodontia and the p.Trp26Arg mutation in *PAX9* [33]. It is suggested that the agenesis of deciduous teeth in patients with *PAX9* mutations may be due to the severity of the mutational consequences or the role of modifying genes and the *PAX9* gene plays a role in the normal development of the teeth.

Interestingly, for in-frame mutations, a missing second molar was commonly found with mutations in the PD, especially those in the linking peptide that showed a 100% absence of second molars. In contrast, missing second molars was rare with mutations in the C-terminus, which instead had the highest prevalence of missing second premolars. Moreover, the total number of missing teeth and the prevalence of absent anterior teeth were lowest in the C-terminus. The variants in the C-terminus were previously revealed to cause less clinical severity than mutations in the N-terminus [9,21]. These finding confirm that the degree of lost *PAX9* function correlates with both severity and type of tooth agenesis.

In conclusion, our study demonstrates that the severity and pattern of TA correlate with the types and locations of the *PAX9* mutations. We provide new and more complete perspectives of PAX9 phenotype-genotype correlation that are of benefit to clinical diagnosis by clinicians, molecular determination by geneticists, and systems biology by researchers.

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Data Availability

Original data generated and analyzed during this study are included in this published article or in the supplementary material

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jdsr.2023.04.001.

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