Supplementary Materials

PAX9 Mutations and Genetic Synergism in Familial Tooth Agenesis

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Contents:

Supplementary Methods

RT-PCR Primer Sets

The following primer sets were designed and used for RT-PCR experiments:

Gene	Forward primer	Reverse primer
PAX9	ATCACCGACCAAGTGAGCG	GGAGCAGCACTGTAGGTCAT
LEF1	ACCCATCCCGAGAACATCAA	GTGAGGATGGGTAGGGTTGC
AXIN2	ACGGACAGCAGTGTAGATGG	CTCGGAGCCCTCTCTCTCTT
BMP4	AGCTTCCACCACGAAGAACA	TCTGCTGGGGGCTTCATAAC
MSX1	CTCAAGCTGCCAGAAGATGC	CTGAGCGAGCTGGAGAACT
DAPDH	GTCTCCTCTGACTTCAACAGCG	ACCACCCTGTTGCTGTAGCCAA

Criteria for Exclusion from Statistical Analyses

A total of 20 publications were excluded from further statistical analyses of genotype-phenotype correlation for following reasons:

Twelve publications were excluded for inconclusive pathogenicity of reported common *PAX9* sequence variants. The dbSNP ID number (rs#) and its allele frequency (AF) are provided when available:

(1) Safari *et al.*, Screening PAX9, MSX1 and WNT10A mutations in 4 Iranian families with non-syndromic tooth agenesis. *Avicenna J Med Biotechnol.* 12, 236-240 (2020). *(rs4904210, AF=0.36)*

(2) Jobbágy-Óvári *et al.*, Complex analysis of multiple single nucleotide polymorphisms as putative risk factors of tooth agenesis in the Hungarian population. *Acta. Odontol. Scand.* 72, 216-227 (2014). *(rs2073246, AF=0.31; rs2073244, AF=0.33)*

(3) Mu *et al.*, Mutational analysis of AXIN2, MSX1, and PAX9 in two Mexican oligodontia families. *Genet. Mol. Res.* 12, 4446-4458 (2013). *(rs12881240, AF=0.18; rs4904210, AF=0.36)*

(4) Isman *et al.*, PAX9 polymorphisms and susceptibility with sporadic tooth agenesis in Turkish populations: a case-control study. *BMC Genomics* 14, 733 (2013). *(rs2073247, AF=0.33; rs2073244, AF=0.33)*

(5) Liu *et al.*, A case-control study of the association between tooth-development gene polymorphisms and non-syndromic hypodontia in the Chinese Han population. *Eur. J. Oral Sci.* 120, 378-385 (2012). *(rs2073244, AF=0.33; rs2073247, AF=0.33; rs4904155, AF=0.37; rs4904210, AF=0.36; rs10141087, AF=0.54)*

(6) Wang *et al.*, Sequence analysis of PAX9, MSX1 and AXIN2 genes in a Chinese oligodontia family. *Arch. Oral Biol.* 56, 1027-1034 (2011). *(rs4904210, AF=0.36)*

(7) Paixão-Côrtes *et al.*, PAX9 and MSX1 transcription factor genes in non-syndromic dental agenesis. *Arch. Oral Biol.* 56, 337-344 (2011). *(rs7143727, AF=0.04; rs12881240, AF=2/233,582; rs4904210, AF=0.36)*

(8) Pinho *et al.*, Mutational analysis of MSX1 and PAX9 genes in Portuguese families with maxillary lateral incisor agenesis. *Eur. J. Orthod.* 32, 582-588 (2010). *(rs12881240, AF=0.18; rs4904210, AF=0.36)*

(9) Pawlowska *et al.*, Mutations in the PAX9 gene in sporadic oligodontia. *Orthod. Craniofac. Res.* 13, 142-152 (2010). *(multiple intronic SNPs)*

(10) Pan *et al.*, PAX9 polymorphisms and susceptibility to sporadic tooth agenesis: a case-control study in southeast China. *Eur. J. Oral Sci.* 116, 98-103 (2008). *(rs2073244, AF=0.33; rs2073245, AF=0.39; rs2073247, AF=0.33; rs4904210, AF=0.36)*

(11) Bianch *et al.*, Association between polymorphism in the promoter region (G/C-915) of PAX9 gene and third molar agenesis. *J. Appl. Oral Sci.* 15, 382-386 (2007). *(rs2073247, AF=0.33)*

(12) Peres *et al.*, Association between PAX-9 promoter polymorphisms and hypodontia in humans. *Arch. Oral Biol.* 50, 861-871 (2005). *(rs2073247, AF=0.33; rs2073244, AF=0.33)*

A manuscript from van den Boogaard *et al.*, reporting 3 disease-causing *PAX9* mutations, was excluded due to a lack of description of dental phenotypes for affected individuals:

(13) van den Boogaard *et al.*, Mutations in WNT10A are present in more than half of isolated hypodontia cases. *J. Med. Genet.* 49, 327-331 (2012).

Following 2 manuscripts were excluded for undetermined pathogenicity:

(14) Shahid *et al.*, Mutations in MSX1, PAX9 and MMP20 genes in Saudi Arabian patients with tooth agenesis. *Eur. J. Med. Genet.* 59, 377-385 (2016). *(Homozygous PAX9 p.N116I mutation was identified in 2 patients with one missing tooth.)*

(15) Idrus *et al.*, PAX9 mutation of non-syndromic hypodontia in a Malaysian family. *UPI Health Med.* 1, 108-111 (2016). *(Segregation analysis in the family was dubious.)*

A manuscript from Frazier-Bowers *et al.* was excluded as no *PAX9* mutation was identified in any analyzed individual:

(16) Frazier-Bowers *et al.*, Mutational analysis of families affected with molar oligodontia. *Connect. Tissue Res.* 43, 296-300 (2002).

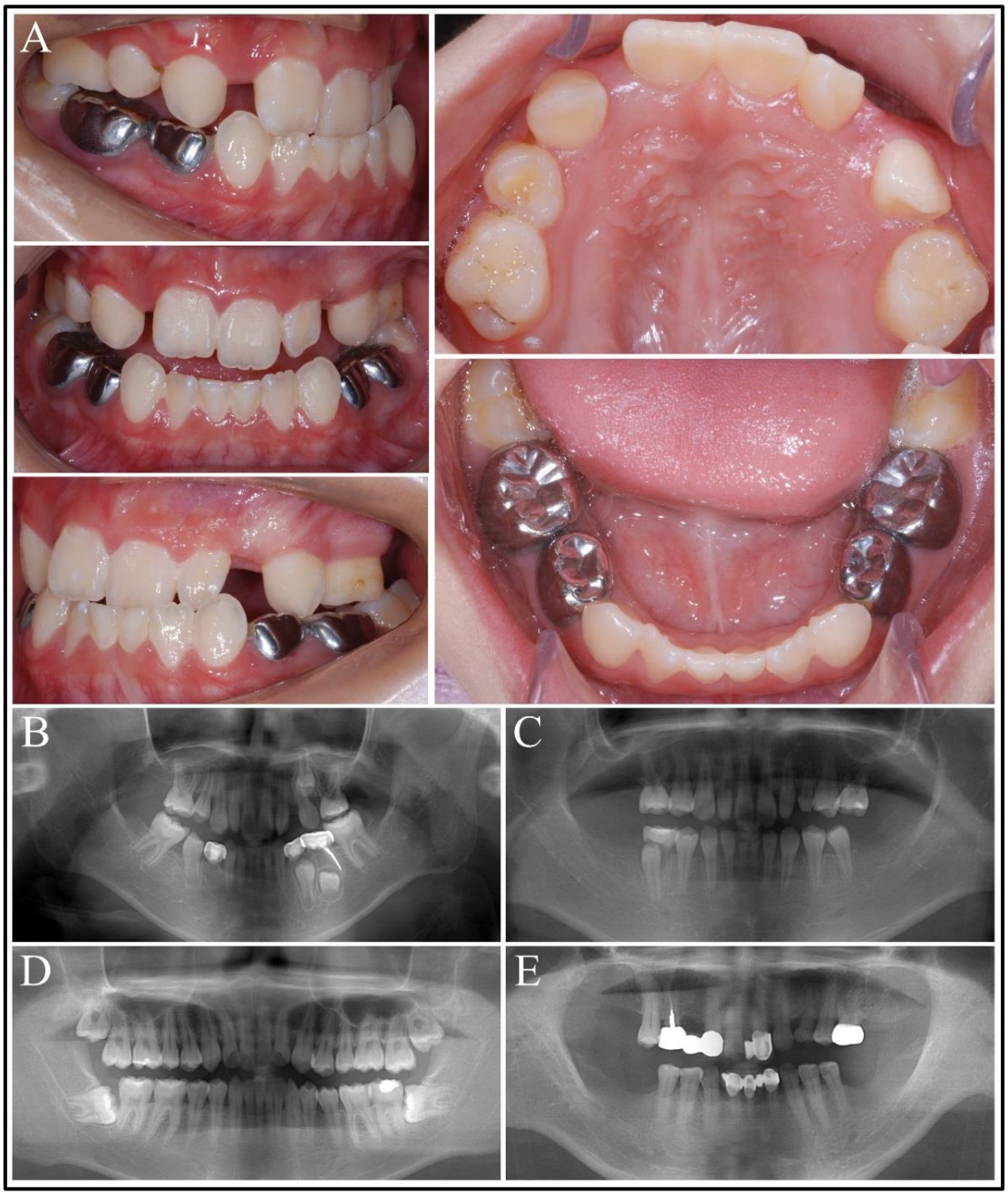
Four papers were excluded because the reported mutations are large deletions which involve genes other than *PAX9*:

(17) Hayashi *et al.*, Identical deletion at 14q13.3 including PAX9 and NKX2-1 in siblings from mosaicism of unaffected parent. *J. Hum. Genet.* 60, 203-206 (2015). *(884-kb 14q13.3 deletion)*

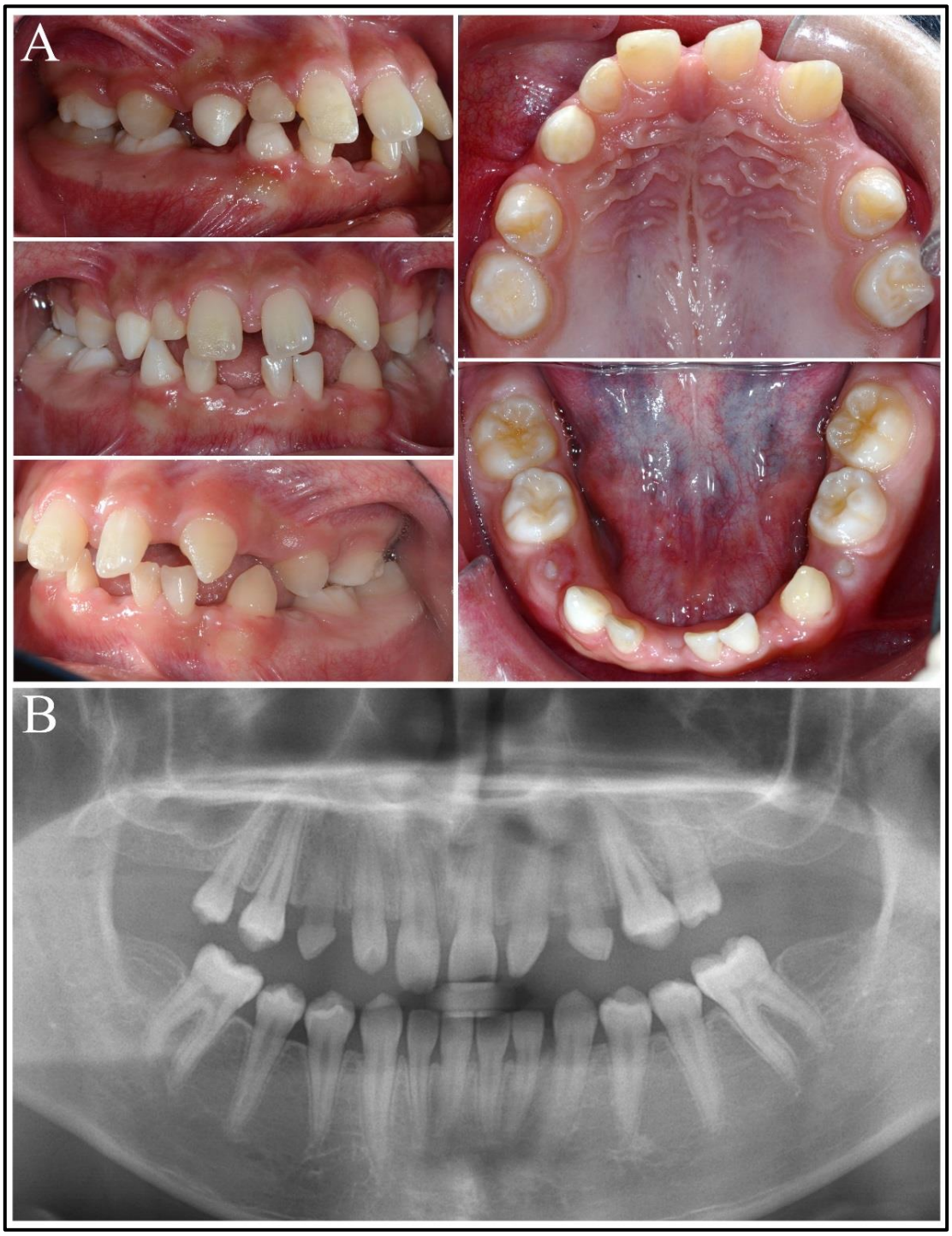
(18) Haldeman-Englert *et al.*, A 223-kb de novo deletion of PAX9 in a patient with oligodontia. *J. Craniofac. Surg.* 21, 837-839 (2010). *(223-kb 14q13.3 deletion)*

(19) Guala *et al.*, Deletion of PAX9 and oligodontia: a third family and review of the literature. *Int. J. Paediatr. Dent.* 18, 441-445 (2008). *(1.2-mb 14q13.1 deletion)*

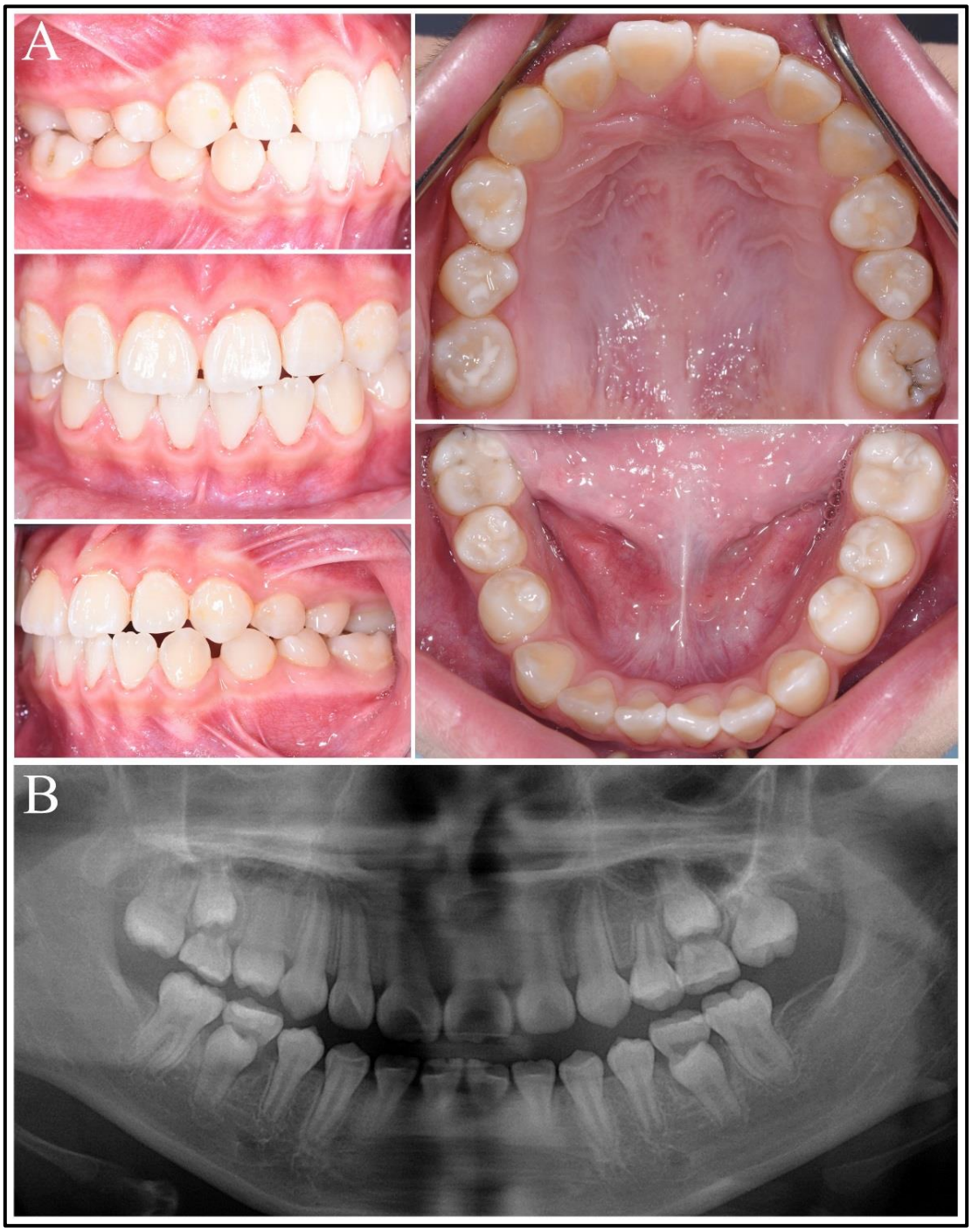
(20) Das *et al.*, Haploinsufficiency of PAX9 is associated with autosomal dominant hypodontia. *Hum. Genet.* 110, 371-376 (2002). *(44~100-kb deletion including PAX9)*



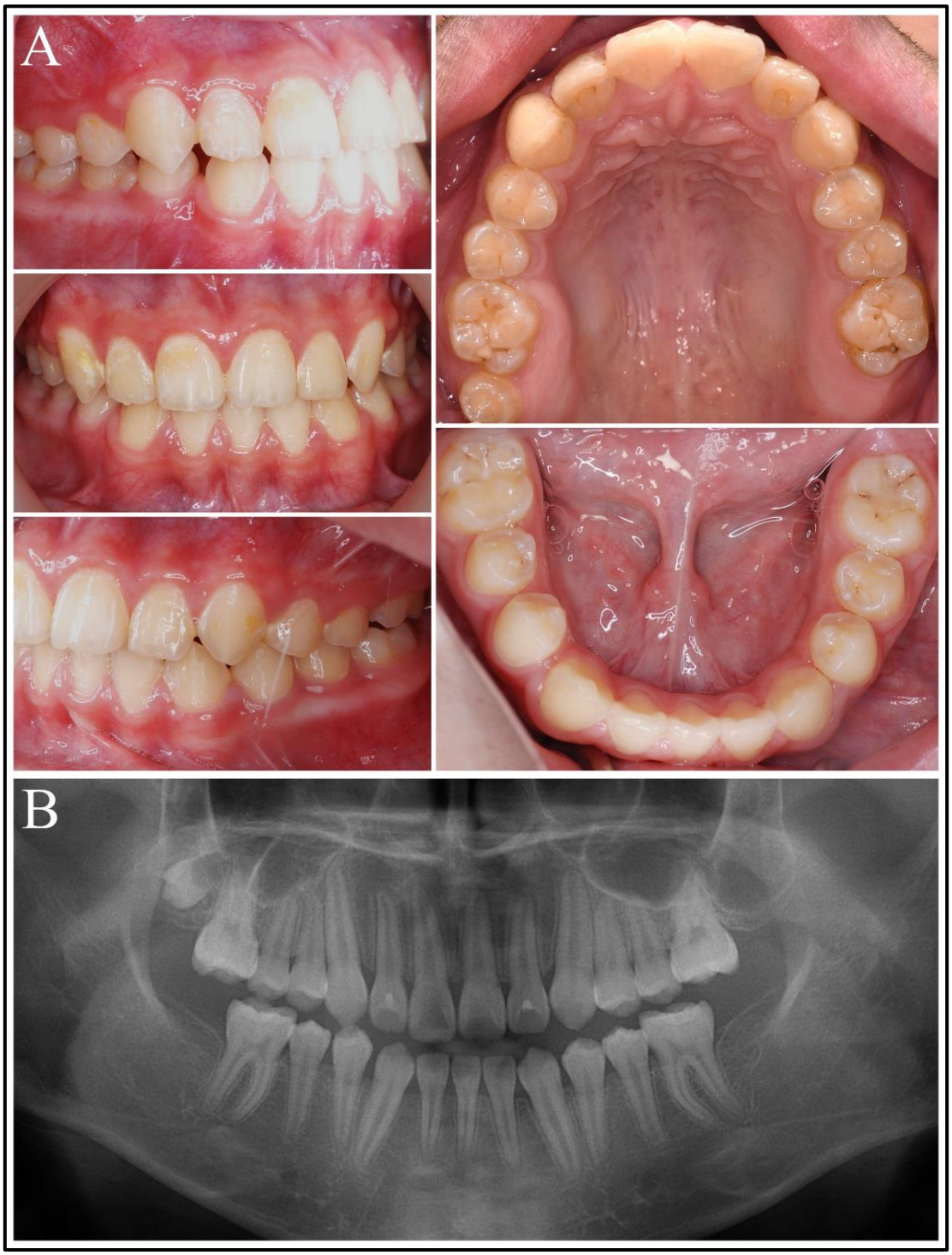
Supplementary Figure 1. Phenotypes of Family 1 (*PAX9* c.771+4A>G) members. (**A**) Clinical photographs of the proband (II:3) at age of 8. He had a mixed dentition with normal tooth morphology and size, except for a peg-shaped lateral incisor (Tooth number 10). Mandibular primary molars were restored with stainless steel crowns due to large carious lesions. (**B**) The panoramic radiograph of the proband (II:3) at age 9.5 showed that he had a total of 11 missing teeth excluding third molars. Bilateral maxillary lateral incisors were both microdontic. (**C**) The panoramic radiograph of proband's sister (II:2) at age 12 revealed absence of 13 permanent teeth. Like the proband, her maxillary lateral incisors were both peg-shaped. (**D**) The older brother's (II:1, age 18) panorex showed that he had a full set of permanent teeth including all third molars. (**E**) The panorex of the father (I:1, age 48) demonstrated that he was oligodontic with 13 missing teeth involving all permanent molars. A primary molar (Tooth letter J) was retained with no apparent root resorption.



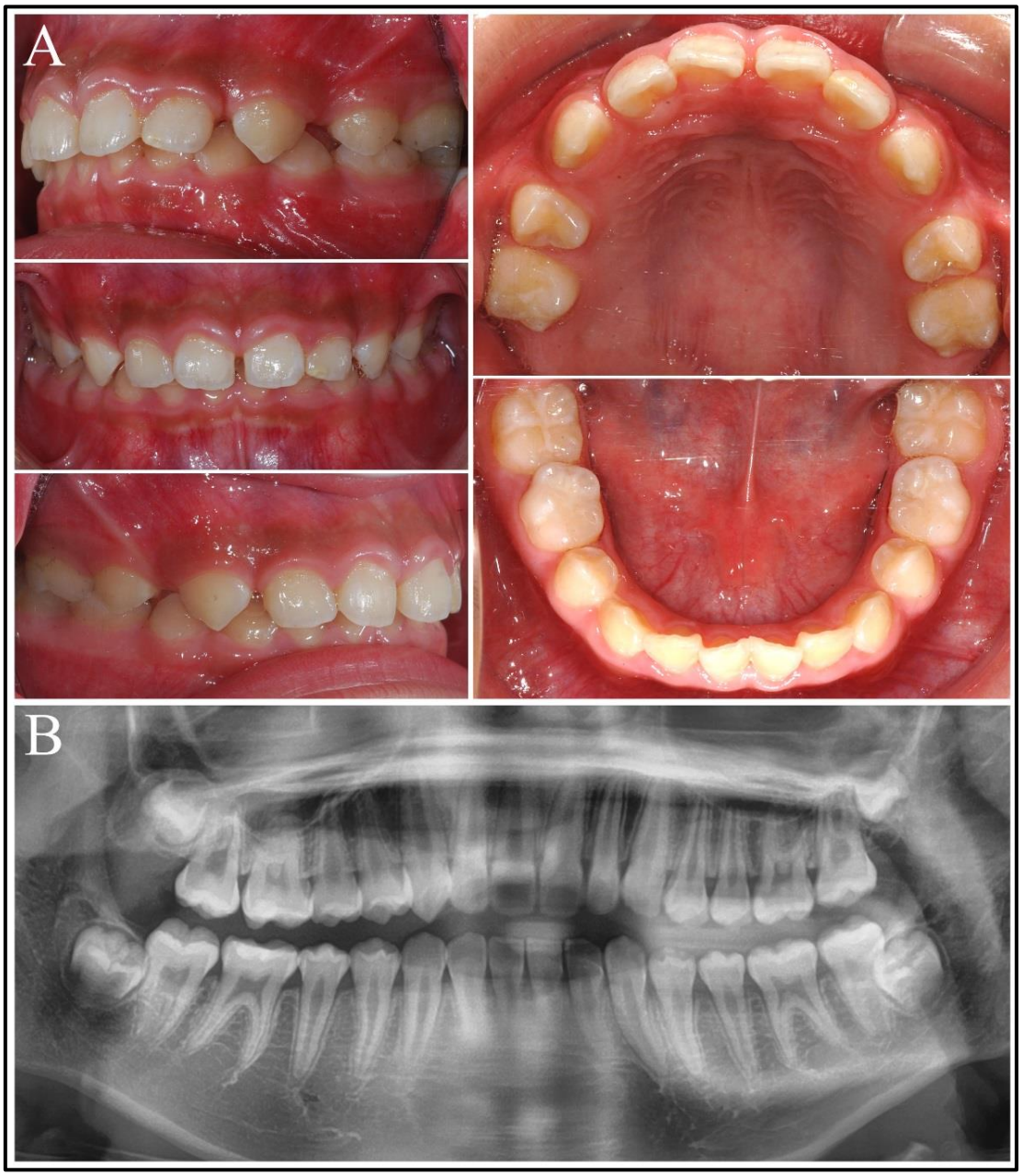
Supplementary Figure 2. Phenotypes of Family 2 (*PAX9* p.Pro118Ser and *WNT10A* c.376+1G>A) members. (**A**) Clinical photographs of the proband (II:2) at age of 11 showed that he had a mixed dentition. While most mandibular teeth were of normal morphology, his maxillary central incisors appeared slender and first molars heart-shaped. Generalized microdontia was evident. (**B**) The panoramic radiograph of proband's brother (II:1, age 14, lacking the *WNT10A* defect) revealed that he had 8 missing teeth, involving mostly maxillary teeth. Like those of the proband, his upper incisors were all slender and microdontic.



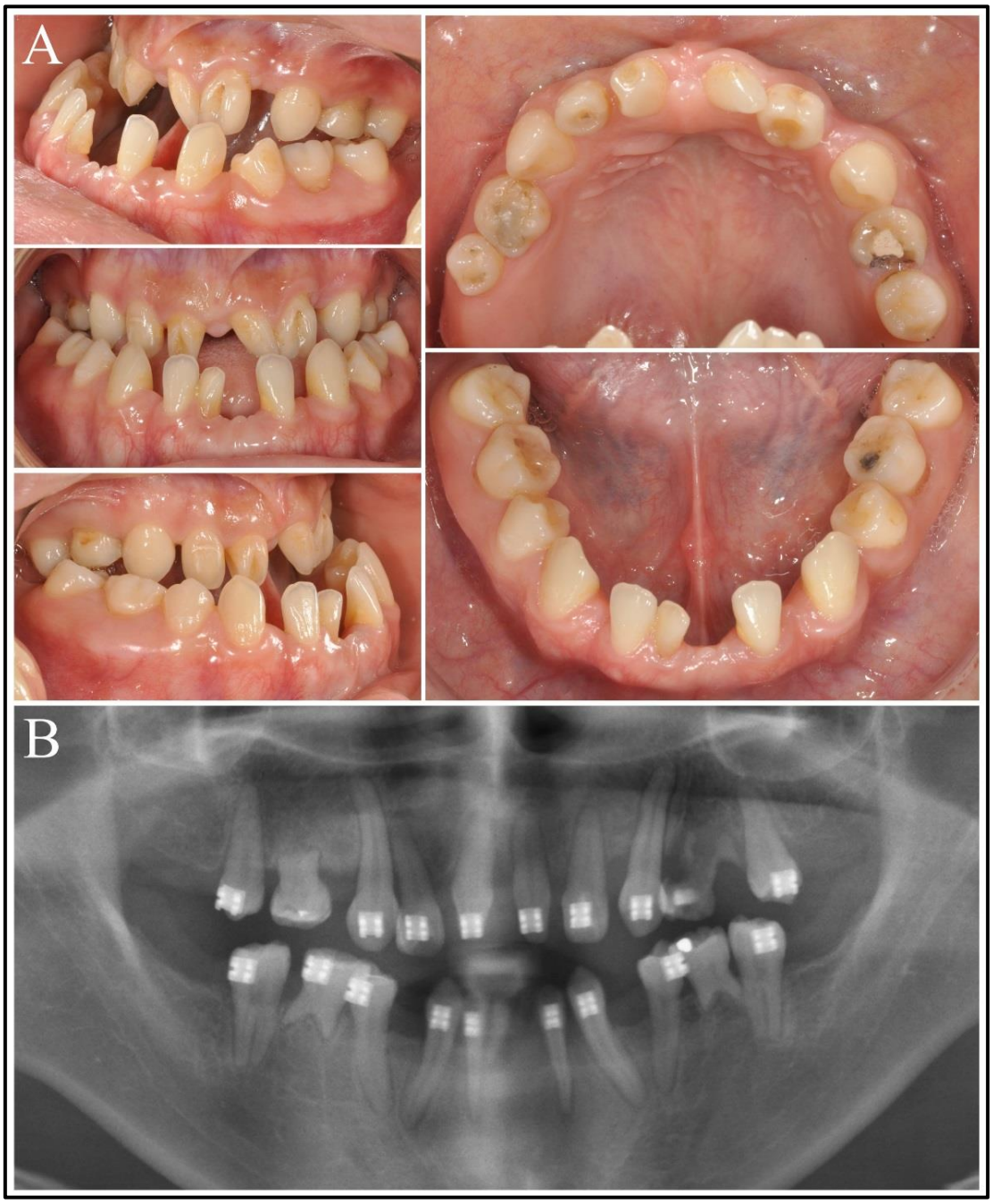
Supplementary Figure 3. Phenotypes of the proband in Family 3 (*PAX9* p.Val83Leu). (**A**) Clinical photographs of the proband (II:2) at age of 15 showed that she had a partial permanent dentition without second and third molars. While most of her teeth were of normal morphology and size, the second bicuspids and first molars all appeared microdontic. Teeth 3 and 14 were dysmorphic with a heart shape. (**B**) Her panoramic radiograph at age 9.5 revealed no detectable tooth germs of second and third molars. The roots of most teeth appeared shorter than normal except for maxillary lateral incisors and canines.



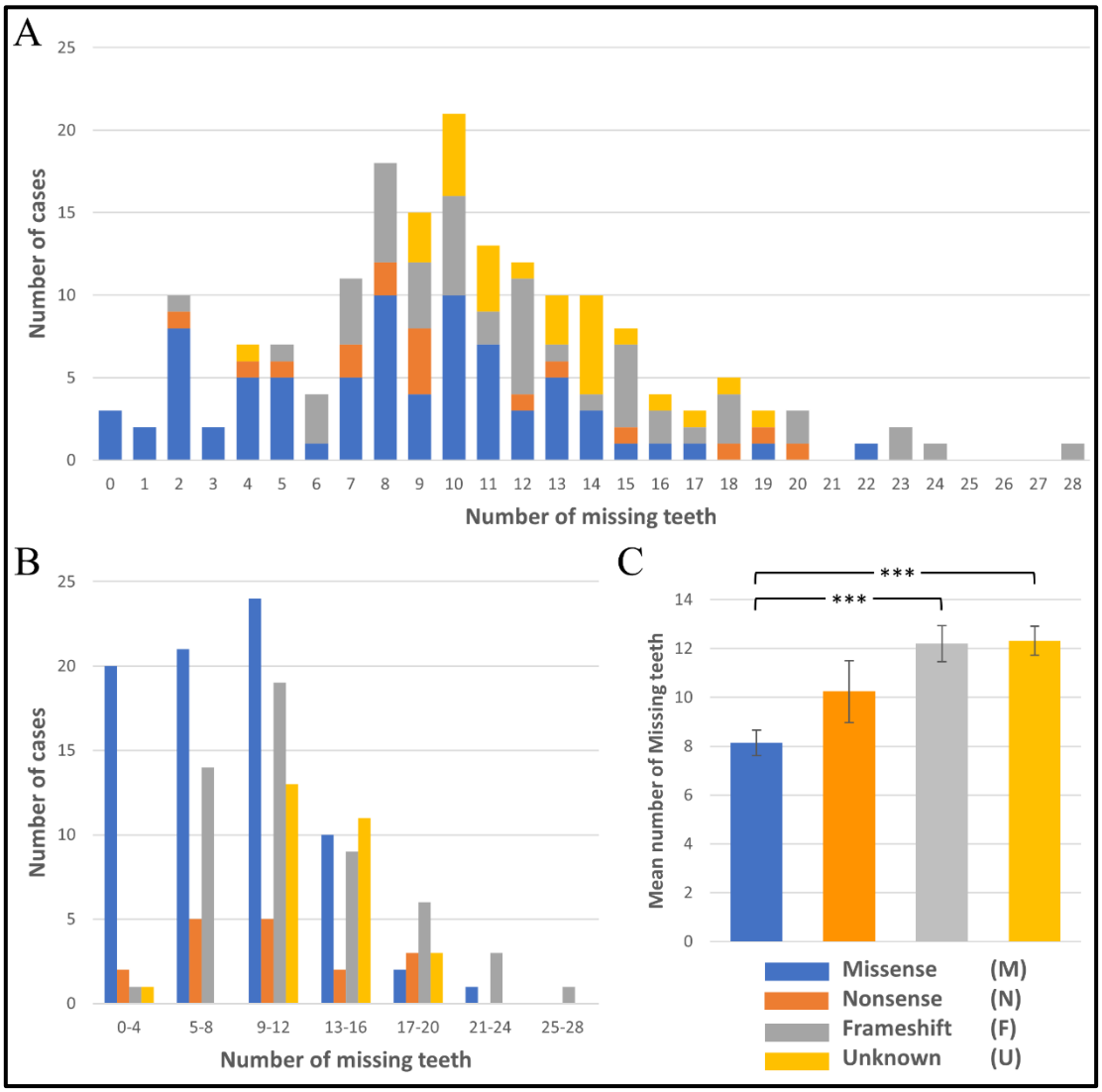
Supplementary Figure 4. Phenotypes of an affected individual in Family 3 (*PAX9* p.Val83Leu). (**A**) Clinical photographs of proband's brother (II:1) at age of 21 showed that he had a partial permanent dentition identical to that of the proband. However, a microdontic third molar (Tooth number 1) was present. All of his teeth had normal morphology and size except the third molar. (**B**) His panoramic radiograph at age 11 revealed no detectable tooth germs of second and third molars except for Tooth number 1. Unlike those of the proband, the roots of his teeth were not particularly short.



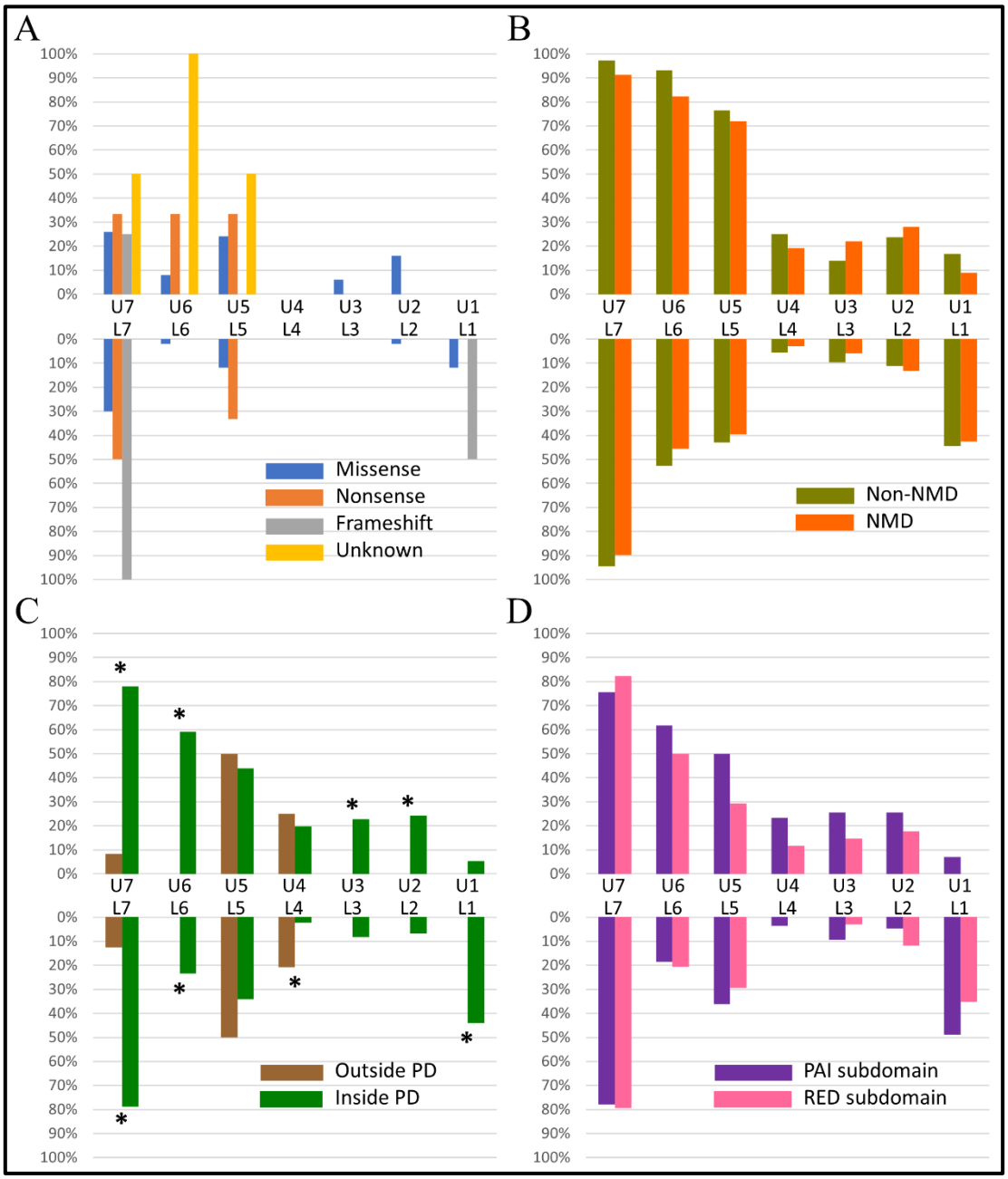
Supplementary Figure 5. Phenotypes of Family 4 (*PAX9* p.Ser197Argfs*23) members. (**A**) Clinical photographs of the proband (II:2) at age of 9 showed that most of his primary teeth had shed except for mandibular primary second molars. Due to absence of maxillary second bicuspids, the canines and first bicuspids have distally drifted. The teeth were generally normal in size and morphology (**B**) The panoramic radiograph of proband's sister (II:1, age 11) revealed that she had a full set of permanent dentition including all third molars.



Supplementary Figure 6. Phenotypes of the proband in Family 5 (*PAX9* p.Glu7Lys and *WNT10A* p.Gly213Ser). (**A**) Clinical photographs of the proband (II:1) at age of 19.5 showed that she had 4 over-retained primary molars. Her teeth were generally microdontic and dysmorphic, particularly the maxillary incisors. The mandibular first molars appeared rounded with an abnormal cusp pattern. A skeletal and dental Class III malocclusion was evident. (**B**) Her panoramic radiograph revealed a total of 12 missing permanent teeth excluding third molars. The roots of most teeth appeared long and slender. She was undergoing orthodontic treatment at the time.



Supplementary Figure 7. Missing tooth numbers in different mutation categories. (**A**) The frequency distribution of missing tooth numbers. (**B**) The frequency distribution of missing tooth numbers grouped into 7 sequential ranges. (**C**) The mean number of missing teeth for each mutation type. Frameshifts and mutations with unknown effects cause ~4 more missing teeth than missense mutations. Key: ***, $p < 0.01$.



Supplementary Figure 8. Patterns of missing teeth in different mutation categories. (**A**) Correlations between the percentage of missing teeth at each tooth position and the mutation category for hypodontia cases. (**B**) Correlations between the percentage of missing teeth at each tooth position and truncation mutations that cause nonsense mediated decay (NMD) or not. (**C**) Correlations between the percentage of missing teeth at each tooth position and missense mutations that are located inside or outside of paired domain (PD). (**D**) Correlations between the percentage of missing teeth at each tooth position and missense mutations that are located in the PAI or RED subdomains. Key: *, $p < 0.05$.

#	Exon	Gene (NG_013357.1)	cDNA (NM_006194.4)	Protein (NP_006185.1)	Ref
1	2	g.9523A>G	c.1A>G	p.(Met1Val)	(1)
2	2	g.9524T>A	c.2T>A	p.(Met1Lys)	(2)
3	2	g.9524T>G	c.2T>G	p.(Met1Arg)	(3)
4	2	g.9525G>A	c.3G>A	g.(Met1Ile)	(4)
5		g.9527G>T	c.4+1G>T	p.(?)	(5)
6	3	g.10341G>A	c.16G>A	p.(Gly6Arg)	(6)
7	3	g.10344G>A	c.19G>A	p.(Glu7Lys)	Family 5
8	3	g.10368T>A	c.43T>A	p.(Phe15Ile)	(7)
9	3	g.10384del	c.59del	p.(Pro20Argfs*65)	(8)
10	3	g.10384C>T	c.59C>T	p.(Pro20Leu)	(9-11)
11	3	g.10387T>C	c.62T>C	p.(Leu21Pro)	(12)
12	3	g.10398_10400del	c.73_75del	p.(Ile25del)	(13)
13	3	g.10401C>T	c.76C>T	p.(Arg26Trp)	(14, 15)
14	3	g.10405T>C	c.80T>C	p.(Leu27Pro)	(16)
15	3	g.10408G>C	c.83G>C	p.(Arg28Pro)	(17)
16	3	g.10411T>C	c.86T>C	p.(Ile29Thr)	(16)
17	3	g.10453_10454delinsAA	c.128_129delinsAA	p.(Ser43Lys)	(6)
18	3	g.10464C>T	c.139C>T	p.(Arg47Trp)	(18)
19	3	g.10465G>C	c.140G>C	p.(Arg47Pro)	(15, 19)
20	3	g.10471del	c.146del	p.(Ser49Cysfs*36)	(15)
21	3	g.10471C>T	c.146C>T	p.(Ser49Leu)	(13)
22	3	g.10476G>A	c.151G>A	p.(Gly51Ser)	(20)
23	3	g.10477G>C	c.152G>C	p.(Gly51Ala)	(21)
24	3	g.10492T>A	c.167T>A	p.(Ile56Asn)	(15)
25	3	g.10492T>C	c.167T>C	p.(Ile56Thr)	(19)
26	3	g.10500C>T	c.175C>T	p.(Arg59*)	(22)
27	3	g.10501_10507delins(288)	c.176_182delins(288)	p.(Arg59Glnfs*120)	(12)
28	3	g.10510_10514dup	c.185_189dup	p.(Gly64Argfs*23)	(15)
29	3	g.10516G>T	c.191G>T	p.(Gly64Val)	(23)
30	3	g.10519C>A	c.194C>A	p.(Ser65*)	(15)
31	3	g.10536dup	c.211dup	p.(Ile71Asnfs*246)	(24)
32	3	g.10543dup	c.218dup	p.(Ser74Glnfs*243)	(15, 25, 26)
33	3	g.10554C>G	c.229C>G	p.(Arg77Gly)	(24)
34	3	g.10555G>A	c.230G>A	p.(Arg77Gln)	(11)
35	3	g.10555_10567del	c.230_242del	p.(Arg77Profs*4)	(21)
36	3	g.10560_10561dup	c.235_236dup	p.(Thr80Leufs*6)	(24)
37	3	g.10563A>G	c.238A>G	p.(Thr80Ala)	(21)
38	3	g.10572G>T	c.247G>T	p.(Val83Leu)	Family 3
39	3	g.10581_10587dup	c.256_262dup	p.(Arg88Profs*231)	(15)
40	3	g.10584A>T	c.259A>T	p.(Ile87Phe)	(27)

41	3	g.10596A>T	c.271A>T	p.(Lys91*)	(15)
42	3	g.10596A>G	c.271A>G	p.(Lys91Glu)	(12)
43	3	g.10620G>C	c.295G>C	p.(Ala99Pro)	(28)
44	3	g.10647dup	c.322dup	p.(Ala108Glyfs*209)	(29)
45	3	g.10647G>C	c.322G>C	p.(Ala108Pro)	(15)
46	3	g.10665A>T	c.340A>T	p.(Lys114*)	(19, 30)
47	3	g.10675T>G	c.350T>G	p.(Val117Gly)	(23)
48	3	g.10675_10678dup	c.350_353dup	p.(Ser119Alafs*199)	(31)
49	3	g.10677C>T	c.352C>T	p.(Pro118Ser)	Family 2
50	3	g.10679del	c.354del	p.(Ser119Profs*2)	(23)
51	3	g.10731C>T	c.406C>T	p.(Gln136*)	(19)
52	3	g.10753A>G	c.428A>G	p.(Tyr143Cys)	(21)
53	3	g.10758C>T	c.433C>T	p.(Gln145*)	(32)
54	3	g.10805C>G	c.480C>G	p.(Tyr160*)	(26)
55	3	g.10819_10838del	c.494_513del	p.(Pro165Glnfs*145)	(33)
56	3	g.10828C>G	c.503C>G	p.(Ala168Gly)	(34)
57	3	g.10891_10913dup	c.566_588dup	p.(Ser197Argfs*23)	Family 4
58	3	g.10917del	c.592del	p.(Val198Serfs*14)	(15)
59	3	g.10917_10921dup	c.592_596dup	p.(Asp200Serfs*14)	(35)
60	3	g.10944_10946delins(24)	c.619_621delins(24)	p.(?)	(36)
61	4	g.13911dup	c.648dup	p.(Tyr217Leufs*100)	(23)
62		g.14038A>G	c.771+4A>G	p.(?)	Family 1
63	5	c.23651_23652insC	c.792_793insC	p.(Val265Argfs*52)	(37)

Supplementary Table 1. *PAX9* disease-causing mutations. Genomic mutation designations start from the beginning of the *PAX9* gene reference (NG_013357.1), which has the ATG translation initiation codon in exon 2 starting at nucleotide 9523. The cDNA designations correspond to the NM_006194.4 reference, with the A of the ATG translation initiation codon assigned as nucleotide 1.

Mutation type	Mutation	No. of hypodontia	No. of oligodontia	Reference
Missense	p.(Gly6Arg)	1	0	(6)
	p.(Phe15Ile)	1	1	(7)
	p.(Pro20Leu)	3	5	(9, 10)
	p.(Leu21Pro)	1	8	(12)
	p.(Ser43Lys)	1	1	(6)
	p.(Ile56Thr)	1	0	(19)
	p.(Val83Leu)	2	0	Family 3
	p.(Lys91Glu)	3	3	(12)
	p.(Ala99Pro)	1	1	(28)
	p.(Val117Gly)	2	0	(23)
	p.(Ala168Gly)	9*	2	(34)
Nonsense	p.(Lys114*)	1	3	(19, 30)
	p.(Gln145*)	2	3	(32)
Frameshift	p.(Ser74Glnfs*243)	1	13	(15, 25, 26)
	p.(Asp200Serfs*14)	1	3	(35)
Unknown	p.(Met1Lys)	1	3	(2)

Supplementary Table 2. *PAX9*-associated hypodontia. While *PAX9* mutations primarily cause oligodontia, 31 hypodontia cases have been documented, including 2 individuals from Family 3 of this study. Among these cases, 25 carry a missense mutation.

*The number includes 3 cases with no missing teeth other than third molars

Mutation type	Subgroup	No. of mutations	No. of individuals
Missense	Outside PAX domain	2	12
	Inside PAX domain *(3 subdomains)*	29	66
	PAI subdomain of PAX	17	43
	Linker region of PAX	5	6
	RED subdomain of PAX	7	17
Nonsense & Frameshift	Non-NMD	8	36
	NMD	17	34

Supplementary Table 3. Descriptive statistics of mutation subgroups. Number of mutations and cases used for statistics and charts in Supplementary Figure 8.

References in Supplementary Materials

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