Original Article

Novel PAX9 Mutations Cause Non-syndromic Tooth Agenesis

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Key Words

nonsyndromic, oligodontia, paired domain, *PAX9*, transcription factor

ABSTRACT

PAX9 is a transcription factor expressed in the tooth mesenchyme during tooth morphogenesis. In *Pax9*-null mice, tooth development is arrested at the bud stage. In humans, heterozygous mutations in *PAX9* have been associated with non-syndromic tooth agenesis, predominantly in the molars. Here, we report two novel mutations in the paired domain of PAX9, a three-nucleotide deletion (73-75delATC) and a missense mutation (C146T), in two unrelated Japanese patients with non-syndromic tooth agenesis. The individual with the 73-75delATC mutation was missing all upper molars and lower second and third molars. The subject with the C146T mutation was missing the lower central incisors, upper second premolars, and first molars, along with all second and third molars. Both mutations affected amino acids that are highly conserved among different species and are critical for DNA binding. When both mutants were transfected to COS7 cells, nuclear localization of PAX9 proteins was not affected. However, reduced expression of the mutant proteins and almost no transcriptional activity of the target *BMP4* gene were observed, suggesting haploinsufficiency of *PAX9* as the cause of nonsyndromic tooth agenesis.

INTRODUCTION

Development of the dentition is coordinated by sequential and reciprocal interactions of the epithelial and mesenchymal cells. These interactions are regulated by transcription factors (TFs), signaling molecules, growth factors, and extracellular matrix components (Thesleff *et al*., 1995), disturbances of which may result in changes in the number, size, morphology, and cytodifferentiation of the teeth (Matalova *et al*., 2008). Tooth agenesis is one of the most common developmental anomalies in humans. The incidence of agenesis of the permanent dentition, excluding the third molars, ranges from 1.6% to 9.6% in the general population (Niswander and Sujaku, 1963; Vastardis, 2000; Endo *et al*., 2006).

Among the genes involved in tooth morphogenesis, mutations in *MSX1*, *PAX9*, *AXIN2*, *WNT10A* and *EDA* have been reported to be associated with non-syndromic tooth agenesis (Vastardis *et al*., 1996; Stockton *et al*., 2000; Lammi *et al*., 2004; Tao *et al*., 2006; Mostowska *et al.*, 2012). *PAX*9 belongs to a gene family encoding TFs. It plays key roles in the formation of tissues and organs during embryonic development. Tooth developmental arrest at the bud stage and secondary cleft palate and other skeletal abnormalities in the head and limbs have been observed in *Pax9*-null mice (Peters *et al*., 1998). *Pax9* is required for the mesenchymal expression of *Bmp4*, *Msx1*, and *Lef1* (Peters *et al*., 1998). In humans, several *PAX9* mutations have been identified in non-syndromic oligodontia preferentially affecting the molars (Frazier-Bowers *et al*. 2002; Kapadia *et al*., 2006; Suda *et al*., 2011).

Here, we report the identification of two novel mutations in the paired domain of PAX9 in Japanese patients with non-syndromic tooth agenesis and the results of the corresponding functional analysis.

MATERIALS & METHODS

Pedigree Construction and Clinical Diagnosis

Participants in this study were from two unrelated Japanese families with tooth agenesis. Pedigrees of the families were made by clinical examinations and interviews with the available family members. The diagnosis of tooth agenesis was verified by panoramic radiographs. Written consent was obtained from all participants. This study was approved by the Ethics Committee of the Tokushima University Hospital (H24-8).

Mutation Detection

Genomic DNA was extracted from saliva samples with use of the DNeasy Blood & Tissue Kit (Qiagen, Germantown, MD, USA). *MSX1*, *PAX9, AXIN2* and *WNT10A* genes were selected for genetic analysis. The coding regions, including exon-intron boundaries were PCR-amplified with the *Ex Taq*™ Hot Start Version Premix (Takara Bio Inc., Otsu, Japan) and specific primer pairs (Table 1, Vastardis *et al*., 1996; Lammi *et al*., 2004; Bohring *et al*., 2009) in an iCycler thermal cycler (BioRad, Hercules, CA, USA). PCR products were purified by 1.5% agarose gel electrophoresis. Both strands of the PCR products were prepared using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and analyzed by ABI PRISM® 3100-*Avant* Genetic Analyzer (Applied Biosystems). Sequencing results were compared by BLAST (http://blast.ddbj.nig.ac.jp/top-j.html). The putative functional consequences of all identified mutations were analyzed *in silico* using PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) and MutationTaster (http://www.mutationtaster.org/).

dbSNP (http://www.ncbi.nlm.nih.gov/SNP/) and 1000 Genomes project (http://www.1000genomes.org/) consulted to verify the allele frequencies.

Plasmid Preparation

The pCMV-*PAX9* expression vector was purchased from OriGene Technologies, Inc. (Rockville, MD, USA). *In vitro* site-directed mutagenesis was performed for the construct ion of pCMV-I25R*PAX9* and pCMV-S49L*PAX9* using the KOD-Plus-Mutagenesis Kit (Toyobo Co., LTD, Osaka, Japan). Full coding sequences of wild-type or mutated *PAX9* were cloned in-frame into the vector pCMV-3Tag-2A (Agilent Technologies Inc., Santa Clara, CA, USA). The entire coding sequence of the mutant construct was verified by ABI PRISM® 3100-*Avant* Genetic Analyzer and a BigDye Terminator v.3.1 Cycle Sequencing Kit. The *BMP4* promoter-reporter construct (*BMP4-2.7 kb Luc*) was obtained by cloning the 2.7-kb genomic PCR product around the transcription start site of *BMP4* into the pGL3 Basic luciferase reporter vector (Promega Corp., Madison, WI, USA). Specific primers for *BMP-4* with restriction sites were 5'-GCTAGCAGGCTGTCCCAAGGGCTCG-3' and 5'-AAGCTTCGCCAGCAGCAGCTCCTG-3'.

Transfection and Western Blot Analysis of Cultured Cells

COS-7 cells were cultured in Dulbecco's modified Eagle's medium (Wako, Tokyo, Japan) supplemented with 10% fetal bovine serum. Transient transfection was performed with Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's instructions. After 48 hr of transfection, cells were analyzed by Western blotting or immunofluorescent analysis.

Western Blot Analysis

Transfected cells underwent lysis with RIPA buffer (Cell Signaling Technology, Danvers, MA, USA) containing protease and phosphatase inhibitor cocktail (Thermo Fisher Scientific, Rockford, IL, USA). Western blot analysis was performed with cell lysates containing 10 μg of proteins. Electrophoretically transferred membranes were probed with an anti-Myc monoclonal antibody (Sigma, St. Louis, MO, USA) or anti-glyceraldehyde- 3-phosphate dehydrogenase (GAPDH; Santa Cruz Biotechnology, Santa Cruz, CA, USA). Bound antibody was detected by ECL Plus Western blotting detection reagents (GE Healthcare Bio-Sciences, Piscataway, NJ, USA), according to the manufacturer's instructions.

Fluorescent Immunocytochemistry

Transfected cells were fixed in 4% paraformaldehyde, permea- bilized with 0.2% Triton X-100, and blocked with 1% bovine serum albumin in PBS. After incubation with an anti-Myc antibody, cells were incubated with a fluorescein Alexa488-conjugated anti- mouse secondary antibody (Invitrogen). Cells were mounted with 4′,6′-diamidino- 2-phenylindole (DAPI) to stain nuclei and observed by fluorescence microscopy.

Reporter Assay

Each expression vector was co-transfected with a *BMP4* promoter-reporter construct (*BMP4-2.7 kb Luc*). The phRL-TK plas- mid (Promega Corp.) was used as an internal control.

Cell extracts prepared 48 hr after transfection were assayed with the Dual-Luciferase® Reporter Assay System (Promega Corp.). Experiments were carried out in triplicate and repeated at least twice. For multiple group comparisons, one-way analysis of variance followed by Scheffé's *post hoc* test was performed. Differences were assessed by a two-sided test and considered significant at $p < .05$.

RESULTS

Clinical Diagnosis

Probands of the two nuclear families showed sporadic forms of tooth agenesis, predominantly in the posterior teeth, with an autosomal-dominant inheritance pattern (Fig. 1). Members of both families had no medical history of abnormalities in their sweat glands, skin, hair, or nails, indicating the non-syndromic type of tooth agenesis. The proband in family A (AII-1; 7-year-old girl) had tooth agenesis of all maxillary molars and mandibular second and third molars. The proband in family B (BII-1; 10-year- old boy) lacked mandibular central incisors, maxillary second premolars, and first molars, as

Mutational Analysis

Two novel mutations in the paired-box sequence of *PAX9* were identified. Sequencing analysis showed 2 different mutations in exon 2 (Fig. 2A). The proband in family A carried a three-nucleotide deletion (c.73-75delATC), which resulted in the deletion of one amino acid (p.I25del). The proband in family B carried a heterozygous C>T transition at nucleotide position 146 (c.C146T), resulting in the substitution of serine by leucine (p.S49L). These mutations were

not observed in the unaffected members of the corresponding families, and no mutation was found in *MSX1, AXIN2*, and *WNT10A*. Both affected PAX9 residues are highly conserved among different species and may play important roles in recognizing DNA sequences for binding (Fig. 2B).

The PolyPhen-2 and MutationTaster analyses predicted that both mutations are disease-causing. Sequence analysis of other exons of *PAX9* did not show any mutation in the affected individuals. The above-mentioned mutations were not detected in any of the unaffected relatives (Fig. 2B). They have not been reported as variants in dbSNP or the 1000 Genomes project. In addition, those 2 novel alterations were not observed in the central resource recently provided to archive and display Japanese genetic variation determined by exome sequencing of 1,208 Japanese individuals (Human Genetic Variation Browser from Japanese genetic variation consortium, http://www.genome .med.kyoto-u.ac.jp/SnpDB), indicating that these 2 alterations are unlikely to be rare population-specific variants.

Characteristic of the I25del and S49L PAX9 Mutants

COS7 cells were transfected with Myc-tagged wild-type or mutated PAX9 expression constructs for examination of the characteristics of the mutant proteins. Western blot analysis revealed lower protein expression in cell lysates of mutated *PAX9*-transfected COS7 cells compared with the wild-type *PAX9*-transfected cells (Fig. 3A). To verify whether the 2 novel mutations could affect the nuclear localization, we transfected COS-7 cells with Myc-tagged *PAX9* wild-type or mutants. Fluorescent immunocytochemistry showed nuclear localization in Myc-tagged PAX9 proteins 48 hr after transfection, and no differences were observed between

wild-type and mutant proteins (Fig. 3B). A dual-luciferase reporter assay was performed to test whether the mutated PAX9 proteins could transactivate the downstream transcriptional target effector gene, *BMP4*. Both mutated PAX9 proteins reduced the transcriptional activity of *BMP4* compared with the wild-type protein (Fig. 3C).

DISCUSSION

We identified 2 novel mutations in the paired domain of PAX9 (c.73-75delATC and c.C146T) in two unrelated Japanese patients with sporadic non-syndromic oligodontia. The probands showed similar phenotypes, with tooth agenesis predominantly in the molar region. The proband in family B also showed tooth agenesis of the maxillary second premolars and mandibular central incisors. *MSX1, AXIN2*, and *WNT10A* were also selected for mutation screening, but no mutation was observed. In the unaffected members of both probands, neither of the mutations described in *PAX9* was observed.

PAX9 is characterized by the presence of a paired domain, an octapeptide motif, and a C-terminal transcriptional regulatory domain (Chi and Epstein, 2002). The paired domain is an evolutionarily conserved 128-amino-acid domain contacting with DNA in a sequence-specific manner. It consists of 2 independent subdomains that structurally resemble a helix-turn-helix motif, composed of 3 α-helices (Xu *et al*., 1999). A homology search of the 2 novel mutations revealed that both affected PAX9 residues are evolutionarily conserved. I25del, identified in the proband of family A, was located at the N-terminus of the first α-helix next to the contact between Arg-26 and the sugar phosphate backbone. To the best of our knowledge, most of the mutations identified in *PAX9* have been single-nucleotide substitutions; no study has identified a

deletion of three nucleotides corresponding to a single amino acid (Boeira and Echeverrigaray, 2012).

The amino acid substitution (p.S49L) identified in the pro- band of family B was located near the N-terminus of the third α-helix, which is considered to be the site of a recognition helix fitting directly into the major groove of the DNA. In wild-type PAX9, the residue immediately preceding this α-helix, Ser-49, makes van der Waals contact with the thymine in the PAX9-binding domain; in the identified mutation, the serine is substituted by leucine (Xu *et al*., 1999). Both mutations were predicted to be disease-causing *in silico*.

Most of the PAX9 mutations have been found to cause agenesis of permanent molars, sometimes in combination with other types of teeth, most commonly second premolars and mandibular incisors (Matalova *et al*., 2008; Nieminen, 2009). It has been reported that missense mutations in the paired-domain clustered near the N-terminal of the first 2 α-helices resulted in the molar and canine agenesis phenotype, suggesting that mutations in this region could affect canine development (Liang *et al*., 2012). Unlike previous reports, the proband in family A, carrying a single-amino-acid deletion (p.I25del) in the N-terminus of the first α-helix, did not show canine agenesis. This demonstrates the variability in number and pattern of tooth agenesis among individuals with different PAX9 mutations and their affected family members carrying the same genotype.

PAX9 is co-expressed with MSX1 in the dental mesenchyme, playing a key role in tooth morphogenesis. Several mutations in *MSX1* have been identified in tooth agenesis patients (Vastardis *et al*., 1996; Lidral and Reising, 2002; Chishti *et al*., 2006). An *in vitro* study demonstrated that PAX9 forms a heterodimeric complex with MSX1 and acts synergistically to

activate the *BMP4* gene promoter (Ogawa *et al*., 2006). Our functional analysis demonstrated that the expression of both mutant proteins was reduced, although nuclear localization was observed.

Both mutants showed complete loss of transcriptional activity of the *BMP4* promoter. These results corresponded with our *in silico* analysis results. The importance of *BMP4* in tooth morphogenesis has also been demonstrated *in vivo*. Homozygous deletion of either *Msx1* or *Pax9* resulted in tooth developmental arrest, accompanied by markedly reduced Bmp4 expression in the dental mesenchyme (Satokata and Maas, 1994; Chen *et al*., 1996; Peters *et al*., 1998). Moreover, $Msx1^{+/-};Pax9^{+/-}$ mice lacking mandibular incisors and third molars have been partially rescued by transgenic expression of BMP4 (Nakatomi *et al*., 2010). On the basis of our results, we suggest that the 2 identified *PAX9* mutations affect DNA binding and *BMP4* promoter transactivation, supporting the mechanism of functional haploinsufficiency as the under- lying cause of tooth agenesis (Wang *et al*., 2009).

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REFERENCES

Boeira BR Jr, Echeverrigaray S (2012). Novel missense mutation in PAX9 gene associated with familial tooth agenesis. *J Oral Pathol Med* 42:99-105.

Bohring A, Stamm T, Spaich C, Haase C, Spree K, Hehr U, et al. (2009) WNT10A mutations are a frequent cause of a broad spectrum of ectodermal dysplasias with sex-biased manifestation pattern in heterozygotes. *Am J Hum Genet* 85:97-105.

Chen Y, Bei M, Woo I, Satokata I, Maas R (1996). Msx1 controls inductive signaling in mammalian tooth morphogenesis. *Development* 122:3035-3044.

Chi N, Epstein JA (2002). Getting your Pax straight: Pax proteins in development and disease. *Trends Genet* 18:41-47.

Chishti MS, Muhammad D, Haider M, Ahmad W (2006). A novel missense mutation in MSX1 underlies autosomal recessive oligodontia with associated dental anomalies in Pakistani families. *J Hum Genet* 51:872-878.

Endo T, Ozoe R, Kubota M, Akiyama M, Shimooka S (2006). A survey of hypodontia in Japanese orthodontic patients. *Am J Orthod Dentofacial Orthop* 129:29-35.

Frazier-Bowers SA, Guo DC, Cavender A, Xue L, Evans B, King T, *et al*. (2002). A novel mutation in human PAX9 causes molar oligodontia. *J Dent Res* 81:129-133.

Kapadia H, Frazier-Bowers S, Ogawa T, D'Souza RN (2006). Molecular characterization of a novel PAX9 missense mutation causing posterior tooth agenesis. *Eur J Hum Genet* 14:403-409.

Lammi L, Arte S, Somer M, Jarvinen H, Lahermo P, Thesleff I, *et al.* (2004). Mutations in AXIN2 cause familial tooth agenesis and predispose to colorectal cancer. *Am J Hum Genet* 74:1043-1050.

Liang J, Song G, Li Q, Bian Z (2012). Novel missense mutations in PAX9 causing oligodontia. *Arch Oral Biol* 57:784-9.

Lidral AC, Reising BC (2002). The role of MSX1 in human tooth agenesis. *J Dent Res* 81:274-278.

Matalova E, Fleischmannova J, Sharpe PT (2008). Tooth agenesis: from molecular genetics to molecular dentistry. *J Dent Res* 87:617-623.

Mostowska A, Biedziak B, Zadurska M, Dunin-Wilczynska I, Lianeri M, Jagodzinski PP. (2012) Nucleotide variants of genes encoding components of the Wnt signalling pathway and the risk of non-syndromic tooth agenesis. *Clin Genet*: 84:429-40.

Nakatomi M, Wang XP, Key D, Lund JJ, Turbe-Doan A, Kist R, *et al*. (2010). Genetic interactions between Pax9 and Msx1 regulate lip development and several stages of tooth morphogenesis. *Dev Biol* 340:438-449.

Nieminen P (2009). Genetic basis of tooth agenesis. *J Exp Zool B Mol Dev Evol* 312(B):320-342.

Niswander JD, Sujaku C (1963). Congenital Anomalies of Teeth in Japanese Children. *Am J Phys Anthropol* 21:56-574.

Ogawa T, Kapadia H, Feng JQ, Raghow R, Peters H, D'Souza RN (2006). Functional consequences of interactions between Pax9 and Msx1 genes in normal and abnormal tooth development. *J Biol Chem* 281:18363-18369.

Peters H, Neubuser A, Kratochwi, K, Balling R (1998). Pax9-deficient mice lack pharyngeal pouch derivatives and teeth and exhibit craniofacial and limb abnormalities. *Genes Dev* 12:2735-2747.

Satokata I, Maas R (1994). Msx1 deficient mice exhibit cleft palate and abnormalities of craniofacial and tooth development. *Nat Genet* 6:348-356.

Stockton DW, Das P, Goldenberg M, D'Souza RN, Patel PI (2000). Mutation of PAX9 is associated with oligodontia. *Nat Genet* 24:18-19.

Suda N, Ogawa T, Kojima T, Saito C, Moriyama K (2011). Non-syndromic oligodontia with a novel mutation of PAX9. *J Dent Res* 90:382-386.

Tao R, Jin B, Guo SZ, Qing W, Feng GY, Brooks DG, *et al*. (2006). A novel missense mutation of the EDA gene in a Mongolian family with congenital hypodontia. *J Hum Genet* 51:498-502.

Thesleff I, Vaahtokari A, Kettunen P, Aberg T (1995). Epithelial–mesenchymal signaling during tooth development. *Connect Tissue Res* 32:9-15.

Vastardis H, Karimbux N, Guthua SW, Seidman JG, Seidman CE (1996). A human MSX1 homeodomain missense mutation causes selective tooth agenesis. *Nat Genet* 13:417-421.

Vastardis H (2000). The genetics of human tooth agenesis: new discoveries for understanding dental anomalies. *Am J Orthod Dentofacial Orthop* 117:650-656.

Wang Y, Groppe JC, Wu J, Ogawa T, Mues G, D'Souza RN, *et al.* (2009). Pathogenic mechanisms of tooth agenesis linked to paired domain mutations in human PAX9. *Hum Mol Genet* 18:2863-2874.

Xu HE, Rould MA, Xu W, Epstein JA, Maas RL, Pabo CO (1999). Crystal structure of the human Pax6 paired domain-DNA complex reveals specific roles for the linker region and carboxy-terminal subdomain in DNA binding. *Gene Dev* 13:1263-1275.

Figure Legends

Figure 1. Two Japanese patients with non-syndromic oligodontia. (**A**) Pedigree of two unrelated Japanese patients with non-syndromic oligodontia. Arrow indicates the proband. (**b**) Summary of permanent tooth agenesis in the probands. Asterisks indicate tooth agenesis. (**c**) Panoramic radiographs of the probands in families A (maxillary) and B (mandibular).

Figure 2. Mutational analysis of the *PAX9* gene. (**A**) Chromatograms of the probands and the unaffected members in families A and B. Arrows indicate the affected bases. (**b**) Diagram of the PAX9 protein. Rectangles represent the paired and octapeptide domains (ODs) and the location of each mutation identified in the probands of families A (c.73-75delATC) and B (c.145C>T). Affected residues are indicated in yellow in different species.

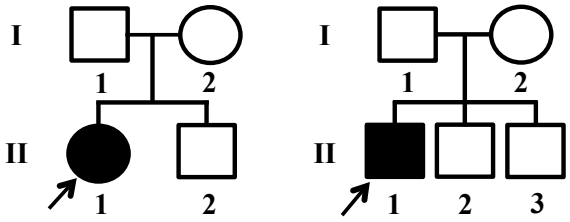
Figure 3. Functional analysis of the PAX9 mutants. (**A**) Western blot analysis of Myc-tagged PAX9 proteins 48 hrs after transfection of COS7 cells. Expression of both mutated proteins was reduced compared with the wild-type (WT) protein. (**b**) Immunofluorescence images of Myc- tagged proteins are shown in the left column. DAPI and merged images are shown in the central and right columns, respectively. (**c**) Luciferase assay to evaluate the role of PAX9 proteins in the transactivation of the *BMP4* promoter. $*p < .05$ *vs*. mock-transfected cells (one- way analysis of variance with subsequent Scheffé's test).

Table 1. Primers used for PCR amplification of human *PAX9* exons and PCR conditions

Exon	Orientation	Primer sequence	Annealing T (°C)
1	Sense	5′-CCCTTCTCCCTTCGAGTCAT-3′	56.5°
	Antisense	5′-GCGTTCACATTGAACTGCTG-3′	
2	Sense	5′-GGGGACAGCCCAGTAGTTA-3′	60°
	Antisense	5′-CACGGTGGGGGTAGTGAC-3′	
3	Sense	5′-GGGAGTAAAACTTCACCAGGC-3′	61°
	Antisense	5′-CCACCTGGCCTGACCCTC-3′	
4	Sense	5′-GGAGAGTAGAGTCAGAGCATTGCTG-3′	61°
	Antisense	5′-GAGACCTGGGAATTGGGGA-3′	

Figure 1

A



Family A Family B

B

		Right								Left							
		8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8
Family A	Maxilla	*	*	*											*	*	*
II-1	Mandible	*	*													*	*
Family B	Maxilla	*	*	*	*									*	*	*	*
II-1	Mandible	*	*						*	*						*	*

C

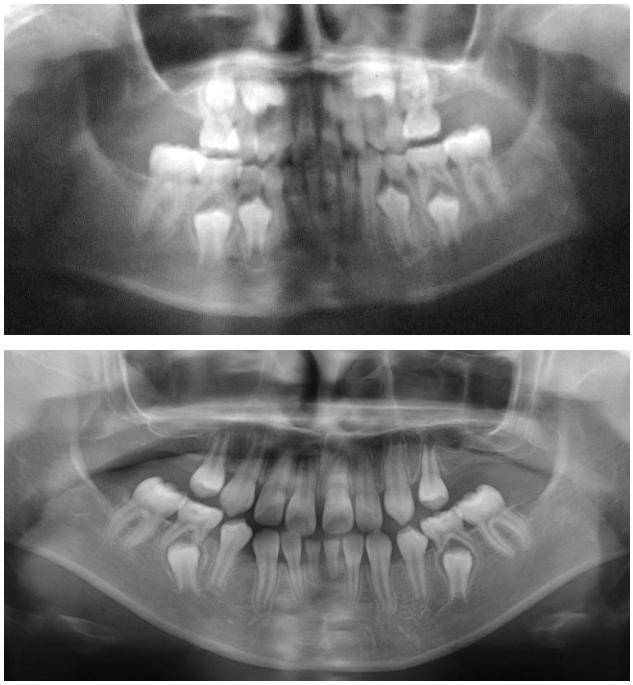
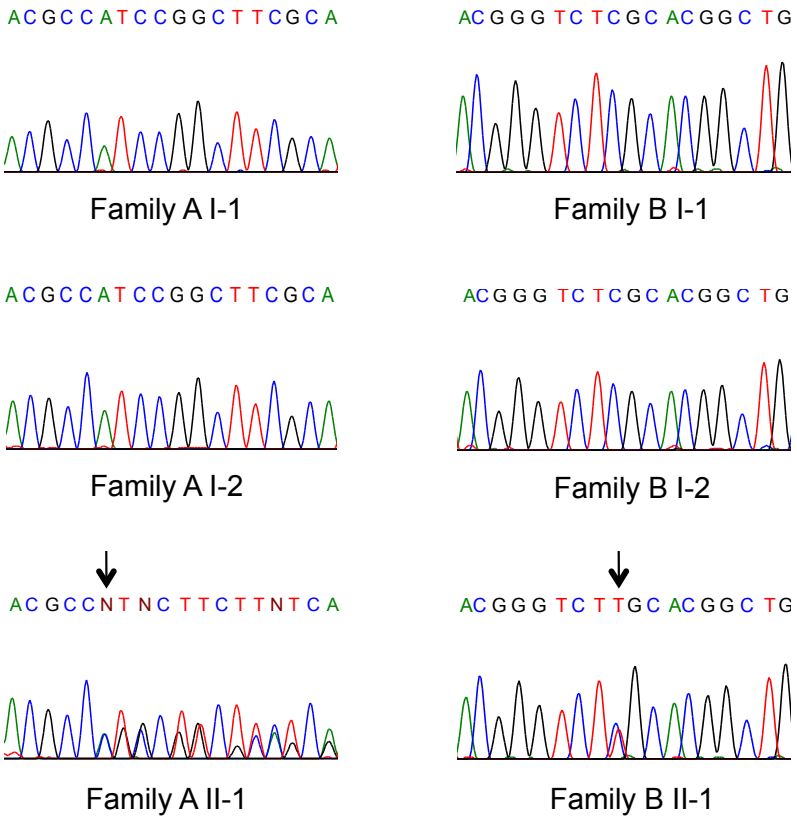


Figure 2

A


ACGCCATCCGGCTTCGCA
Family A I-1
ACGGGTCTCGCACGGCTG
Family B I-1
ACGCCATCCGGCTTCGCA
Family A I-2
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Family B I-2
ACGCCNTNCTTCTTNTCA
Family A II-1
ACGGGTCTTGCACGGCTG
Family B II-1

B

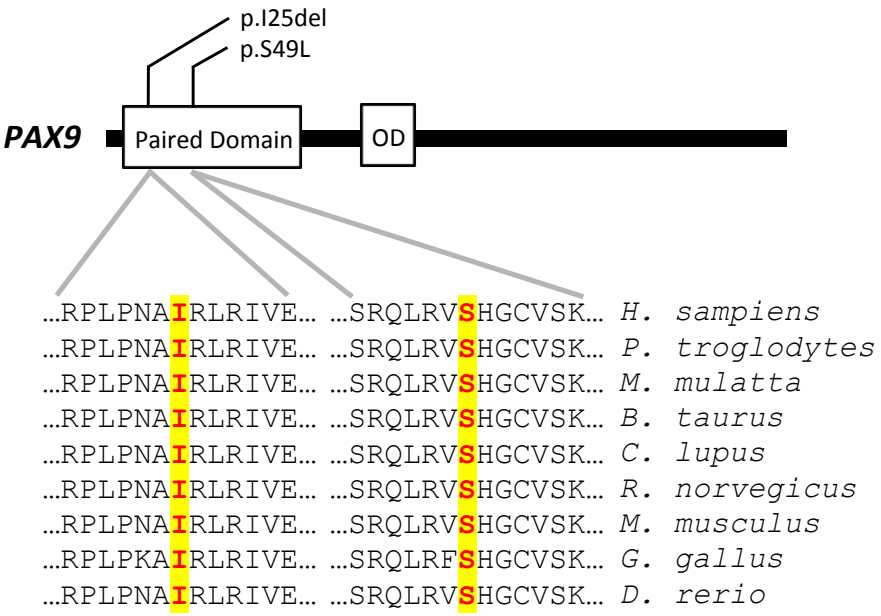
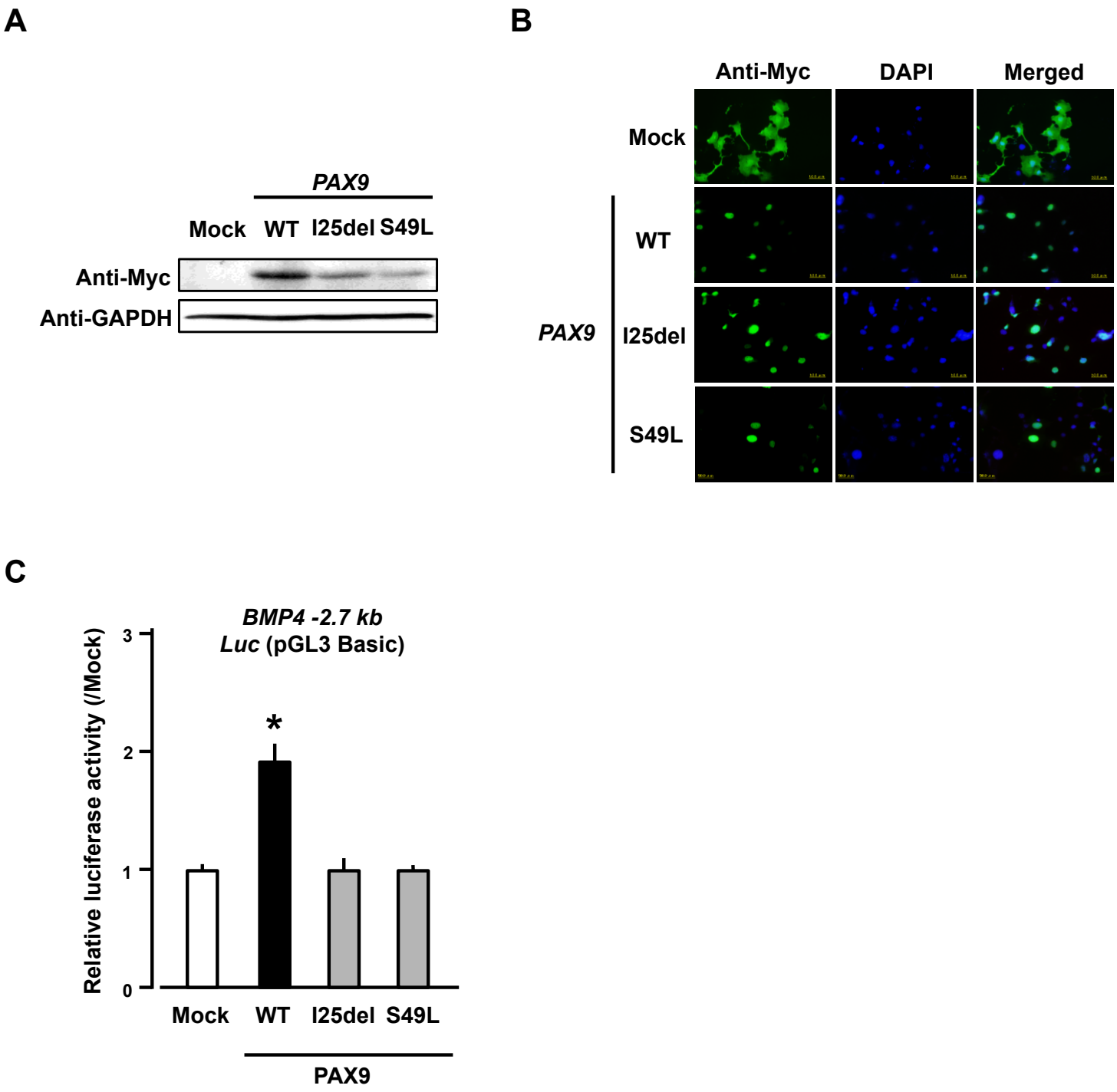

p.I25del
p.S49L
PAX9
Paired Domain
OD
…RPLPNAIRLRIVE… …SRQLRVSHGCVSK… H. sampiens
…RPLPNAIRLRIVE… …SRQLRVSHGCVSK… P. troglodytes
…RPLPNAIRLRIVE… …SRQLRVSHGCVSK… M. mulatta
…RPLPNAIRLRIVE… …SRQLRVSHGCVSK… B. taurus
…RPLPNAIRLRIVE… …SRQLRVSHGCVSK… C. lupus
…RPLPNAIRLRIVE… …SRQLRVSHGCVSK… R. norvegicus
…RPLPNAIRLRIVE… …SRQLRVSHGCVSK… M. musculus
…RPLPKAIRLRIVE… …SRQLRFSHGCVSK… G. gallus
…RPLPNAIRLRIVE… …SRQLRVSHGCVSK… D. rerio

Figure 3


A
PAX9
Mock
WT
I25del
S49L
Anti-Myc
Anti-GAPDH
B
Anti-Myc
DAPI
Merged
Mock
PAX9
WT
I25del
S49L
C
BMP4 -2.7 kb
Luc (pGL3 Basic)
Relative luciferase activity (/Mock)
3
2
1
0
*
Mock
WT
I25del
S49L
PAX9