Genetic Analysis

for Non-Syndromic Peg lateralis

Using Whole Exome Sequencing

Junglim Choi

The Graduate School

Yonsei University

Department of Dentistry

Genetic Analysis

for Non-Syndromic Peg lateralis

Using Whole Exome Sequencing

A Dissertation

Submitted to the Department of Dentistry

and the Graduate School of Yonsei University

in partial fulfillment of the

requirements for the degree of

Doctor of Philosophy in Dental Science

Junglim Choi

June 2024

This certifies that the Doctoral Dissertation

of Junglim Choi is approved.

Thesis Supervisor: Jae Hoon Lee

Jee-Hwan Kim

Sung-Won Cho

Je Seon Song

Sanguk Kim

The Graduate School

Yonsei University

June 2024

감사의 글

먼저, 본 논문이 완성되기까지 아낌없이 가르쳐주신 이재훈 교수님께 깊은 감사의 마음을 드리고 싶습니다. 연구과정 내내 조언을 해주신 김상욱 교수님, 그리고 같이 연구하고 치열하게 토론하며 같이 연구를 완성시켰던 안현수 선생님, 김현수 선생님께 감사함을 전달합니다. 또한, 본 논문을 위해 귀중한 시간을 내어 꼼꼼하게 살펴봐 주시고 훌륭한 조언과 격려를 해주신 조성원 교수님, 송제선 교수님 그리고 김지환 교수님께도 깊은 감사의 말씀을 올립니다.

평생 제 편이 되어 제가 어떤 일을 하든지 믿고 응원해주시는 엄마, 아빠 너무 사랑하고 감사합니다. 늦은 나이까지 뒷바라지 해주시며 아낌없이 늘 주심에 이렇게 결과물을 얻을 수 있었습니다. 한없이 어렸지만, 이제는 한 가정의 가장으로 한 아이의 아빠로 너무 열심히 살고 있는 내 동생 그리고 올케와 너무나 예쁜 조카 민지에게도 감사의 마음을 전합니다. 누구보다도 나를 아끼시고 내 편이 되어 주시는 어머님과 아가씨, 너무 귀여운 조카 준희와 서희에게도 고마움을 전하고 싶습니다.

언제나 저를 응원해주시는 단국대학교 통합치의학과 교수님들께도 감사인사 드립니다. 인생의 멘토 같은 유태민 교수님, 항상 본받고 싶은 도레미 교수님, 그리고 저의 곁에서 위로해주시고 응원해주시는 송승례 교수님, 김경은 교수. 논문이 나오기까지 꽤 오랜 시간 동안 지지해주시고 응원해 주심에 감사합니다.

그리고, 항상 나를 응원하고 위로해주고, 한껏 안아주는, 힘이 되어준 나의 가장 소중한 친구 내 남편. 언제나 사랑하고 감사합니다. 물심양면으로 도와준 저의 소중한 사람들에게 이 논문을 받칩니다

최정임

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Genetic Analysis for Non-Syndromic Peg lateralis Using Whole Exome Sequencing

Junglim Choi, D.D.S.

Department of Dentistry, Graduate School, Yonsei University
(Directed by Prof. Jae Hoon Lee, D.D.S., M.S.D.., Ph.D.)

Although peg-shaped lateral incisors are a common dental anomaly, the genetic mechanisms underlying peg lateralis are poorly understood, particularly in cases without associated anomalies. The present study aimed to identify potential candidate genes contributing to the development of non-syndromic peg lateralis, by performing whole-exome sequencing (WES). Saliva samples were collected from 20 cases of unrelated Korean individuals that were; not associated with other anomalies. WES was conducted on these samples, and variants were filtered using criteria of a *p*-value < 0.05, a false discovery rate < 10^{-10}, and an odds ratio > 1. In silico mutation impact analysis was performed using Polymorphism Phenotyping v2, sorting intolerant from tolerant, and integrated score of co-evolution and conservation algorithms. We identified a heterozygous allele, which are candidate genes unknown gene, a *AL132642* and *OTOP1* which encodes the Otopetrin-1 protein, a proton channel, in all 20 individuals. Gene

Ontology analysis revealed an association between candidate genes and peg lateralis. We further confirmed that the peg lateralis candidate variants of the same genotype were found in the family member of three subjects. The results suggest a possible function of new identified genes, which is yet to be studied, and identified it as new candidates contributing to the development of peg lateralis. This study provides new insights into the genetic basis of non-syndromic peg lateralis and has important implications for further studies on the role of new genes in peg lateralis.

Keywords: Calcium flux; Dental anomalies; Otopetrin; Peg-shaped maxillary lateral incisors; Whole exome sequencing

Genetic Analysis for Non-Syndromic Peg lateralis Using Whole Exome Sequencing

Department of Dentistry

Graduate School, Yonsei University

(Directed by Professor Jae Hoon Lee, D.D.S., M.S.D., Ph.D.)

Junglim Choi

I. INTRODUCTION

More than 350 genes, most of which encode signaling molecules that play crucial roles in molecular and cellular interactions, have been identified to be involved in odontogenesis (Townsend et al., 2012). The epithelial-mesenchymal interactions mediated by these proteins regulate the different stages of tooth formation, including initiation, morphogenesis, and differentiation (Tucker and Sharpe, 2004). Tooth anomalies, which appear as alterations in the number, size, morphology, or structure of a single tooth or multiple teeth, occur in isolation or in association with other syndromes (Cobourne and Sharpe, 2013). Congenital tooth anomalies cause functional, esthetic, and psychological problems, sometimes leading to significant facial changes

(Suda et al., 2010; Taju et al., 2018; Townsend et al., 2005). Thus, early diagnosis is essential to allow for early intervention at appropriate time, and studies on biomarkers for dental abnormalities may be necessary.

In 1987, Granhene defined peg lateralis as a tooth with incisal mesiodistal width shorter than cervical width (Fig. 1). These tapered shaped tooth often appears in maxillary lateral incisors and is thus called peg lateralis (Davis, 1987). The prevalence of peg lateralis varies from 0.6% to 9.9% depending on ethnicity, sex, and region; Mongolians have a significantly higher prevalence rate than other ethnic groups. However, the overall prevalence rate is 1.8% according to meta-analyses (Hua et al., 2013). Although non-syndromic teeth abnormalities are inherited as an autosomal trait, the literature suggests that peg shaped teeth are 1.35 times more prevalent in women (Polder et al., 2004). Both unilateral and bilateral peg lateralis seem to have a similar prevalence, but the frequency of left-side unilateral peg lateralis are almost twice that of right-side ones (Magnusson, 1977; Meskin and Gorlin, 1963).

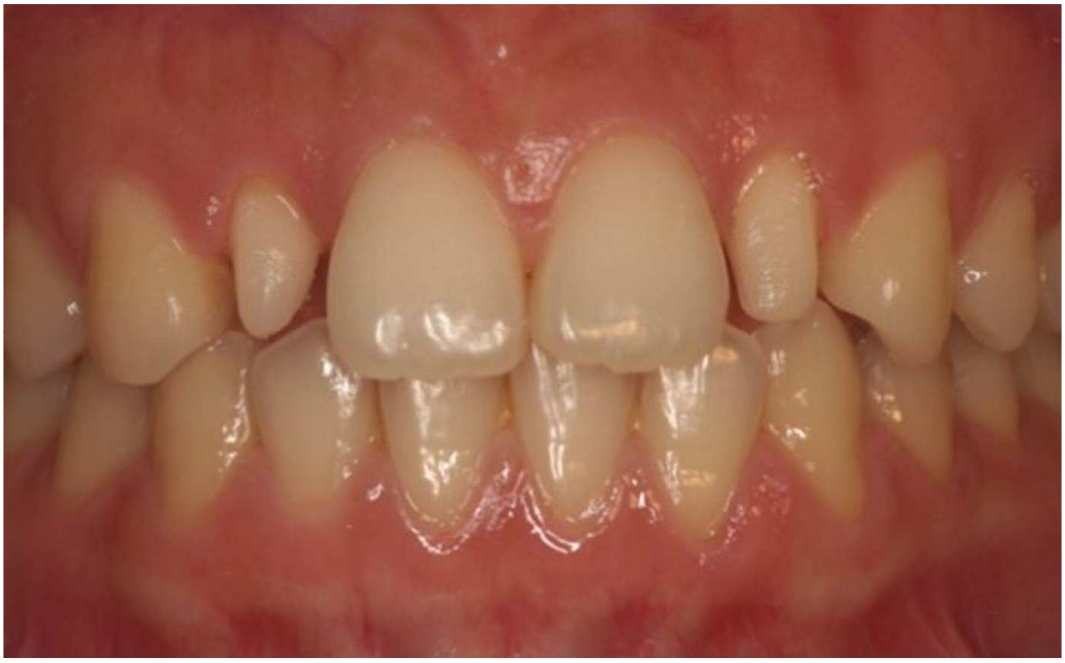

Figure 1. Peg lateralis on both maxillary lateral incisors.

Moreover, peg lateralis has a higher incidence of being associated with other tooth anomalies, such as hypodontia, palatal displacement of maxillary canines, dens invaginatus, tooth transposition, premolar rotations, and supernumerary teeth. These anomalies are considered as the same genotype exhibiting different phenotypes (Baccetti, 1998). According to Brook's etiological model, he suggested the relationship between alterations in number and size (Brook, 1984). These associations have been considered as different phenotypes of an identical genotype, leading to variations in tooth number and size. Brook suggested an etiological model wherein the absence of tooth may be a quasi-continuous phenotype of tooth size distribution (Brook, 1984; Liuk et al., 2013). Dental diseases are not determined by a single gene malfunction, but by the interaction of several genes and environmental factors. Previous studies have identified mutation in genes, such as *MSX1*, *AXIN2*, *FGF3*, *EDA*, *WNT5A*, *WNT10A*, and *Pax9* as related to the occurrence of both microdontia and hypodontia (Brook et al., 2014; Cai et al., 2011; Laurikkala et al., 2001). This pleiotropic and multifactorial nature of tooth anomalies, due to a complex mechanism of odontogenesis, makes it difficult to identify a specific single gene involved in a specific tooth anomaly. Because of these obstacles, genetic studies of non-syndromic isolated peg lateralis have rarely been reported. Therefore, this study analyzes peg lateralis without any syndromes or other tooth anomalies to investigate its genotypes.

We analyzed DNA from subjects with a peg-shaped incisor. Whole exome sequencing (WES) is a next-generation sequencing technique that analyzes the exon region, which codes for proteins and accounts for only 2% of the total genome (Rabbani, Tekin and Mahdieh, 2014). Only approximately 23,000 genes in the human genome are protein-coding genes. Interestingly, about 85% of diseases-related genes are associated with these exon sites. Therefore, while whole genome sequencing (WGS) analyzes genes, including non-coding genes, WES is an easier and a more effective tool for investigating genes contributing to the pathogenesis of various diseases. In this study, we applied WES to investigate the special genotype associated with the phenotype of peg lateralis in pilot study.

II. MATERIALS AND METHODS

1. Sample collection and DNA extraction

The study protocol was approved by the Institutional Review Board of Yonsei University College of Dentistry (Yonsei IRB No. 2-2020-0045). The subjects were recruited from 2020 to 2022 at Yonsei University Dental Hospital and Dankook University Dental Hospital. The experimental group consisted of 20 individuals with peg lateralis who voluntarily provided consent to participate in this clinical trial. Subjects were limited to patients with a family member who had peg lateralis; patients with systemic disease or other tooth anomalies were excluded. Consent was obtained from all participants before sample collection and was documented. The subject demographics are presented in Table 1 and Figure 2. Additionally, saliva samples were collected from the family members of three subjects with peg lateralis, who voluntarily agreed to participate, to additionally validate the association between candidate variants and peg lateralis.

For DNA analysis, saliva samples were collected from the subjects using a DNA self-collection kit (Genotek, Ottawa, Ontario, Canada). Each subject provided 2 mL saliva in the collection kit containing a 2 mL DNA-preserving solution in the lid. After collecting saliva, the lid on the kit was closed, and the DNA-preserving solution was mixed with the saliva. The genetic information of the control group was analyzed with the Ansan cohort's information provided by the Korea Disease Control and Prevention Agency. This can be considered a standardization of Koreans as a population-based cohort, which was established for general population groups aged 40 or older, and a "gene-environment model cohort" to identify risk factors for genetic and environmental interaction in chronic diseases.

Table 1. Demographics of the study participants

Pt.	Sex	Age	Site	Family history
1	M	23	Both	Mother
2	F	22	Both	Mother
3	M	44	Both	Son
4	F	18	Right	Mother
5	F	61	Left	Sister, Son
6	F	23	Right	Father
7	F	42	Right	Daughter
8	F	33	Right	Sister
9	M	24	Right	Father
10	F	19	Right	Mother
11	F	20	Both	Aunt
12	F	40	Both	Cousins
13	F	24	Both	Aunt, Cousins
14	M	25	Both	Parents
15	M	17	Right	Mother
16	F	44	Both	Son, Mother
17	M	21	Both	Brother
18	F	54	Both	Son
19	M	23	Right	Uncle
20	F	30	Left	Mother, Brother

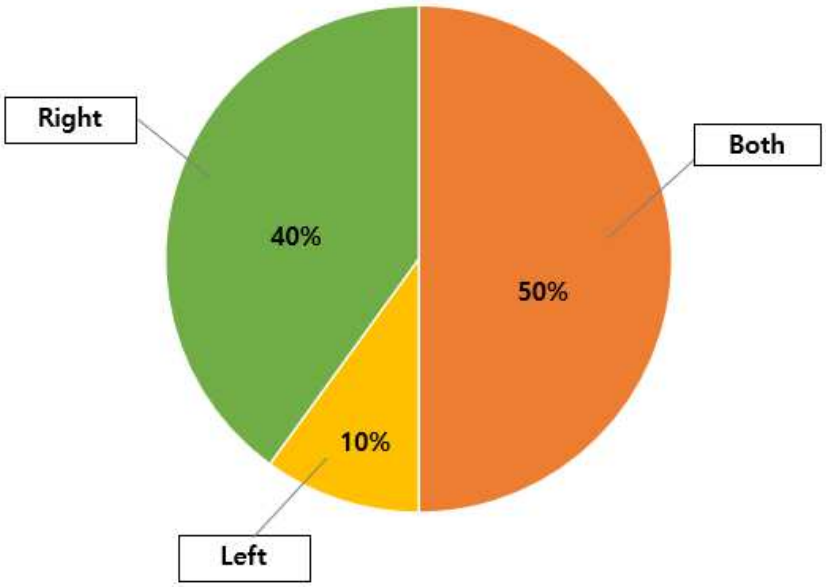

Figure 2. Rate of occurrence of peg lateralis site to the study participants

2. WES data variant filtering using statistical analysis

Variants were identified from the WES results, with only variants in protein-coding transcripts being considered. Variants that exhibited a *p*-value < 0.05, a false discovery rate (FDR) < 10^{-10}, and an odds ratio > 1 were identified using Fisher's exact test when comparing them to those in the Ansan's cohort. Variants with minor allele frequency (MAF) < 1% in the East Asian genome or with no reported MAF were subsequently filtered out using the genome aggregation database (gnomAD) v2.1.1 dataset (Karczewski et al., 2020). Additional filters were applied to exclude low-mutational impact variants, such as those annotated as LOW and MODIFIER impact in SnpEff, and low-mutational impact variants predicted with in-silico mutation-impact analysis (Cingolani et al., 2012). A list of candidate variants is summarized in Table 2.

Table 2. List of peg lateralis-related variants after filtration with statistical analysis and in-silico mutation-impact analysis. Statistical analysis used the Ansan's cohort as the control. The impacts of the mutation on canonical transcripts were predicted using SIFT, PolyPhen2, CES, and SnpEff.

Gene	dbSNP_id	UniProt ID	CHR - POS - REF - ALT	Mutation Type
AL132642	No_id	No_id	14 – 94410275 - CAA - CA, C	frameshift variant p.Lys57fs
OTOP1	rs199742451	Q7RTM1	4 – 4190576 - C - G	Missense variant p.Arg598Pro

PolyPhen2	SIFT	CES	SnpEff Impact	EST ASN MAF	MAF
			HIGH	ND	ND
PD	D	I	Moderate	0.01047	0.006169

dbSNP_id, variant ID in dbSNP; UniProt ID, protein ID in UniProt; Transcript ID, canonical transcript ID; CHR, chromosome; POS, base pair position; REF, reference allele; ALT, alternative allele; Effect, mutation type, types of mutation in protein; PolyPhen2, mutation impact predicted by PolyPhen2. 'PD' implies probably damaging; SIFT, mutation impact predicted by SIFT. 'D' implies damaging; CES, mutation impact predicted by CES. 'I' implies Intolerant; SnpEff Impact, categorized mutation impact pre-defined by sequence ontology effect; EST ASN MAF, alternative allele frequency in gnomAD v2.1.1 East Asian descendent samples; MAF, alternative allele frequency in whole gnomAD v2.1.1 samples.

3. In silico mutation impact analysis

To identify potentially pathogenic mutations in patients with peg lateralis, an in-silico mutation-impact analysis was performed. Missense variants in all possible non-redundant protein sequences of Ensembl GRCh37.p13 were analyzed using Polymorphism Phenotyping v2 (PolyPhen2), sorting intolerant from tolerant (SIFT), and an integrated score of co-evolution and conservation (CES) (Adzhubei et al., 2010; Kim et al., 2019; Vaser et al., 2016). These methods predict the effects of mutations by analyzing evolutionary patterns in protein sequences. We obtained PolyPhen2 and SIFT scores for candidate variants from SnpEf, which uses pre-calculated scores from the DbNspf database (Cingolani et al., 2012; Liu et al., 2020). The CES score was measured using pre-calculated scores provided at the CES website (https://sbi.postech.ac.kr/w/CE). The results of mutation-impact analysis are summarized in Table 2.

4. In-silico analysis of mutation impact on protein structure

To investigate the impact of the Arg598Pro (Arginine 598 to proline) mutation on the protein structure of Otopetrin-1 (OTOP1), we generated a 3D structure of OTOP1 using AlphaFold2, a state-of-the-art approach for predicting protein structure (Jumper et al., 2021). We selected interacting residues in which the shortest distance from Arg598 was less than 4 Å. Naccess was used to quantify the relative solvent accessibility (RSA) of the residues. Naccess calculates the atomically accessible surface by simulating the motion of a solvent molecule over a van der Waals surface (Lee and Richards, 1971). Changes in the energy of residues ($\Delta\Delta G$) upon mutation were assessed using the FoldX and PyRosetta computational methods (Chaudhury, Lyskov and Gray, 2010;

Schymkowitz et al., 2005). For FoldX, we employed the BuildModel function in its mutation engine to predict stability changes, and the default scoring function was used for PyRosetta.

III. RESULT

1. Identification of candidate variants discovered by WES analysis

We identified two candidate variants through WES analysis of 20 individuals with peg lateralis (Table 2). One of these variants was a rare variant, rs199742451, in *OTOP1*, with a MAF of 0.0105 in the East Asian population. All 20 peg lateralis individuals had a heterozygous genotype for this variant, while none of the 100 control individuals carried this variant. The *P*-value of fisher's exact test was $1.18X10^{-18}$ and the FDR corrected p-value was $2.19X10^{-15}$. The other candidate variant, a frameshift variant in *AL132642*, was not analyzed because of lack of studies.

2. In silico mutation impact analysis of the *OTOP1* variant

In-silico mutation-impact analysis predicted that the missense variant in *OTOP1* (c.1793G>C, p.Arg598Pro, rs199742451) is deleterious. Three different tools were used for the analysis: Polyphen2 (Adzhubei et al., 2010), SIFT (Vaser et al., 2016), and CES (Kim et al., 2019). All three tools predicted that the *OTOP1* variant is probably damaging and intolerant (Table 2). These predictions suggest that the variant is evolutionarily conserved and may be under a strong selective pressure. These evolutionary analyses imply that the observed variant will likely cause OTOP1 protein dysfunction. For instance, in Figure 2a, the missense variant is located in exon 1 of *OTOP1*, and arginine at amino acid position 598 in this exon is well conserved in mammalian orthologs (Fig. 3b).

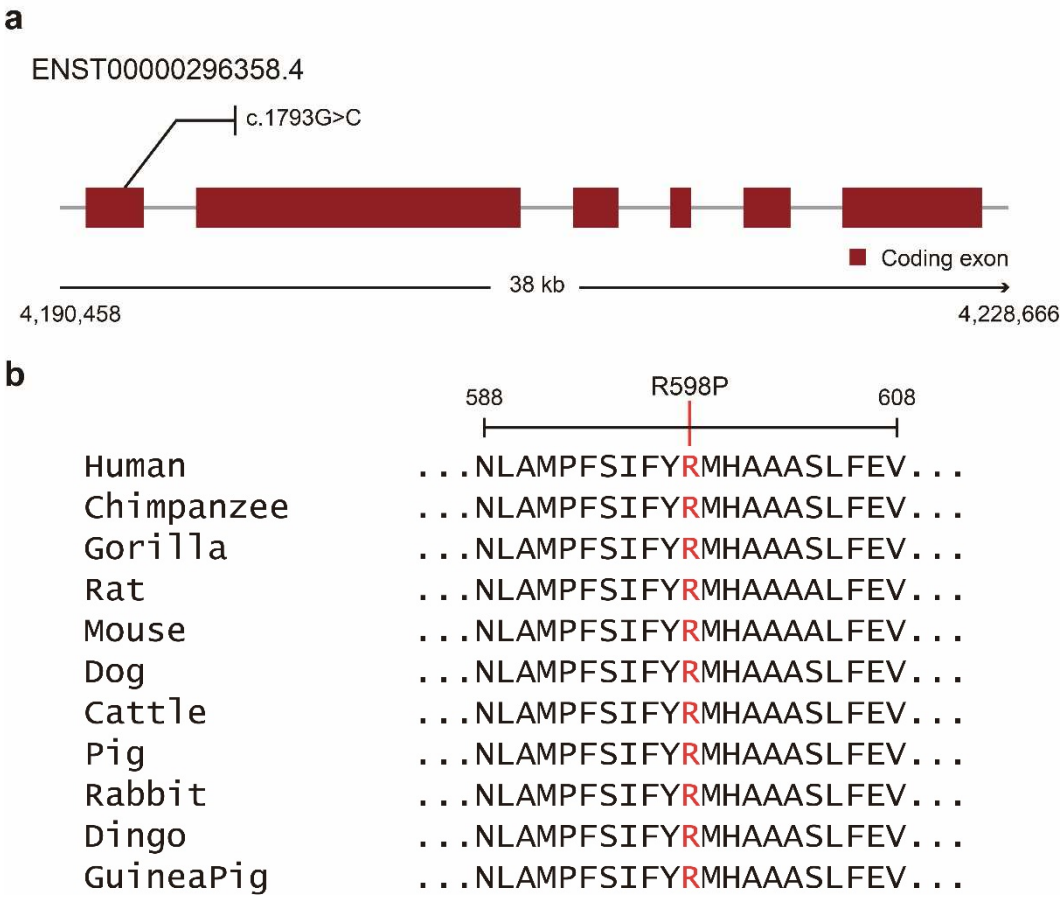

Figure 3. Schematic view and multiple sequence alignments of *OTOP1*.

a. Coding exons are shown as red boxes and introns are shown as gray lines. The missense variant (c.1793G>C, p.Arg598Pro, rs199742451) identified in the present is shown above the exons. **b.** Multiple sequence alignments for *OTOP1* homologs in various mammal species. The mutated Arg598 residue is indicated using red font.

3. Potential effect of OTOP1 mutation on protein 3D structure

In-silico structural analysis indicated that the Arg598Pro mutation is likely to have a deleterious impact on protein function. Analysis based on the tertiary structure of the protein showed that the Arg598 residue had limited exposure to solvents and interacted with 16 other amino acids (Fig. 4a, yellow sticks). The RSA value of Arg598 was low (Fig. 4b), suggesting that the residue is usually located in the inner region of the protein and has limited exposure to solvent. Therefore, the Arg598Pro substitution, where Arg is substituted by an amino acid with very different physicochemical properties at a low RSA position, may interfere with proper protein folding. In addition, the Arg598Pro variant was predicted to reduce the stability of the protein. Both Rosetta and FoldX, which calculate the stability change produced by mutations using the 3D structure of the protein, predicted that the Arg598Pro mutation makes OTOP1 unstable. The total stability change predicted by Rosetta and FoldX was 184.533 rosetta energy unit and 3.6573 kcal/mol, respectively (Fig. 4d,e). Furthermore, the reduced protein stability caused by mutations can potentially interfere with protein function.

The Arg598Pro substitution in OTOP1 disrupts a direct interaction with Glu435, which may impair the protein function. The interaction between the aligned sites with Arg598 and Glu435 was critical for the proton transport activity of zebrafish Otop1 (Saotome et al., 2019). A single mutation that interferes with the electrostatic interaction between Arg598 and Glu435 greatly diminished the current amplitude observed from Otop1. The tertiary structures of human OTOP1 and zebrafish Otop1 are well conserved, and the conformation and interactions of Arg598 and Glu435 are maintained in both species (Fig. 4c). This suggests that changes in the interaction between Arg598 and Glu435 may also lead to the loss of protein function in human OTOP1.

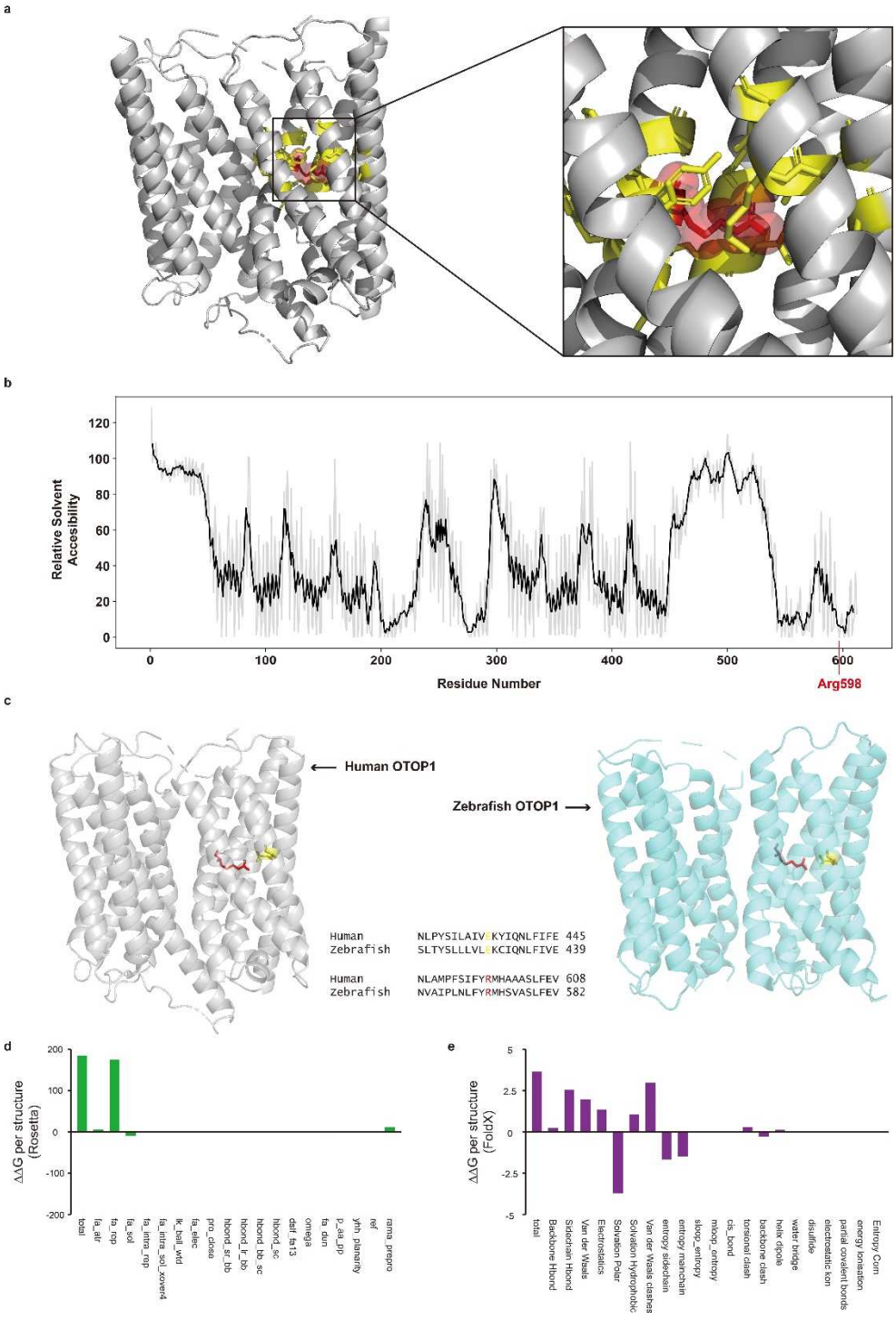

Figure 4. Protein structural analysis of the OTOP1 Arg598Pro variant.

a. Three-dimensional structure of OTOP1 predicted by AlphaFold. The structure was rendered using PyMO (residues with pLDDT<50 are hidden). The red spheres and yellow sticks indicate arginine 598 (Arg598) and its interacting amino acid residues, respectively. The Arg598 residue interacts with 16 residues. **b.** Relative solvent accessibility (RSA) plot of residues in OTOP1. The black line depicts the mean RSA of -2 to +2 residues. **c.** Comparison of structural characteristics of the OTOP1 Arg598Pro variant and sequence alignment between human and zebrafish homologs. Gray structure is the three-dimensional protein structure of human OTOP1 predicted by AlphaFold. Blue structure is the three-dimensional protein structure of zebrafish OTOP1 (PDB ID: 6NF4). The red and yellow residues in human OTOP1 indicate arginine 598 (Arg598) and glutamic acid 435 (Glu435), respectively. The red and yellow residues in zebrafish OTOP1 are the corresponding sites of human OTOP1 Arg598 and Glu435, respectively. The structure was rendered using PyMOL. **d.** Energy changes calculated with Rosetta and broken down by each score term. **e.** Energy changes calculated with FoldX and broken down by each score term.

fa_atr, Lennard-Jones attractive between atoms in different residues; fa_rep, Lennard-Jones repulsive between atoms in different residues; fa_sol, Lazaridis-Karplus solvation energy; fa_intra_rep, Lennard-Jones repulsive between atoms in the same residue; fa_intra_sol_xover4, Intra-residue Lazaridis-Karplus solvation energy; lk_ball_wtd, Asymmetric solvation energy; fa_elec, Coulombic electrostatic potential with a distance-dependent dielectric; pro_close, Proline ring closure energy and energy of psi angle of preceding residue; hbond_sr_bb, Backbone-backbone hbonds close in primary sequence; hbond_lr_bb, Backbone-backbone hbonds distant in primary sequence; hbond_bb_sc, Sidechain-backbone hydrogen bond energy; hbond_sc, Sidechain-sidechain hydrogen bond energy; dslf_fa13, Disulfide geometry potential; omega, Omega dihedral in the

backbone. A harmonic constraint on planarity with standard deviation of Omega dihedral in the backbone; fa_dun, Internal energy of sidechain rotamers as derived from Dunbrack's statistics; p_aa_pp, Probability of amino acid, given torsion values for phi and psi; yhh_planarity, A special torsional potential to keep the tyrosine hydroxyl in the plane of the aromatic ring; ref, Reference energy for each amino acid. Balances internal energy of amino acid terms. Plays role; rama_prepro, Ramachandran preferences (with separate lookup tables for pre-proline positions and other posit.). (Explanation for the x axes is from the Rosetta and FoldX official websites)

4. Functional analysis of *OTOP1*

Functional analysis of *OTOP1* suggested a possible association with peg lateralis. The Gene Ontology (GO) terms associated with *OTOP1*, obtained from the AmiGO database, included biomineral tissue development, which is critical for tooth development (Gene Ontology, 2021; Moradian-Oldak and George, 2021). This suggests a possible relationship between *OTOP1* and the development of peg lateralis.

5. Validation of the *OTOP1* variant in additional patients

To further validate the relationship between the candidate genes and peg lateralis, the genotype of the loci was additionally identified in the family members of study participants, as shown in Figure 4. Sequencing the family members of three subjects (the sister of No. 8, cousin of No. 12, and mother of No. 18) revealed the presence of the variants in all three. The variants were expressed in a heterozygous condition similar to that in the 20 study participants. This finding indicated the possibility that the variants are associated with the occurrence of peg lateralis within the family (Fig. 5).

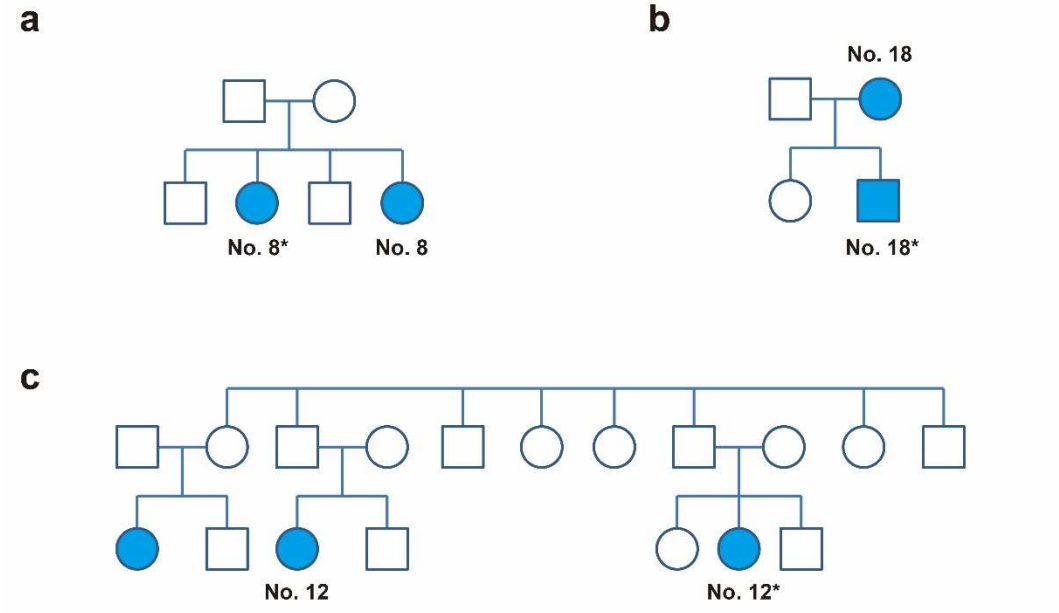

Figure 5. *AL132642* and *OTOP1* genotype of family members of three subjects.

a-c. Pedigrees of family No. 8 (a), No. 12 (b), and No. 18 (c).

6. Known hypodontia variants in Korean peg lateralis patients

The presence of peg lateralis is highly linked to other dental irregularities, including hypodontia, the genetics of which is relatively well studied (Baccetti, 1998). None of the study participants in this study exhibited any association with variants known to cause hypodontia. Analysis of variants related to hypodontia obtained from the DisGeNet database did not show any significant association with this study's participants (Table 3) (Pinero et al., 2020). This suggests that the peg lateralis in our cohort is not related to previously revealed hypodontia-related gene.

Table 3. Statistical significance of known hypodontia variants in a Korean peg lateralis cohort. Known hypodontia variants were collected from DisGeNET.

dbSNP_id, variant ID in dbSNP; CHR, chromosome; POS, base pair position; REF, reference allele; ALT, alternative allele; Fisher_p, p-value obtained in the Fisher Exact test; con_ref, reference allele count among 100 controls; con_alt, alternative allele count among 100 controls; pat_ref, reference allele count among 20 patients; pat_alt, alternative allele count among 20 patients

Gene	dbSNP_id	CHR	REF	ALT	Fisher_p	Allele Count			
						con_ref	con_alt	pat_ref	pat_alt
WNT10A	rs147680216	2	G	A	0.19	197	3	38	2
GLI3	rs929387	7	G	A	0.81	31	167	7	33
PAX9	rs12881240	14	C	T	1.00	151	43	31	9
PAX9	rs4904210	14	G	C	0.61	102	94	19	21
PLD2	rs3764897	17	G	A	0.22	180	20	39	1
AXIN2	rs2240308	17	G	A	0.71	138	62	26	14

IV. DISCUSSION

In this study, all patients with peg lateralis had a heterozygous mutation. Two candidate variants were identified; the *AL132642,* which is not a known gene, and a missense mutation in exon 1 of *OTOP1* (c.1793G>C, p.Arg598Pro, rs199742451) (Fig. 3). OTOP1 belongs to the Otopetrin family of multi-transmembrane domain proteins that are highly conserved in mammals (Hurle et al., 2003). OTOP1 is required for the formation of otoconia and otoliths, and mutations in OTOP1 in mice and zebrafish lead to non-syndromic otoconia agenesis. The Otopetrin proteins are a proton selective ion channel activated by purinergic stimuli in vestibule-supporting cells and play a crucial role in regulating intracellular calcium concentration (Hughes et al., 2007). Since otoconia are essential for mechanosensory transduction of linear acceleration and gravity in the inner ear, their degeneration or displacement can result in dizziness and progressive loss of balance. OTOP1 expression is continuously observed in adult mice and is involved in the maintenance and restoration of otoconia mineralization. However, whether OTOP1 expression persists in humans remain unclear (Kim et al., 2010). Although the biochemical role of OTOP1 was recently identified, it has not been implicated in odontogenesis. Nevertheless, our results suggest that *OTOP1* and an unknown gene, *AL132642,* likely directly or indirectly affect the development a peg shaped tooth.

During dental development, ion channels regulate or control various physiological and biological activities, such as pH control, calcium flux, and gene expression (Duan, 2014). The loss-of-function mutations of OTOP1 identified in the present study might cause an influx of calcium ion and disrupt calcium homeostasis, potentially leading to peg lateralis (Fig. 4). One study found that mutations in *CACNA1S,* which encodes the voltage-

dependent calcium channel, leads to the formation of multiple cusps in molars this study analyzed the genes and demonstrated that defects in calcium signal interaction could cause variations in tooth morphology (Laugel-Haushalter et al., 2018).

As OTOP1 is a proton-selective ion channel, it is activated by acidic conditions and directly gated by protons (Teng et al., 2022). In Tain's study, it was activated by alkaline conditions, and mutations in OTOP1 affected its activation in alkaline conditions but had no influence in acidic conditions (Tian et al., 2023). The alkali-activated OTOP1 is permeable to protons and cations but not calcium ions. The mechanism of pH regulation in odontogenesis remains unknown, but the characteristics of mutant OTOP1 might affect abnormal tooth morphology by altering calcium ion concentration.

Tooth development progresses through the initiation, bud and bell stages and genetically controlled through several molecular signaling pathways. An important part of tooth development is the folding the tooth epithelium at the cap and bell stage, regulated by primary and secondary enamel knots (Jernvall and Thesleff, 2012). During tooth morphogenesis, the enamel knot serves as a signaling center that expresses several signals, including SHH, BMP, FGF, and WNT families, regulating tooth size and shape by controlling the growth and folding of the internal dental epithelium (Jernvall et al., 1994). In addition, apoptosis in the enamel knot determines tooth size or morphology by regulating the duration of signaling interactions and is crucial in determining the size and patterning of cusps (Jernvall et al., 1998). In Kim's study, inhibiting apoptosis of enamel knots reduced the crown height (Kim et al., 2006). The enamel knot determines the number, shape, size, and location of cusps, thus determining the shape of the occlusal surface of the molars. Consequently, the final shape of the crown is already determined at the bell and cap stages (Jernvall and Thesleff, 2012).

CACNA1S is expressed in epithelial cells around the secondary enamel from the initial

bud to the postnatal stage. Altering the inflow of calcium near the enamel knots suggests that the molars caused additional cusps. *CACNA1S* is also expressed in the Hertwig's epithelial root sheath during postnatal development and is implicated in mal-shaped roots (Kantaputra et al., 2023). Thus, the morphology of teeth is affected by the time and location of gene expression. Furthermore, while the molar exhibited more complex cusps, the premolar had a less complexity due to mutation. The author suggests that the difference could be due to variations in tissue sensitivity to mutations (Kantaputra et al., 2023). Therefore, whether the same incisors express the mutant phenotype may depend on difference in tissue sensitivity to mutation. However, animal studies are needed to investigate the timing and location of the candidate gene expressions found in this study to confirm their effects on the mechanism of generating peg shaped tooth.

Unlike previous studies, this study only analyzed the DNA of patients with peg lateralis unassociated with other anomalies or syndromes using WES. We identified *OTOP1* and *AL132642* as candidate genes contributing to the development of peg lateralis. Moreover, additional analysis within the same family identified that close family members carried the same genetic variant, which further supported the finding from the initial study cohort (Fig. 5). Notably, this finding differs from previous findings that suggested that dental anomalies within the same genotype have different phenotypes. This difference is likely because this study exclusively selected isolated peg lateralis subjects, even though peg lateralis is more commonly associated with other abnormalities (Kim, Choi and Kim, 2017). Although the role of OTOP1 and another gene in dental development have not been clearly defined, our results suggest the possibility of interrelation between two candidate genes and peg shaped tooth. The potential function of *OTOP1* in tooth morphogenesis indicates the need for future research on genes in odontogenesis.

While the variants had significant associations in the 20 Korean patients, the sample

size was relatively small to definitively confirm a significant association between the variant and peg-lateralis. Analyzing the entire family, including members without peg lateralis, could provide stronger evidence for the relationship between genes and phenotype. Additionally, in-silico analysis had limitations in establishing a causal relationship. Despite these limitations, as this study presents the relationship between candidate genes and peg lateralis for the first time, we hope that the findings herein will serve as a cornerstone for further experimental studies, such as animal mutation or molecular studies, to determine the cause and pathogenesis of peg-lateralis.

V. CONCLUSION

This study aimed to identify genotypes associated with non-syndromic and isolated peg lateralis in Koreans using whole-exome sequencing (WES). In all patients, heterozygous alleles were found, and two candidate genes were identified: *AL132642*, an unknown gene and *OTOP1* which encodes a calcium ion channel. However, WES and in-silico analysis have limitations in confirming strong relationships between candidate genes and peg lateral. This study is a preliminary exploration of genotypes associated with peg shaped teeth and may help design future studies on tooth development. Further studies with larger populations and animal studies focusing on the candidate genes are needed to identify the genetic basis of peg lateralis.

VI. REFERENCES

Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, Kondrashov AS, Sunyaev SR. A method and server for predicting damaging missense mutations. Nat Methods 2010;7(4):248-9.

Baccetti T. A controlled study of associated dental anomalies. Angle Orthod 1998;68(3):267-74.

Brook AH. A unifying aetiological explanation for anomalies of human tooth number and size. Arch Oral Biol 1984;29(5):373-8.

Brook AH, Jernvall J, Smith RN, Hughes TE, Townsend GC. The dentition: the outcomes of morphogenesis leading to variations of tooth number, size and shape. Aust Dent J 2014;59 Suppl 1:131-42.

Cai J, Mutoh N, Shin JO, Tani-Ishii N, Ohshima H, Cho SW, Jung HS. Wnt5a plays a crucial role in determining tooth size during murine tooth development. Cell Tissue Res 2011;345(3):367-77.

Chaudhury S, Lyskov S, Gray JJ. PyRosetta: a script-based interface for implementing molecular modeling algorithms using Rosetta. Bioinformatics 2010;26(5):689-91.

Cingolani P, Platts A, Wang le L, Coon M, Nguyen T, Wang L, Land SJ, Lu X, Ruden DM. A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of Drosophila melanogaster strain w1118; iso-2; iso-3. Fly (Austin) 2012;6(2):80-92.

Cobourne MT, Sharpe PT. Diseases of the tooth: the genetic and molecular basis of inherited anomalies affecting the dentition. Wiley Interdiscip Rev Dev Biol 2013;2(2):183-212.

Davis PJ. Hypodontia and hyperdontia of permanent teeth in Hong Kong schoolchildren. Community Dent Oral Epidemiol 1987;15(4):218-20.

Duan X. Ion channels, channelopathies, and tooth formation. J Dent Res 2014;93(2):117-25.

Gene Ontology C. The Gene Ontology resource: enriching a GOld mine. Nucleic Acids Res 2021;49(D1):D325-D34.

Hua F, He H, Ngan P, Bouzid W. Prevalence of peg-shaped maxillary permanent lateral incisors: A meta-analysis. Am J Orthod Dentofacial Orthop 2013;144(1):97-109.

Hughes I, Saito M, Schlesinger PH, Ornitz DM. Otopetrin 1 activation by purinergic nucleotides regulates intracellular calcium. Proc Natl Acad Sci U S A 2007;104(29):12023-8.

Hurle B, Ignatova E, Massironi SM, Mashimo T, Rios X, Thalmann I, Thalmann R, Ornitz DM. Non-syndromic vestibular disorder with otoconial agenesis in tilted/mergulhador mice caused by mutations in otopetrin 1. Hum Mol Genet 2003;12(7):777-89.

Jernvall J, Aberg T, Kettunen P, Keranen S, Thesleff I. The life history of an embryonic signaling center: BMP-4 induces p21 and is associated with apoptosis in the mouse tooth enamel knot. Development 1998;125(2):161-9.

Jernvall J, Kettunen P, Karavanova I, Martin LB, Thesleff I. Evidence for the role of the enamel knot as a control center in mammalian tooth cusp formation: non-dividing cells express growth stimulating Fgf-4 gene. Int J Dev Biol 1994;38(3):463-9.

Jernvall J, Thesleff I. Tooth shape formation and tooth renewal: evolving with the same signals. Development 2012;139(19):3487-97.

Jumper J, Evans R, Pritzel A, Green T, Figurnov M, Ronneberger O, Tunyasuvunakool K, Bates R, Zidek A, Potapenko A, Bridgland A, Meyer C, Kohl SAA, Ballard AJ, Cowie A, Romera-Paredes B, Nikolov S, Jain R, Adler J, Back T, Petersen S, Reiman D, Clancy E, Zielinski M, Steinegger M, Pacholska M, Berghammer T, Bodenstein S, Silver D, Vinyals O, Senior AW, Kavukcuoglu K, Kohli P, Hassabis D. Highly accurate protein structure prediction with AlphaFold. Nature 2021;596(7873):583-9.

Kantaputra P, Butali A, Eliason S, Chalkley C, Nakornchai S, Bongkochwilawan C, Kawasaki K, Kumchiang A, Ngamphiw C, Tongsima S, Ketudat Cairns JR, Olsen B, Intachai W, Ohazama A, Tucker AS, Amendt BA. CACNA1S mutation-associated dental anomalies: A calcium channelopathy. Oral Dis 2023.

Karczewski KJ, Francioli LC, Tiao G, Cummings BB, Alfoldi J, Wang Q, Collins RL, Laricchia KM, Ganna A, Birnbaum DP, Gauthier LD, Brand H, Solomonson M,

Watts NA, Rhodes D, Singer-Berk M, England EM, Seaby EG, Kosmicki JA, Walters RK, Tashman K, Farjoun Y, Banks E, Poterba T, Wang A, Seed C, Whiffin N, Chong JX, Samocha KE, Pierce-Hoffman E, Zappala Z, O'Donnell-Luria AH, Minikel EV, Weisburd B, Lek M, Ware JS, Vittal C, Armean IM, Bergelson L, Cibulskis K, Connolly KM, Covarrubias M, Donnelly S, Ferriera S, Gabriel S, Gentry J, Gupta N, Jeandet T, Kaplan D, Llanwarne C, Munshi R, Novod S, Petrillo N, Roazen D, Ruano-Rubio V, Saltzman A, Schleicher M, Soto J, Tibbetts K, Tolonen C, Wade G, Talkowski ME, Genome Aggregation Database C, Neale BM, Daly MJ, MacArthur DG. The mutational constraint spectrum quantified from variation in 141,456 humans. Nature 2020;581(7809):434-43.

Kim D, Han SK, Lee K, Kim I, Kong J, Kim S. Evolutionary coupling analysis identifies the impact of disease-associated variants at less-conserved sites. Nucleic Acids Res 2019;47(16):e94.

Kim E, Hyrc KL, Speck J, Lundberg YW, Salles FT, Kachar B, Goldberg MP, Warchol ME, Ornitz DM. Regulation of cellular calcium in vestibular supporting cells by otopetrin 1. J Neurophysiol 2010;104(6):3439-50.

Kim JH, Choi NK, Kim SM. A Retrospective Study of Association between Peg-shaped Maxillary Lateral Incisors and Dental Anomalies. J Clin Pediatr Dent 2017;41(2):150-3.

Kim JY, Cha YG, Cho SW, Kim EJ, Lee MJ, Lee JM, Cai J, Ohshima H, Jung HS. Inhibition of apoptosis in early tooth development alters tooth shape and size. J Dent Res 2006;85(6):530-5.

Laugel-Haushalter V, Morkmued S, Stoetzel C, Geoffroy V, Muller J, Boland A, Deleuze JF, Chennen K, Pitiphat W, Dollfus H, Niederreither K, Bloch-Zupan A, Pungchanchaikul P. Genetic Evidence Supporting the Role of the Calcium Channel, CACNA1S, in Tooth Cusp and Root Patterning. Front Physiol 2018;9:1329.

Laurikkala J, Mikkola M, Mustonen T, Aberg T, Koppinen P, Pispa J, Nieminen P, Galceran J, Grosschedl R, Thesleff I. TNF signaling via the ligand-receptor pair ectodysplasin and edar controls the function of epithelial signaling centers and is regulated by Wnt and activin during tooth organogenesis. Dev Biol

2001;229(2):443-55.

Lee B, Richards FM. The interpretation of protein structures: estimation of static accessibility. J Mol Biol 1971;55(3):379-400.

Liu X, Li C, Mou C, Dong Y, Tu Y. dbNSFP v4: a comprehensive database of transcript-specific functional predictions and annotations for human nonsynonymous and splice-site SNVs. Genome Med 2020;12(1):103.

Liuk IW, Olive RJ, Griffin M, Monsour P. Associations between palatally displaced canines and maxillary lateral incisors. Am J Orthod Dentofacial Orthop 2013;143(5):622-32.

Magnusson TE. Prevalence of hypodontia and malformations of permanent teeth in Iceland. Community Dent Oral Epidemiol 1977;5(4):173-8.

Meskin LH, Gorlin RJ. Agenesis and Peg-Shaped Permanent Maxillary Lateral Incisors. J Dent Res 1963;42:1476-9.

Moradian-Oldak J, George A. Biomineralization of Enamel and Dentin Mediated by Matrix Proteins. J Dent Res 2021;100(10):1020-9.

Pinero J, Ramirez-Anguita JM, Sauch-Pitarch J, Ronzano F, Centeno E, Sanz F, Furlong LI. The DisGeNET knowledge platform for disease genomics: 2019 update. Nucleic Acids Res 2020;48(D1):D845-D55.

Polder BJ, Van't Hof MA, Van der Linden FP, Kuijpers-Jagtman AM. A meta-analysis of the prevalence of dental agenesis of permanent teeth. Community Dent Oral Epidemiol 2004;32(3):217-26.

Rabbani B, Tekin M, Mahdieh N. The promise of whole-exome sequencing in medical genetics. J Hum Genet 2014;59(1):5-15.

Saotome K, Teng B, Tsui CCA, Lee WH, Tu YH, Kaplan JP, Sansom MSP, Liman ER, Ward AB. Structures of the otopetrin proton channels Otop1 and Otop3. Nat Struct Mol Biol 2019;26(6):518-25.

Schymkowitz J, Borg J, Stricher F, Nys R, Rousseau F, Serrano L. The FoldX web server: an online force field. Nucleic Acids Res 2005;33(Web Server issue):W382-8.

Suda N, Hattori M, Kosaki K, Banshodani A, Kozai K, Tanimoto K, Moriyama K. Correlation between genotype and supernumerary tooth formation in cleidocranial dysplasia. Orthod Craniofac Res 2010;13(4):197-202.

Taju W, Sherriff M, Bister D, Shah S. Association between severity of hypodontia and cephalometric skeletal patterns: a retrospective study. Eur J Orthod 2018;40(2):200-5.

Teng B, Kaplan JP, Liang Z, Krieger Z, Tu YH, Burendei B, Ward AB, Liman ER. Structural motifs for subtype-specific pH-sensitive gating of vertebrate otopetrin proton channels. Elife 2022;11.

Tian L, Zhang H, Yang S, Luo A, Kamau PM, Hu J, Luo L, Lai R. Vertebrate OTOP1 is also an alkali-activated channel. Nat Commun 2023;14(1):26.

Townsend G, Bockmann M, Hughes T, Brook A. Genetic, environmental and epigenetic influences on variation in human tooth number, size and shape. Odontology 2012;100(1):1-9.

Townsend GC, Richards L, Hughes T, Pinkerton S, Schwerdt W. Epigenetic influences may explain dental differences in monozygotic twin pairs. Aust Dent J 2005;50(2):95-100.

Tucker A, Sharpe P. The cutting-edge of mammalian development; how the embryo makes teeth. Nat Rev Genet 2004;5(7):499-508.

Vaser R, Adusumalli S, Leng SN, Sikic M, Ng PC. SIFT missense predictions for genomes. Nat Protoc 2016;11(1):1-9.

ABSTRACT (KOREAN)

전장 엑솜 시퀀싱을 이용한

증후군과 연관없는 왜소측절치의 유전학적 분석

최 정 임

연세대학교 대학원

치의학과

(지도교수 이 재 훈)

왜소측절치 (peg lateralis)은 흔한 치아 형태 이상이지만, 이에 관한 유전적 메커니즘은 잘 알려져 있지 않다. 이에 따라 본 연구는 전장엑솜시퀀싱 (Whole exome sequencing, WES)을 통해 증후군과 상관없는 왜소치 발생에 기여하는 잠재적인 후보 유전자를 식별하는 것을 목표로 하였다.

다른 치아 이상을 가지지 않으며, 증후군과 관련이 없는 독립된 20명의 한국인으로부터 타액을 채취하였다. 전장엑솜시퀀싱 분석을 하여 100명의 정상

한국인 그룹과 비교하였다. 정상 그룹과 비교하여 환자 그룹에서 특이적으로 많이 발견되는 변이를 p-value < 0.05, false discovery rate < 10^{-10}, 그리고 odds ratio > 1을 기준으로 선별하였다. 선별된 변이들을 Polymolphism Phenotyping v2, Sorting Intolerant From Tolerant, 그리고 integarated score of co-evolution and conservation 3가지 알려진 도구와, in-silico 단백질 구조 분석을 통해 변이로 인한 단백질의 기능이상을 판별하였다.

본 연구에서는 20명의 모든 개인에서 양성자 채널인 otopetrin-1 단백질을 encoding 하는 이형 *OTOP1* 과 잘 알려져 있지 않은 *AL132642* 두 개의 대립 유전자 후보가 확인되었다. 또한, 유전자 온톨로지 분석 결과 후보 유전자들과 왜소치 사이의 기능적 연관성이 확인되었다. 추가적으로 실험자의 가족에서 왜소치를 가진 다른 구성원들에게도 동일한 유전자형의 왜소치 후보 변이체들이 발견되었다. 이 결과는 아직 연구되지 않은 두 개의 후보 유전자들의 새로운 기능의 가능성을 제시하고, 왜소측절치 발생에 기여하는 새로운 후보로 확인되었다. 본 연구는 증후군과 관련 없는 왜소치의 유전적 기초에 대한 새로운 이해를 제공하고, 새로운 유전자의 치아 발생 과정 중의 역할에 대한 후속 연구를 위한 배경이 될 수 있을 것으로 생각된다.

핵심되는 단어: 칼슘이온 흐름, 치아 이상, 왜소측절치, 전장엑솜시퀀싱 분석