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MOLECULAR GENETIC STUDY OF HUMAN CONGENITAL TOOTH AGENESIS

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Synopsis

Congenital tooth agenesis is one of the most common congenital anomalies in humans. It has been demonstrated that WNT/β-catenin signaling is crucial for determining final permanent tooth numbers in adult humans. Though haploinsufficiency of WNT10A, for example, is identified as the most frequent cause of human non-syndromic tooth agenesis, the etiology in many patients still remains unknown. Thus, we have performed a genetic analysis of Japanese patients using whole exome sequencing to discover causative genes for congenital human tooth agenesis. In this study, we isolated four variants in the LRP6 gene, known as a co-receptor for WNT ligands and as one of the pathogenic genes for tooth agenesis. Though one of the four variants was common and predicted as benign by the ClinVer database, the remaining three variants were very rare. In addition, SIFT and PolyPhen-2 scores predicted the pathogenicity of two of the three variants.

Key words: LRP6, tooth agenesis, whole exome sequencing

Introduction

The number of permanent teeth is generally 32 in humans. However, patients with missing teeth are often encountered in clinical practices, and congenitally missing teeth is known to be the most frequent human anomaly[1)]. The number of missing teeth is used for classifying congenital tooth agenesis: hypodontia is defined as five or fewer missing teeth, excluding the wisdom teeth, whereas oligodontia is defined as six or more missing teeth[2)]. The frequency of hypodontia is 6.8% (95% confidence interval: 6.1–7.7%) and that of oligodontia is 0.1% (95% confidence interval: 0.04–0.3%) in the Japanese population. We previously demonstrated that the sibling recurrence risk ratio of oligodontia is 43.8%, suggesting that oligodontia exhibits a dominant mode of inheritance in most cases[3)].

One line of study has revealed that stout teeth development requires a variety of growth factors or morphogens produced by the oral ectodermal epithelium, such as fibroblast growth factors, bone morphogenic proteins (BMP), and the wingless-type MMTV integration site (WNT) ligand family[4)]. WNT10A is the most frequent cause of human non-syndromic tooth agenesis including in Japan (STHAG-4, MIM 150400). The WNT signal stabilizes intracellular β-catenin, which activates lymphoid enhancer factor/T-cell factor protein. In tooth germ cells, the WNT/β-catenin signal induces the expression of essential transcriptional factors for human tooth development, such as muscle segment homeobox 1 (MSX1), paired box 9 (PAX9), runt-related transcription factor 2 (RUNX2), and BMP[5-8)]. However, the genetic causes remain unknown in more than 50% of human tooth agenesis cases.

Therefore, we performed whole-exome analysis of 15

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Japanese patients affected by tooth agenesis to identify the causative gene for the congenital disease. This study reports some gene variants in low-density lipoprotein receptor-related protein 6 (LRP6). LRP6 is a co-receptor for WNT ligand family members, of which heterozygous functional null variant causes human selective tooth agenesis (STHAG7: Phenotype MIM number, 616724) [9,10].

Patients and Methods

Patient recruitment

The subjects were the unrelated probands and their family members who presented to the Department of Maxillofacial Surgery, Aichi Gakuin University Dental Hospital, and were diagnosed with non-syndromic tooth agenesis by interview, intraoral examination, and imaging tests. Informed consent was obtained from all participants. After we obtained informed consent from 15 subjects aged 7 to 40-year-old, genomic DNA was extracted from the saliva for whole exome sequencing (WES) studies. This study was approved by the Aichi Gakuin University Committee on the Ethics of Human Cell Tissue Gene Immunology (Approval No. 94).

Genomic DNA extraction

Genomic DNA was isolated from saliva of the probands as previously described[11]. Saliva samples were collected with an Oragene® DISCOVER kit (DNA Genotek, Ottawa, Canada) according to the manufacturer's protocol.

Whole exome sequencing

Genomic DNA samples were sequenced by using the Illumina HiSeq X Ten or NovaSeq 6000 platform with paired-end reads of 151 bp following with the manufacturer's instructions. Read sequences were mapped to the human reference genome (GRCh37). The accuracy of the results was confirmed by Sanger sequencing.

Results

Variations detected by WES sequencing

In the 15 samples of patients' genomes, we identified four non-synonymous LRP6 variants, c.1099T>C, c.2450C>G, c.3184G>A, c.3184G>A, c.3277C>T in five unrelated patients (Table 1, Figure 1). LRP6 encodes a co-receptor for WNT ligands, and variants with loss of function of the gene cause human congenital tooth agenesis. All detected variants in the current study were with single nucleotide substitutions including homozygote of a very common variant, c.3184G>A, in two probands. The other three probands were with a heterozygous state of a rare variant. The Sorting Intolerant from Tolerant (SIFT: https://sift.bii.a-star.edu.sg) program and Polymorphism Phenotyping v2 (PolyPhen-2: http://genetics.bwh.harvard.edu/pph2/) were used to predict the effect of the change on the protein function of these variants detected by WES.

Computational pathogenicity predictions by PolyPhen-2 and SIFT

PolyPhen-2 and SIFT are the most highly used predictors. These applications accept single FASTA protein sequences, protein accession numbers, and genomic coordinates. We applied three rare variants of LRP6 sequences to the software to predict the pathogenicity of these gene variant products. The results of SIFT and PolyPhen-2 for c.1099T>C, c.2450C>G, c.3277C>T were as follows for (SIFT/PolyPhen-2); 0/0.999 (probably damaging, c.1099T>C; p.Tyr367His), 0.17/0.723 (possibly damaging, c.2450C>G; p.Ser817 Cys), and 0.03/1.0 (probably damaging, c.3277C>T; p.Arg1093Trp), respectively (Table 2).

Web-based gene pathogenicity analysis

We evaluated the pathogenicity of detected variants using databases such as ClinVer (https://www.ncbi.nlm.nih.gov/clinvar/), which is an archive of publications on the relationships between gene variations and phenotypes in human.

The variant c.3184G>A (p.Val1062Ile) was judged as benign/non-pathogenic by ClinVer since the allelic frequency of the variant was too high, 0.851, c.1099T>C (p.Tyr367His), and c.3277C>T (p.Arg1093Trp) was not found in the database.

We then analyzed these variants using the Genome Aggregation Database (gnomAD: https://gnomad.broadinstitute.org), a huge database aggregating exome data from several large-scale sequencing projects in various countries. Surprisingly, c.1099T>C (p.Tyr367His) LRP6 is not listed even in this enormous database, indicating the rarity of this variant. The allelic frequency of the other two variants, c.3277C>T (p.Arg1093Trp) is 0.0000119, and c.2450C>G

(p.Ser817Cys) is 0.000879.

Discussion

SIFT is based on sequence homology that detects variation from an alignment of homologous genes in various organisms. A SIFT score against an amino acid substitution predicts how severely a mutation affects protein function. Therefore, protein conformational changes are ignored in a pathogenicity prediction generated by SIFT[12]. On the other hand, the PolyPhen-2 program predicts the effect of an amino acid substitution on structural components, including the polarity of the structure, potentially changing the hydrophobic core of a protein, and whether its interactions are with itself or with other proteins[13]. Taking this into account, we evaluated 4 variants of LRP6 genes isolated from congenital tooth agenesis patients with SIFT and PolyPhen-2. While one of the variants, c.3184G>A (p.Val1062Ile) should not be a pathogenic variant, because it was prevalent (SIFT sore, 1; PolyPhen-2

Table 1. Phenotypes of analyzed patients with tooth agenesis

Pedigree No.	Gender	Age		Tooth phenotype R 7 6 5 4 3 2 1 1 2 3 4 5 6 7 L	Classification
1	M	22	Upper	●	Hypodontia
			Lower		
2	F	20	Upper		Hypodontia
			Lower	● ●	
3	F	26	Upper	● ●	Hypodontia
			Lower	● ●	
4	M	18	Upper	● ●	Hypodontia
			Lower	● ●	
5	M	24	Upper		Hypodontia
			Lower	● ●	
6	F	23	Upper	●	Hypodontia
			Lower	●	
7	F	40	Upper	● ● ● ● ●	Hypodontia
			Lower		
8	F	7	Upper	● ●	Hypodontia
			Lower	● ●	
9	M	7	Upper		Hypodontia
			Lower	● ●	
10	F	17	Upper	● ●	Hypodontia
			Lower	● ●	
11	M	20	Upper	●	Hypodontia
			Lower		
12	F	20	Upper	● ● ● ● ● ● ● ●	Oligodontia
			Lower	● ● ● ●	
13	F	14	Upper	● ●	Hypodontia
			Lower	● ●	
14	F	11	Upper	● ● ● ●	Oligodontia
			Lower	● ● ● ●	
15	F	26	Upper	● ● ● ● ● ●	Oligodontia
			Lower	● ●	

● : Congenital permanent tooth defect

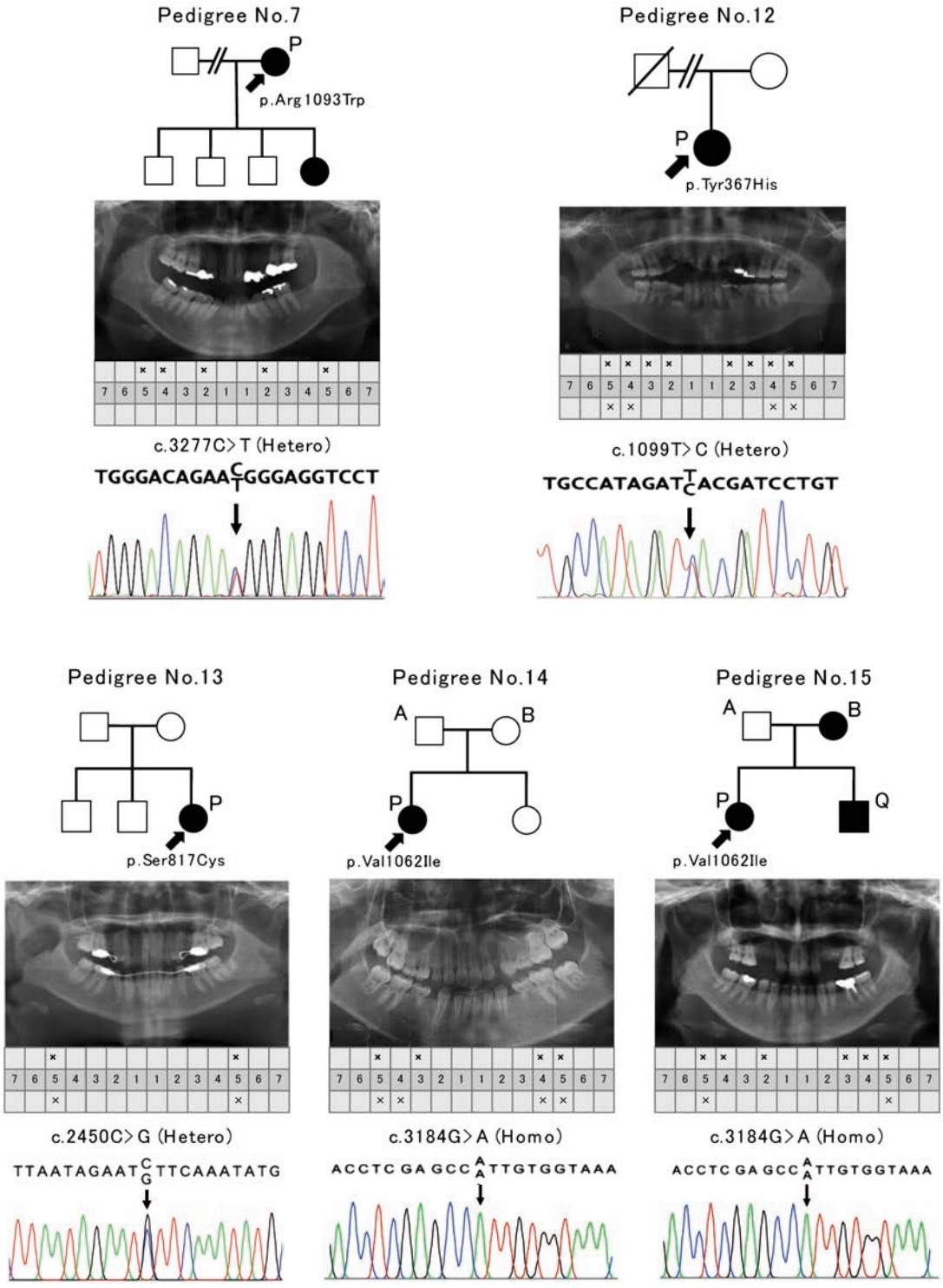

Figure 1. Pedigree trees, Sanger sequencing and oral phenotypes of patients with LRP6 variant

Squares indicate male family members and circles indicate female family members. The filled arrow indicates the proband. The black symbols indicate individuals who were clinically diagnosed with isolated tooth agenesis. Panoramic radiographs and tooth phenotypes of patients in families 7, 12, 13, 14, and 15. An (x) marks the congenital missing tooth. Sequencing results for the LRP6 gene of tooth agenesis patient. Heterologous peaks of the nucleotide sequence were detected in the patient LRP6 gene.

Table 2. Variants in the LRP6 gene and their predicted effects on WNT signaling

LRP6(NM_002336.3)											
Variant ID	Nuc. posion	AA. posion	Mutation	Zygosity	Allelic frequency gnomAD	ClnVer: Clinical significance	rsID	Number of Homozygote	PolyPhen-2		Sift
12-12181317 A>G	c.1099T>C	p.Tyr367His	missense	Hetero	Variant not found	?	-	-	0.999	PROBABLY DAMAGING	0
12-12159794 G>C	c.2450C>G	p.Ser817Cys	missense	Hetero	0.0008790	Benign	rs2302686	5	0.723	POSSIBLY DAMAGING	0.17
12-12148964 C>T	c.3184G>A	p.Val1062Ile	missense	Homo	0.8510000	Benign	rs2302685	102342	0	BENIGN	1
12-12148964 C>T	c.3184G>A	p.Val1062Ile	missense	Homo	0.8510000	Benign	rs2302685	102342	0	BENIGN	1
12-12300420 G>A	c.3277C>T	p.Arg1093Trp	missense	Hetero	0.0000119	?	rs752896710	0	1	PROBABLY DAMAGING	0.03

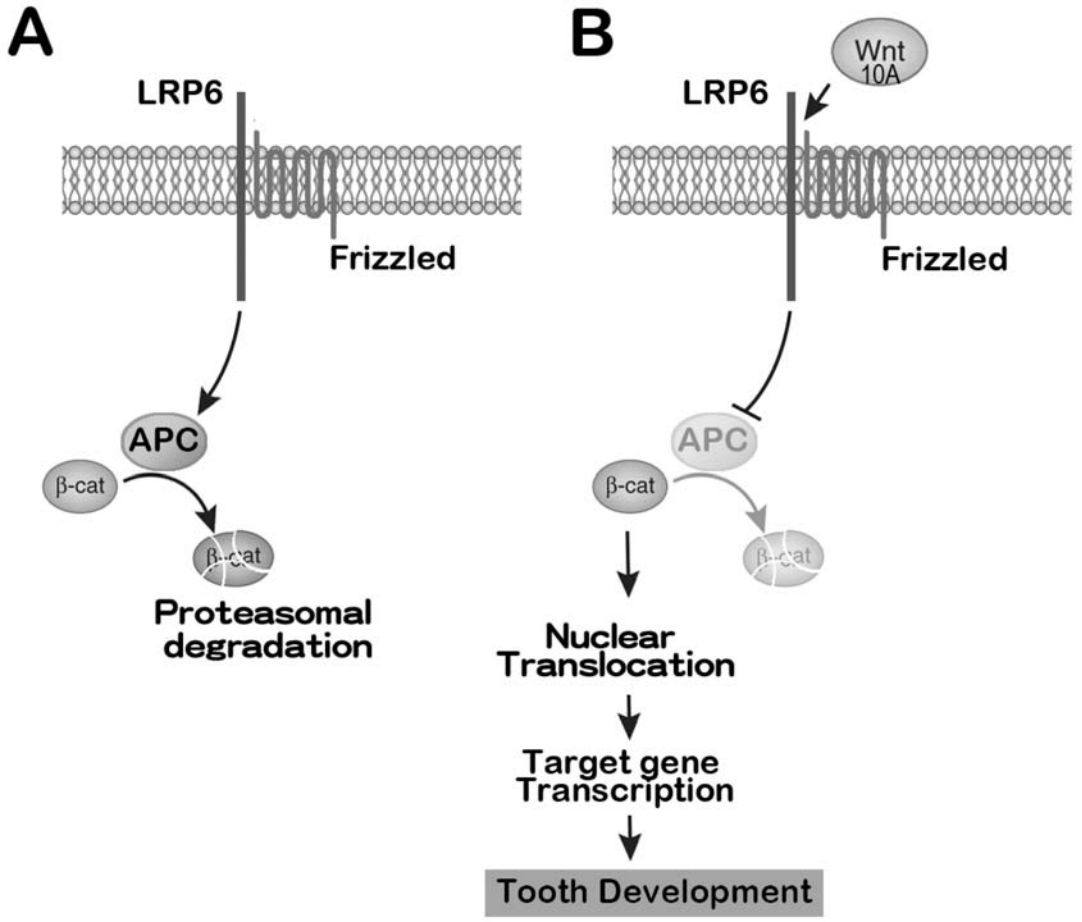

Figure 2. Canonical WNT/β-catenin signaling pathway

A: In the absence of a WNT ligand, β-catenin is proteasomal degraded by APC complex (APC) including Axin, synthase kinase 3 and casein kinase 1.

B: A WNT ligand recruits the destruction of APC complex. Stabilized β-catenin translocates to the nuclei and interacts with transcription factors of TCF/LEF.

score, 0; allelic frequency, 0.851 in gnomAD database), other variants could be rare causative variants of LRP6; c.2450C>G, p.Ser817Cys, allelic frequency 0.000879; c.3277C>T, p.Arg1093Trp, allelic frequency 0.0000119. In particular, the variant of c.1099T>C, p.Tyr367His is not listed in the gnomAD databases (Table 2). However, there are 4 individuals with c.3277C>T (p.Arg1093Trp) among 141336 individuals (282672 alleles), and 239 individuals with c.2450C>G (p.Ser817Cys) among 141392 individuals (282784 alleles), respectively. Since tooth agenesis may not be recognized as a human congenital disease, rare pathogenic gene variants related to non-syndromic tooth agenesis are sometimes listed among the healthy controls. As mentioned above, this record would not rule out the pathogenicity of this variant.

As functionally null variants of WNT10A are most frequently detected in tooth agenesis cases, WNT/β-catenin signaling is considered crucial for regulating human tooth number. LRP5/6 are single transmembrane type co-receptors for WNT ligands, which can induce the intracellular signaling of WNT to stabilize cytosolic β-catenin proteins (Figure 2) [14-17]. The amount of β-catenin signal is crucial to tooth development and tooth number. LRP5 is reported as the cause of osteoporosis-pseudoglioma syndrome (OPPG [MIM 259770]) [18], whereas LRP6 plays a pivotal role in tooth development and is responsible for STHAG7 (Phenotype MIM number; 616724).

To date, 25 pathogenic LRP6 variants have been reported, all of which are located in the extracellular domain, except one [9, 10, 19-27]. Based on the results, these sites in LRP6 might play a role on WNT ligand interactions, and each amino acid residue substitution might impair the overall structural integrity of the extracellular domain. Taken together, these results strongly suggest that the c.1099T>C (p.Tyr367His), c.2450C>G (p.Ser817Cys), and c.3277C>T (p.Arg1093Trp) variants may cause tooth agenesis in families. Our current finding contributes to the elucidating of LRP6 structure and function associated with isolated tooth agenesis. However, further analysis, including in vitro WNT responsive promoter assay, is required to clarify these variants' pathogenicity.

Conclusion

We performed a whole exome sequence analysis of 15 patients with congenital permanent tooth agenesis and identified four LRP6 variants (c.1099T>C, c.2450C>G, c.3184G>A, c.3277C>T) from five patients.

Among these, the three variants c.1099T>C, c.2450C>G, c.3277C>T were classified as probably damaging, whereas c.3184G>A was of uncertain significance on the basis of present evidence. Taken together, our study extends the mutation spectrum in patients with non-syndromic tooth agenesis and provides helpful data for genetic counselling. However, further functional studies are required to elucidate the molecular pathogenesis of these mutations.

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