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ORIGINAL ARTICLE

Genetic analysis of non-syndromic familial multiple supernumerary premolars

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ABSTRACT

Objective: Supernumerary teeth, a term describing a condition where patients have an abnormally large number of teeth, can be associated with non-syndromic or syndromic phenotypes. PDGFRs are cell surface tyrosine kinase receptors, and are involved in several aspects of tooth development. The purpose of this study was to identify causative genes of familial supernumerary teeth and the molecular pathogenesis of tooth number abnormalities through genetic analysis of a family that showed supernumerary premolars in two successive generations.

Material and methods: We recruited a Korean family with supernumerary premolars and performed mutational analyses to identify the underlying molecular genetic aetiology.

Results: Targeted exome sequencing identified a missense mutation in *PDGFRB* (c.C2053T, p.R685C). Sanger sequencing confirmed that three affected individuals in the patient's family were heterozygous for the mutation.

Conclusions: This is the first report of a Korean family that carries a *PDGFRB* mutation potentially responsible for supernumerary premolars. Our results demonstrate the power of next-generation sequencing in rapidly determining the genetic aetiology of numerical tooth abnormalities.

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Introduction

Supernumerary teeth refer to extra teeth that exceed the normal number in dentition [1]. The incidence of supernumerary teeth is about 3%. Among them, single supernumerary tooth accounts for 76–86% of cases, double supernumerary teeth comprise 12–23%, and three or more supernumerary teeth represent less than 1% [2,3]. In most cases, multiple supernumerary teeth are associated with other syndromes or developmental disorders such as cleft palate and cleft lip, cleidocranial dysplasia, and familial adenomatous polyposis [4–6]. Multiple supernumerary teeth, without any associated syndromes or conditions, are very rare [7]. In other words, in most cases, multiple supernumerary teeth appear as the result of a genetic trait. This indicates that there is strong evidence for a genetic basis of supernumerary teeth.

The beginning stage of tooth development is a key step in determining tooth number. Studies on odontogenesis-regulating genes have revealed that over 300 genes are associated with tooth development. Transcription factors such as Barx, Dlx, Gli, Lef and Lhx, and secreted proteins such as Bmp, Fgf, Hgf, and Shh affect gene expression during tooth development [8,9].

Although the aetiology of supernumerary teeth has not been well-documented, it has been proposed that genetic factors are associated with the process. Genes related to some syndromes that cause multiple supernumerary teeth have been identified, such as *RUNX2*, *APC*, *Tenascin-XB*, *NHS*, *EVC*, *TRPS1*, and *ROR2*, but genes associated with non-syndromic supernumerary teeth have not yet been identified [4,10–15].

Most reported cases related to non-syndromic familial multiple supernumerary teeth were related to mesiodens, and genetic analysis has never been performed on non-syndromic premolar patients [16]. To date, most genetic studies of the teeth have been carried out using mice. However, mice do not develop both canine and premolar teeth. Therefore, genetic studies on supernumerary premolars in the human family can reveal information that cannot be determined with *in vivo* mouse studies.

The aim of this study was to identify genetic mutations correlated with non-syndromic supernumerary premolars. Genetic analyses were executed through exome sequencing of a Korean family that presented with supernumerary premolars in two successive generations.

Materials and methods

Subjects

The index patient (III-1) and his younger brothers (III-2, III-3) presented at the Department of Pediatric Dentistry, Yonsei University, Wonju Severance Christian Hospital for extraction

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Supplemental data for this article can be accessed here.

of supernumerary teeth and non-eruption of permanent teeth, respectively. Panoramic radiography of the index patient (III-1) revealed the presence of four total supernumerary teeth adjacent to the premolars on both sides (Figure 1(B)). His brothers also showed supernumerary teeth (Figures 1(D,E)).

During health history examination, the father (II-3) reported the familial history of supernumerary teeth, which included himself (II-3) and his father (I-1). There were no syndromic features of the body, or other relevant developmental or medical history. Panoramic radiographs were collected from other family members. As a result, a total of four family members were confirmed to have supernumerary teeth (Figures 1(B–E)), and the index patient's grandfather was suspected to have had supernumerary teeth. Characteristically, supernumerary teeth were commonly found in the lower premolar area.

Multiple supernumerary teeth developed in several members in one family and in the same area in each family member, despite an extremely low incidence of non-syndromic multiple supernumerary teeth in the general population. For this reason, we suspected that there was a genetic cause, and genetic analyses of family members were planned to identify the causative genes of these multiple supernumerary teeth.

Written informed consent was obtained from all the participants or their legal guardians. All clinical investigations were conducted according to the principles expressed in the Declaration of Helsinki. This study was approved by the Institutional Review Board at Yonsei University Wonju College of Medicine (YWDR-14-9-097).

Genetic analysis

Genomic DNA from saliva was extracted using Oragene DNA kits (OG-500) (DNA Genotek, Ontario, Canada) according to the manufacturer's instructions. For mutation analysis, 101 candidate genes were selected based on genes and pathways involved in regulation of tooth development (Supplementary Table). For targeted sequencing, DNA fragments were enriched by solution-based hybridization capture and followed by sequencing with an Illumina Hiseq2500 platform. Analyses of NGS data were performed using an in-house analysis pipeline. Specifically, sequencing reads from the HiSeq2500 raw data were sorted by index and barcode sequences. Sorted fastq files were aligned to the hg19 reference genome using the Burrows-Wheeler Aligner algorithm (BWA; ver. 0.7.5a) [17]. Output SAM files were converted into BAM files and sorted using SAMtools (ver. 0.1.18) [18]. Duplicate removal was performed with the Picard tools (ver. 1.95) MarkDuplicates. Realignment around known indel sites and base quality score recalibration (BQSR) were performed using GATK (ver. 2.6–5) to create final BAM files [19].

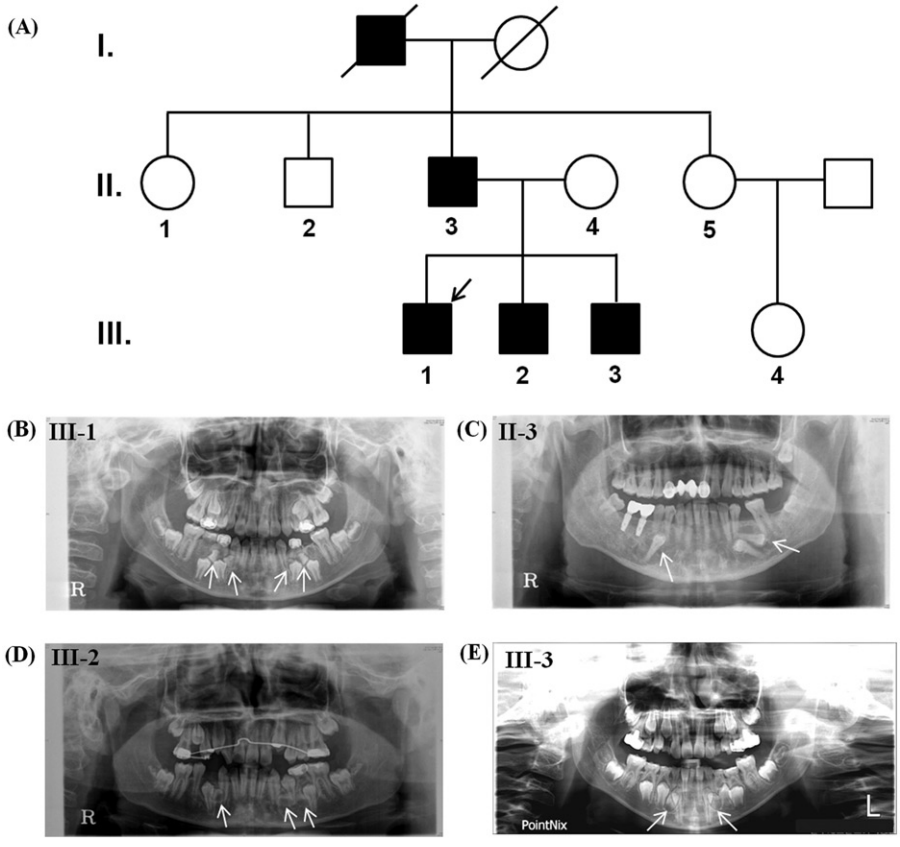

Figure 1. Pedigree and panoramic radiographs of the family. (A) Pedigree of the family. Family members who participated in this study are denoted by a number under the symbol. The proband is indicated with a black arrow. (B) Panoramic radiograph of subject III-1 showing 4 supernumerary premolars. (C) Panoramic radiograph of subject II-3 showing 2 supernumerary premolars. (D) Panoramic radiograph of subject III-2 showing 3 supernumerary premolars. (E) Panoramic radiograph of subject III-3 showing 2 supernumerary premolars and 2 missing permanent incisors.

Variants were identified using the GATK v2.6 Unified Genotyper algorithm for loci with a sequencing depth greater than or equal to 20X. Variants were annotated with ANNOVAR (ver. 2013-06-21) [20]. Functional effect prediction for single-nucleotide variants (SNVs) was performed by PolyPhen-2 (v2.2.2), SIFT, and Mutation Taster. Polymorphisms found in the Korean population ($N = 405$) and public databases (1000 Genome project SNP (2012 April release, ESP)) from both Asian and all-population databases were also filtered. All selected variants were validated by Sanger sequencing.

Results

Clinical findings and family pedigree

Clinical and radiological examinations of the family members showed that the father (family member II-3) and his three sons (family members III-1, III-2, III-3) had common supernumerary premolars in the lower premolar region (Figure 1). Initially, the youngest brother (family member III-3) had been considered to have transposed incisors rather than supernumerary teeth. However, if they were lower incisors, their roots should be fully formed. Considering their root development, the youngest brother seemed to have both missing incisors and supernumeraries. No evidence of any other systemic anomalies was observed in the family members. Of nine members from whom DNA was extracted, four were affected (all males). Therefore, we determined that the supernumerary tooth mutation seemed to be inherited in an autosomal dominant manner.

Mutation identification

Using a targeted capture approach, we sequenced 101 candidate genes involved in tooth development regulation. The average depth of coverage for the targeted regions was 871.7X. Greater than 20X coverage was obtained for 98.1% of the bases sequenced. Targeted exome sequence data of the two subjects affected (II-3 and III-1) showed several shared variants. A frequency cutoff was set considering the incidence of supernumerary premolars. The variants, which appeared more than the frequency cutoff, were excluded from candidate genes, and *PDGFRB*, *MSX2,* and *FGFR2* were considered candidates (Table 1).

Sanger sequencing screening was performed on other family members (III-2, III-3), a *PDGFRB* mutation was detected in one family member (III-2) but not in the other family member (III-3), and *MSX2* and *FGFR2* mutations were not detected in either family member (III-2, III-3) (Figure 2). This indicated that the *PDGFRB* mutation was incompletely segregated, and *MSX2* and *FGFR2* mutations were not segregated with supernumerary teeth in this family; therefore, the *PDGFRB* mutation is the most likely cause of the supernumerary teeth.

Discussion

Associations between *PDGFRB* mutations and supernumerary premolars were proposed by targeted exome sequencing results. However, the location of the sequence variants in *PDGFRB* is not given, so the effect on protein function cannot be exactly inferred. There is also missing sequence information for II-3 and III-1. A gene editing experiment on humans could overcome these weaknesses; however such experiments are not ethically acceptable and, even if allowed, would take a very long time. The phenotype was almost the same among the affected family members, but the number of supernumerary teeth varied. This could be attributed to modifier genes that have not been considered in this study.

It is known that *PDGFRB* is important in vasculature support cell development. According to Klinghoffer et al. [21] and Hellstrom et al. [22], *PDGFRB* mutations can cause cardiac hypertrophy, glomerulosclerosis, and proliferative retinopathy in mice. The role of *PDGFRB* in organogenesis has been defined in mice, but not in humans. Considering that the family had no cardiac hypertrophy, glomerulosclerosis, or proliferative retinopathy, *PDGFRB* may have a different role in human organogenesis. While its precise role in tooth development remains unclear even in mice, some involvement of *PDGFRB* in tooth development has been confirmed. PDGF ligands and their receptors are expressed throughout the initial stages of tooth development, indicating their involvement in that process [23]. Particularly, PDGFRB proteins are mainly expressed in the dental mesenchyme during initial tooth development and PDGF-BB signalling is important for dental mesenchymal cell proliferation [23]. PDGF-BB serves as a mitogen for dental follicles [24]. Morphogenesis is controlled by interactions between epithelial cells and mesenchymal cells. There is a possibility that the mitogenetic ability of PDGF-BB induces excessive mesenchymal expression, leading to inductive ability of the epithelium.

Table 1. Rare, non-synonymous, exonic variants detected by targeted sequencing in two affected family members.

Chr	Gene	Changes	SIFT	PolyPhen-2	Mutation Taster	1000G MAF	ESP MAF	Korean MAF ($N = 405$)	II-3	III-1
chr5	*PDGFRB*	NM_002609 c.C2053T p.R685C	Deleterious (score: 0.95)	Possibly damaging (score: 0.894)	Disease causing	NA	NA	0.01	Hetero	Hetero
chr5	*MSX2*	NM_002449 c.A703G p.I235V	Tolerated (score: 0.91)	Benign (score: 0.004)	Neutral	NA	NA	0	Hetero	Hetero
Chr10	*FGFR2*	NM_000141 c.T557C p.M186T	Tolerated (score: 0)	Benign (score: 0)	Polymorphism	0.06	0.10	0.06	Hetero	Hetero

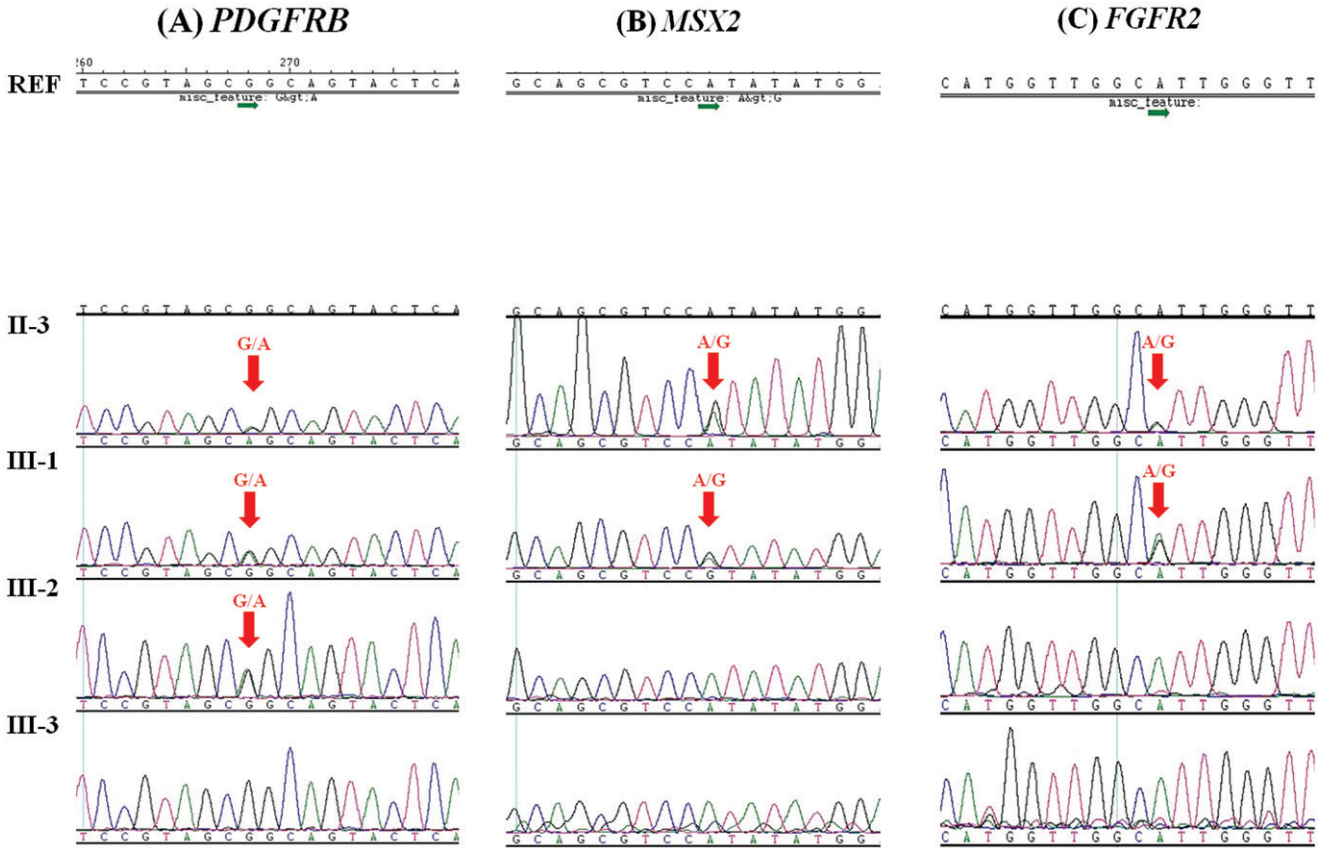

Figure 2. Mutation analysis. Sanger sequencing chromatograms of the family members are shown. Nucleotide sequences are shown above the chromatograms. Each red arrow indicates a mutation (A) *PDGFRB* (c.C2053T, p.R685C), (B) *MSX2* (c.A703G, p.I235V) and (C) *FGFR2* (c.T557C, p.M186T).

Excessive mesenchymal expression by *PDGFRB* mutation is a possible cause of supernumerary tooth formation.

The majority of reported mutations in *PDGFRB* have shown an autosomal dominant pattern, as was found in the family in this study. Therefore, this study assumed Mendelian inheritance, which is based on the premise that affected subjects have the same mutations. However, there is the possibility of non-Mendelian inheritance in cases of supernumerary teeth. To analyze more complex inheritance patterns, more samples are needed to identify genetic mutations and diagnostic markers. If non-syndromic supernumerary teeth are inherited with a non-Mendelian inheritance pattern, many additional samples are needed, which could prove to be difficult because the incidence of non-syndromic supernumerary teeth is extremely low [7]. Additional study is planned to be performed when other families with non-syndromic supernumerary teeth are found.

PDGFRB was considered the single potential causative gene in this study, but some phenotypes are caused by double mutations, such as *MSX1/MSX2* or *DLX1/DLX2*. There is a possibility that non-syndromic supernumerary teeth are not caused by a *PDGFRB* single mutation, but a *PDGFRB* mutation in combination with another mutation of a gene such as *Spry2* and *AXIN2*, which were excluded based on the frequency cutoff, or *Gas1* which was not included in the candidate genes.

This is the first report of the association between supernumerary premolars and *PDGFRB* mutations. Further molecular studies are required to clarify the causative genes of non-syndromic supernumerary premolars. Finally, next-generation sequencing could be applied as a powerful tool in future studies.

Disclosure statement

The authors report no conflicts of interest.

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