The Effect of MMP-13, MMP-12, and AMBN on Gingival Enlargement and Root Deformation In a New Type of Gingival Fibromatosis

Chung-Min Kang* / Jae-Ho Lee**/ Mijeong Jeon***/ Je Seon Song****/ Seong-Oh Kim*****

This case compared gene-expression between a new type of idiopathic gingival fibromatosis (IGF) and normal gingiva, to clarify the nature of the gingival overgrowth and dental anomaly. A 6-year-old girl with generalized gingival overgrowth and root deformations was diagnosed with IGF. Gene expression profiles were compared between normal gingiva (N=9) and one IGF gingiva using cDNA microarray. Genes related to regulation of cell proliferation and proteolytic degradation were expressed strongly in IGF. MMP-13 and MMP-12 expression were 120 times and 96 times lower in IGF, respectively, whereas AMBN expression was 79 times higher. RT-PCR and immunohistochemical staining supported the microarray results. Reduced proteolytic activity due to low MMP-13 and MMP-12 expression appears to be a potential mechanism for gingival overgrowth. Genetic investigations, such as expression levels of MMP-13, MMP-12, and AMBN, may enable classification of a new syndrome characterized by gingival enlargement with abnormal root development.

Key words*: gingival fibromatosis; cDNA microarray; gingival overgrowth; MMP-13; MMP-12; ameloblastin*

INTRODUCTION

Idiopathic gingival fibromatosis (IGF), also known as gingival hyperplasia and gingival overgrowth, is believed to be due to an imbalance between synthesis and degradation of extracellular matrix molecules or an alteration in fibroblast proliferation. A possible mechanism of IGF pathogenesis is impairment in extracellular matrix degradation. Collagen turnover in gingival tissues is high and degradation occurs by two main pathways: fibroblast phagocytosis and degradation of the extracellular matrix (ECM) by members of the matrix metalloproteinase (MMP) family of proteases. There are conflicting results concerning the activity of MMPs that mediate the degradation of ECM in IGF. Some research reported that decreased levels of MMPs might be responsible for gingival overgrowth[1-3]. In contrast, other studies have recognized that the decreased expression of MMPs did not explain the collagen accumulation of ECM in IGF[4].

Ameloblastin (*AMBN*) encodes enamel matrix protein, which may be important in enamel matrix formation and mineralization. Mutations in this gene are known to be associated with amelogenesis imperfecta (AI)[5]. Although AI is considered to primarily affect the enamel, further alterations could include un-erupted teeth, crown resorption, high cementum deposition, and root deformation[6,7]. Gingival overgrowth with AI was described in a few isolated case reports[8,9]. Here, we present gene-expression analysis of an IGF case, which supports a new syndrome associated with root anomalies.

*Chung-Min Kang, DDS, PhD, Department of Pediatric Dentistry, College of Dentistry, Yonsei University, Oral Science Research Center, College of Dentistry, Yonsei University, Seoul, Republic of Korea

**Jae-Ho Lee, DDS, PhD, Department of Pediatric Dentistry, College of Dentistry, Yonsei University, Oral Science Research Center, College of Dentistry, Yonsei University, Seoul, Republic of Korea.

*** Mijeong Jeon, PhD, Oral Science Research Center, College of Dentistry, Yonsei University, Seoul, Republic of Korea.

****Je Seon Song, DDS, PhD, Department of Pediatric Dentistry, College of Dentistry, Yonsei University,Oral Science Research Center, College of Dentistry, Yonsei University, Seoul, Republic of Korea

*****Seong-Oh Kim, DDS, PhD, Department of Pediatric Dentistry, College of Dentistry, Yonsei University, Oral Science Research Center, College of Dentistry, Yonsei University, Seoul, Republic of Korea.

Send all correspondence to:
Pf. Seong-Oh Kim,
Department of Pediatric Dentistry, College of Dentistry, Yonsei University,
50 Yonsei-ro, Seodaemun-gu, Seoul 120-752, Republic of Korea
Phone: (82-2) 2228-3171
E-mail: KSODDS@yuhs.ac

Case Report

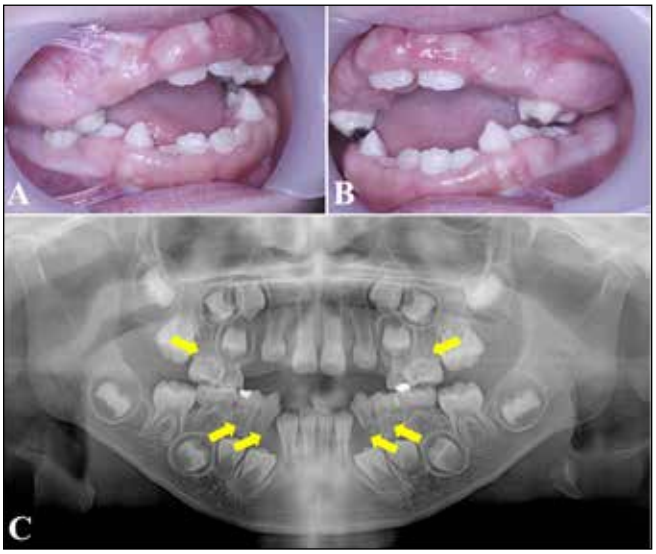
A six-year-old female had generalized gingival overgrowth involving attached gingiva, marginal gingiva, and interdental papilla. The posterior areas were deformed by gingival tissue that was sufficient to inhibit tooth eruption (Fig. 1A, B). Panoramic radiographs revealed delayed root development of the maxillary primary second molars and mandibular primary canines without abnormal clinical appearance of enamel (Fig. 1C). The patient was diagnosed with IGF because there was no evidence of genetic transmission when she was referred to the department of clinical genetics and pediatrics. In addition, her family and medical history was not indicative of drug-induced gingival enlargement or hormonal disturbances. Blood and gingiva samples were collected and karyotypes were analyzed, but no abnormalities were detected. Normal gingiva was collected from nine patients (five males and four females, mean age of 8.7-years) who underwent surgical gingival resection for extraction of a supernumerary tooth and odontoma. This study protocol was approved by the Institutional Review Board of Yonsei University Dental Hospital, and informed consent to participate was obtained from all subjects and their parents (approval no. 2-2013-0009).

Total RNA was isolated using the RNeasy Fibrous Tissue Mini kit (Qiagen, CA, USA) according to the manufacturer's instructions. The Agilent 2100 bioanalyzer using the RNA 6000 Nano chip (Agilent technologies, Amstelveen, The Netherlands) was used for analysis of RNA quality, and RNA quantity was determined using a NanoDrop ND-2000 device (Thermo Scientific, MA, USA). The RNA samples used in this study had 260/280 nm ratios of at least 1.8. Global gene-expression was performed using Affymetrix GeneChip® Human Gene 1.0 ST oligonucleotide arrays (Affymetrix Inc., CA, USA) following the manufacturer's instructions. Highly expressed genes that showed over 3-fold differences were selected for further investigation. These genes were classified based on the gene function in the Kyoto Encyclopedia of Genes and Genomes Pathway database. Some genes were verified through quantitative RT-PCR (qPCR). Specific antibodies (Abcam, Cambridge, UK) were used for immunohistochemical staining (IHC) in IGF and healthy gingival tissues.

Complementary DNA microarray technology was used to compare multiple gene-expression profiles representative of normal and IGF gingival tissue. Table 1 and 2 show important genes among the results. Fig. 2A shows the gene distribution based on molecular activity. Genes related to ion binding, structural molecules, DNA and RNA binding, receptor activity, growth-factor activity, and sugar binding were more strongly expressed in IGF than in normal gingiva. In qPCR analysis, the expressions of *AMBN*, *PI15*, *COL1A1*, and *COL1A2* were up-regulated in IGF, with that of *AMBN* being particularly marked. *MMP-13* and *MMP-12* expressions were markedly higher in normal gingival tissue, and the fold difference was 120 times and 96 times, respectively (Fig. 2B).

In IHC results, *COL1A1* was stained more densely within individual fibers in IGF connective tissue than in normal gingiva (Fig. 3B, F). *MMP-13* and *MMP-12* were strongly expressed in normal gingiva, but were not specifically expressed in IGF connective tissue (Fig. 3C, D, G, H). In normal gingiva, the epithelium was dark brown for *MMP-13* and *MMP-12*, which represents that the cytoplasm as well as nucleus was stained (Fig. 3C, D). Cell nuclei in the connective tissue were also stained brown. IHC staining performed for *AMBN* and *PI15* did not show clear differences.

Figure 1. Extraoral photograph of a patient with idiopathic gingival fibromatosis. (A, B) Note the severity of generalized gingival overgrowth involving both arches. (C) Panoramic radiograph showing abnormal root development in the maxillary primary second molars and mandibular primary canines (yellow arrow).



DISCUSSION

There has been controversy surrounding the mechanism of collagen accumulation in IGF gingiva: IGF is the result of increased collagen synthesis and/or decreased collagen degradation, or alteration in fibroblast proliferation[10]. The present case report was therefore undertaken to establish whether such a correlation exists at the gene function level, using a microarray procedure.

Genes that were strongly expressed in IGF gingival tissue were related to cell differentiation, development, inhibition of protein degradation, and structural organization, such as *COLAI* and *COLA2*. Interstitial collagen accumulation is one of the main features of IGF, and we found that *COLA1* and *COLA2* were strongly expressed in IGF gingiva with changes of 2.13 and 2.04 fold, respectively (qPCR). In addition, the expressions of *COLIII*, *COLVI*, and *COLXII* were higher in IGF than in normal gingiva. These findings concur with those of previous studies of IGF[4,11].

Destruction of the ECM contributes to the turnover rate of gingival tissue, and is known to result from the elaboration of extracellular proteinases, MMP activity, and phagocytosis and intracellular destruction of ECM components by lysozomal enzymes[12,13]. One study has shown a reduced level of *MMP-1* and *MMP-2* mRNA in HGF fibroblasts, leading to the accumulation of collagen[1]. Among different types of MMPs, this report focused on decreased *MMP-13* and *MMP-12* activity in IGF tissue, favoring gingival enlargement.

We found that down regulated *MMP-13* expression may be responsible for decreased tissue breakdown in IGF, indicating a potential imbalance between degradation and synthesis of ECM. *MMP-13* or collagenase-3 presents the most strictly regulated expression by gingival fibroblasts, especially in healing human gingiva. *MMP-13* is a collagenase with broad substrate specificity, whereas *MMP-1* is a collagenase that is more restricted to hydrolyzing collagens[14]. Various studies showed increased expression of *MMP-13* in periodontal tissues during physiological events related

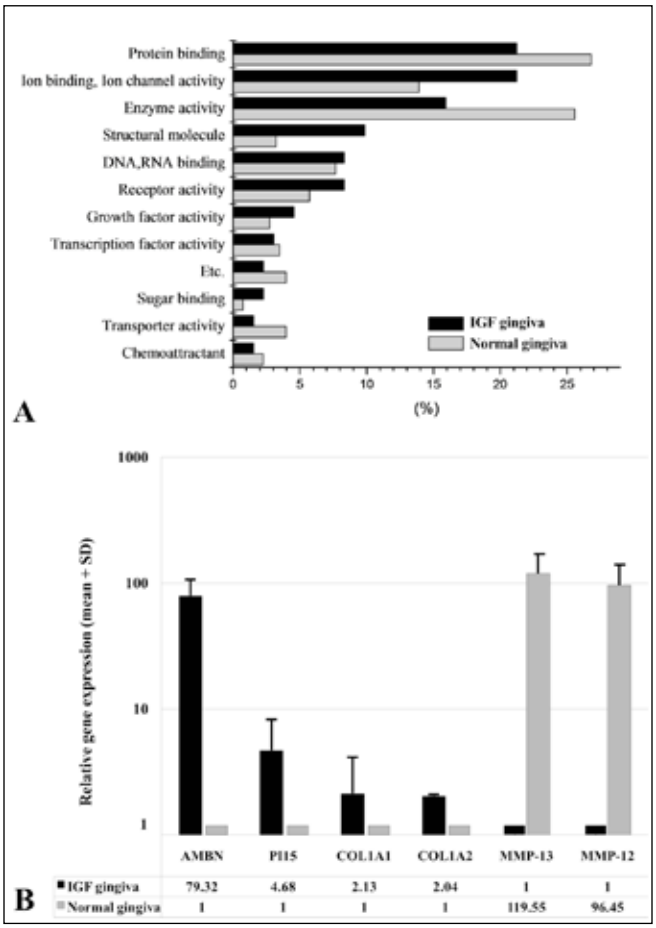
Table 1. Up-regulated genes in gingival tissue of an idiopathic gingival fibromatosis patient (compared to normal gingiva)

Name	Gene symbol	Absolute fold change	Gene accession	Cytoband
Ameloblastin (enamel matrix protein)	AMBN	8.745797835	NM_016519	4q21
Amelotin	AMTN	7.145836972	NM_212557	4q13.3
Hemoglobin, beta	HBB	4.603103877	NM_000518	11p15.5
Peptidase inhibitor 15	PI15	3.870384777	NM_015886	8q21.11
Osteoglycin	OGN	3.695926329	NM_033014	9q22
Collagen, type I, alpha 2	COL1A2	3.401707277	NM_000089	7q22.1
Calbindin 1, 28kda	CALB1	3.367686866	NM_004929	8q21.3-q22.1
Chemokine receptor-like 1	CCRL1	3.330777538	NM_178445	3q22
Transglutaminase 7	TGM7	3.318506269	NM_052955	15q15.2
Collagen, type I, alpha 1	COL1A1	3.297080245	NM_000088	17q21.33
Collagen, type III, alpha 1	COL3A1	2.654720544	NM_000090	2q31
Integrin, beta-like 1	ITGBL1	2.378340045	NM_004791	13q33
Microtubule-associated protein 1B	MAP1B	2.311983337	NM_005909	5q13
Collagen, type XII, alpha 1	COL12A1	2.145515019	NM_004370	6q12-q13
Chemokine (C-C motif) ligand 2	CCL2	2.061190716	NM_002982	17q11.2-q12
Epidermal growth factor	EGF	1.750283961	NM_001963	4q25
Collagen, type VI, alpha 1	COL6A1	1.70467534	NM_001848	21q22.3
Fibroblast growth factor receptor 2	FGFR2	1.651732432	NM_000141	10q26
Insulin-like growth factor 2	IGF2	1.598657627	NM_000612	11p15.5
Transforming growth factor, beta receptor III	TGFBR3	1.585046319	NM_003243	1p33-p32

Table 2. Up-regulated genes in normal gingiva (compared to gingival tissue of idiopathic gingival fibromatosis)

Name	Gene symbol	Absolute fold change	Gene accession	Cytoband
Matrix metallopeptidase 13	MMP-13	11.47562639	NM_002427	11q22.3
Odontogenic, ameloblast asssociated	ODAM	10.26153748	NM_017855	4q13.3
Amphiregulin	AREG	10.11155519	NM_001657	4q13-q21
Matrix metallopeptidase 12	MMP-12	8.521893370	NM_002426	11q22.3
Filaggrin	FLG	7.254795762	NM_002016	1q21.3
Extracellular matrix protein 1	ECM1	6.772164417	NM_004425	1q21
Cytidine deaminase	CDA	6.633999944	NM_001785	1p36.2-p35
Interleukin 1, beta	IL1B	6.317264595	NM_000576	2q14
Keratin 2	KRT2	5.722577118	NM_000423	12q11-q13
Chemokine ligand 13	CXCL13	5.507932960	NM_006419	4q21
Membrane metallo-endopeptidase	MME	4.395197515	NM_007288	3q25.1-q25.2
Heparin-binding EGF-like growth factor	HBEGF	4.145749653	NM_001945	5q23
Chemokine ligand 20	CCL20	3.593899336	NM_004591	2q33-q37
Interleukin 1, alpha	IL1A	3.319746316	NM_000575	2q14
IGF-like family member 1	IGFL1	3.238581387	NM_198541	19q13.32
Interleukin 1 family, member 6	IL1F6	2.858463761	NM_014440	2q12-q14.1
Chemokine ligand 1	CXCL1	2.565869861	NM_001511	4q21
Tumor necrosis factor, alpha-induced protein 2	TNFAIP2	2.420176055	NM_006291	14q32
Transforming growth factor, alpha	TGFA	2.283902399	NM_003236	2p13
Fibroblast growth factor binding protein 1	FGFBP1	2.175647758	NM_005130	4p15.32

Figure 2. (A) Main gene categories specifically expressed in normal and idiopathic gingival fibromatosis (IGF) based on molecular activity. (B) Relative difference in mRNA expression of six differentially expressed genes between IGF and normal gingiva using quantitative RT-PCR. Data are presented as means + standard deviation and expressed as the relative change by applying the equation $2^{-\Delta Ct}$; ΔCt (cycle threshold) = Ct of the gene minus Ct of the 18S rRNA. Y-axis: a log scale measure.

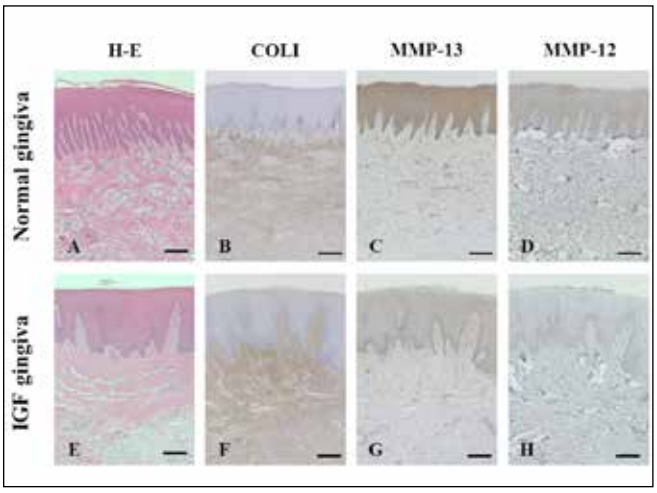


to bone remodeling and ECM turnover. *MMP-13* was reported to be expressed mainly by gingival sulcular epithelial cells and macrophage-like cells and was expressed highly in gingival crevicular fluid in destructive periodontitis[3].

This study also suggests that limited expression of *MMP-12* affects hydrolyzation of proteins in IGF gingival tissue. *MMP-12* known as human macrophage metalloelastase is a unique MMP that possesses elastolytic activity and is expressed in alveolar macrophages[15]. In addition, *MMP-12* has been suggested to be antiangiogenic, as it generates angiostatin from plasminogen[16]. Angiostatin inhibits endothelial cell proliferation and growth, and it could be hypothesized that enlarged gingiva of IGF express less *MMP-12*. However, the association between *MMP-12* and periodontal diseases could not be documented.

The patient represents a very interesting demonstration of abnormal root formation with generalized gingival overgrowth. There are some reports demonstrating the association between AI and gingival enlargement[8,9]. However, this report may discover

Figure 3. Hematoxylin-eosin staining in normal (A) and idiopathic gingival fibromatosis (IGF) (E) gingiva. Immunohistochemical (IHC) staining for collagen type I (COL1) in normal (B) and IGF (F) gingiva. IHC staining for matrix metalloproteinase (MMP)-13 in normal (C) and IGF (G) gingiva. IHC staining for MMP-12 in normal (D) and IGF (H) gingiva. Scale bars: 200 μm.



a new type of IGF because typical characteristics of AI such as enamel hypoplasia or hypomineralization were not clearly seen in this case. Intrapulpal calcification, pericoronal radiolucency, and root dilacerations which were reported in similar cases were also absent. Relative gene expression of *AMBN* was abnormally 79 times higher in this case. Although *AMBN* has generally been believed to be located in ameloblasts, recent studies reported that *AMBN* increased proliferation in periodontal ligament cells, osteoblasts, and Hertwig's epithelial root sheath (HERS)[17,18]. The overexpression of *AMBN* inhibited proliferation through suppression of negative cell cycle regulators[19] and may have an effect on regulating root development in HERS.

The existence of autosomal-dominant AI phenotypes without genetic linkage to any of these most commonly cited AI candidate genes—amelogenin, *AMBN*, enamelin, *MMP-20*, or *KLK-4*—suggests the existence of additional genes that are crucial for amelogenesis[20,21]. In this study, amelotin were also expressed at significantly higher levels in gingival tissue of GF. Previous findings suggested that amelotin is a novel factor produced by ameloblasts that plays a critical role in the formation of enamel[21,22]. Although the specific role of enamelin remains to be elucidated, mutations in this protein have been associated with various forms of AI in human[23]. This case describes the over expression of *AMBN* and amelotin appears to be associated with a new syndrome within the spectrum of GF.

CONCLUSION

This case reports a new syndrome characterized by GF associated with abnormal root development. Genetic investigations such as different expression level of *MMP-13*, *MMP-12,* and *AMBN* may clarify the defect behind syndrome associating gingival enlargement and dental anomaly.

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