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Case Report

Periodontal treatment of two siblings with juvenile hyaline fibromatosis

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Abstract

Background and Aim: Juvenile hyaline fibromatosis (JHF) is an autosomal recessive disease that presents with multiple subcutaneous nodular tumours, gingival fibromatosis, flexion contractures of the joint and hyaline material accumulation in extracellular area. Recently, the causative gene for JHF, capillary morphogenesis protein 2 (CMG2) was identified. In this case report, periodontal status, treatment and follow-up together with histopathologic evaluation of gingival tissue specimens and mutation screening of two JHF cases are presented.

Case Reports: A 10-year-old female (case 1) and her 3-year-old brother (case 2) were first examined in our department with a complaint of gingival hyperplasia in 1991. Symptoms of the disease were detected in two of four siblings in the family. Several gingivectomy operations were carried out over 11 years with hygiene motivation and initial phase therapy. After the last gingivectomy operation in 2002, the patients were reviewed frequently.

Results and Conclusions: Although there was linear marginal gingival inflammation, no remarkable enlargement was noted at last appointment. Histopathological findings showed increased amounts of subepithelial nodular connective tissue, thinned epithelial mucosa, separated inter-cellular bridges and decreased numbers of connective tissue cells in gingival tissue samples. Electron microscopic examinations supported the histopathological findings. Mutation screening of *CMG2* demonstrated that the siblings were homozygous for a pathogenic missense mutation, V386F. Our clinical findings demonstrate that gingivectomy is useful and frequent periodontal visits are important for maintaining oral hygiene and decreasing growth rate of gingiva in JHF.

Key words: capillary morphogenesis protein 2; gingivectomy; histopathologic and electron microscopy; juvenile hyaline fibromatosis; periodontal maintenance

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Juvenile hyaline fibromatosis (JHF) is a rare inherited condition, characterized by multiple subcutaneous skin nodules, gingival hypertrophy, joint contractures and hyaline deposition in the extracellular matrix. It was first reported as ''Molluscum fibrosum in children'' by Murray (1873). Several reports have used different names for this disease such as Puretic syndrome, systemic hyalinosis, fibromatosis hyalinica multiplex but ''JHF'' is preferred for describing this condition (Puretic et al. 1962, Alfi et al. 1975, Stringer et al. 1981, Finlay et al. 1983, Remberger et al. 1985).

The skin nodules are observed generally on the hands, toes fingers, knees, forehead, scalp, chin, ears and around of nose (Kitano et al. 1972, Woyke et al. 1984, Hallock 1993). The skin lesions are painless but they prevent functional movement of the extremities and severe limitation of mobility may result (Suzuki et al. 1992). Lesions, which are papular and/or nodular, may require recurrent excision and tend to increase during adolescence. Gingival fibromatosis is observed in affected patients and gingival overgrowth may also need recurrent excision.

The diagnosis of JHF is confirmed by demonstration of perivascular hyaline deposition in the dermis and gingiva (Piattelli et al. 1996, Keser et al. 1999). Although the origin of this amorphous hyaline material is not known, it appears to contain glycoproteins, glycosaminoglycans and collagens (Ishikawa et al. 1979). Recently, mutations of capillary morphogenesis protein 2 (*CMG2*) were shown to cause JHF and the related condition infantile systemic hyalinosis (ISH) (Dowling et al. 2003, Hanks et al. 2003). *CMG2* is a transmembrane protein induced during

capillary morphogenesis. Identification of *CMG2* mutations in JHF suggests that defects in basement membrane matrix assembly resulting in leakage of plasma components into the perivascular space are responsible for the characteristic hyaline deposition seen in JHF.

To date, approximately 60 JHF cases have been reported in the literature (Caylakli et al. 2003). In this case report we present two new JHF cases together with the histopathologic and electron microscopic (EM) findings, *CMG2* screening results and periodontal management.

Case Reports

A 10-year-old girl and her 3-year-old brother were first referred to the Department of Periodontology from the Plastic Surgery Department of Selcuk University in 1991 with a diagnosis of JHF. The patients complained of gingival overgrowth and had mastication problems because of gingival enlargement. The children were the offspring of healthy consanguineous parents. Neither the parents nor two other siblings were affected. The pedigree is shown in Fig. 1. Periodontal examination displayed generalized fibrotic and diffuse gingival enlargement in siblings (Fig. 2a, b). Since 1991, their treatment including scaling, root planing, hygiene motivation and gingivectomy were conducted but follow-up could not be performed regularly because of socioeconomic difficulties. The children had recurrent gingival enlargement and subsequent gingivectomy operations were performed three times during 11 years. Two years ago the cases were referred to our department again for treatment. This time gingival hyperplasia was milder when compared with earlier visits (Fig. 3a, b). Dentition showed minimal enamel hypoplasia in each sibling. Radiographical examination demonstrated no periodontal destruction in either case (Fig. 4a, b). Mild retardation of physical growth, subcutaneous nodular tumours of the face, ears, hands and feet were detected in Case 1 (Fig. 5a–c). Extraoral examination displayed major skin tumours in both ear and skull of Case 2 (Fig. 6a–e). Minor skin nodules were noted on the nose, hands and feet of both cases. These nodules limited walking and hand skill including toothbrushing and resulted in poor oral hygiene. The last gingivectomy operations were carried out in 2002 and wound healing was uneventful. After the last surgical operation, frequent control visits were undertaken. Maintenance therapy including scaling and polishing was performed every 2 months to support oral hygiene. At last recall, we observed localized areas of recurrent gingival enlargement with marginal inflammation (Fig. 7a, b).

Microscopic findings

Histopathologic examinations

The tissue specimens obtained during the gingivectomy operations were stored immediately in 10% buffered formaldehyde, prepared in an autotechnicon, embedded in paraffin and sectioned with a microtome. The sections (5 μM) were

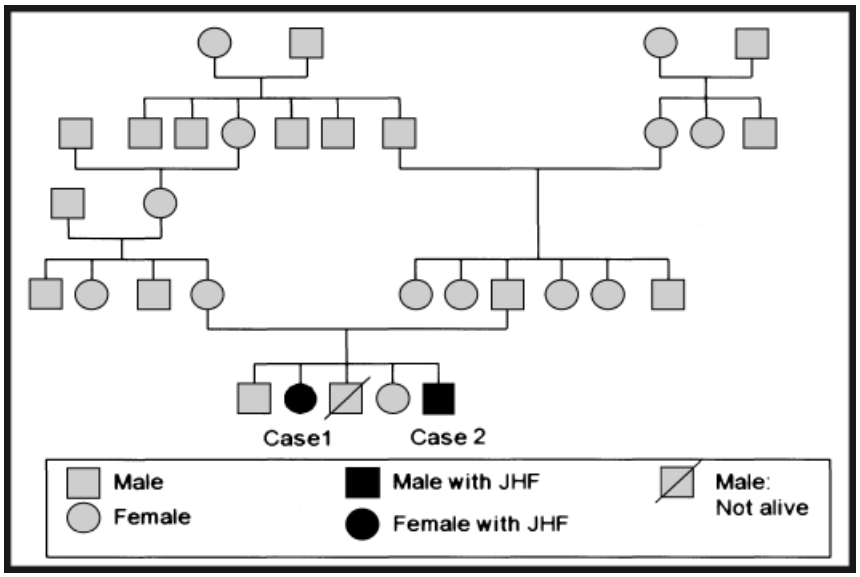

Fig. 1. Pedigree chart of the consanguineous family.

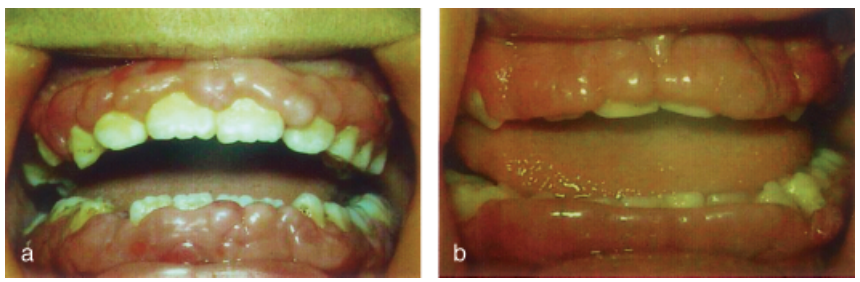

Fig. 2. (a) Ten year-old girl (Case 1) and (b) 3-year old boy (Case 2) with juvenile hyaline fibromatosis at first appointment (in 1991). Note severe gingival hyperplasia.

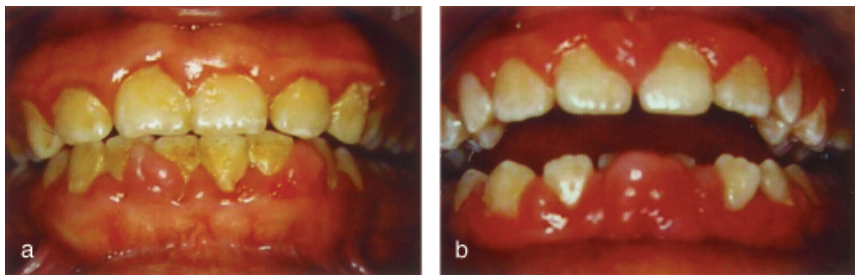

Fig. 3. (a) The intra-oral view of 21 year-old girl and (b) 14 year-old boy in 2002. The gingival hyperplasia of cases were slight when compared with first appointment. Note poor oral hygiene.

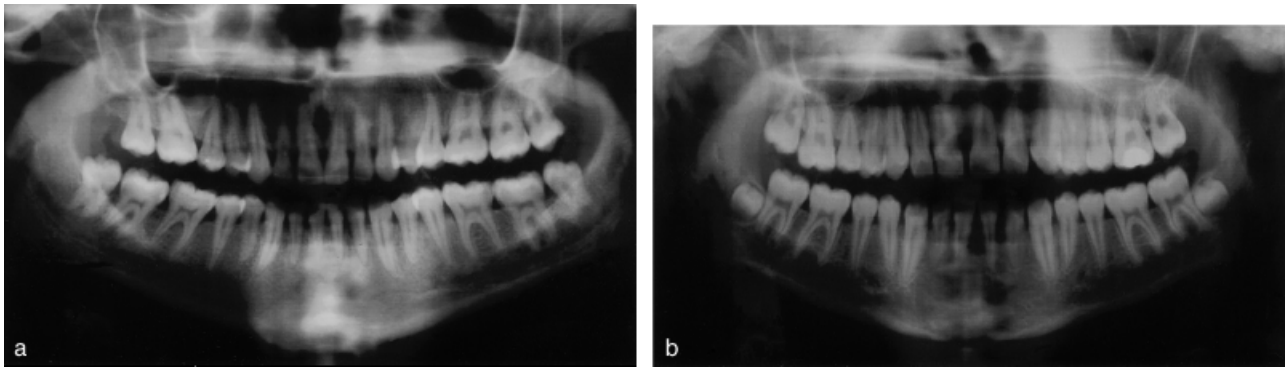

Fig. 4. (a) Panoramic radiography of Case 1 and (b) panoramic radiography of Case 2.

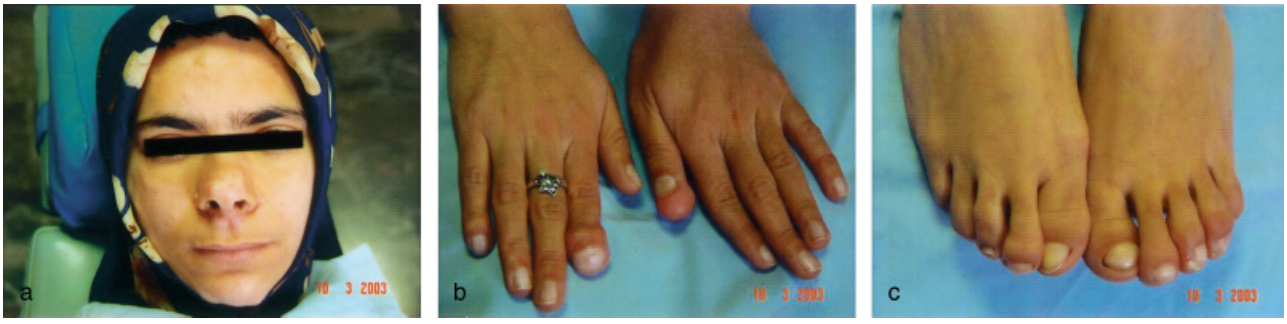

Fig. 5. (a–c) Nodules on face, hands and feet of Case 1.

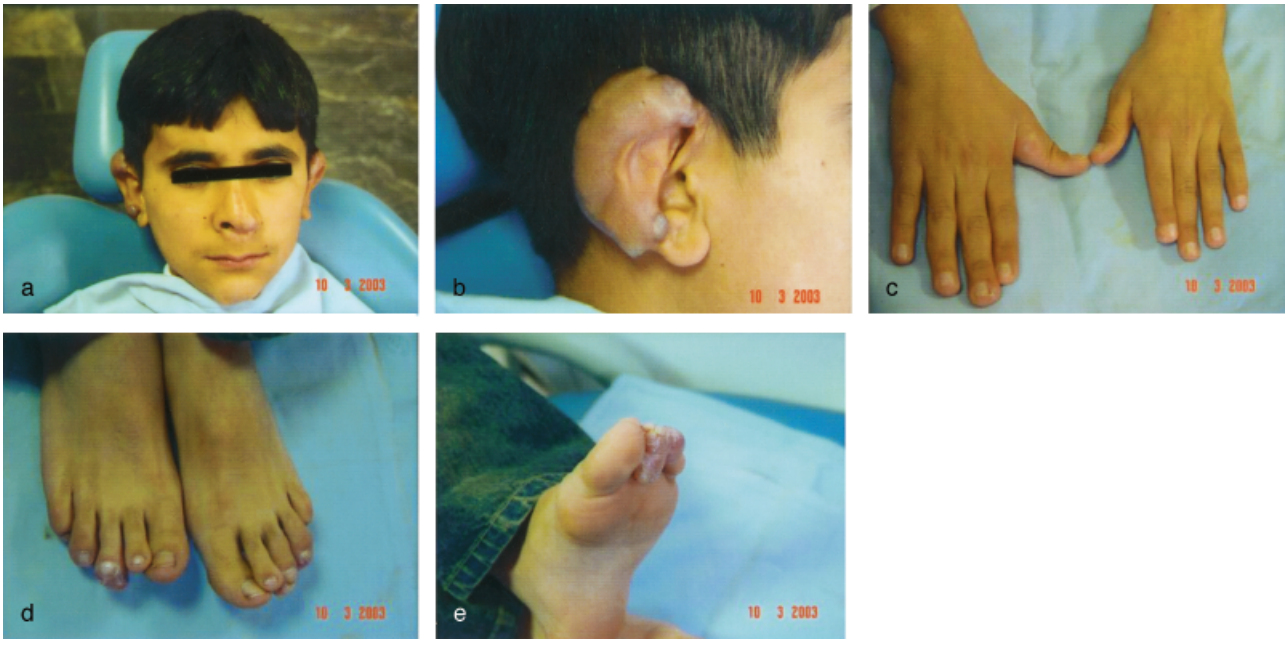

Fig. 6. (a–e) Nodules on face and scars belong to previous surgery and note tissue enlargement of ear and nodules on hands, feet and fingers.

stained with haematoxylin–eosin (HE), Van Gieson's and Masson's Trichrome for collagen detection. Immunohistochemical examination was performed for laminin and Collagen Type IV. Light microscopic examination clearly showed increased amounts of subepithelial nodular connective tissue, thinned epithelial mucosa, separated inter-cellular bridges and decreased numbers of connective tissue cells (Fig. 8a, b). Dense collagen fibres were observed in the connective tissue, but there was no Collagen Type IV expression in collagen bundles (Fig. 9a, b). In addition, microcystic spaces were observed because of destruction of inter-cellular junctions in the epithelium. These separations and intact inter-cellular junction were frankly seen with laminin immunostaining (Fig. 9c).

EM examination

For EM examinations, specimens were fixed in 3% glutaraldehyde in 0.2 M phosphate buffer (pH 7.4), then prepared using conventional EM procedures. Ultra-thin sections (70 nm) were stained with uranyl acetate and lead citrate and photographed using a Leo 906 E (80 kV) Electron Microscope (Leo, Oberkohen, Germany). Although hemidesmosomes were detected in the epithelial basal layer region, the basal lamina was not continuous. Anchoring fibrills and microfibrillar bundles that usually extend from basal lamina to connective tissue were not observed (Fig. 10a). In the epithelial spinous layer, dense bundles of filaments in the cytoplasm terminated in the dense plaques of desmosomes and short interdigitating processes were reduced significantly (Fig. 10b).

CMG2 mutation screening

Peripheral blood samples were obtained from affected and non-affected children and their healthy parents after signing a consent form approved by the Ethics Committee of Selcuk University. DNA was isolated and the 17 exons of *CMG2* were screened in both patients and parents by Conformation Sensitive Gel Electrophoresis and direct sequencing as previously described (Hanks et al. 2003). A single base substitution in exon 14, 1156G>T was identified. This results in a missense alteration V386F in the highly conserved intracellular domain. Both parents were heterozygous for this mutation and both affected children were homozygous.

Discussion

JHF displays multiple slowly or rapidly growing subcutaneous nodules. Nodules show tumour-like deposits of amorphous hyaline ground substance with delicate staining properties situated partly between cellular and vascular areas (Quintal et al. 1985; Mayer-da-Silva et al. 1988). Ultrastructural properties of the nodules include cystic, dilated rough endoplasmatic reticulum and cystic Golgi vesicles which contain a fine fibrillar material that is found in the ground substance (Iwata et al. 1980). Based on these findings, we wanted to evaluate the oral manifestations of JHF and to describe light microscopic, EM, and immunohistochemical features of gingival tissue samples from two siblings with JHF.

Our EM observations demonstrated that the basal lamina was not continuous and the anchoring filaments and microfibrillar bundles that usually extend from basal lamina to connective tissue were not observed. Type IV collagen expression was not detected among these fibres and inter-cellular bridge defects were present in our cases. In the literature, there were several reports describing the abnormality in extracellular matrix components synthesis and degradation in JHF cases. Haleem et al. (2002) demonstrated the characteristic chondroid appearance of large peripheral vesicles in the stromal cell cyto-

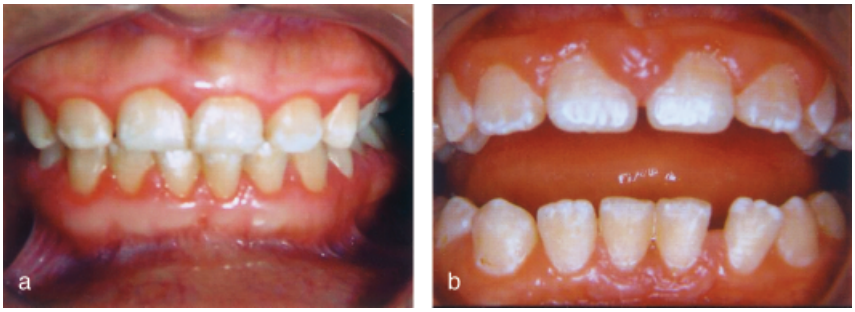

Fig. 7. Intra-oral view of Case 1 (a) and Case 2 (b) after last gingivectomy operation. In case 2, gingivectomy operation was performed only in mandibular anterior region. Maxiller gingivectomy was delayed because of lack of cooperation.

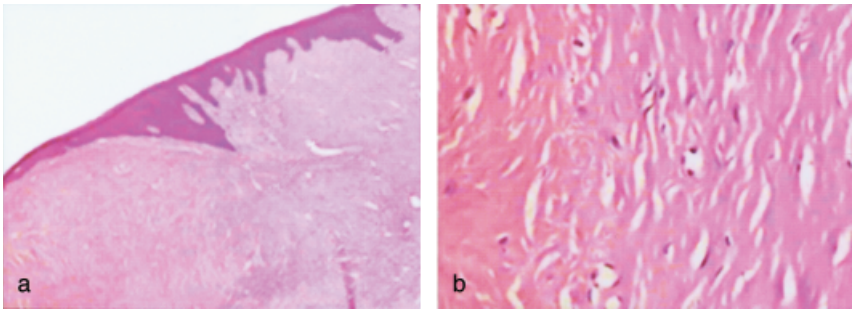

Fig. 8. (a and b) Dens collagen fibres mass and hyaline accumulation are observed under the gingival epithelium (H-E).

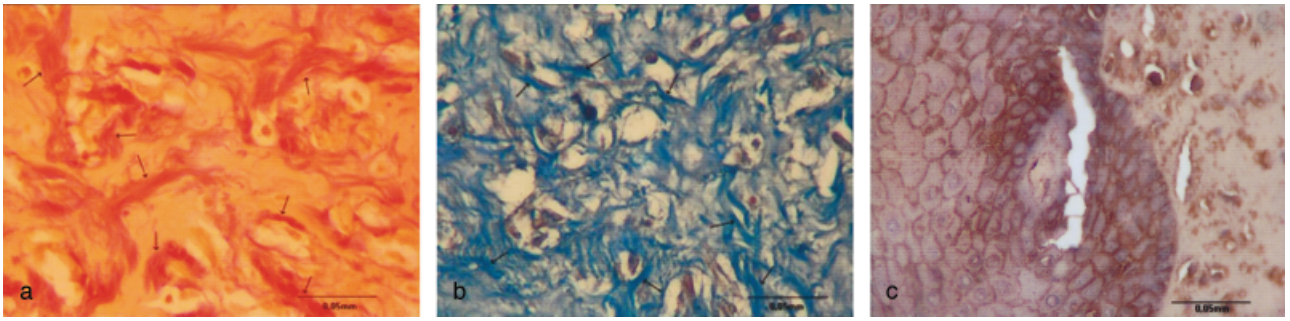

Fig. 9. (a) Collagen fibres were seen (arrows) (Van Gieson's; bar = 0.05 mm). (b) Collagen fibres were seen (arrows) (Masson's Trichrome; bar = 0.05 mm (c) Inter-cellular bridges (arrows) and inter-cellular space (s) were seen (Laminin; bar = 0.05 mm).

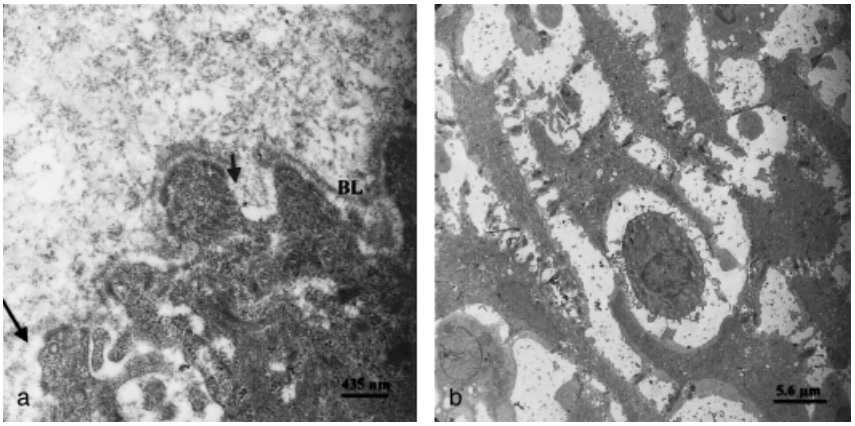

Fig. 10. (a) Epithelial–subepithelial junction by electron microscopy. Hemidesmosomes (*) of the undulating lower basal cell surface and interruptive basal lamina (↑) were observed. (b) Electron micrograph of stratum spinosum, unexpected big spaces between the adjoining cells and significant decrease of the inter-digitating processes were noted.

plasm, multinucleated histocytic giant cells, defective synthesis and deposition of collagen as fibrillogranular material in the extracellular matrix of subcutaneous nodules. By quantitative biochemical investigation, the presence of collagen type I and III and their normal ratio in subcutaneous nodules were shown, but type II and type IV collagen were absent (Remberger et al. 1985). Post-mortem immunochemical studies on collagens showed the absence of pro-α2 (I) chain and collagen type III in skin but not in the other organs of a child with JHF (Lubec et al. 1995). Moreover, in skin fibroblasts cell cultures obtained from healthy and JHF individuals, increased synthesis and degradation of type I collagen and reduced-type III collagen overall metabolism were noted when compared with healthy controls (Breier et al. 1997).

Regarding intra-oral findings and treatment, Piattelli et al. (1996) reported a girl with multiple skin modules, masses and hypertrophy of gingiva. They histopathologically observed normal epithelium with the presence in the subepithelial connective tissue of large amounts of dense hyalinized material, and intact basal membrane with no infiltration by the lesional tissue. We also observed similar findings in our HE staining, but contrast to their observation regarding intact basal membrane, our immunostaining results demonstrated that because of lack of type IV collagen expression and laminin defect, basal membrane was affected and electron microscopy findings also confirmed immunostaining results in our cases. Generally, gingival hyperplasia is observed in JHF and cases without gingival hyperplasia have only been rarely reported (Ugras et al. 2000). Occasionally inter-proximal alveolar bone loss occurs in conjunction with gingival hyperplasia (Aldred et al. 1987). However, there was no associated bone loss in our cases.

A genome-wide linkage search in five JHF families was performed and identified a region of homozygosity on chromosome 4q21 (Rahman et al. 2002). After this study, Hanks et al. (2003) identified 15 different mutations *CMG2* in 17 families with JHF or ISH. *CMG2* is a transmembrane protein that is induced during capillary morphogenesis and that binds laminin and collagen IV via a von Willebrand factor type A domain. Dowling et al. (2003), confirmed that *CMG2* mutations cause JHF and ISH and also showed that *CMG2* mutations abrogate normal cell–extracellular matrix interaction. Mutation analysis in our family revealed a homozygous missense *CMG2* alteration in both cases. This mutation alters an amino acid that is conserved in all known *CMG2* paralogues and orthologues and that is in the vicinity of recognized pathogenic *CMG2* missense mutations identified in other JHF families (Hanks et al. 2003). It is therefore most likely to be causative in our family and provides molecular confirmation of the clinical diagnosis of JHF.

Advancement in our knowledge of the pathogenesis of JHF will facilitate understanding of the mechanisms and signalling pathways of overgrowth and other tissue abnormalities. Further research is now required to clarify the collagen types and other extracellular matrix and adhesion molecules that are aberrantly expressed in JHF.

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