



## Dental Findings and Mutational Analysis of a Case with Ehlers-Danlos Syndrome

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### Abstract

Ehlers Danlos syndrome (EDS) is a rare hereditary disorder characterized by abnormalities of skin, joints and ligament of the connective tissue. Several gene mutations were reported in different forms of EDS. Twelve years old male was referred to us from Department of Genetics with EDS type I (classic type) diagnosis. The patient was first child of healthy non-consanguineous parents. According to medical history, his skin tears and bruises easily after minor trauma. Skin fragility and extensive scar formation, several widened atrophic scars all over his body especially in his face and legs and joint hypermobility in the fingers were observed in the case. Abnormal teeth root in mandibular anterior region and variations in pulp shape, pulp calcifications were noted radiographically. Histological examination demonstrated abnormalities in the structure and arrangement of collagen fibrils in extracellular matrix. Mutational analysis on a highly conserved region within exon 62 of COL5A1 gene demonstrated no substitution of cysteine (TGC) by serine (TCC) in position 1,181 of the pro  $\alpha 1(V)$  collagen chain.

**Keywords:** Classic ehlers-danlos syndrome; Dental; Mutation analysis; Collagen type I; Collagen type V

### Introduction

In 1901, Ehlers described the condition as a hyperelasticity of the skin and tendency to easily bruising. Danlos introduced a condition with a pseudo-tumor of a molluscoid or fibrous type in 1908. Firstly, Pommeau-Delille and Soussie described this condition as Ehlers Danlos syndrome (EDS) in 1934. Other terms like 'elastic or rubber man/woman' were also used for description of syndrome [1]. EDS is a genetically heterogeneous group of connective tissue disorders characterized by abnormalities of skin, joints and ligament. Although several classifications were found in the literature, the latest classification of EDS in to 6 major types has been defined by Beighton et al. [2] based on clinical, genetic and biochemical criteria. Only 4 types of EDS including IV, VI, and VII and X can be confirmed by biochemical and molecular level tests. Since there are 19 types of collagen in human, it is especially difficult to establish a precise diagnosis regarding types of EDS. The classic type of EDS is an autosomal dominant disorder which is characterized by skin hyperextensibility, abnormal wound healing, joint hypermobility and generalized tissue fragility. Based on phenotypic severity, previous classification of classic EDS comprised type I ('gravis') and type II ('mitis') [3,4]. Several mutations were identified in the genes encoding  $\alpha 1(I)$ ,  $\alpha 2(I)$ ,  $\alpha 1(III)$ ,  $\alpha 1(V)$  and  $\alpha 2(V)$  chains of procollagens, lysyl hydroxylase and tenascin X in different forms of EDS [5-7]. Colige et al. [8,9] also demonstrated that EDS type VIIC result from mutations in the procollagen I N-proteinase (pNPI) gene and ADAMTS2 gene. The molecular basis of classic type of EDS has remained unclear for a long time. In 1995, transgenic mice experiments presented the first evidence on the causal involvement of type I collagen (COLI) in this disorder. Mice with a homozygous deletion in ColV  $\alpha 2$  gene provided clinical and ultrastructural model for classic EDS like in humans. To clarify the genetic basis of classic EDS, linkage studies were performed and several ( $\approx 10$ ) mutations within ColV  $\alpha 1$  and ColV  $\alpha 2$  genes were reported [10-18]. Based on linkage and transgenic mice studies, it is known that Collagen V (COL V) is causally involved in human EDS I and II. COLV is co expressed with COLI in many tissues and plays an important role in COLI fibrillogenesis. It consists of three different polypeptide chains including pro  $\alpha 1V$ , pro  $\alpha 2V$  and pro  $\alpha 3V$ , encoded by COLVA1, COLVA2 and COLVA3 genes respectively. Linkage studies in

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**Figure 1:** Note abnormal wound healing with widened atrophic scarring on his forehead.



**Figure 2:** Excessive scar formation on the knees and hyperpigmented scars on the legs of case.



**Figure 3:** A and B) Closer view of Figure 2.



**Figure 4:** Hyperelasticity of the skin.

the families with EDS II and EDSI/II have demonstrated linkage to the COLVA1 gene. The mutation causes the substitution of the most 5' cysteine residue by a serine within a highly conserved sequence of the pro  $\alpha$ 1V C-propeptide domain and causes reduction of COLV by preventing incorporation of the mutant pro  $\alpha$ 1V chains in the COLV trimers. Several articles describe skin and joint problems associated with EDS, but few describe oral and dental or periodontal findings of this condition [19-23]. Here, we report the clinical and



**Figure 5:** Joint hypermobility in the fingers.



**Figure 6:** Intra-oral view of the case. Clinically, there was no abnormal condition related to gingiva and alveolar mucosa.



**Figure 7:** Panoramic radiography of the case. Note variations in pulp shape and pulp calcifications in mandibular canine teeth.



**Figure 8:** Peri-apical radiography of the case. Note abnormal teeth root in mandibular incisors.

histopathological features and the mutational analysis of COLVA1 gene of a 12-year-old case with classical type of EDS.

## Case Presentation

### Medical history

Twelve years old male was referred to us from Department of Genetics with EDS type I diagnosis. Oral health and periodontium of case was assessed including alterations of dental hard and soft tissues clinically. The patient was first children of healthy non-consanguineous parents. According to medical history, his skin tears and bruises easily after minor trauma.

## Clinical Manifestations of Case

### Extraoral findings

Extra-oral examination demonstrated that the case has marked skin fragility and extensive scar formation, eventually leads to formation of several widened atrophic scars all over his body, especially in his face and legs (Figures 1-4). Joint hypermobility was observed in the fingers of case (Figure 5).

### Intraoral findings

**Periodontal tissues:** Clinical appearance of gingiva and oral mucosa were not different in this case when compared to age and gender-matched healthy individual and his clinical diagnose was marginal gingivitis (Figure 6). Abnormal scars or gingival/mucous membrane findings were not noted. The mobility scores of anterior teeth were Grade III.

**The teeth:** Clinically no morphologic pathology was noted in the teeth crown. Radiographically, there was aplasia of root development in mandibular anterior region Figure 7 and pulp stones in mandibular canine teeth were also observed in this case (Figure 7 and 8). Right and left maxillary canine and left mandibular canine did not erupted so far.

### Histopathological evaluation

Gingival specimen was evaluated histopathologically. Histological examination demonstrated abnormalities in the structure and arrangement of collagen fibrils in extracellular matrix. The abnormality was defined by thickness, array and shape of collagen fibrils (Figure 9).

### Dental treatment approach

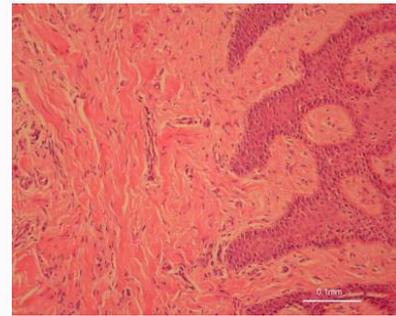
Periodontal treatment including scaling and polishing was performed and oral hygiene was motivated. Since he may lose anterior incisor due to abnormal root type of teeth in a short term, regular dental visits were planned and orthodontic and prosthetic consultations were performed. It is decided to initiate the orthodontic treatment of case with well-controlled forces. After orthodontic treatment, implant therapy or prosthetic restorations were planned in a long term.

### Mutational analysis

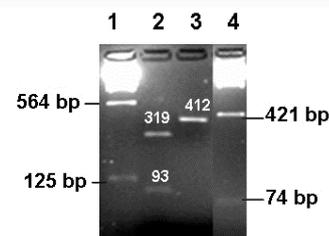
A mutation in Type V Col gene was evaluated in this case. Two ml blood was taken for RNA isolation. All procedures were performed with appropriate informed consent from the parents and the protocol was approved by the Ethics Committee of the Selcuk University, Faculty of Dentistry (2007/8-2). cDNA synthesis was performed according to Superscript™ First-Strand Synthesis System (Invitrogen™ Life Technologies, USA) for RT-PCR procedure.

### RT-PCR

Amplification reactions were performed in a final volume of 25 µl containing 0.5 µl cDNA, 25 mM of each dNTPs (Larova, Biochemie GmbH, Germany), 50 pmol of each forward (5'-TGGGCACG CAGCAGAAC-3') and reverse (5'-AGCGGAGGGCCTTGTCGTAGC-3') primers, 1.5 mM MgCl<sub>2</sub> (Bioron GmbH, Germany), 1X reaction buffer (160 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 670 mM TrisHCl (pH 8.8), 0.1% Tween-20), and 1U of Taq DNA polymerase (Bioron GmbH, Germany). The RT-PCR products were resolved by electrophoresis in 1.5% agarose gels stained with ethidium bromide.



**Figure 9:** Abnormal arrangement of collagen fibrils in extracellular matrix of gingival specimen.



**Lane 1:** λ DNA EcoRI/HindIII ladder  
**Lane 2:** StyI restriction digested PCR product  
**Lane 3:** Nondigested PCR product  
**Lane 4:** λDNA Eco130I ladder

**Figure 10:** StyI restriction pattern of cDNA fragment flanking the potential mutation site localized within exon 62 of the COL5A1 gene. After digestion of the fragment via StyI, a 62 bp and a 31 bp band which is related with mutation were not noted on the gel.

### Gel extraction

Gel slice with DNA fragment flanking the region of putative mutation was cut on a UV transilluminator and DNA was extracted from the gel using QIA quick Gel Extraction Kit (Germany) according to its procedure.

### Enzyme digestion

10 µl of gel purified DNA fragment was digested for 20 h at 37°C with 10 units of Eco130I (StyI) (Fermentas, Lithuania) within 1X Buffer O, supplied with the enzyme, followed by enzyme inactivation at 65°C for 20 min. As positive control, the fragment had a second recognition site for the enzyme. λDNA was also digested in parallel and run on the same gel. The size of the fragment was precisely determined by using molecular size markers flanking the size of the digested fragments run on a 2.5% agarose gel.

### Mutational evaluation

cDNA was amplified using primers flanking the position 1,181 of the pro α1(V) collagen chain within exon 62, generating a 412-bp fragment. Upon restriction digestion of the fragment via StyI two bands were generated, size of which were 319 bp and 93 bp. However, the digestion seen in Figure 10 was not due to the potential mutation resulting in EDSI, determined previously by De Paepe et al. [10]. This was because of another downstream StyI recognition site normally present within pro α1(V) collagen chain. Otherwise we should have seen the smaller fragment split into two, generating a 31 bp and a 62 bp band. Hence, the enzyme digestion determined in our sample has no relation with the mutation resulting in EDSI phenotype.

## Discussion

Classic EDS (Type I and II) should be discriminated from other types of EDS including dermatosparaxis, kyphoscoliotic and arthrocalasis. Generalized joint hypermobility with complication such as repetitive dislocations and chronic articular pain and more severe skin abnormalities were the clinical manifestation of other types [3]. In our case, moderate skin abnormalities and wound healing and slight joint hyper mobility were observed. The most common reported molecular mechanism in classic EDS is the functional loss of one allele of COLVA1 which encodes pro  $\alpha 1(V)$  chains of type V collagen. Type V collagen is a minor fibrillar collagen and it is widely distributed in numerous tissues including skin, tendon, bone, cornea, placenta and fetal membranes. COLV plays important roles in extracellular matrix organization and in COLI fibril nucleation [24]. Abnormal fibril nucleation and dysfunctional fibril growth with potential disruption of cell-directed fibril organization result in connective tissue dysfunction associated with EDS. Increased lethality (at embryonic day 10) of ColVA1 knock-out mice suggested that COLV may have important role in early organogenesis as well [24]. Wenstrup et al. [25-28] demonstrated a 50% reduction in type V collagen in heterozygous mice for COLVA1 mutation. This reduction leads to abnormal connective tissue biosynthesis in EDS. The COLVA1 gene, located at 9q34.2-q34.3, is a large gene comprising 66 exons distributed over 150 kb of genomic DNA. COLVA2 gene located at 2q31, comprises 52 exons distributed over 67 kb and COLVA3 is located at 19p13.2. To date, mutations in COLVA1 and COLVA2 genes are reported in at least half of the patients with classic EDS. No mutation was reported in COLVA3 gene so far. In approximately 1/3 of classic EDS patients nonsense, frame shift or splice site mutations that introduce a premature termination codon are responsible for a non-functional COLVA1 allele [29]. However, one of the point mutation, identified in COLVA1, is a unique mutation that changes a highly conserved cysteine residue to a serine in the C terminal propeptide of the  $\alpha 1(V)$  collagen chain. Sequencing of the PCR product showed a G-to-C transversion that resulted in the substitution of cystein (TGC) by serine (TCC) in position 1,181 of the pro  $\alpha 1(V)$  collagen chain. This mutation created a restriction site for the endonuclease *StyI*. Although this is a rare mutation it is important since it resides within a region that is highly conserved throughout evolution, among the different fibrillar collagens as well as among fibrillar collagens in different species. This is because this residue is essential for intrachain disulphide bonding prior to assembly of the pro  $\alpha$ -chains and initiation of triple-helix folding of fibrillar collagen molecules [30]. In this study, we have determined that the moderate EDSI phenotype of the patient given dental findings was not related to a mutation on COLVA1 at the highly conserved cysteine in the C-propeptide domain in fibrillar collagens in chain assembly. However, advance in molecular testing increase understanding of classical EDS since 1997, genetic heterogeneity in type V collagen might not allow finding associations with the abnormalities [31]. De Coster et al. [20] assessed oral health of 31 EDS patients (16 with hypermobility EDS, 9 with classical EDS and 6 with vascular EDS). They observed abnormal pulp shape in 13% and pulp calcifications in 78% of EDS patients. Poor oral hygiene and high caries incidence due to increased mucosal fragility and restraint of wrist joint mobility were examined and 62% of EDS patients need periodontal treatment (CPITN=2). Their study demonstrated that oral health of EDS patients may be at risk. Slow and deficient healing after tooth extraction and periodontal treatment, delay in forming of new bone in sockets and

soft tissue scarring, TMJ hypermobility with recurrent dislocation was reported in the literature. During orthodontic treatment have been reported further complications such as rapid migration and greater teeth mobility during the movement phase due to poorly organized collagen fibrils in the periodontal ligament. Non-traumatic approach in the treatment of EDS patients is very important. Arun et al. [32] reported satisfactory results in the orthodontic treatment of EDS type VI by taking appropriate precautions during orthodontic treatment. In our case, after genetic and orthodontic consultations, it was decided to initiate the orthodontic treatment with well-controlled forces and regular periodontal visits were arranged to optimize oral hygiene and prevent of dental caries. Periodontal conditions have been reported with EDS Type I, VII and VIII in the literature. Pope et al. [33] reported dental findings of two EDS I cases with defective dentinogenesis and aplasia or hypoplasia in mandibular incisors and a bulbous enlargement of the roots together with pulp stones. Similar dental features were examined in our case. Perez et al. [23] and Rahman et al. [34] presented EDSVIII cases with periodontal involvement. While it was reported predisposition for localized periodontal disease in EDSI, and EDSVIII, in our case we did not observe periodontal breakdown. Careful decision and well-interaction between the periodontist, oral surgeon, endodontist, orthodontist and prosthodontist is crucial in establishing the dental treatment plan for EDS patient. Collaboration between the geneticist and dentist is also important since the dental treatment should be performed and preventions should be taken based on the type of EDS.

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