

CASE REPORT

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Classic Ehlers–Danlos syndrome presenting as atypical chronic haematoma: a case report with novel frameshift mutation in *COL5A1*

Wei-Ching Chiu¹, Shu-Huey Chen^{1,2,3}, Mei-Chen Lo^{1,4} and Yung-Ting Kuo^{1,2*} 

Abstract

Background: Ehlers–Danlos syndrome (EDS) is a group of inherited connective tissue disorders characterized by skin hyperextensibility, joint hypermobility and soft tissue vulnerable to blunt injury. Early recognition and diagnosis are crucial to patients to provide appropriate treatment, as well as to screen for life-threatening conditions such as aortic dissection and hollow organ perforation. The diagnosis of EDS is made based on clinical presentations, skin biopsy, and electron microscopy findings. To date, mutations in at least 20 genes have been found to cause the Ehlers–Danlos syndromes. However, EDS is still underestimated due to lack of awareness of its variable clinical presentations. Here we reported an EDS case with atypical initial presentation and a novel genetic mutation.

Case presentation: This 4-year-old Taiwanese male patient presented with easy bruising, multiple ecchymoses, joint hypermobility, hyperextensible skin, and prolonged pretibial haematoma. He was initially suspected of a bleeding tendency due to coagulation disorders. The coagulation test results were normal. DNA sequencing was performed for molecular diagnosis. Subsequently, the diagnosis of classical EDS was made by identifying a novel frameshift mutation in *COL5A1* [NM_000093.4:c.4211_4212delAG, p.Gln1404Arg]. This mutation in the type V collagen gene *COL5A1* contributes to the phenotype of classical EDS. This novel frameshift mutation may disturb the structural stability of collagen V and interfere with its heparin binding capacity, explaining the chronic haematoma.

Conclusion: The reported case showed the unusual features of chronic haematoma. This novel frameshift mutation and its phenotype correlation can provide useful information for practitioners about early recognition in Ehlers–Danlos syndrome.

Keywords: Ehlers–Danlos syndrome classical type, Collagen type V alpha-1 chain (*COL5A1*) gene, *COL5A1* frameshift mutation, Collagen type V heparin-binding, Bleeding diathesis

* Correspondence: pedkuoyt@tmu.edu.tw

¹Department of Pediatrics, Shuang Ho Hospital, Ministry of Health and Welfare, Taipei Medical University, No. 291, Zhongzheng Rd., Zhonghe District, New Taipei 23561, Taiwan

²Department of Pediatrics, School of Medicine, College of Medicine, Taipei Medical University, No. 250, Wu-Hsing Street, Taipei 110, Taiwan

Full list of author information is available at the end of the article



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Background

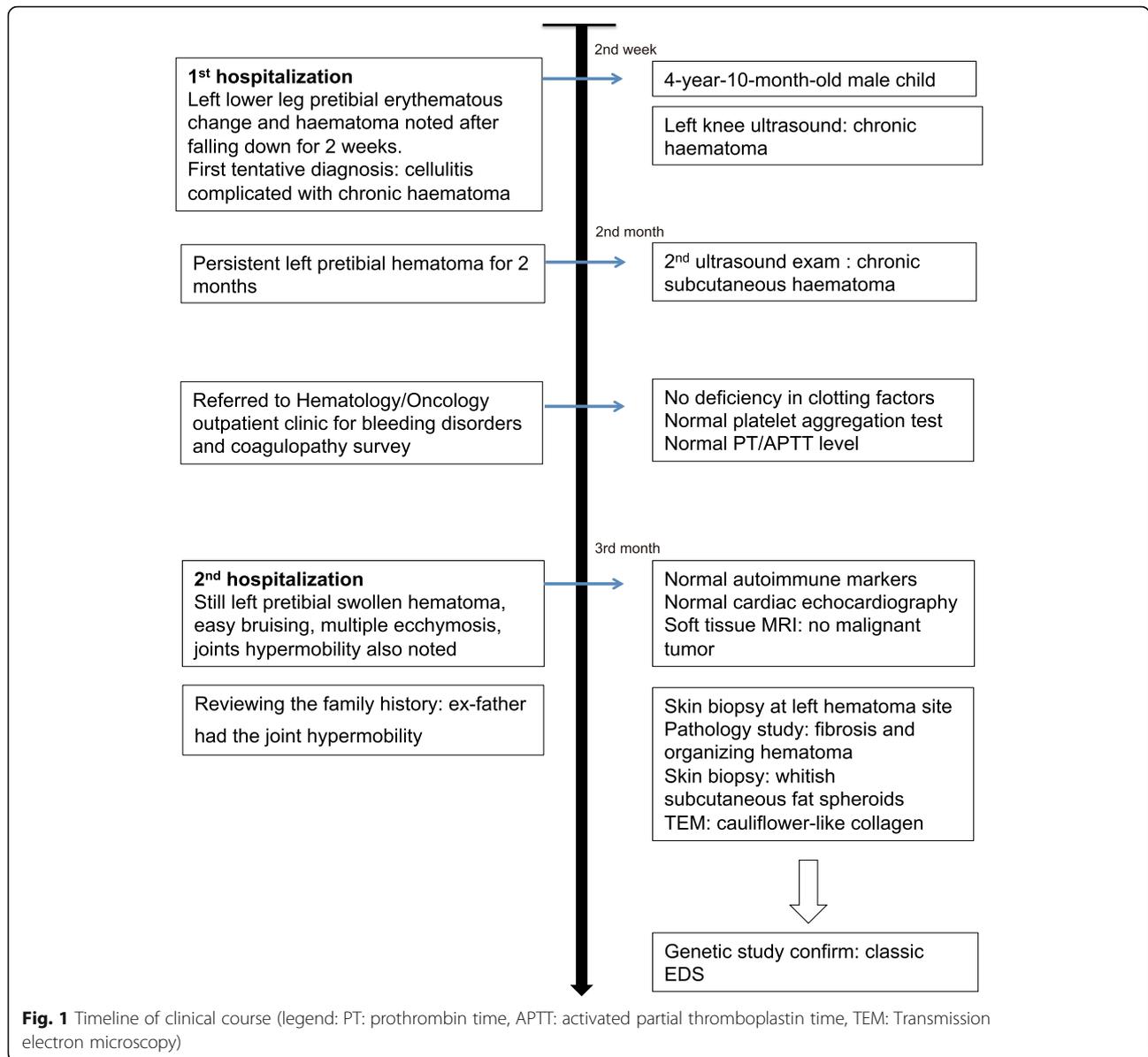
Ehlers–Danlos syndrome (EDS) is a broad-spectrum hereditary disorder of connective tissue. In patients with EDS, accurate diagnosis is crucial to screen for life-threatening complications, such as vascular and hollow organ perforation, ligamentous laxity, and joint subluxation that can lead subsequent pain and long-term disability [1]. EDS was once recognized as a rare disease with the prevalence of all forms of EDS was previously estimated at 1:150,000 in the previous studies [2]. In the United Kingdom, a national electronic cohort study suggested a higher prevalence was estimated at 194.2 per 100,000 in 2016/2017 or roughly 10 cases in 5000 patients [3]. These prevalence differences can arise from poor recognition in children and consideration of initial symptoms as normal and irrelevant episode. Based on the latest 2017 international classification of EDS, there are 13 subtypes of EDS that are identified based on clinical characteristics, inheritance pattern, and molecular characteristics: classical, classical-like, cardiac-valvular, vascular, hypermobile, arthrochalasia, dermatosparaxis, kyphoscoliotic, Brittle cornea syndrome, spondylodysplastic, musculocontractural, myopathic, and periodontal [4]. Classical EDS (cEDS) is autosomal dominant genetic disorder, and cEDS together with the hypermobility type accounts for approximately 90% of all cases of EDS. It arises due to mutations in type V collagen genes (*COL5A1* and *COL5A2*) and is characterized by marked joint hypermobility, hyperextensible skin, hemosiderotic scars, and cutaneous pseudotumors [5]. A definite diagnosis can be made by recognizing a pathognomonic mutation in *COL5A1* or *COL5A2*. However, their symptoms overlap between various types of EDS and other hereditary connective tissue disorders including osteogenesis imperfecta and Marfan syndrome, thus making the timely diagnosis of EDS challenging. Many cases are undiagnosed or misdiagnosed, and delayed diagnosis will cause impedance of appropriate treatment and preventive strategies [3, 6]. Here, we report a Taiwanese patient who was diagnosed with cEDS and who harbored a novel frameshift mutation in *COL5A1* [NM_000093.4:c.4211_4212delAG, p.Gln1404Arg] that was detected on Sanger sequencing.

Case presentation

We presented the case of a 5-year-1-month-old boy who was referred to our outpatient clinic when he was 4-year-10-month-old (timeline of clinical course—Fig. 1 illustrates the clinical course).

The patient was examined because of prolonged unresolved pretibial haematoma (Fig. 2a) that measured $6.5 \times 6.5 \text{ cm}^2$ after falling down to the floor with initial impression of cellulitis. Easy bruising, multiple ecchymoses (Fig. 2b), joint hypermobility (Fig. 2c) and poly-

arthralgia were noted since childhood; however, no specific medical advice was sought before the clinic visit. Clinical examination revealed body height 108 cm (23th percentile) and body weight 21 kg (75th percentile). The patient had joint hypermobility, Beighton score 7 (including bilaterally passive dorsiflexion of fifth finger beyond 90, score 2; bilaterally passive flexion of thumb to forearm, score 2; hyperextension of the elbow beyond 10 degree, score 0; bilateral hyperextension of the knee beyond 10 degree, score 2; forward flexion of the trunk with knees fully extended and palms resting on the floor, score 1) [7], poly-arthralgia without inflammatory signs, mildly hyperextensible skin, and ecchymoses over multiple sites. Among the minor diagnostic criteria for cEDS, muscle hypotonia, delayed gross motor development, easy bruising without obvious causes, and positive family history (similar symptoms of joint hypermobility in his father and aunt) were also observed. Chest wall deformity, kyphoscoliotic posture, periodontal lesions were not observed during physical examination. No tumors and vasculopathy were observed on ultrasound and magnetic resonance imaging of the pretibial mass. Echocardiographic assessment revealed no mitral valve prolapse and aortic root dilatation. The myopathy was less likely due to normal range creatine kinase. Hematological studies for coagulation and platelet disorders and study for autoimmune diseases all provided normal results (Supplementary Table. 1). With aforementioned presentation, the patient was suspected of having EDS. Skin biopsy was performed, and whitish subcutaneous fat spheroids were observed during the biopsy (Fig. 2d). Light microscopy revealed fibrosis in the subcutaneous fat and organizing haematoma without malignancy. Transmission electron microscopy of the skin biopsy revealed irregular interfibrillar spaces, collagen fibers with variable diameter, and collagen cauliflower (Fig. 2e) [8]. Molecular evaluation of genomic DNA extracted from a blood sample was performed. Whole exome sequencing and conventional Sanger sequencing were performed using the CytoOnearray sequencing panel (Phalanx Biotech, Taiwan). These analyses identified the frameshift mutation NM_000093.4(*COL5A1*):c.4211_4212delAG in exon 54, which resulted in a glutamine to arginine substitution at codon 1404 [NP_000084.3: p.Gln1404ArgfsTer77] (Fig. 3). Based on disease-causing mutation databases, such as Leiden Open Variation Database (LOVD) [9] and ClinVar [10], this frameshift mutation identified in our patient is a novel finding. At least 45 pathologic variants of a frameshift mutation in cEDS have been found; however, no pathological variants and mutation loci were detected, as in our patient. This is a novel frameshift mutation in *CO5A1* first identified in this patient with cEDS. This patient was regularly followed in the



multidisciplinary team and accepted the rehabilitation program in outpatient clinic.

Discussion and conclusion

Considering chronic haematoma as another disease entity
Unreasonable ecchymoses and increased bleeding diathesis are crucial clinical characteristics of several coagulation and platelet disorders, such as von Willebrand disease and hemophilia A and B. However, these characteristics can also be observed in hereditary collagen disorders, which belong to a heterogeneous group of genetic diseases that are caused by mutations in structural collagen genes or in genes that code for enzymes involved in their post-translational modifications. EDS may be regarded as one of the differential diagnoses in such cases [11].

COL5A1 mutations involved in cEDS

The main etiology of cEDS involves structural defects in type V collagen. Type V collagen is widely distributed in tissues, and it is a quantitatively minor fibrillar collagen. Together with type I collagen, it plays a main role in collagen fibrillogenesis [12]. The commonest isoform of type V collagen is a heterotrimer composed of two α 1-propeptide chains and one α 2-propeptide chain encoded by *COL5A1* and *COL5A2*, respectively. The α 1-propeptide chain is composed of three domains: N-propeptide, α -helix, and C-propeptide [13]. *COL5A1* cDNA comprises 66 exons distributed over more than 150 kb of genomic DNA. The most common null mutations detected in *COL5A1* consist of frameshift, nonsense, and splice site mutations that result in a premature

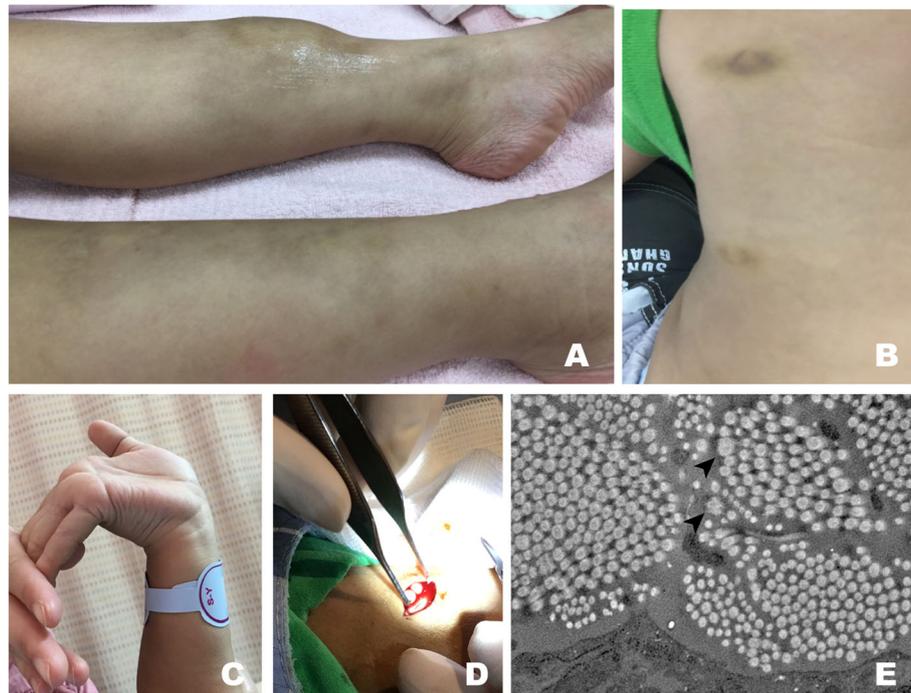


Fig. 2 Clinical presentations and electron microscopic examination. **a.** Chronic haematoma in the lower pretibial area. **b.** Multiple bruising ecchymosis on the anterior upper chest and flank areas. **c.** Hypermobility of the wrist joint and hyperextension of the proximal digital joints. **d.** Subcutaneous fat spheroids observed in the skin biopsy. **e.** “Collagen followers” (closed black arrow, >) in electron microscopic examination

termination codon (PTC) [14, 15], which generates unstable mutant *COL5A1* mRNA that is rapidly degraded, resulting in the overall reduction of type V collagen [15]. In approximately 40–50% of individuals with cEDS, nonsense or frameshift variants are responsible for a nonfunctional *COL5A1* allele [16]. *COL5A1* haploinsufficiency mutations are the predominant mutations, and they result in the reduction of type V collagen, whereas a minority of

mutations result in the production of structurally abnormal type V collagen [17].

Disruption of type V collagen and heparin binding site interaction

Type V collagen binds to DNA, heparan sulfate, thrombospondin, heparin, and insulin. Previous studies have reported the structural requirements and molecular

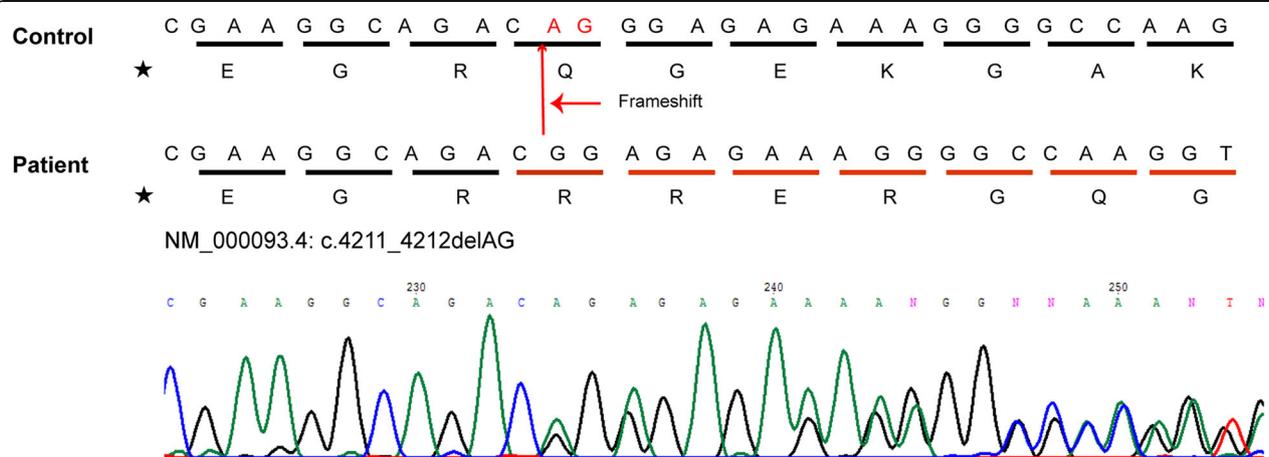


Fig. 3 Genetic sequence of *COL5A1* mutation. Genetic studies revealed a novel frameshift mutation in exon 54. This mutation replaces glutamine (Q) with arginine (R) and the following amino acid codons. ★, amino acids are represented by the letters used in the international nomenclature

mechanisms of the interactions of heparin and heparan sulfate with collagen V [18–20]. A fragment (Ile824-Pro950) of the collagen 1(V) chain, called HepV, binds to heparin through a cluster of three major basic residues—Arg912, Arg918, and Arg921—and two additional residues—Lys905 and Arg909 [21]. Triple-helical molecules with the homotrimeric or heterotrimeric collagen V molecules form very stable complexes with heparin and heparan sulfate [20]. Structural disability of collagen V interferes with heparin binding, resulting in dysfunction of cell–matrix interactions and contributing to the molecular pathogenesis of cEDS.

In our case, the patient demonstrated the characteristics of easy bruising and multiple ecchymoses in addition to skin fragility, hyperextensibility, and joint hypermobility. Skin biopsy revealed subcutaneous spheroids due to herniation of the subcutaneous fat; these spheroids were small, hard, and cyst-like nodules. With fragile collagen matrix protein in the surrounding tissue, these fat spheroids are freely movable over the bony protuberances along the extremities. The dermis in our patient contained large collagen fibrils of irregular diameters (collagen “cauliflowers”), which are indicative of disturbed collagen fibrillogenesis [6]. Genetic studies have revealed the frameshift mutation c.4211_4212delAG in exon 54, which resulted in the replacement of glutamine with arginine at position 1404 [p.(Gln1404Arg)]. Arginine is one of the destabilizing residues observed in the Gly position of the Gly-X-Y pattern that is repeated in the collagen triple helix [22, 23]. Collagen represents approximately 40% of the total protein in a vessel wall, and type V collagen is one of the widely distributed types of collagen in vessels. Collagen is the only matrix protein that supports both platelet adhesion and complete activation, and it has an imperative role in initiating homeostasis. If the collagen structure is abnormal, the vessel wall is more vulnerable to injury, and the homeostasis function is altered. This frameshift mutation detected in our patient may explain the phenomena of chronic haematoma, multiple ecchymoses, and easy bruising, which are due to the disturbance of the structural integrity of the triple helix and interference with its heparin-binding capacity.

Conclusions

This reported case showed initial symptoms with chronic pretibial haematoma. We identified a novel frameshift mutation in *COL5A1* in a patient with cEDS. This mutation disturbed the structural stability of collagen V and interfered with its heparin-binding capacity; this is one of the molecular mechanisms contributing to cEDS. The patient’s clinical symptoms, skin biopsy, in combination with molecular test can help practitioners increase the awareness of this disease entity and take

stepwise diagnostic approaches to screen risky complications.

Supplementary information

The online version contains supplementary material available at <https://doi.org/10.1186/s12887-020-02386-1>.

Additional file 1: Table S1. Crucial laboratory findings. (TIF 58 kb)

Additional file 2. (TIF 103 kb)

Abbreviations

EDS: Ehlers-Danlos syndrome; cEDS: Classic Ehlers-Danlos syndrome; COL5A1: Collagen Type V Alpha 1 Chain; COL5A2: Collagen Type V Alpha 2 Chain; DNA: Deoxyribonucleic acid; PTC: Premature termination codon

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Authors’ contributions

SHC and YTK disclosed the patent’s clinical manifestations and initiated the overall project. SHC and YTK screened the correlation between phenotype and genotype. WCC and YTK were responsible for skin biopsy, interpretation of the examination data, and wrote the first draft of the paper. MCL performed the technique of electron microscopy examination. WCC and YTK revised the manuscript for important intellectual contest. SHC and YTK provided professional advice. All authors contributed to and approved the final version.

Authors’ information

YTK: MD, PhD, pediatrician, pediatric neurologist, attending physician in Department of Pediatrics, Shuang Ho Hospital, Ministry of Health and Welfare, Taipei Medical University, Head of Neuromuscular Center in Taipei Medical College, member of Taiwan Society for Mitochondrial Research and Medicine, director of Pediatric Child Neurology Society.

SHC: MD, pediatrician, pediatric hematologist-oncologist, attending physician in Department of Pediatrics, Shuang Ho Hospital, Ministry of Health and Welfare, Taipei Medical University; chief director of Hemophilia Center of Shuang Ho Hospital, Ministry of Health and Welfare.

WCC: MD, resident of pediatrics, department of Pediatrics, Shuang Ho Hospital, Ministry of Health and Welfare, Taipei Medical University, New Taipei City, Taiwan.

MCL: PhD, medical researcher, mitochondrial medicine, Medical technology, intracellular communication, diabetes complications, member of Asian Society for Mitochondrial Research and Medicine

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Availability of data and materials

The datasets used and analyzed during the current report are included in this published article and its supplementary information files.

Ethics approval and consent to participate

Not applicable. We did not perform any additional scientific studies on the patient. Informed consents were obtained from the patient’s parents on each stage of the diagnostic process.

Consent for publication

The parents have signed informed consent to publish this information, and the proof of consent can be requested at any time.

Competing interests

The authors declare that they have no competing interests.

Author details

¹Department of Pediatrics, Shuang Ho Hospital, Ministry of Health and Welfare, Taipei Medical University, No. 291, Zhongzheng Rd., Zhonghe District, New Taipei 23561, Taiwan. ²Department of Pediatrics, School of Medicine, College of Medicine, Taipei Medical University, No. 250, Wu-Hsing

Stree, Taipei 110, Taiwan. ³Taipei Cancer Center, Taipei Medical University, Taipei, Taiwan. ⁴Department of Nursing, Central Taiwan University of Science and Technology, Taichung, Taiwan.

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References

- Sobey G. Ehlers-Danlos syndrome: how to diagnose and when to perform genetic tests. *Arch Dis Child*. 2015;100(1):57–61.
- Mulvey MR, Macfarlane GJ, Beasley M, et al. Modest association of joint hypermobility with disabling and limiting musculoskeletal pain: results from a large-scale general population-based survey. *Arthritis Care Res*. 2013;65(8):1325–33.
- Demmler JC, Atkinson MD, Reinhold EJ, et al. Diagnosed prevalence of Ehlers-Danlos syndrome and hypermobility spectrum disorder in Wales, UK: a national electronic cohort study and case-control comparison. *BMJ Open*. 2019;9:e031365. <https://doi.org/10.1136/bmjopen-2019-031365>.
- Malfait F, Francomano C, Byers P, Belmont J, Berglund B, Black J, et al. The 2017 international classification of the Ehlers-Danlos syndromes. *Am J Med Genet C Semin Med Genet*. 2017;175(1):8–26.
- Bowen JM, Sobey GJ, Burrows NP, Colombi M, Lavallee ME, Malfait F, Francomano CA. Ehlers-Danlos syndrome, classical type. *Am J Med Genet C Semin Med Genet*. 2017;175(1):27–39.
- Watanabe M, Nakagawa R, Naruto T, Kohmoto T, Suga K, Goji A, et al. A novel missense mutation of COL5A2 in a patient with Ehlers-Danlos syndrome. *Hum Genome Var*. 2016;15(3):16030.
- Beighton P, Horan F. Orthopaedic aspects of the Ehlers-Danlos syndrome. *J Bone Joint Surg Br*. 1969;51(3):444–53.
- de Almeida HL Jr, Bicca E, Rocha NM, de Castro LA. Light and electron microscopy of classical Ehlers-Danlos syndrome. *Am J Dermatopathol*. 2013;35(1):102–5.
- Ehlers Danlos Syndrome Variant Database: collagen type V alpha 1 chain (COL5A1). [Internet] Dagleish Laboratory, Department of Genetics, University of Leicester, UK. 2020. https://eds.gene.le.ac.uk/variants.php?select_db=COL5A1&action=search_all&search_Variant%2FType=Deletion. Accessed 23 Sept 2020.
- ClinVarCOL5A1 [gene] [Internet] National Center for Biotechnology Information, U.S. National Library of Medicine, USA. <https://www.ncbi.nlm.nih.gov/clinvar/?term=COL5A1%5Bgene%5D>. Accessed 23 Sept 2020.
- De Paepe A, Malfait F. Bleeding and bruising in patients with Ehlers-Danlos syndrome and other collagen vascular disorders. *Br J Haematol*. 2004;127(5):491–500.
- Birk DE. Type V collagen: heterotypic type I/V collagen interactions in the regulation of fibril assembly. *Micron*. 2001;32(3):223–37.
- Takahara K, Hoffman GG, Greenspan DS. Complete structural organization of the human alpha 1 (V) collagen gene (COL5A1): divergence from the conserved organization of other characterized fibrillar collagen genes. *Genomics*. 1995;29(3):588–97.
- Ritelli M, Dordoni C, Venturini M, Chiarelli N, Quinzani S, Traversa M, et al. Clinical and molecular characterization of 40 patients with classic Ehlers-Danlos syndrome: identification of 18 COL5A1 and 2 COL5A2 novel mutations. *Orphanet J Rare Dis*. 2013;8:58.
- Mitchell AL, Schwarze U, Jennings JF, Byers PH. Molecular mechanisms of classical Ehlers-Danlos syndrome (EDS). *Hum Mutat*. 2009;30(6):995–1002.
- Malfait F, Coucke P, Symoens S, Loeys B, Nuytinck L, De Paepe A. The molecular basis of classic Ehlers-Danlos syndrome: a comprehensive study of biochemical and molecular findings in 48 unrelated patients. *Hum Mutat*. 2005;25(1):28–37.
- Malfait F, Wenstrup R, De Paepe A. Classic Ehlers-Danlos syndrome. 2007 May 29 [updated 2018 Jul 26]. In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, editors. *GeneReviews*® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2020. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK1244/>.
- Symoens S, Malfait F, Renard M, André J, Hausser I, Loeys B, Coucke P, De Paepe A. COL5A1 signal peptide mutations interfere with protein secretion and cause classic Ehlers-Danlos syndrome. *Hum Mutat*. 2009;30(2):E395–403.
- Delacoux F, Fichard A, Geourjon C, Garrone R, Ruggiero F. Molecular features of the collagen V heparin binding site. *J Biol Chem*. 1998;273(24):15069–76.
- Yaoi Y, Hashimoto K, Koitabashi H, Takahara K, Ito M, Kato I. Primary structure of the heparin-binding site of type V collagen. *Biochim Biophys Acta*. 1990;1035(2):139–45.
- Ricard-Blum S, Beraud M, Raynal N, Farndale RW, Ruggiero F. Structural requirements for heparin/heparan sulfate binding to type V collagen. *J Biol Chem*. 2006;281(35):25195–204.
- Delacoux F, Fichard A, Cogne S, Garrone R, Ruggiero F. Unraveling the amino acid sequence crucial for heparin binding to collagen V. *J Biol Chem*. 2000;275(38):29377–82.
- Persikov AV, Pillitteri RJ, Amin P, Schwarze U, Byers PH, Brodsky B. Stability related bias in residues replacing glycines within the collagen triple helix (Gly-Xaa-Yaa) in inherited connective tissue disorders. *Hum Mutat*. 2004;24(4):330–7.

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