HOMOZYGOUS *CHST14* MUTATION IN AN ADOLESCENT GIRL WITH MUSCULOCONTRACTURAL EHLERS-DANLOS SYNDROME: A FIRST REPORT IN THAILAND

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Abstract

Musculocontractural Ehlers-Danlos Syndrome (mcEDS) is a rare subtype of EDS, recognized as a multisystem congenital malformation characterized by multiple congenital contractures, distinctive craniofacial features, and characteristic skin manifestations. Most mcEDS is caused by biallelic loss-of-function mutations in the carbohydrate sulfotransferase 14 (*CHST14*) gene. This study reports a 15-year-old girl with classical clinical features of mcEDS who was first presented at Phramongkutklao Hospital in Thailand. The whole exome sequencing identified a homozygous frameshift, c.1033dup or p. Arg345ProfsTer10, mutation in exon 1 of the *CHST14* gene. Sanger sequencing verified that this mutation was inherited from second-degree consanguineous parents. This study is the first to establish this frameshift mutation as a pathogenic mutation, thereby contributing to the literature on the *CHST14* gene and enhancing our understanding of this rare condition. Our patient represents the first reported case of mcEDS in Thailand.

Keywords: Musculocontractural EDS, *CHST14*, Ehlers-Danlos Syndrome

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Introduction

Musculocontractural EDS (mcEDS) is a rare subtype of EDS caused by biallelic loss of function mutations in either carbohydrate sulfotransferase 14 *(CHST14)* gene (mcEDS-*CHST14*; OMIM #601776) or dermatan sulfate epimerase (*DSE*) gene (mcEDS-DSE; OMIM #615539).[1–3] The *CHST14* gene encodes dermatan 4-O-sulfotransferase 1 (D4ST1). D4ST1 and DSE enzymes are necessary for the biosynthesis of dermatan sulfate (DS) proteoglycan, a component of various connective tissues. Deficiency of DS proteoglycan results in abnormal regulation of collagen fibril assembly and leads to mcEDS.[4] The mcEDS is a multisystem congenital malformation defined by three major clinical criteria, including congenital multiple contractures, distinct craniofacial features, and characteristic cutaneous manifestations such as skin hyperextensibility, easy bruising, skin fragility with atrophic scarring, and increased palmar wrinkling.[1,5]

To our knowledge, fewer than 70 patients in 50 families with mcEDS-*CHST14* have been reported.[5–8] Here, we report the first case of a 15-year-old girl with mcEDS in Thailand. Molecular analysis demonstrated a homozygous frameshift c.1033dup (p.Arg345ProfsTer10) mutation in exon 1 of the *CHST14* gene inherited from both carrier parents. This mutation has been reported as pathogenic for the first time in our patient.

Case presentation

A 15-year-old girl was first presented to Phramongkutklao Hospital due to generalized ligamentous laxity and recurrent knee dislocations. She was a product of second-degree consanguineous parents. She was born at term with an uneventful pregnancy. At birth, congenital equinovarus deformity was detected and treated with braces, and the patient underwent Achilles tendonectomy at 3 years of age. Her motor milestone was delayed. She started sitting at 12 months and could walk with support at 3 years of age. Her cognitive development was normal. She began suffering from bilateral progressive visual loss and recurrent dislocation of bilateral patellae at the age of 6 and 9 years, respectively.

An examination at the age of 15 years showed a hyposthenic-built girl with a height of 153 cm. (P10-25) and a weight of 36 kg (<P3). Craniofacial features revealed a drooping face, hypertelorism, down-slanting palpebral fissures, prominent nasolabial folds, long and smooth philtrum with thin upper lips, high-arched palate, and low-set and posteriorly rotated ears **(Figure 1).** Skin hyperextensibility with neither subcutaneous hematoma nor bruise was detected. The musculoskeletal exam revealed hypermobility of elbow joints, knee joints, small hand joints, slender and tapering fingers with fine palmar creases, bilateral pes planus, and levoscoliosis, which was also demonstrated in skeletal radiographs **(Figures 1 and 2).** Eye examination by an ophthalmologist discovered microcornea, malposition of both pupils, and diffuse retinal atrophy of both eyes. The echocardiogram demonstrated mild mitral valve regurgitation and aortic valve regurgitation with a left ventricular ejection fraction (LVEF) of 65 percent. The audiogram showed high-frequency sensorineural hearing loss in both ears. Pulmonary function test established restrictive lung disease from narrowing of the chest wall. Routine laboratory tests were unremarkable, including complete blood count, serum electrolytes, and renal and liver function tests. Serum creatinine kinase (CK) was 4,131 U/L (normal 25-200). Chromosome study revealed normal female karyotype (46, XX). A comparison of patient characteristics with the 2017 International Classification of Ehlers-Danlos Syndrome criteria is presented in **Table 1.**[1]

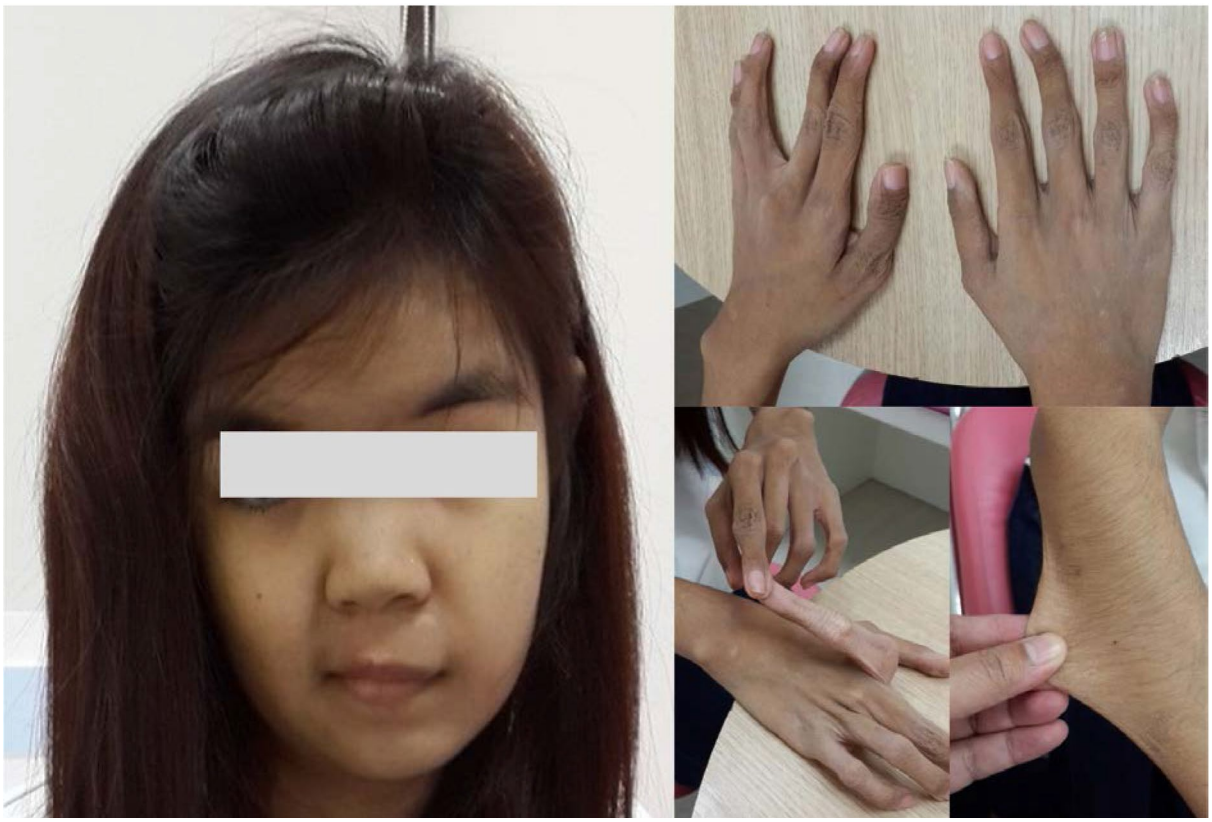

Figure 1. Characteristic facial abnormalities (left) long and slender fingers, skin hyperextensibility and hypermobility of small hand joints (right) in a 15-year-old girl with mcEDS.

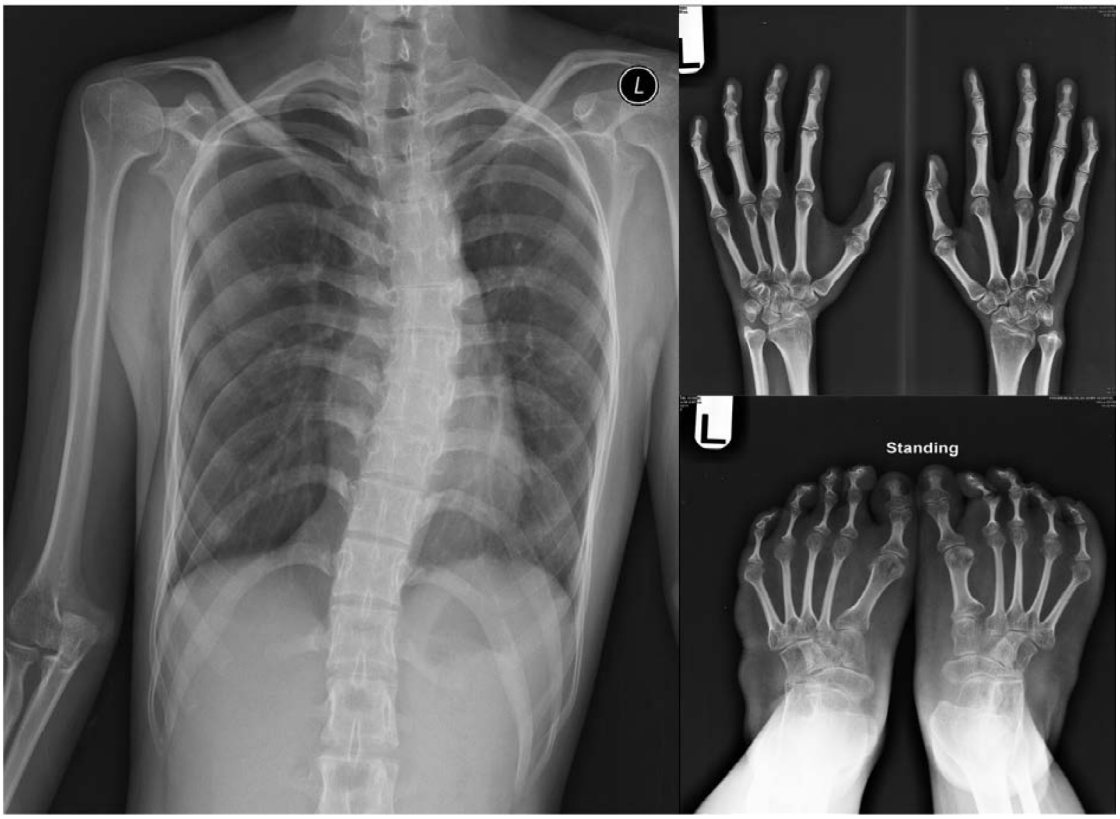

Figure 2. The skeletal radiograph reveals levoscoliosis (left), long and slender fingers, and equinovarus foot deformities (right) in the patient.

Table 1. The mc-EDS diagnostic criteria based on the 2017 International Classification of Ehlers-Danlos Syndrome

Criteria	Positive (✓)
Major criteria	
Congenital multiple contractures, characteristically adduction-flexion contractures, and/or talipes equinovarus (clubfoot)	✓
Characteristic craniofacial features, which are evident at birth or in early infancy	✓
Characteristic cutaneous features include skin hyperextensibility, easy bruisability, skin fragility with atrophic scars, increased palmar wrinkling.	✓
Minor criteria	
Recurrent/chronic dislocations	✓
Pectus deformities (flat, excavated)	
Spinal deformities (scoliosis, kyphoscoliosis)	✓
Peculiar fingers (tapering, slender, cylindrical)	✓
Progressive talipes deformities (valgus, planus, cavum)	✓
Large subcutaneous hematomas	
Chronic constipation	
Colonic diverticula	
Pneumothorax/pneumohemothorax	
Nephrolithiasis/cystolithiasis	
Hydronephrosis	
Strabismus	✓
Refractive errors (myopia, astigmatism)	
Glaucoma/elevated intraocular pressure	
Minimal criteria suggestive for mcEDS diagnosis	
At birth or in early childhood: Major criteria (1) Congenital multiple contractures AND (2) characteristic craniofacial features	✓
In adolescence and adulthood: Major criteria (1) Congenital multiple contractures AND (3) characteristic cutaneous features	✓

mc-EDS, musculocontractural Ehlers–Danlos syndrome

Molecular Analysis

The Institutional Review Board of the Royal Thai Army approved this study. After informed consent was obtained, genomic DNA from the patient and both parents were extracted from peripheral blood leukocytes according to the manufacturer's protocol. Whole exome sequencing (WES) was performed by Macrogen Inc. (Seoul, South Korea). DNA was captured on SureSelect Human All Exon V7 (Agilent Technologies, Santa Clara, CA, USA) and then sequenced on the Novaseq 6000 platform (Illumina, San Diego, CA, USA). Alignment and variant calling were performed by BWA and GATK, respectively. The reference of the genome version was UCSC hg19, and the reference sequences were NM_130468.4 and NP_569735.1 for *CHST14* cDNA and *CHST14* amino-acid position, respectively. Sanger sequencing was performed to verify any identified mutations.

Results

A homozygous frameshift c.1033dup (p.Arg 345ProfsTer10) mutation in exon 1 of the *CHST14* gene was identified in the patient's DNA and was inherited from both heterozygous parents **(Figure 3)**. According to the ACMG classification guideline,[9] This mutation was classified as "Pathogenic" according to PVS1, PM2, and PM3 criteria. Hence, the mcEDS was made as the final diagnosis in this patient.

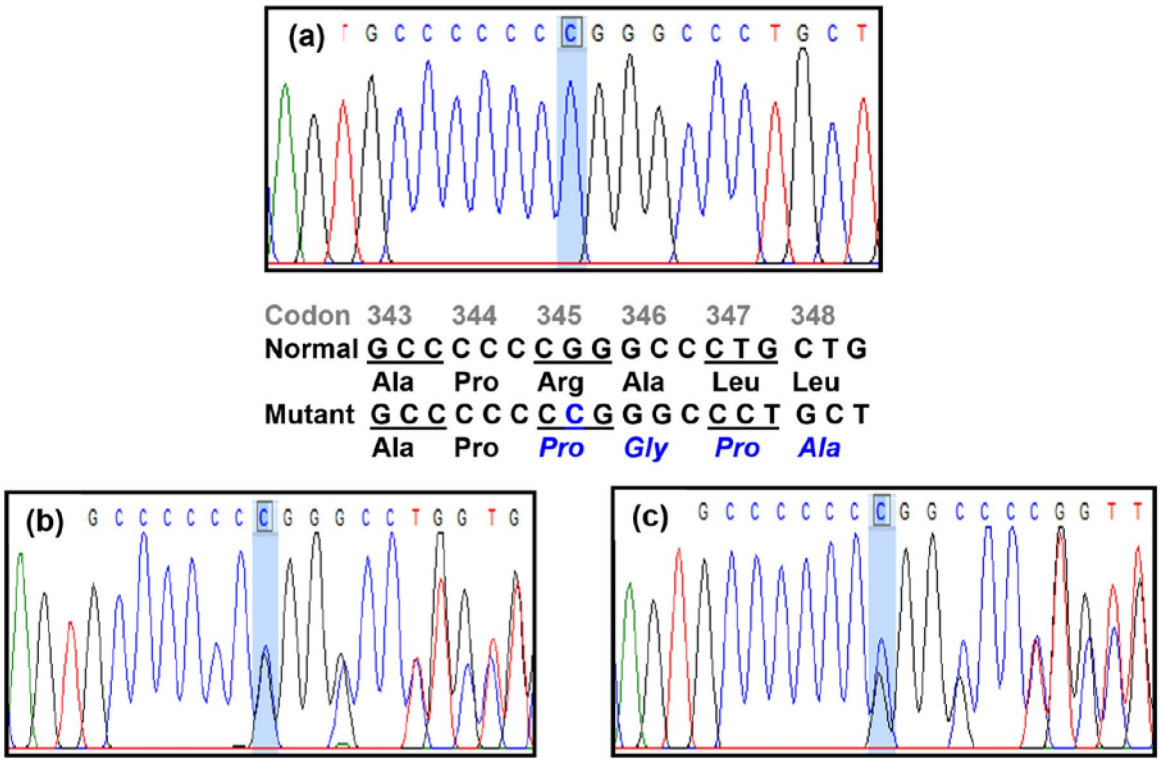

Figure 3. Electropherogram of the patient and the family. A homozygous frameshift c.1033dup (p.Arg345ProfsTer10) mutation in exon 1 of the *CHST14* gene was identified in the patient's DNA **(a)**, which was inherited from both heterozygous parents **(b)** and **(c)**.

Discussion

Our patient presented multiple congenital malformations, including congenital multiple contractures, mainly talipes equinovarus, craniofacial dysmorphism evident in early childhood, and skin hyperextensibility. These phenotypes are major clinical features important for diagnosing mcEDS.[1,5,8,10] In addition, other mcEDS-related manifestations, including motor developmental delay, joint hypermobility, recurrent joint dislocations, progressive kyphoscoliosis with restrictive lung disease, ocular abnormalities, hearing impairment, and cardiac valvular diseases, were also detected in our patients. Although the diagnosis of mcEDS mainly relied on clinical features, molecular analysis is very important for diagnostic confirmation because of the overlap of clinical phenotypes between different EDS subtypes, including kyphoscoliosis, spondylocheirodysplastic, FKBP14-related, and tenascin-X-deficient EDS.

Whole exome sequencing is the method of choice for our patient. A novel pathogenic homozygous frameshift c.1033dup (p.Arg 345ProfsTer10) mutation in exon 1 of the *CHST14* gene was identified, which can cause mcEDS. This mutation was classified as "Pathogenic" according to ACMG classification for several reasons. First, a homozygous duplication of C at position 1033 of the *CHST14* gene results in the substitution of arginine at position 345 by proline. It is predicted to create a premature termination codon at position 355. This mutation results in premature termination of a normal 376-amino acids D4ST1 protein, leading to the loss of the sulfotransferase domain and its role in dermatan sulfate biosynthesis. To the best of our knowledge, at least 27 mutations of the *CHST14* gene have been reported, with the most recent case documented in June 2024.[11] However, no apparent genotype-phenotype correlation has been demonstrated[5]. Furthermore, the rare c.1033dup variant has been exclusively reported in the Global populations with an estimated allelic frequency of 0.000066 (1/120358) in the Exome Aggregation Consortium (ExAC)

database and was identified only in the heterozygous state (PM2). Homozygosity of this rare variant in our patient was confirmed to be inherited from both carrier parents by Sanger sequencing. Thus, when two heterozygous variants are identified in a gene associated with a recessive disorder, and one variant is known to be pathogenic, determining that the other variant in trans can be considered moderate evidence for the latter variant's pathogenicity (PM3). This evidence confirms the pathogenicity of this rare homozygous frameshift variant identified in our patient. Previous reported pathogenic variants comprise 13 missense mutations, seven frameshift mutations, five nonsense mutations, and one in-frame deletion. Most missense variants occurred within the middle of the sulfotransferase two domain, while truncating variants were usually located outside this domain or at both ends of the gene[5]. Interestingly, our patient's frameshift mutation is located at the most telomeric end of the *CHST14* gene compared to the reported truncating variants.

DS proteoglycans are ubiquitously expressed in human tissues, including blood vessels, skin, tendons, sclerae, cartilage, and undifferentiated mesenchymal tissues.[12] The tissue fragility in mcEDS is attributed to the loss of DS and/or replacement of DS by chondroitin sulfate (CS) in the glycosaminoglycan chains of decorin, resulting in abnormal regulation of collagen fibril assembly.[13, 14] Concerning progressive fragility-related multisystem manifestations in mcEDS, systemic surveillance is essential to detect multiple anomalies and complications associated with mcEDS, and regular evaluations of the musculoskeletal, cardiovascular, respiratory, ophthalmologic, otologic, and dermatologic systems are recommended from childhood to adulthood.[5] Currently, treatment for mcEDS primarily focuses on symptomatic management, including management of acute severe complications and surgical interventions for comorbidities.

Conclusion

We present a 15-year-old girl who had characteristic clinical features of mcEDS. Molecular analysis by WES and Sanger sequencing identified a novel pathogenic homozygous frameshift: c.1033dup (p.Arg345ProfsTer10), a mutation in the *CHST14* gene inherited from second-degree consanguineous parents. Although the diagnosis of mcEDS relies on characteristic clinical manifestations, a definite diagnosis depends on genetic testing. Our study demonstrated the efficiency of WES in diagnosing this rare genetically heterogeneous disease.

Data availability

Data is available on request from the authors.

Conflict of interest

All authors report no conflict of interest for this article.

Acknowledgments

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Ethics Approval and Consent to Participate

This case report was approved by the Medical Department Ethics Review Committee for Research in Human Subjects, Institutional Review Board, Royal Thai Army (RTA) (Approval no. S035h/66_Exp), following international guidelines such as the Declaration of Helsinki, the Belmont Report, CIOMS Guidelines, and the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use - Good Clinical Practice. Informed consent documentation was obtained from the subject with permission from the Institutional Review Board, RTA Medical Department.

Author contributions

SL, PR, ST, GS, and BB contributed to drafting and writing the case report manuscript. PR and BB were involved in the patient's clinical care. SL, PR, and BB performed DNA extraction and were engaged in genetic studies. All authors approved the final version of the manuscript.

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