Original Research

A novel variant in the *DSE* gene leads to Ehlers-Danlos musculocontractural type 2 (mcEDS-*DSE*) in a Pakistani family

Running title: A novel variant in DSE gene

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Abstract

The Ehlers-Danlos syndrome (EDS) is a group of heritable connective tissue disorders. Common features of EDS include skin hyperextensibility, articular hypermobility, and tissue fragility. It is classified into thirteen subtypes, caused by variations of more than 19 different genes. Among these two subtypes termed as EDS musculocontractural type 1 (EDSMC1/mcEDS-*CHST14*; MIM# 601776) is caused by biallelic mutations in the *CHST14* gene (MIM# 608429) on chromosome 15q14 and EDS musculocontractural type 2 (EDSMC2/mcEDS-*DSE*;MIM#615539) is caused by a mutation in *DSE*(MIM# 605942)on chromosome 6q22. In this study, clinical and molecular diagnosis has been performed for a consanguineous Pakistani (Pakhtun) family with five affected siblings, presenting mcEDS-*DSE* phenotype. Whole exome sequencing (WES) analysis identified a novel homozygous *DSE* variant (NM_001080976.1;

c.2813T>A, p.Val938Asp) in the proband. Sanger sequencing in all available affected members and their obligate carriers confirmed autosomal recessive segregation of the diseased allele. To the best of our knowledge this variant identified is novel and expands the *DSE* pathogenicity leading to Ehlers-Danlos syndrome, musculocontractural type 2. The result obtained has the potential to help in early diagnosis, genetic counseling and possible therapeutic inventions.

Keywords
EDSMC2; *DSE* gene; Homozygous variant; Whole exome sequencing (WES); Pakhtun family.

1. Introduction

Ehlers-Danlos syndrome (EDS) is a group of hereditary connective tissue disorders classified in thirteen subtypes based on their clinical manifestation[1], characterized by distinct craniofacial features, multisystem congenital malformations and progressive fragility of connective tissues.[2,3]EDS caused by variations of more than 19 kinds of genes, involved in collagen synthesis and extracellular matrix synthesis (ECM) and maintenance has been recognized to contribute to different forms of EDS.[1,4]

The most common symptoms of mcEDS-*DSE* are hyperextensible skin, fragile skin, long and slender finger, reduce muscle tone and strength, joint and muscle pain, dental crowding, prominent ears, adducted thumb, congenital bilateral, talipesequinovarus (club foot).Delayed development and weak muscle tone (hypotonia) may be evident in infancy. Cognitive development is normal and the expected total prevalence of EDS is 1 in 5000.[5,6] The hypermobile and classical forms are most common and their frequencies are 1 out of 5,000 to 20,000 and 1 in of 20,000 - 40,000 respectively. Other forms of Ehlers-Danlos syndrome are very rare (https://medlineplus.gov).

Musculocontractural EDS(mcEDS) can be caused by genetic alterations in*CHST14*(MIM# 608429) and *DSE* (MIM# 605942), which are involved in the synthesis of dermatan sulfate (DS) by changing of glucuronic acid to iduronic acid (IdoA).[7,8]Patients with this type of disease experience joint hypermobility, joint pain and clicking joints, loose and unstable joints, hyper extensible skin, delayed wound healing, distinct facial features, multiple congenital contractures, and congenital defects in cardiovascular, gastrointestinal, renal, ocular and central nervous

systems.[2,9,7]No treatment for mcEDS is currently available but through molecular diagnostics one can find causative variants and can adopt management strategies.

Here, we present detailed clinical and molecular results in five more patients with mcEDS-*DSE* originating from the North-Western area of Pakistan.

2. Material and methods

2.1 Ethical approval & Patients

Written consent forms were signed from the patients and their legal guardians. Ethical approval has been provided by the Institutional Bioethics Committee (IBC), Islamia College University Peshawar (Ref.No. 602/ORIC/ICP) for sample collection, clinical information, genetic analysis and patient's data publication. Blood samples were collected in EDTA tubes and stored in a freezer till further processing.

2.2 Whole Exome sequencing (WES)

Genomic DNA was extracted from the peripheral blood and was processed for making libraries which were sequenced on HiSeq2500 (Illumina, San Diego, CA, USA). All the sequence reads were then assessed for quality check using FastQ to get improved reads. The obtained reads were then aligned to the reference human genome (GRCH37/hg19) using the Burrows Wheeler Aligner (BWA) tool, while duplication was removed by Picard. All kind of variants were called by using GATK. Functional annotation of all the passed non-synonymous variants were performed using ANNOVAR(www.annovar.openbioinformatics.org) and were screened against databases like SIFT (www.sift.bii.a-star.edu.sg), Polyphen-2 (www.genetics.bwh.harvard.edu/pph2), MutationTaster (www.mutationtaster.org) , dbSNP (www.ncbi.nlm.nih.gov/snp), 1000 Genomes (https://www.internationalgenome.org/category/phase-3) and gnomAD (https://gnomad.broadinstitute.org/) databases. Sanger sequencing of *DSE* in all available family members was performed using standard methods for the autosomal recessive segregation of the affected allele in the family.

2.3Sanger sequencing (SS)

The pathogenic mutation was confirmed by standard Sanger sequencing for which primers were designed using Primer 3 plus(https://bioinfo.ut.ee/primer3) (Forward primer: 5′-GTTTGGACAGGCACGGATG-3′; Reverse primer: 5′-TCTCCACGTTCCTGCTTTCT-3′). Sequencing was done using ABI-3730 DNA analyzer and the data obtained was visualized with Chromas 2.6.6.

2.4 Protein modeling and Visualization

Amino acid sequence was uploaded to I-TASSER (https://zhanglab.dcmb.med.umich.edu/I-TASSER)server for predicting protein model using fold recognition method. Wild and mutated structures were visualized by Discovery Studio Visualizer.

3. Results

3.1 Clinical report

We investigated a Pakhtun family residing in the north-western areas (Swat valley) of Pakistan. There are five affected siblings from two consanguineous marriages in one large family (Figure 1). Siblings of IV-1 and IV-2 had two males (V-1 and V-3) and a female (V-2), and siblings of IV-3 and IV-4 had two females (V-4 and V-5) with affected phenotypes (Figure 2). An unaffected father IV-2 was not available for investigation as he was out of the country. The five available affected individuals (V-1, V-2, V-3, V-4 and V-5) were of the ages 17, 11, 7, 4 and 1respectively, at the time of this study. All were born with the same affected phenotype. They had club foot at the time of birth and later on surgery was performed to correct club foot and align the foot in normal position. They had features like hypertelorism, small mouth, thin upper lip, broad tall nasal bridge, long slender fingers, mild hypertropia, feeling extreme fatigue was observed in (V-1, V-2 and V-3),overly flexible elbow and knee joints. Patient (V-1) and (V-2) also had myopia. The clinical findings are showed in Figure 2 and summarized in Table 1.

3.2 Molecular investigation

WES analysis of two affected (V-1 and V-2) and unaffected (IV-1) individual was performed to investigate the causative gene mutation responsible for phenotype in the family. In total 123,953 variants in V-1 and 105,432 variants in V-2 were identified. Variants with depth quality higher than 20 were 56,194 in V-1 and 49,521 in V-2the rest were filtered out. Applying filter of

synonymous and deep intronic regions, 11,429 variants left in V-1 and 10,239 in V-2inwhich 6,803 were heterozygous in V-1 and 6,201 in V-2 and 4,626 were homozygous in V-1 and 4,030 in V-2 respectively. Similarly removing variants with MAF higher than 0.001 in 1000 genomes (https://www.internationalgenome.org/) and ExAc65000 (http://exac.broadinstitute.org/) databases only 184 variants left in V-1 and 144 in V-2. At the end shortlisted variants were filter out using multiple prediction tools, which pick out variant (NM_001080976.1, c.2813T>A, p.Val938Asp) in *DSE* in both patients. Pathogenic scores of the variant was analyzed by screening against SIFT (Damaging: 0.001), FATHMM-MKL (Damaging: 0.9608) MutationTaster (Disease Causing), Polyphen 2_HumVar (Possibly Damaging: 0.533), PhD-SNP (Deleterious) and PROVEAN (Neutral: -0.663)pinpointed, that the variant (NM_001080976.1, c.2813T>A, p.Val938Asp) in *DSE* is the most probable cause of the phenotype in this family and according to ACMG classification[11] the variant is uncertain significance because its homozygous allele is absent in public databases (PM2; Moderate) and gnomAD missense Z-score is 2.03 as compared to synonymous Z-score 1.08 (PP2; Supporting).The MAF of variant is 1 in 251208 (0.00000398) in heterozygous form and not reported in homozygous form according to (https://gnomad.broadinstitute.org) and (https://varsome.com).

We performed Sanger sequencing of eight family members including three phenotypically normal parents (IV-1, IV-3, IV-4) are heterozygous and affected proband (V-1) including his affected siblings (V-2,V-3,V-4 and V-5) are homozygous. The Sanger sequencing chromatographs of eight family members are shown in Figure3. On the basis of the above findings, it is concluded that the variant c.2813T>A, p.Val938Asp) in *DSE* segregated in the family and is likely to be pathogenic. The mutated and wild type mutations have been visualized in the protein model (Figure 4).

4. Discussion

In the current study, WES analysis was performed to investigate a consanguineous Pakistani (Pakhtun) family from the district of Swat in the North-West area of Pakistan. There were five affected individuals with a novel homozygous missense *DSE* variant (NM_001080976.1; c.2813T>A; p.Val938Asp) causing mcEDS-*DSE*.

Previously, two Spanish siblings have been reported with a musculocontractural type of Ehlers-Danlos syndrome, identified with a homozygous missense variant (c.799A>; p.Arg267Gly) in *DSE*, that segregated with disease in the family.[7]Another homozygous missense variant (c.803C>T; p.Ser268Leu) in *DSE* was found in a two year old Indian boy with the mcEDS. While their parents and one sibling were heterozygous for the disease.[9] Similarly, a homozygous pathogenic variant in *DSE* was found (c.960T>A; p.Tyr320*) in a Turkish patient and a frameshift variant (c.996dupT; p.Val333Cysfs*4)in Indian patient, while (c.1763A>G; p.His588Arg) in Pakistani family and (c.1150_1157delp.Pro384Trpfs*9) in Portuguese.[2,8]Apatient has been reported to have delayed wound healing and atrophic scarring of the skin after clubfoot surgery.[7,8,9,10]

Five new patients with a novel pathogenic variant in *DSE* associated with mcEDS-*DSE* is reported here which will add detailed clinical and molecular descriptions to the existing literature. The results obtained in this study will help us in better understanding this disease.

Authors' contribution

MI, HH and HA designed the experiments and supervised the research. IU, MA, AA and ND did the experimental work. FA and HH did the clinical study. MA, IU, WU and MA wrote the paper. MI, MJ, MI, RM, SE and HH critically reviewed and improved the manuscript. All authors have read and approved the manuscript.

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Conflict of interest:

The authors declare that they have no conflict of interest

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Table 1: Clinical features in all patients with *DSE* associated mcEDS.

FIGURE LEGENDS

Figure 1: Family pedigree showing autosomal recessive mode of inheritance for the disease phenotype.

Figure 2:(A-E) shows distinctive facial features with prominent forehead, bulbous nose and thick lips; (F-H)fingers were long and slender with joint laxity and a swan-neck deformity; (K-O)shows long toes with mild finger webbing and bilateral pescavus feet.

Figure 3: Chromatograms of Sanger sequencing of the c.2813T>A in Exon 6 of *DSE* gene.

Figure 4: (A) Protein model of the DSE protein predicted by I-TASSER. (B) Visualization of wild amino acid. (C) Visualization of mutated amino acid by Discovery Studio Visualizer.