Supplemental material

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Supplementary Information S1

Clinical and molecular features of 66 patients with musculocontractural Ehlers−Danlos syndrome caused by pathogenic variants in *CHST14* (mcEDS-*CHST14*)

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Molecular investigation was performed according to the protocol described by Koitabashi et al (2018). Genomic DNA was extracted from the patient's peripheral blood lymphocytes, using standard techniques. A next generation sequencing-based custom panel analysis for heritable connective tissue disorders was performed on Ion PGM System (Life Technologies, Carlsbad, CA, USA). The panel was an Ion AmpliSeq Custom Panel designed by Ion AmpliSeq Designer (https://ampliseq.com/browse.action), for most of the coding regions of 52 genes associated with hereditary connective tissue disorders as shown in the table below.

The sequencing data was mapped to human genome hg19 using Torrent Suite software (Thermo Fisher Scientific), and single nucleotide variants and small insertions/deletions were detected from the mapped data using the Torrent Variant Caller plug-in. The variants detected were annotated using wANNOVAR (http://wannovar.wglab.org/), including validation with allele frequencies in public databases such as gnomAD, 1000 Genomes Project, Exome Aggregation Consortium (ExAC), NHLBI-ESP 6500 exomes and Complete Genomics 46 (CG46); dbSNP annotation, ClinVar annotation, and 15 prediction scores for non-synonymous variants such as SIFT, PolyPhen-2, and GERP++. We also referred to Human

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Genetic Variation Database (HGVD) (http://www.hgvd.genome.med.kyoto-u.ac.jp/) to exclude SNPs specific to Japanese. An identified variant was confirmed through Sanger sequencing, performed on an ABI3130xl Genetic Analyzer using a BigDye Direct Cycle Sequencing Kit (Thermo Fisher Scientific).

A gene list in the present custom gene panel

Ehlers-Danlos syndrome: *COL5A1, COL5A2, TNXB, COL3A1, PLOD, COL1A1, COL1A2, ADAMTS2, CHST14, DSE, ZNF469, PRDM5, FKBP14, SLC39A13, B4GALT7, B3GALT6*

Marfan syndrome: *FBN1*

Loeys-Dietz syndrome: *TGFBR1, TGFBR2, SMAD3, TGFB2*

Arterial tortuosity syndrome: *SLC2A10*

Familial thoracic aortic aneurysms and aortic dissections: *MYH11, ACTA2, MYLK*

Beals syndrome: *FBN2*

Occipital horn syndrome: *ATP7A*

Shprintzen-Goldberg syndrome: *SKI*

***FLNA*-related periventricular nodular heterotopia/Otopalatodigital syndrome**: *FLNA*

Camurati-Engelmann disease: *TGFB1*

Osteogenesis imperfecta: *PLS3, CRTAP, LEPRE1, PPIB, BMP1, FKBP10, SERPINH1, SERPINF1, WNT1, SP7, TMEM38B, IFITM5, CREB3L1, PLOD2*

Cutis laxa: *ALDH18A1, FBLN5, EFEMP2, ATP6V0A2, ELN, GORAB, PYCR1, LTBP4, RIN2, SLC2A10, PTDSS1*