OPEN ACCESS

Original research

Genetic complexity of diagnostically unresolved Ehlers-Danlos syndrome

Anthony M Vandersteen,[1,2,3] Ruwan A Weerakkody ,[3,4,5] David A Parry,[3] Christina Kanonidou,[6] Daniel J Toddie-Moore,[3] Jana Vandrovcova,[7] Rebecca Darlay,[8] Javier Santoyo-Lopez ,[9] Alison Meynert,[10] NIHR BioResource,[11] Hanadi Kazkaz,[12] Rodney Grahame,[12] Carole Cummings,[13,14] Marion Bartlett,[13,14] Neeti Ghali ,[13,14] Angela F Brady,[13,14] F Michael Pope,[13,14] Fleur S van Dijk,[13,14] Heather J Cordell,[8] Timothy J Aitman [3]

► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/jmg-2023-109329).

For numbered affiliations see end of article.

Correspondence to
Professor Timothy J Aitman, Centre for Genomic and Experimental Medicine, University of Edinburgh, Edinburgh, UK;
tim.aitman@ed.ac.uk
Dr Anthony M Vandersteen; anthony.vandersteen@dal.ca

Received 10 April 2023
Accepted 18 September 2023

To cite: Vandersteen AM, Weerakkody RA, Parry DA, *et al*. *J Med Genet* Epub ahead of print: [please include Day Month Year]. doi:10.1136/jmg-2023-109329

ABSTRACT

Background The Ehlers-Danlos syndromes (EDS) are heritable disorders of connective tissue (HDCT), reclassified in the 2017 nosology into 13 subtypes. The genetic basis for hypermobile Ehlers-Danlos syndrome (hEDS) remains unknown.

Methods Whole exome sequencing (WES) was undertaken on 174 EDS patients recruited from a national diagnostic service for complex EDS and a specialist clinic for hEDS. Patients had already undergone expert phenotyping, laboratory investigation and gene sequencing, but were without a genetic diagnosis. Filtered WES data were reviewed for genes underlying Mendelian disorders and loci reported in EDS linkage, transcriptome and genome-wide association studies (GWAS). A genetic burden analysis (Minor Allele Frequency (MAF) <0.05) incorporating 248 Avon Longitudinal Study of Parents and Children (ALSPAC) controls sequenced as part of the UK10K study was undertaken using TASER methodology.

Results Heterozygous pathogenic (P) or likely pathogenic (LP) variants were identified in known EDS and Loeys-Dietz (LDS) genes. Multiple variants of uncertain significance where segregation and functional analysis may enable reclassification were found in genes associated with EDS, LDS, heritable thoracic aortic disease (HTAD), Mendelian disorders with EDS symptomatology and syndromes with EDS-like features. Genetic burden analysis revealed a number of novel loci, although none reached the threshold for genome-wide significance. Variants with biological plausibility were found in genes and pathways not currently associated with EDS or HTAD.

Conclusions We demonstrate the clinical utility of large panel-based sequencing and WES for patients with complex EDS in distinguishing rare EDS subtypes, LDS and related syndromes. Although many of the P and LP variants reported in this cohort would be identified with current panel testing, they were not at the time of this study, highlighting the use of extended panels and WES as a clinical tool for complex EDS. Our results are consistent with the complex genetic architecture of EDS and suggest a number of novel hEDS and HTAD candidate genes and pathways.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ The genetic basis for hypermobile Ehlers-Danlos syndrome (EDS) remains unknown.

WHAT THIS STUDY ADDS

⇒ We report the results of whole exome sequencing for 174 patients with complex, genetically undiagnosed EDS.

⇒ Using rare variant and genetic burden analysis, we identified new clinical diagnoses, variants of uncertain significance close to likely pathogenic classification and multiple novel candidate loci.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The study demonstrates the diagnostic utility of whole exome sequencing in diagnostically unresolved, complex EDS and adds to present knowledge of the genetic architecture of the Ehlers-Danlos Syndromes.

INTRODUCTION

The Ehlers-Danlos syndromes (EDS) are heritable disorders of connective tissue (HDCT) that share key clinical features of generalised joint hypermobility (GJH), skin hyperextensibility and tissue fragility. The 2017 EDS nosology classifies 13 subtypes including primary disorders of collagen structure, processing, folding and cross-linking, disorder of the myomatrix, glycosaminoglycan synthesis, complement pathway and other unknown intracellular processes.[1] There are several other syndromes with EDS-like features including Loeys-Dietz syndrome (LDS), Ehlers-Danlos syndrome classic-like-2 (MIM 618000), lysyl hydroxylase 3 deficiency (*PLOD3*, MIM 612394) and inborn errors of metabolism such as homocystinuria. Newly identified genes that are associated with EDS-like syndromes but awaiting confirmation include *ALDH18A1* and *EFEMP1*.[2 3] Diagnostic genetic testing has high clinical utility when a rare EDS type is suspected, differentiating EDS subtypes with varying risks of vascular involvement and inheritance patterns from other EDS-like conditions.

The genetic basis for hypermobile EDS (hEDS) remains unknown, although heterozygous *TNXB* mutations have been reported in association with features of hEDS in female patients.[4] GJH is a common population trait: 5% of 14 year olds had a Beighton score >=6 in the ALSPAC cohort.[5] A genome-wide association study (GWAS) using self-reported Beighton scores >5 identified 18 loci with p values between 8.7×10^{-7} and 1.1×10^{-12}.[6] Here, we have used WES and genetic burden analysis to investigate the genetic basis of EDS in patients with diagnostically unresolved, complex EDS.

MATERIALS AND METHODS

Patient recruitment and ethics approval

One hundred seventy-four patients from the national EDS diagnostic service (LNWUH) and specialist EDS rheumatology clinic (UCLH) were recruited. Patients had complex or suspected monogenic EDS, with arterial aneurysm(s) in proband and/or family member(s) and complex and/or severe symptoms. Patients consented to exome sequencing under approved protocols for Mendelian Disease research (Ethics Protocol Reference 11/LO/0883 (West London Research Ethics Committee)) and the NIHR BioResource project (Cambridgeshire 2 Research Ethics Committee Reference 04/Q0108/44). Patients were clinically categorised using the Villefranche criteria prior to skin biopsy and/or molecular testing.[7] The cohort comprised classical EDS (cEDS) (4 male/16 female), vascular EDS (vEDS) (5 female), hEDS (22 male/ 87 female), kyphoscoliotic EDS (kEDS) (2 male), (online supplemental tables 1-4). Patients not fulfilling the Villefranche criteria for a specific EDS subtype were categorised as HDCT (16 male/22 female; online supplemental table 5). At the time of recruitment, diagnostic gene sequencing for EDS-associated genes was available (LNWUH clinic); however, *B3GALT6, B4GALT7, C1R, C1S, COL6A1, COL6A2, COL6A3, COL12A1, DSE, PRDM5, ZNF469* and *LDS* genes were not offered. Patients with confirmed molecular diagnoses of monogenic HTAD or EDS were excluded. Patients reported previously by our research group, who had undergone non-diagnostic panel gene sequencing for EDS and HTAD, were enrolled.[8]

DNA sequencing

DNA extraction was carried out as reported previously.[8] WES was performed in the Edinburgh Genomics and Cambridge sequencing laboratories.

Variant analysis

WES data were filtered for variants with population frequency <0.1% (rare variants) and Combined Annotation Dependent Depletion (CADD) score >15 for further analysis using Varsome and Franklin, and were classified using the ACMG criteria and the Association for Clinical Genomic Science (ACGS) Best Practice Guidelines.[9 10] WES data were also analysed with the exomiser tool using HPO terms in the 2017 EDS nosology.[1]

Genetic burden analysis

WES data (~100-fold coverage) were analysed from 128 unrelated EDS cases of Caucasian ancestry together with whole-genome sequence data (2-fold to 20-fold coverage) from 248 ALSPAC controls[11] sequenced as part of the UK10K study.[12] The software package TASER[13] was used for burden analysis. This recalls variants in both cases and controls and constructs a test statistic while allowing for systematic differences in read depth (online supplemental method). WES data from 46 individuals of non-Caucasian or unknown ethnicity were excluded from this analysis.

RESULTS

Variants in known EDS, HTAD, GJH associated syndromes and known Mendelian entities with EDS symptomatology were correlated with phenotypic data for each patient. We identified a small number of clearly pathogenic (P) and likely pathogenic (LP) variants.

New diagnoses of EDS and HTAD

We identified 10 diagnostic P or LP variants in genes that are known causes of EDS and HTAD (table 1, online supplemental table 6). Two novel heterozygous pathogenic *COL12A1* variants

Table 1 Diagnostic variants meeting the American College of Medical Genetics (ACMG) criteria for pathogenic and likely pathogenic classification

Patient ID	Variant ID	Age (years)	Gender	Clinical diagnosis	Gene/NM	Protein	ACMG classification
33	1	40–49	F	HDCT	*TGFB3* NM_003239.4 c.463C>T	p.Arg155Trp	LP
34	2	30–39	F	HDCT	*COL5A1* NM_000093.4 c.4068G>A	Splice	LP
402	4	30–39	M	hEDS	*COL12A1* NM_004370.6 c.5097+1G>A	Splice	LP
479	8	20–29	F	HDCT	*SMAD2* NM_001003652.3 c.842A>T	p.Glu281Val	LP
564	9	20–29	M	HDCT	*TGFB2* NM_001135599.3 c.989G>A	p.Arg330His	P
755	10	40–49	F	hEDS	*COL12A1* NM_004370.6 c.8321G>A	p.Gly2774Glu	P
814	14	30–39	F	HDCT	*TGFBR2* NM_001024847.2 c.1613T>C	p.Val538Ala	LP
1420	17	0–9	M	HDCT	*ALPL* NM_000478.6 c.394G>A	p.Ala132Thr	P
1484	18	50–59	F	hEDS	*COMP* NM_000095.3 c.2048G>T	p.Arg683Leu	LP
1528	19	30–39	M	cEDS	*COL5A1* NM_001278074.1 c.3397C>T	p.Arg1133Ter	P

Additional variant annotation is given in online supplemental table 6.
cEDS, classical Ehlers-Danlos syndrome; HDCT, heritable disorders of connective tissue; hEDS, hypermobile Ehlers-Danlos syndrome; LP, likely pathogenic; P, pathogenic.

were considered diagnostic. Splice site variant 4 was identified in patient 402 (bilateral congenital hip dislocation): the variant was found in one other individual in gnomAD and had high in silico prediction of pathogenicity (ADA score 0.999). *COL12A1* variant 10 resulted in a helical glycine substitution in patient 755 with multiple features suggestive for myopathic EDS (mEDS), including neonatal hypotonia and kyphoscoliosis.

Variant 19 resulted in loss of function in *COL5A1* in patient 1528, who had previously declined clinical diagnostic testing (ClinVar ID 280931). Patient 34 with hyperextensible skin, distal joint hypermobility and a carotid artery dissection had an overlapping HDCT/cEDS phenotype and carried the synonymous variant 2 in *COL5A1*. We had previously classified this as a variant of uncertain significance (VUS).[8 14] The variant impacts the last nucleotide of exon 51, with high in silico pathogenicity, and we now consider this likely pathogenic (ClinVar ID 212971). This patient also carried a pathogenic variant in *ITGB3* (variant 3) (autosomal recessive Lanzmann thrombasthenia MIM 173470), a gene that has been found to be abnormally expressed in skin fibroblasts from patients with hEDS,[15] and a novel variant in candidate gene *PGTER4* (see below).

HDCT patient 814 carried novel LP *TGFBR2* variant 14 in the Ser/Thr kinase domain, without known vascular involvement. A recent report of this variant and accompanying functional data support LP classification.[16] HDCT patient 564, with pectus carinatum and aortic root dilatation, carried a *TGFB2* pathogenic variant 9 (CADD=34). A different variant at the same nucleotide was reported as LP in association with syndromic aortic aneurysm (ClinVar ID 440982). Two patients (patient 33 and patient 479) had complex HDCT phenotypes and LP variants in *TGFB3* (variant 1) and *SMAD2* (variant 8). hEDS patient 1484 had LP variant 18 in *COMP* (multiple epiphysial dysplasia type 1, MIM 600310). HDCT patient 1420 had LP variant 17 in *ALPL* causative for hypophosphatasia (MIM 171760).

VUS in EDS, LDS, HTAD and other syndromic genes with potential for pathogenicity reclassification

Thirty variants met the ACGS criteria where further segregation/functional work may enable reclassification as pathogenic or LP (online supplemental table 7).[17] Two patients with a clear cEDS phenotype harboured variants in *COL5A1* exon/intron 64, which encodes two transcripts in the C-propeptide domain, with alternate splicing in different tissue.[18] Patient 583 with *COL5A1* LoF variant 29 had cEDS major features: skin hyperextensibility, widened atrophic scars, generalised and small joint hypermobility with additional features of hEDS. cEDS patient 806 has a novel variant 35 at position +6 of intron 64. While a single multi-exon deletion including exon 64 (exons 63i-65i) has been reported as pathogenic, other exon 64 variants remain VUS (https://databases.lovd.nl/shared/genes/COL5A1).

cEDS patient 595 with missense *TGFB3* variant 31 (CADD=25) had Mitral Valve Prolapse (MVP) and a high arched palate. hEDS patient 107, with a second-degree relative with an aneurysm, carried an *ULK4* splice variant 23. Loss of Function (LoF) variants in *ULK4* have been reported to increase the risk of aortic thoracic dissection in a single small study.[19] In syndromes with EDS-like features, patient 1530 (female) had splice variant 45, a VUS* in the *UPF3B* gene, Lujan syndrome (MIM 309520, intellectual development disorder X linked, associated with Marfanoid habitus).[20] hEDS patient 107 carried variant 22, a VUS* in *KCNH1* (MIM 135500, Zimmerman-Laband syndrome), which may have cartilage abnormalities and gingival hyperplasia as associated features. hEDS patient 967 carried variant 36, a VUS* in *FLCN1* (MIM 607273, Birt-Hogg-Dube syndrome), associated with recurrent pneumothoraces and an increased risk of renal carcinoma.

We identified variants in genes associated with a skeletal dysplasia phenotype. cEDS patient 1451 had *COL9A3* variant 40, a glycine substitution in the triple helical domain (MIM 120270, AD multiple epiphysial dysplasia type 3 with and without proximal myopathy) and also carried two VUS in COL5A1 (online supplemental table 9). cEDS patient 1002 carried a novel cysteine substitution (variant 37) in *MAP3K7* (cardiospondylocarpofacial syndrome, MIM 157800) within the protein kinase domain.

We interrogated our data for Mendelian causes of symptomatology associated with EDS. Erythromelalgia is a *SCN9A* channelopathy associated with abnormal pain sensation and small fibre neuropathy (MIM 133020). We identified a novel *SCN9A* variant 27, at a transmembrane domain mutation hotspot, in patient 482 with a vEDS-like phenotype with thin skin and tissue fragility.

We identified patients with two or more rare/novel variants, for example, HDCT patient 72, with terminal digital and nail anomalies and a family history of HTAD had missense variant in *WNT10A* (variant 21, CADD=30, odontoonychodermal dysplasia/tooth agenesis MIM 606268)) and a VUS in *ROBO4* (aortic valve disease 3 MIM 618496) (online supplemental table 10). Multiple patients in the cohort had complex symptoms, signs and/or family histories, suggesting possible enrichment for patients with more than one rare Mendelian disorder.

Variants of uncertain significance in genes associated with risk of ICA

We identified multiple variants in genes previously reported as associated with risk of intracranial aneurysm (ICA) (online supplemental tables 7; 8). hEDS patient 65 with a femoral artery aneurysm and family history of ICA carried *ROBO4* VUS and a second VUS in the fibrinogen-like domain of *ANGPTL6*. Rare variants in this domain have been reported as associated with familial ICA risk.[21] Variant 42 (VUS*) in *PCNT* was found in hEDS patient 1495 who was not known to have a personal or family history of ICA; this variant has been previously reported in familial ICA.[22]

Autosomal recessive disorders

A further eight heterozygous LP/P variants were identified in autosomal recessive EDS genes and other autosomal recessive genes overlapping with EDS symptomatology, *ZNF469*, *LAMA2*, *ITGB3*, *ELP1*, *ADAM22*, *C1QC* and *PRSS56* (table 1, online supplemental tables 6; 7; 9–11). Seven heterozygous VUS* were identified in LAMA2, TNFSF11, TONSL, RYR3, SLC2A10 and CANT1. Multiple VUS in ZNF469, PRDM5, DSE, CHST14, ELP1, AEBP1, CCN6, RYR3, DYSF and LAMA2 (data not shown). HDCT patient 620 with an occipital horn syndrome phenotype, and consanguineous parents, was homozygous for a VUS in *SDSL* (NM_138432.3 c.626C>T, p.Ala209Val) (MIM 618752, severe congenital neutropenia type 8). Phenotypic review did not show haematological abnormalities: these variants were therefore considered unlikely to be causative.

VUS in EDS, HTAD, myopathy and inborn errors of metabolism genes

Additonal VUS were identified in genes associated with EDS, HTAD, myopathy and inborn errors of metabolism (online supplemental tables 7; 9–11). A VUS in *BGN* was identified in

hEDS patient 1393 (female) with increased arm span to height ratio and talipes, and aortic root dilatation; loss of function mutations in this gene have been reported to result in Meester-Loeys.[23] A number of patients carried ultrarare variants in genes associated with non-syndromic HTAD (*ROBO4, PRKG1, SMAD6, ULK4, MAT2A, SMAD2, MFAP5*). HDCT patient 453 with carotid dissection had a 64 bp insertion predicted to result in out of frame/loss of function transcript in *PRKG1* (pLi=1). hEDS patient 1629 without known cardiovascular involvement had a novel *SMAD6* VUS in the MH1 domain. hEDS patient 1443 had a family history of abdominal aortic aneurysm in maternal relatives and ICA in a paternal relative carried novel VUS in *SMAD6*. Patient 526 had MVP and a family history of multiple individuals with cardiac valvular disease, with novel VUS in *IFIH* (CADD=31), in the helicase domain (MIM 606951, Singleton-Merten syndrome, acroosteolysis and aortic valve calcification).[24] HDCT patient 79 carried *EMILIN1* VUS at amino acid residue 28, close to residue 22, thought to affect N terminal signal peptide cleavage.[25] HDCT patient 422, with camptodactyly and Asperger's syndrome, carried a novel VUS, resulting in an in-frame deletion mutation in *MED12*.

We found a single VUS* variant 43, and multiple VUSs in EDS and Bethlem myopathy genes (online supplemental table 9), HTAD (online supplemental table 10), myopathy, inborn errors of metabolism and dysautonomia genes (online supplemental table 11), many of which are similarly classified in ClinVar. These patients did not have specific clinical features (eg, contractures for Bethlem myopathy, cauliflower ears for Beals syndrome or aggressive periodontal disease for pEDS) which might contribute to ACMG criteria PP4.

EDS gene candidates based on linkage and skin fibroblast gene expression studies

We reviewed our data for germline variants in loci previously reported in a linkage study of a large family with hEDS, which identified *LZTS1* as a candidate gene (online supplemental tables 12–16).[26] A single patient with hEDS in our cohort (patient 703) had a *LZST1* missense variant, with limited in silico evidence of pathogenicity (CADD=23). We also identified multiple rare variants (CADD >15) in genes within the reported region of linkage (online supplemental table 12). These included *SORBS3* (vinculin binding domain) reported to regulate extracellular matrix (ECM) stiffness in vitro,[27] *ADAM7, ADAM27* (variants in protease domains), multiple variants in the *CCAR1* gene (a regulator of cell division) and *DOCK5* (mouse model has reduced skeletal muscle, zebrafish has abnormal fast muscle.[28] In addition, we identified multiple rare variants in genes previously reported in a linkage study of Pelvic Organ Prolapse,[29] for example, *LAMC1, ROBO2* (online supplemental table 13, online supplemental methods).

Gene expression data from skin fibroblasts for patients with hEDS, cEDS and vEDS have been published, suggesting candidacy for several dysregulated genes.[15 30 31] We identified multiple rare germline variants with CADD >15, in several of these genes (online supplemental methods and online supplemental tables 14-16). These included integrin signalling, innate immune system function, TRAIL and TRAIL receptor genes, reported to affect integrin signalling in the ECM, controlling vascular remodelling.[32] We identified multiple rare heterozygous variants in *HSPG2* (Perlecan) (online supplemental table 15). Homozygous variants in *HSPG2* cause AR Schwartz-Jampel syndrome (MIM 142461) via disordered cartilage maintenance, osteonecrosis and endomysial dysfunction via a channelopathy mechanism. A knock-in *HSPG2* mouse model demonstrated disordered acetylcholinesterase endplate morphology with abnormal patch clamp and a fatigability phenotype.[33] Two *POSTN* variants were found in FAS1 domains (online supplemental table 16): periostin is reported as contributing to tissue repair after injury via upregulating collagen (I) and multiple other ECM component proteins.[34]

Rare variants in loci associated with GJH/self-reported Beighton score, rotator cuff injury and knee pain GWASs

We identified multiple rare variants with CADD >15 in genes associated ($p<5\times10^{-8}$) with self-measured Beighton score >5 in a published GWAS[6]: These included the PIEZO Type Mechanosensitive Ion Channel Component 1 (*PIEZO1*) and NEDD4 E3 ubiquitin protein ligase (*NEDD4*) (online supplemental table 17). PIEZO1 is a mechanotranducer protein, important in the cellular responses to shear stress, maintenance of the vascular endothelium and mechanosensation in chondrocytes and epithelium .[35] NEDD4 is a mediator of abnormal fibroblast proliferation in keloid scarring.[36]

HTAD candidate genes

Multiple patients in this cohort had a personal or family history of HTAD, carotid, intracranial and other aneurysmal disease . Careful review of all novel variants with CADD >15 in non-annotated genes revealed a small number of variants with high CADD scores (>20) in candidate genes with published data supporting a role in vascular disease and remodelling (online supplemental table 18). HDCT patient 1625 with a dilated aortic root and megacolon had a novel missense variant 63, in transforming growth factor beta 1–induced transcript 1 gene (*TGFB1/1*). This gene is regulated by TGF beta signalling; mice lacking its homologue, hic5, show deficient smooth muscle cell response to vascular injury (MIM 602353).[37] This variant at TGFB1/1 Arg 67, neighbours phosphoserine 68, hence may disturb signal transduction. kEDS patient 1396 carried variant 59, a nonsense mutation *INO80D* (MIM 610169). Homozygous missense variants in *INO80D* were reported in a single family with aortic hypoplasia, aggressive atherosclerotic disease and periodontal disease,[38] pLi=1. Patient 34, with HDCT and carotid artery dissection, harboured variant 50 in prostaglandin E receptor 4 (*PTGER4) (MIM 601586*). Dysregulated expression of *PTGER4* has been reported in abnormal wound healing, regulation of vascular tone and blood pressure, in abdominal and thoracic aortic aneurysm and the regulation of cerebral blood flow.[39]

Reviewing murine and functional studies reported for Marfan syndrome, we identified germline variants in *TMBIM1* (MIM 610364), *SCUBE3, IRF7, IGFBP2* and *TMEM176B and MMP2*.[40] hEDS patient 1491 with kyphosis and a high arched palate carried *FBN3* variant 61 in the TGFbeta binding domain, disruption of the equivalent domain in *FBN1* cause Marfan syndrome. hEDS patient 1695 had a loss of function variant 64 in *NOTCH4*, (LOEUF=0.32), with livedo reticularis and a maternal aunt with pulmonary artery atresia. This gene is known to affect vascular morphogenesis in mice, but has not been associated with disease in humans.[41] HDCT patient 446 with carotid dissection carried four variants, including novel variant 54 in *NFAT5* (MIM 604708). Osmoregulatory stimulus has previously been found to upregulate *NFAT5* expression, resulting in abdominal aortic aneurysm and dysregulated immune function.[42] Two other *NFAT5* variants were also identified, in hEDS patients 1595 and 922 without aneurysms (online supplemental table

19). We identified an hEDS patient 566 with Marfanoid habitus, arterial rupture and collagen fibril irregularity, who carried a novel loss of function variant in the *SYAP1* gene (variant 56); a knockout mouse model for this gene has a highly distinctive motor deficit phenotype[43] (the pLi score is 0.94).

Matrisome genes

We searched for rare variants with CADD >15 in genes known to interact with fibrillar collagen biosynthesis and signalling, chondroitin synthesis and modification (https://reactome.org/PathwayBrowser) (online supplemental table 19). Collagenases I/II/III (MMP1, 8, 13 and 4) are known regulators of the fibrillar collagens in the ECM. Variant 60 substituted a histidine residue of Zinc binding site in MMP8, which was previously reported in GWAS as associated with premature rupture of the membranes (MIM 120355). The patient had hEDS with a family history of recurrent miscarriage. Heterozygous missense variant 51 in MMP25 (608482) (online supplemental table 18) was identified in a patient with hEDS: this gene is functional in the innate immune system and abnormal expression has been associated with tendinopathy in a mouse model.[44 45] We also noted multiple heterozygous VUS in autosomal recessive skeletal dysplasia genes, *CANT1*, *TONSL*, *OSTM1* (data not shown).

Biallelic pathogenic variants in *ADAMTS2* cause dermatosparaxis type EDS. We identified a patient with HDCT (patient 446) with heterozygous Variant 52 in *ADAMTS5* and variant 53 in *ADAMTS16*. Both variants were in the spacer domains, known to regulate aggrecanase activity. Heterozygous missense variants were also identified in *ADAMTS20, ADAMTS22, ADAMTS23, ADAMTS28*. Pathogenic variants in C1R/C1S cause pEDS, by gain of function on as-yet unidentified targets,[46] we found multiple rare variants in other (non-annotated) serine proteases (online supplemental table 19).

Integrins, ephrin, ciliopathy, *TSPAN*s, *DOCK*, circadian rhythm pathways

Within the entire cohort, we noted clusters of variants in genes not currently associated with EDS and in novel genes and pathways with biologically plausible links to EDS, including integrins (*ITGA3, ITGB4, ITGA8, ITGAV* and *ITGB1BP1*) (online supplemental table 19). Integrin-collagen interactions are integral to wound healing, inflammation, innate immunity and via TGFBeta signalling and other pathways.[47] We identified multiple rare variants in ephrins and their receptors (data shown for *EPHA8, EFNA1*), known to regulate vascular endothelial and corneal proliferation, tissue fibrosis, wound healing and catecholamine synthesis.[48] Ciliopathies are generally associated with complex phenotypes; however, variants in *IFT88* and *NFATC3* were recently reported with bicuspid aortic valve.[49] We identified two novel variants in these genes. Wound healing is known to be under circadian rhythm control through local and central mechanisms.[50] We identified a small number of variants in *PER1 (MIM 602260), PER2 (MIM 603426)* and *ZFHX3 (MIM 104155)*. It is possible that abnormal wound healing seen in patients with EDS is due to the disruption of these control mechanisms. We identified multiple variants in *DOCK5* (MIM 616904), in the linked region for hEDS. While it has not yet been annotated as causative of disease in humans, a mouse model has a reduced skeletal muscle phenotype and a zebrafish model has abnormal fast muscle.[28] We also identified multiple variants in various *TSPANS*. *TSPAN2* regulates TGFB1/SMAD expression in vascular endothelium (MIM 613133).

Genetic burden analysis

In view of the large number of rare variants identified in multiple pathways, a formal burden analysis was carried out to seek statistically significant associations. Burden analysis was carried out using the TASER software[13] (table 2). While *LOC283685* was close to meeting the criteria for significance (p=2.34e-6, adjusted p=7.41e-6), we identified that the coding sequence of the final exon of *GOLGA6L2* transcript ENST00000312015 (Glu308-Ter415), annotated separately in USC GRCh38, probably overlaps the C-terminal sequence of *LOC283685* (Glu61-Ter168). The overall burden of rare variants in *GOLGA6L2* including this terminal region did not meet significance (p=2.67e-3, adjusted p=4.36e-3). The lack of statistically significant results of this analysis is likely related to the small sample size. A number of the top scoring loci, however, had biological plausibility. The LRTTM4-HSPG (heparan sulfate proteoglycome) complex has been proposed a tetrapartite model for synaptic plasticity involving interactions with the ECM and *HSPG* has been noted in the vEDS transcriptome. *GOLGA6L2* is of unknown function; golgins are a large group of vesicle tethering proteins with tissue-specific effects, other golgins are known to result in reduced bone mineral density and neuromuscular phenotypes (GOLGA2 MIM 602580). *ANKFY1* is involved in transport to the Golgi apparatus. *ADCY1* (MIM 103072) causes autosomal recessive deafness with abnormalities of circadian rhythm.[50]

DISCUSSION

In this study, we generated WES in 174 patients with several EDS clinical subtypes: cEDS (n=20), vEDS (n=5), kEDS (n=2),

Table 2 Results of genetic burden analysis using TASER methodology, with 128 cases and 248 controls

Gene	Chr (position)	L	M_s	M_st	M_p	New.SB_p	New.STB_p	Adjusted p value
LOC283685	15 (23684612–23685207)	21	7	7	7	2.34E-06	2.34E-06	7.41E-06
OR4C45	11 (48366903–48373999)	14	9	9	9	7.72E-06	7.72E-06	2.18E-05
KCNJ12	17 (21279699–21323179)	178	36	36	35.5	9.63E-06	9.63E-06	2.67E-05
PSMD2	3 (184017022–184026675)	74	6	6	6	5.65E-05	5.65E-05	1.32E-04
BX648489	20 (25825303–25834657)	18	10	10	10	6.34E-05	6.34E-05	1.47E-04
ANKFY1	17 (4066665–4167025)	71	8	8	8	6.79E-05	8.15E-05	1.84E-04
FRG1B	20 (29612306–29631629)	50	14	14	14	9.94E-05	9.94E-05	2.21E-04
LRRTM4	2 (76974850–77749502)	47	5	5	5	1.06E-04	1.06E-04	2.34E-04
MLLT10P1	20 (29637584–29638138)	21	20	20	20	1.41E-04	1.41E-04	3.03E-04
ADCY1	7 (45613739–45703971)	30	1	1	1	1.81E-04	1.81E-04	3.80E-04

Adjusted p value, p value after applying genomic control correction (inflation factor λ=1.11) to the New.STP_p χ^2 test statistic; L, number of variant sites that are considered 'rare' (alternate allele read count frequency AACF <0.05); M_p, estimated number of SNVs in the dataset; M_s, number of variant sites screened in; M_st, number of variant sites screened in and passing threshold AACF >1/(2n), where n=128+248 (the cohort size); New.SB_p, p value of the 'New-SB' test (based on M_s); New.STP_p, p value of the 'New-STB' test (based on M_st).

(hEDS n=109) and HDCT (n=38) from two specialised clinical EDS services. Patients underwent extensive clinical diagnostic and research testing for known EDS/HTAD genes prior to being recruited into this study. Those with a confirmed genetic diagnosis in the clinical laboratory or in our previous research study were excluded.[8] Ten patients previously without a genetic diagnosis were given a new diagnosis: two patients were diagnosed with mEDS, two with cEDS and four with LDS. The pathogenic and LP variants in these patients were subsequently confirmed in the clinical diagnostic laboratory. A molecular diagnosis may be important for clinical management and may facilitate assessment of vascular risk. Although many of the pathogenic (P) and likely pathogenic (LP) variants reported in this cohort would be identified with current panel testing, they were not at the time of this study, highlighting the use of extended panels and WES as a clinical tool for complex EDS.

We also identified a number of high priority VUS in genes for EDS (n=3), LDS/ HTAD (n=3), Lujan syndrome (n=1), Birt-Hogg-Dube syndrome (n=1), skeletal dysplasia and bone metabolism (n=4), erythromyalgia (n=1) with compelling supporting clinical and in silico criteria for pathogenicity, according to ACGS criteria, segregation and functional work may enable reclassification to LP. These findings reflect the overlap between the clinical features of EDS, LDS, HTAD and Mendelian disorders associated with EDS symptomatology. Further, a small number of patients were identified as carrying more than one such variant, suggestive of two separate Mendelian disorders, which may explain the complex phenotypes observed in these patients.

We identified single patients with novel variants with CADD >15 in genes not previously reported as associated with a Mendelian phenotype (*PGTER4*, *TGFB1/1*, *INO8D*, *SYAP1*), with biological plausibility based on published in vitro and animal models of vascular disease and EDS phenotypes. A large number of rare variants with CADD >15 were identified in genes previously identified in EDS GWAS and transcriptome studies (eg, *HSPG2*, *PIEZO1*, *COL27A1*). We note that these included a number of genes reported as causes of autosomal recessive skeletal dysplasia and other pathways implicated in the repair and maintenance of the ECM: Integrins, Ephrins and DOCK genes.

While a formal burden analysis did not identify any genome-wide statistically significant associations, several plausible candidate loci were identified that will benefit from further investigation.

One limitation of this study was the inability to identify chromosomal CNVs, which are implicated in HTAD, *TNXB* and familial mast cell disorders, leading to potential under-ascertainment of these abnormalities in this cohort.[4] Finally, the occurrence of GJH as a normal trait and unknown prevalence of symptomatic hypermobility/hypermobility spectrum disorders (HSD) and hEDS presents a challenge to assessment of the expected prevalence of rare variants in relation to disease.[5]

CONCLUSIONS

We report WES analysis for a large cohort of patients with complex and unresolved EDS phenotypes to have undergone deep phenotyping and WES. This study suggests that large panel-based sequencing and WES will have clinical utility in patients with complex presentations that are unresolved by clinical examination and EDS panel gene sequencing, by making new molecular diagnoses for rare Mendelian disorders that had not been previously suspected in earlier detailed investigation. In addition, multiple heterozygous variants were identified in genes associated with skeletal dysplasia, myopathy and integrins, although these are not as yet proven to be causative for EDS. A smaller number of variants in non-annotated genes with biological plausibility were also identified. Our results are consistent with the complex genetic architecture of EDS and have suggested a number of novel hEDS and HTAD candidate genes and pathways that are worthy of further investigation.

Author affiliations
[1]Maritime Medical Genetics Service, IWK Health Centre, Halifax, Nova Scotia, Canada
[2]Faculty of Medicine, Department of Pediatrics, Dalhousie University, Halifax, Nova Scotia, Canada
[3]Centre for Genomic and Experimental Medicine, Institute of Genetics and Cancer, University of Edinburgh, Edinburgh, UK
[4]Institute of Clinical Sciences, Imperial College London, London, UK
[5]Department of Vascular Surgery, Royal Free Hospital, London, UK
[6]Department of Clinical Biochemistry, Queen Elizabeth University Hospital, NHS Greater Glasgow and Clyde, Glasgow, UK
[7]Department of Neuromuscular Diseases, UCL Queen Street Institute of Neurology, University College London, London, UK
[8]Population Health Sciences Institute, Newcastle University, Newcastle upon Tyne, UK
[9]Edinburgh Genomics, University of Edinburgh, Edinburgh, UK
[10]MRC Human Genetics Unit, University of Edinburgh, Edinburgh, UK
[11]Cambridge University Hospitals, Cambridge Biomedical Campus, Cambridge, UK
[12]Department of Rheumatology, University College London Hospitals NHS Foundation Trust, London, UK
[13]Ehlers-Danlos Syndrome National Diagnostic Service, London North West University Healthcare NHS Trust, Northwick Park Hospital, Harrow, UK
[14]Department of Metabolism, Digestion and Reproduction Section of Genetics and Genomics, Imperial College London, London, UK

Twitter Ruwan A Weerakkody @rweerakk

Acknowledgements The study was supported by the National Institute for Health Research England (NIHR) for the NIHR BioResource project (grant number RG65966). We thank NIHR BioResource volunteers for their participation, and gratefully acknowledge NIHR BioResource Centres, NHS Trusts and staff for their contribution. We thank the National Institute for Health and Care Research, NHS Blood and Transplant, and Health Data Research UK as part of the Digital Innovation Hub Programme. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health and Social Care. We acknowledge Julie Leary and Cherida Watkins (NWLH) for their assistance with recruitment and administrative support. This study also makes use of data generated by the UK10K Consortium, derived from samples from ALSPAC; a full list of the investigators who contributed to the generation of this data is available from www.UK10K.org. Funding for the UK10K was provided by the Wellcome Trust under award WT091310.

Collaborators We acknowledge collaborator support from Willem Ouwehand and Kathy Stirrups.

Contributors The study was designed by RAW, TJA, JV and AMV. Patients were clinically ascertained at the EDS diagnostic service (AMV, FMP, NG, AFB, CC, MB) and at the UCLH hypermobility clinic (HK, RG). DNA extraction and sequencing was completed at Imperial College and in Edinburgh (RAW, JS-L) and the NIHR in Cambridge (NIHR BioResource). WES filtering and data analysis was carried out by DAP, JV, AMV, DJT-M and AM, phenotype summary and review by AMV, CK, RAW, DJT-M, FMP and FSvD; TASER analysis by RD and HJC. The paper was written by AMV and TJA. TJA acts as guarantor.

Funding The study was supported by NIHR grant RG65966 and Wellcome Trust grant UK10K WT091310 for BRIDGE-EDS; and Wellcome Trust Clinical Fellowship (WCMA_P43883) to RAW.

Competing interests TA is co-founder and director of the company BioCaptiva. There are no other competing interests.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by the West London Research Ethics Committee, Reference 11/LO/0883, Cambridgeshire 2 Research Ethics Committee, Reference 04/Q0108/44. Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request.

ORCID iDs
Ruwan A Weerakkody http://orcid.org/0000-0002-6717-7383
Javier Santoyo-Lopez http://orcid.org/0000-0003-1988-5059
Neeti Ghali http://orcid.org/0000-0003-2847-1376
Timothy J Aitman http://orcid.org/0000-0002-7875-4502

REFERENCES

1 Malfait F, Francomano C, Byers P, *et al*. The 2017 International classification of the Ehlers-Danlos syndromes. *Am J Med Genet C Semin Med Genet* 2017;175:8–26.
2 Alazami AM, Al-Qattan SM, Faqeih E, *et al*. Expanding the clinical and genetic heterogeneity of hereditary disorders of connective tissue. *Hum Genet* 2016;135:525–40.
3 Driver SGW, Jackson MR, Richter K, *et al*. Biallelic variants in Efemp1 in a man with a pronounced connective tissue phenotype. *Eur J Hum Genet* 2020;28:445–52.
4 Zweers MC, Bristow J, Steijlen PM, *et al*. Haploinsufficiency of TNXB is associated with Hypermobility type of Ehlers-Danlos syndrome. *Am J Hum Genet* 2003;73:214–7.
5 Tobias JH, Deere K, Palmer S, *et al*. Joint Hypermobility is a risk factor for musculoskeletal pain during adolescence: findings of a prospective cohort study. *Arthritis Rheum* 2013;65:1107–15.
6 Pickrell JK, Berisa T, Liu JZ, *et al*. Detection and interpretation of shared genetic influences on 42 human traits. *Nat Genet* 2016;48:709–17.
7 Beighton P, Paepe A, Steinmann B, *et al*. n.d. Ehlers-Danlos syndromes: revised Nosology, Villefranche,447 1997. Ehlers-Danlos national foundation (USA) and Ehlers-Danlos support group (UK). *Am J Med Genet*:31–7.
8 Weerakkody RA, Vandrovcova J, Kanonidou C, *et al*. Targeted next-generation sequencing makes new molecular diagnoses and expands genotype-phenotype relationship in Ehlers-Danlos syndrome. *Genet Med* 2016;18:1119–27.
9 Richards S, Aziz N, Bale S, *et al*. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American college of medical Genetics and Genomics and the Association for molecular pathology. *Genet Med* 2015;17:405–24.
10 Kopanos C, Tsiolkas V, Kouris A, *et al*. Varsome: the human Genomic variant search engine. *Bioinformatics* 2019;35:1978–80.
11 Boyd A, Golding J, Macleod J, *et al*. Cohort profile: the 'children of the 90S'--The index offspring of the Avon longitudinal study of parents and children. *Int J Epidemiol* 2013;42:111–27.
12 UK10K Consortium, Walter K, Min JL, *et al*. The Uk10K project identifies rare variants in health and disease. *Nature* 2015;526:82–90.
13 Hu Y-J, Liao P, Johnston HR, *et al*. Testing rare-variant Association without calling Genotypes allows for systematic differences in sequencing between cases and controls. *PLoS Genet* 2016;12:e1006040.
14 Angwin C, Brady AF, Colombi M, *et al*. Absence of collagen flowers on electron microscopy and identification of (likely) pathogenic *Col5A1* variants in two patients. *Genes (Basel)* 2019;10:762.
15 Zoppi N, Chiarelli N, Binetti S, *et al*. Dermal fibroblast-to-Myofibroblast transition sustained by Αvß3 integrin-ILK-Snail1/slug signaling is a common feature for Hypermobile Ehlers-Danlos syndrome and Hypermobility spectrum disorders. *Biochim Biophys Acta Mol Basis Dis* 2018;1864(4 Pt A):1010–23.
16 Luo X, Deng S, Jiang Y, *et al*. Identification of a pathogenic Tgfbr2 variant in a patient with Loeys-Dietz syndrome. *Front Genet* 2020;11:479.
17 Ellard S, Baple EL, Callaway A, *et al*. ACGS best practice guidelines for variant classification in rare disease. 2020. Available: https://www.acgs.uk.com/media/11631/uk-practice-guidelines-for-variant-classification-v4-01-2020.pdf
18 Mitchell AL, Judis LM, Schwarze U, *et al*. Characterization of tissue-specific and developmentally regulated alternative splicing of Exon 64 in the Col5A1 gene. *Connect Tissue Res* 2012;53:267–76.
19 Guo D-C, Grove ML, Prakash SK, *et al*. Genetic variants in Lrp1 and Ulk4 are associated with acute aortic Dissections. *Am J Hum Genet* 2016;99:762–9.
20 Tejada MI, Villate O, Ibarluzea N, *et al*. Molecular and clinical characterization of a novel nonsense variant in Exon 1 of the Upf3B gene found in a large Spanish Basque family (Mrx82). *Front Genet* 2019;10:1074.
21 Hostettler IC, O'Callaghan B, Bugiardini E, *et al*. Angptl6 genetic variants are an underlying cause of familial intracranial aneurysms. *Neurology* 2021;96:e947–55.
22 Lorenzo-Betancor O, Blackburn PR, Edwards E, *et al*. PCNT point mutations and familial intracranial aneurysms. *Neurology* 2018;91:e2170–81.
23 Meester JAN, Vandeweyer G, Pintelon I, *et al*. Loss-of-function mutations in the X-linked Biglycan gene cause a severe Syndromic form of Thoracic aortic aneurysms and Dissections. *Genet Med* 2017;19:386–95.
24 Takeichi T, Katayama C, Tanaka T, *et al*. A novel Ifih1 Mutation in the Pincer domain underlies the clinical features of both Aicardi-Goutieres and Singleton-Merten syndromes in a single patient. *Br J Dermatol* 2018;178:e111–3.
25 Capuano A, Bucciotti F, Farwell KD, *et al*. Diagnostic Exome sequencing identifies a novel gene, Emilin1, associated with Autosomal-dominant hereditary connective tissue disease. *Hum Mutat* 2016;37:84–97.
26 Syx D, Symoens S, Steyaert W, *et al*. Ehlers-Danlos syndrome, Hypermobility type, is linked to Chromosome 8P22-8P21.1 in an extended Belgian family. *Dis Markers* 2015;2015:828970.
27 Hino N, Ichikawa T, Kimura Y, *et al*. An Amphipathic Helix of Vinexin alpha is necessary for a substrate stiffness-dependent conformational change in Vinculin. *J Cell Sci* 2019;132:jcs217349.
28 Benson CE, Southgate L. The DOCK protein family in vascular development and disease. *Angiogenesis* 2021;24:417–33.
29 Nikolova G, Lee H, Berkovitz S, *et al*. Sequence variant in the laminin Gamma1 (Lamc1) gene associated with familial pelvic organ Prolapse. *Hum Genet* 2007;120:847–56.
30 Chiarelli N, Carini G, Zoppi N, *et al*. Molecular insights in the pathogenesis of classical Ehlers-Danlos syndrome from Transcriptome-wide expression profiling of patients' skin fibroblasts. *PLoS One* 2019;14:e0211647.
31 Chiarelli N, Carini G, Zoppi N, *et al*. Transcriptome analysis of skin fibroblasts with dominant negative Col3A1 mutations provides molecular insights into the Etiopathology of vascular Ehlers-Danlos syndrome. *PLoS One* 2018;13:e0191220.
32 Patil MS, Cartland SP, Kavurma MM. TRAIL signals, extracellular matrix and vessel remodelling. *Vasc Biol* 2020;2:R73–84.
33 Stum M, Girard E, Bangratz M, *et al*. Evidence of a dosage effect and a physiological Endplate acetylcholinesterase deficiency in the first Mouse models mimicking Schwartz-Jampel syndrome Neuromyotonia. *Hum Mol Genet* 2008;17:3166–79.
34 Kudo A, Kii I. Periostin function in communication with extracellular Matrices. *J Cell Commun Signal* 2018;12:301–8.
35 Murthy SE, Dubin AE, Patapoutian A. Piezos thrive under pressure: mechanically activated ion channels in health and disease. *Nat Rev Mol Cell Biol* 2017;18:771–83.
36 Chung S, Nakashima M, Zembutsu H, *et al*. Possible involvement of Nedd4 in Keloid formation; its critical role in fibroblast proliferation and collagen production. *Proc Jpn Acad Ser B Phys Biol Sci* 2011;87:563–73.
37 Kim-Kaneyama J, Wachi N, Sata M, *et al*. Hic-5, an Adaptor protein expressed in vascular smooth muscle cells, modulates the arterial response to injury in vivo. *Biochemical and Biophysical Research Communications* 2008;376:682–7.
38 Shameer K, Klee EW, Dalenberg AK, *et al*. Whole Exome sequencing Implicates an Ino80D Mutation in a syndrome of aortic hypoplasia, premature Atherosclerosis, and arterial stiffness. *Circ Cardiovasc Genet* 2014;7:607–14.
39 Gilman KE, Limesand KH. The complex role of prostaglandin E2-EP receptor signaling in wound healing. *Am J Physiol Regul Integr Comp Physiol* 2021;320:R287–96.
40 Bhushan R, Altinbas L, Jäger M, *et al*. An integrative systems approach identifies novel candidates in Marfan syndrome-related pathophysiology. *J Cell Mol Med* 2019;23:2526–35.
41 Krebs LT, Xue Y, Norton CR, *et al*. Notch signaling is essential for vascular Morphogenesis in mice. *Genes Dev* 2000;14:1343–52.
42 Scherer C, Pfisterer L, Wagner AH, *et al*. Arterial wall stress controls Nfat5 activity in vascular smooth muscle cells. *J Am Heart Assoc* 2014;3:e000626.
43 von Collenberg CR, Schmitt D, Rülicke T, *et al*. An essential role of the Mouse Synapse-associated protein Syap1 in circuits for spontaneous motor activity and Rotarod balance. *Biol Open* 2019;8.
44 Trella KJ, Li J, Stylianou E, *et al*. Genome-wide analysis identifies differential promoter methylation of Leprel2, Foxf1, Mmp25, Igfbp6, and Peg12 in murine Tendinopathy. *J Orthop Res* 2017;35:947–55.
45 Soria-Valles C, Gutiérrez-Fernández A, Osorio FG, *et al*. MMP-25 Metalloprotease regulates innate immune response through NF-kappaB signaling. *J Immunol* 2016;197:296–302.
46 Kapferer-Seebacher I, Pepin M, Werner R, *et al*. Periodontal Ehlers-Danlos syndrome is caused by mutations in C1R and C1S, which Encode Subcomponents C1R and C1S of complement. *Am J Hum Genet* 2016;99:1005–14.
47 Zeltz C, Gullberg D. The integrin-collagen connection - a glue for tissue repair? *J Cell Sci* 2016;129:1284.
48 Wu B, Rockel JS, Lagares D, *et al*. Ephrins and Eph receptor signaling in tissue repair and fibrosis. *Curr Rheumatol Rep* 2019;21:23.
49 Toomer KA, Fulmer D, Guo L, *et al*. A role for primary cilia in aortic valve development and disease. *Dev Dyn* 2017;246:625–34.
50 Chang J, Garva R, Pickard A, *et al*. Circadian control of the Secretory pathway maintains collagen homeostasis. *Nat Cell Biol* 2020;22:74–86.

Genetic Complexity of Diagnostically Unresolved Ehlers-Danlos Syndrome

Anthony Vandersteen [1,2], Ruwan A. Weerakkody [2,3], David Parry [2], Christina Kanonidou [4], Daniel J. Toddie-Moore[2], Jana Vandrovcova[5], Rebecca Darlay[6], Javier Santoyo-Lopez[7], Alison Meynert[8], NIHR BioResource[9], Hanadi Kazkaz[10], Rodney Grahame[10], Carole Cummings[11], Marion Bartlett[11], Neeti Ghali[11], Angela F. Brady[11], F. Michael Pope[11], Fleur S. Van Dijk[11], Heather J. Cordell[6], Timothy J. Aitman[2].

Supplementary Methods

Whole exome sequencing and variant analysis

Genomic DNA from 89 individuals was processed using the SureSelectXT2 Human All Exon V5 capture kit (Agilent) and sequenced with 75 base paired-end reads on a HiSeq 4000 (Illumina) and from 85 samples with 100 base paired-end reads on a HiSeq 2500 (Illumina). Raw sequence data will be made available on reasonable request to the study's data access committee, chaired by TJA.

FASTQs were aligned to the human genome reference (GRCh37) using bwa mem (0.7.12). Alignments were post-processed using Picard (v2.1.1) for identification of duplicate reads and the Genome Analysis ToolKit (GATK, 3.5-0-g36282e4) for indel realignment and base recalibration. Genotype likelihoods for each sample were calculated using the GATK HaplotypeCaller and resulting GVCF files were called jointly using GATK's GenotypeGVCFs function. Functional annotations were added using Ensembl's Variant Effect Predictor (v90). VASE (v0.1, https://github.com/david-a-parry/vase) was used to perform dominant and recessive segregation filtering of variants. Variants with a frequency greater than 0.1% (for dominant filtering) or 0.5 % (for recessive filtering) in gnomAD or dbSNP150 or those not annotated as either high or moderate impact variants or as splice region variants were removed. Splice region variants not overlapping the canonical +/-2 donor/acceptor intron positions were only retained if they had an ada score and rf score from dbscSNV (https://doi.org/10.1093/nar/gku1206) of 0.8 or higher. Genotype calls were filtered if PHRED scale genotype quality scores were below 20, based on fewer than 5 reads or if the ratio of variant reads compared to total depth was below 0.25.

Vandersteen AM, *et al. J Med Genet* 2023;0:1–7. doi: 10.1136/jmg-2023-109329

Additionally, variants were processed using the G2P plugin for VEP (https://www.ebi.ac.uk/gene2phenotype/g2p_vep_plugin) and the Genomics England Panel App (Ehlers-Danlos Syndrome(https://panelapp.genomicsengland.co.uk/api/v1/panels/53/?version=2.0).

A further 'exomiser' based analysis using all the HPO terms currently identified as clinical criteria in the 2017 EDS nosology [1]. Variants were reviewed for known EDS genes [1], mendelian disorders with EDS features or symptoms, HTAD [2], genes abnormally expressed in skin fibroblast from patients with vEDS, cEDS and hEDS [3-5]. Variant calls were searched for genes associated with the previously linked region for hEDS reported by Syx et al [6],pelvic organ prolapse [7], genome wide association studies for GJH, knee pain, rotator cuff injury and pelvic organ prolapse (https://www.ebi.ac.uk/gwas/) [8 9].

Database searches and variant assessment

Mendelian Disorders: Dominant and autosomal recessive variant datasets were searched using OMIM annotations. Variants with CADD score > 15 were selected for further review to assess for the updated ACMG criteria for pathogenicity [10-13] using the annotation tool Varsome [14]: (https://varsome.com/) and Franklin by Genoox (https://franklin.genoox.com). This included ClinVar reports, functional annotation, previous published reports of specific variants, occurrence of the variant in a specific protein domain and reported allele frequency (https://gnomad.broadinstitute.org/).

A specific search for variants in EDS genes from the 2017 nosology [1] was completed: classical EDS (cEDS): *COL5A1, COL5A2, COL1A1*, classical like EDS (clEDS): *TNXB*, cardiac valvular EDS (cvEDS): *COL1A2*, vascular EDS (vEDS): *COL3A1, COL1A1*, dermatosparaxis EDS (dEDS*): ADAMTS2*, kyphoscoliotic EDS (kEDS): *PLOD1, FKBP14*, Brittle Cornea Syndrome (BCS): *PRDM5, ZNF469*, spondylodysplastic EDS (spEDS): *B4GALT7, B3GALT6, SLC39A13*, Musculocontractural EDS (mcEDS): *CHST14, DSE*, myopathic EDS (mEDS): *COL12A1*, periodontal EDS (pEDS):*C1R, C1S*.

Further searches were completed for rare variants in disorders associated with EDS like phenotypes: including Ehlers-Danlos syndrome classic-like-2: *AEBP1*, Bethlem myopathy: *COL6A1, COL6A2, COL6A3* and Zimmerman-Laband Syndrome: *KCNH1, ATP6V1B2, KCCN3.*

We searched for rare variants in Mendelian disorders associated with EDS symptomatology, including dysautonomia: *SPTLC1, WNK1* and *IBKAP*, familial mast cell disorders, *TPSAB1, KIT* and erythermalgia *SCN9A.*

We searched for rare variants in Mendelian disorders with multisystem manifestations which are rarely associated with aneurysm: Neurofibromatosis type I (MIM 613113) *NF1,* Tuberous Sclerosis (MIM 191100) *TSC1, TSC2*, Birt-Hogg-Dube syndrome (MIM 135150) *FLCN* and Singleton Merten Syndrome (MIM 182250) *IFIH1, DDX58*.

We completed a review of rare variants in genes causative for Inborn errors of metabolism with features of hereditary disorders of connective tissue, these may be underdiagnosed: homocystinuria: *CBS*, Wilson disease: *ATP7B*, Occipital horn syndrome/ Menke's disease: *ATP7A* and hypophosphatasia: *ALPL*.

We searched for HTAD genes using the ClinGen criteria [2] (https://clinicalgenome.org/docs/clinical-validity-of-genes-for-heritable-thoracic-aortic-aneurysm-and-dissection/ for genes strongly associated with HTAD: *ACTA2, COL3A1, FBN1, MYH11, MYLK, SMAD3, TGFB2, TGFBR1, and TGFBR2.* Potentially diagnostic: *EFEMP2, ELN, FBN2, FLNA, NOTCH1, SLC2A10, SMAD4, and SKI.* Gene with limited evidence of causality: *COL4A5, CBS, PKD1, and PKD2,* genes with no evidence/ experimental data only for causality: *ACVRL1, ADAMTS10, B3GAT3, COL1A1, COL1A2, COL4A1, COL5A1, COL5A2, COL9A1, COL9A2, COL11A1, COL18A1, EMILIN1, ENG, GATA5, GJA1, JAG1, MED12, PLOD1, PLOD3, SMAD6, UPF3B, and VCAN.* Newly identified genes*: BGN, FOXE3, HCN4, MAT2A, MFAP5, SMAD2, and TGFB3.*

Mendelian Disorders awaiting confirmation: We searched for rare variants in Mendelian entities with EDS like features, awaiting confirmation with autosomal recessive inheritance: *PLOD3*, *ALDH18A1, ATP6V0D2, ATP6V1E1, CAPN3, GORAB, OBSL1, IFT122, PLP1, SPARC* and *EFEMP* [15 16].

Similarly, we searched for Mendelian entities with EDS-like features awaiting confirmation: autosomal dominant connective tissue disorder with peripheral neuropathy: *EMILIN1,* cardiospondylocarpofacial syndrome: *MAP3K7*, multisystem connective tissue disorder*: LAMA5,* nemaline myopathy *RYR3.*

We searched for rare variants in genes reported in association with risk of intracranial aneurysm [17] (family studies reviewed in PMID: 32367296): *ADAMTS15, ANGPTL6, ARGHGEF16, LOXL2, PCNT, RNF213, THSD1, TMEM132B, NEK4, EDIL3, EDNRB, DNAH9 and GGA3.*

Genes reported as abnormally expressed in EDS linkage studies: We searched for rare variants in genes within the linked region for hEDS [6]: *BMP1, CNOT7, CSGALNACT1, LOXL2, LPL, SLC39A14, HR, NPM2, DOCK5, ADAMDEC1, ADAM7, GNRH1, STC1, ADAM28, FGF17, SORBS3, NKX3-1, SFTPC, NEFL, FGF20, ADAM28, FGL1, ASAH1 PDLIM2, CCAR2 LZTS1 NKX2-6, NAT1, DOK2, TNFRSF10B DMTN, EGF17, KTCD9, NPM2, PDLIM2, ENTPP4, SLC18A1, SFTPC, ATP6V1B2, PDGFRL, PCM1, PFLIM2, TNFRSF10D, GFRA2, NEFM, SLC7A1, BIN3, POLR3D, VSP37A, C8orf20.*

Genes reported as abnormally expressed in skin fibroblast studies: We searched for rare variants (germline) with CADD>15 in genes abnormally expressed in skin fibroblasts from cEDS patients [4]: *SPP1, POSTN, EDIL3, PAPPA, IGFBP2, C3, DNAJB7, CCPG1, ATG10, SVIP, ALG13, VIPAS39, HIF4A, CDKN1A, CCNE2, ASF1B, CLSPN, DTL, DDIAS.*

We searched for rare variants (germline) with CADD>15 in genes abnormally expressed in skin fibroblasts from vEDS patients with confirmed *COL3A1* mutations [5]: *FBN2, TNFAIP6, PTCH2, HIST1H4L*, *ITGA3*, *HSPG2*, *MMP24*, *EDNRA*, *LOXL3*, *P4HA2*, *P4HA3*.

We searched for rare variants (germline) with CADD>15 in genes abnormally expressed in skin fibroblasts from hEDS patients [3]: *CDH11, MMP9, CCN1, CCN2, ITGB3, ILK, PINCH, PARVA, PARVB, PARVG, PXN, AKT1, AKT2, AKT3, GSK3, NFKB1, CDH1, MMP 2, SNAI1, SNAI2.*

Genes reported as associated with features of EDS in GWAS: We reviewed our data for rare variants (MAF<0.1% and CADD>15) in GWAS Loci for one of the diagnostic criteria for hEDS: self-reported Beighton score >5 with $P < 5 \times 10^{-8}$ [8]: *STON1*, (MIM 605357), *EFEMP1* (MIM 601548, Doyne honeycomb degeneration of retina #126600), *C2orf54* (Not annotated), *ABI3BP* (MIM 606279), *VCAN* (MIM 118661, Wagner syndrome #143200), *NOTCH4* (MIM 164951), *XKR6* (Not annotated), *NEDD4* (MIM 602278), *PIEZO1* (MIM 611184, Dehydrated hereditary stomatocytosis with or without pseudohyperkalemia and/or perinatal edema #3194380, Lymphoedema (AR, LoF).

We reviewed our data for rare variants (MAF<0.1% and CADD>15) in GWAS Loci for pelvic organ prolapse with $P < 5 \times 10^{-8}$ [9]: *WNT4, GDF7, EFEMP1, FAT4, IMPDH1, TBX5, SALL1.*

We reviewed our data for rare variants (MAF<0.1% and CADD>15) in GWAS Loci for knee pain and rotator cuff injury associated loci (https://www.ebi.ac.uk/gwas/), with $P < 5 \times 10^{-8}$: *COL27A1* (MIM 608461, Steel syndrome), *GDF5* (MIM 601146, multiple phenotypes), *DENND2C, SASH1, ESRRB, FGFR1, TNC* and *DEFB1*.

Assessment of Candidate genes: We reviewed our data for rare variants expected to result in loss of function, identifying genes with OMIM annotation. For non-annotated genes we reviewed the probability of loss of function intolerance scores (pLi) and biological plausibility, looking for published evidence of expression or impact on the extracellular matrix, collagen synthesis or function, aneurysm formation in human tissue studies and reported EDS or HTAD like phenotypes in animal models https://www.alliancegenome.org/. Similarly, we reviewed our data for novel missense, splice and synonymous variants (gnomAD frequency = 0). Variants with high CADD scores (>20) were selected for further review as above. The entire dataset were reviewed for the same or further rare variants in the same gene.

Genetic burden analysis

Analysis of sequence data where there are systemic differences in coverage between cases and controls typically leads to inflated type I errors, but discarding those samples with insufficient read depth can result in a loss of power. TASER is a program for testing association using sequencing reads without calling genotypes, which is robust to a wide range of differential sequencing qualities between cases and controls. TASER uses the total number of reads mapped to a variant, and the number carrying the minor allele, to calculate a score statistic at each position in a gene of interest, thus providing an assessment of the association of each individual variant with the disease phenotype. A burden statistic is then calculated for each gene as the sum of the score statistics for each of the variants within that gene, allowing identification of genes that have a higher or lower accumulation of rare variants in the cases than might be expected, compared to controls. A bootstrap procedure is used for assessing the significance of the burden statistic. TASER includes a screening procedure to screen-in loci based on allele counts (not on assigned genotypes) where: 1) Alternate allele read count frequency (AACF) in the entire cohort < 0.05 (can be adjusted if required); 2) AACF is not less than 1/(2n) where n is the sample size of the overall cohort tested[18].

For each of the sequences, we split the DNA sequence into non-overlapping exons, where the gene was the unit of the burden test, in genomic order. Each chromosome was split into 100 gene "processing" blocks based on the GRCh37, resulting in the analysis of 16560 genes in 240 blocks. Only bases called with a quality score >30 were added to the read count at each position within each exon, and only if the resultant read depth was greater than 2. The upper MAF limit for analysis was set at 0.05 in the base population. The top scoring loci from this analysis are shown in Table 2. Since analysis of rare variant burden was performed in 16560 genes, a p value of $0.05/16560 = 3 \times 10^{-6}$ would be considered genome-wide evidence for statistical significance. Examination of QQ plots from the overall set of 16560 χ^2 test statistics derived from the bootstrap p values showed a slight inflation (genomic control inflation factor λ=1.11) so we adjusted the p values by dividing the χ^2 test statistics by 1.11 and recalculating the implied p values.

References

1. Malfait F, Francomano C, Byers P, et al. The 2017 international classification of the Ehlers-Danlos syndromes. *Am J Med Genet C Semin Med Genet* 2017;175(1):8-26. doi: 10.1002/ajmg.c.31552
2. Renard M, Francis C, Ghosh R, et al. Clinical Validity of Genes for Heritable Thoracic Aortic Aneurysm and Dissection. *J Am Coll Cardiol* 2018;72(6):605-15. doi: 10.1016/j.jacc.2018.04.089
3. Zoppi N, Chiarelli N, Binetti S, et al. Dermal fibroblast-to-myofibroblast transition sustained by alphavss3 integrin-ILK-Snail1/Slug signaling is a common feature for hypermobile Ehlers-Danlos syndrome and hypermobility spectrum disorders. *Biochim Biophys Acta Mol Basis Dis* 2018;1864(4 Pt A):1010-23. doi: 10.1016/j.bbadis.2018.01.005 [published Online First: 20180105]
4. Chiarelli N, Carini G, Zoppi N, et al. Molecular insights in the pathogenesis of classical Ehlers-Danlos syndrome from transcriptome-wide expression profiling of patients' skin fibroblasts. *PLoS One* 2019;14(2):e0211647. doi: 10.1371/journal.pone.0211647 [published Online First: 20190204]
5. Chiarelli N, Carini G, Zoppi N, et al. Transcriptome analysis of skin fibroblasts with dominant negative COL3A1 mutations provides molecular insights into the etiopathology of vascular Ehlers-Danlos syndrome. *PLoS One* 2018;13(1):e0191220. doi: 10.1371/journal.pone.0191220 [published Online First: 20180118]
6. Syx D, Symoens S, Steyaert W, et al. Ehlers-Danlos Syndrome, Hypermobility Type, Is Linked to Chromosome 8p22-8p21.1 in an Extended Belgian Family. *Dis Markers* 2015;2015:828970. doi: 10.1155/2015/828970 [published Online First: 20151004]
7. Nikolova G, Lee H, Berkovitz S, et al. Sequence variant in the laminin gamma1 (LAMC1) gene associated with familial pelvic organ prolapse. *Hum Genet* 2007;120(6):847-56. doi: 10.1007/s00439-006-0267-1 [published Online First: 20061005]
8. Pickrell JK, Berisa T, Liu JZ, et al. Detection and interpretation of shared genetic influences on 42 human traits. *Nat Genet* 2016;48(7):709-17. doi: 10.1038/ng.3570 [published Online First: 20160516]
9. Olafsdottir T, Thorleifsson G, Sulem P, et al. Genome-wide association identifies seven loci for pelvic organ prolapse in Iceland and the UK Biobank. *Commun Biol* 2020;3(1):129. doi: 10.1038/s42003-020-0857-9 [published Online First: 20200317]
10. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 2015;17(5):405-24. doi: 10.1038/gim.2015.30 [published Online First: 20150305]
11. Abou Tayoun AN, Pesaran T, DiStefano MT, et al. Recommendations for interpreting the loss of function PVS1 ACMG/AMP variant criterion. *Hum Mutat* 2018;39(11):1517-24. doi: 10.1002/humu.23626 [published Online First: 20180907]
12. Brnich SE, Abou Tayoun AN, Couch FJ, et al. Recommendations for application of the functional evidence PS3/BS3 criterion using the ACMG/AMP sequence variant interpretation framework. *Genome Med* 2019;12(1):3. doi: 10.1186/s13073-019-0690-2 [published Online First: 20191231]
13. Pejaver V, Byrne AB, Feng BJ, et al. Calibration of computational tools for missense variant pathogenicity classification and ClinGen recommendations for PP3/BP4 criteria. *Am J Hum Genet* 2022;109(12):2163-77. doi: 10.1016/j.ajhg.2022.10.013 [published Online First: 20221121]
14. Kopanos C, Tsiolkas V, Kouris A, et al. VarSome: the human genomic variant search engine. *Bioinformatics* 2019;35(11):1978-80. doi: 10.1093/bioinformatics/bty897
15. Alazami AM, Al-Qattan SM, Faqeih E, et al. Expanding the clinical and genetic heterogeneity of hereditary disorders of connective tissue. *Hum Genet* 2016;135(5):525-40. doi: 10.1007/s00439-016-1660-z [published Online First: 20160329]

16. Driver SGW, Jackson MR, Richter K, et al. Biallelic variants in EFEMP1 in a man with a pronounced connective tissue phenotype. *Eur J Hum Genet* 2020;28(4):445-52. doi: 10.1038/s41431-019-0546-7 [published Online First: 20191202]
17. Sauvigny T, Alawi M, Krause L, et al. Exome sequencing in 38 patients with intracranial aneurysms and subarachnoid hemorrhage. *J Neurol* 2020;267(9):2533-45. doi: 10.1007/s00415-020-09865-6 [published Online First: 20200504]
18. Hu YJ, Liao P, Johnston HR, et al. Testing Rare-Variant Association without Calling Genotypes Allows for Systematic Differences in Sequencing between Cases and Controls. *PLoS Genet* 2016;12(5):e1006040. doi: 10.1371/journal.pgen.1006040 [published Online First: 20160506]

Supplementary Table 1. Phenotypic data for cEDS Patients.

Patient ID	Age	Sex	Beighton Score	cEDS Major criteria Minor criteria	vEDS Major criteria Minor criteria	hEDS Major criteria Minor criteria	kEDS Major criteria Minor criteria	Vascular/cardiac complications	Family History
75	30-39	F	8	A, C d, i	– n, q	H, I t, u	– –	VV	**Father:** GJH **2 Sisters:** GJH
136	60-69	F	–	A, C a, i	– –	H, I u	– –	–	**Daughter, Grandson:** cEDS
383	20-29	F	7	A, C d, i	– r	H, I s, u	– –	–	**Mother:** GJH, SCAD **Maternal grandmother:** GJH **Sister:** MVP **Others:** ICA.
396	50-59	F	–	A, C a	– –	H, I u	J –	Aneurysm (subclavian artery)	**Daughter:** GJH, MVP
409	40-49	F	–	A, C d, e	– –	H, I u	– –	AoR	**Son:** GJH, Dev delay, AoR, **Daughter**: AoR
431	30-39	F	7	C, d, g, i	D q	H u	J –	–	**Mother:** GJH
534	30-39	F	9	A, B, C f, g, i	F	H, I u	– –	–	**Father:** JHM **Mother:** GJH **Children:** GJH
583	10-19	F	8	A, B, C d, f, g, i	– –	H, I s, t, u	J –	–	**Father. Sister, Paternal uncle Paternal grandmother:** cEDS
595	30-39	M	6	A, C a, d, g	– k, q	H, I	– –	MVR	**Father:** TS **Mother:** Keratoconus **Sister:** Ischemic stroke
611	30-39	M	7	A, C i	– –	H, I u	– –	–	**Daughter:** hEDS
653	20-29	F	9	A, C a, e, i	–	H, I s, t, u	– –	–	**Mother**, **Brother Maternal aunt, Maternal cousin :** GJH
717	20-29	F	8	A, C a, d, f	– –	H, I u	– –	–	**Father:** GJH
718	30-39	F	5	C a, d	D, G –	H, I u	– –	–	**Father:** **3 paternal aunts:** **Brother** SVT. **Mother:** GJH **Children:** GJH
803	20-29	F	8	A, C d	– –	H, I s, u	J –	–	**Son:** GJH
806	10-19	M	–	B, C e, i	– –	H u	J –	–	**Mother:** GJH, **Brother:** GJH
1002	50-59	F	7	A, C d, i	–	H, I s, u	– –	–	**Mother:** mitochondrial myopathy **Father:** GJH
1365	20-29	F	9	A, B, C d, f, i	– k, r	H, I s, u			**Mother:** GJH **Father:** GJH, HS
1451	10-19	F	9	A,C d, g, i	–	H, I t	– –	–	**Father:** TS, Bru, AAA-NOS, AoR, classical EDS phenotype with cauliflower fibres on EM; **Paternal grandmother**: TS, Bru **Paternal Great grandfather**: TS, Bru, AAA
1524	50-59	F	3	C d, e, f, g	D r	H, I –	– –	–	**Mother:** GJH, intestinal rupture
1528	30-39	M	–	A, C d, f, g	– k, q	H, I s, u	– –	–	**Son:** Fragile skin, GJH

Key: EDS Diagnostic Criteria (Villefranche 1997)

cEDS Major: A. Hyperextensible skin; B. Atrophic scars; C. Joint Hypermobility.

cEDS Minor: a. Smooth, velvety skin; b. Molluscoid pseudotumors; c. Subcutaneous spheroids; d. Complications of joint hypermobility (e.g., sprains, dislocations/subluxations, pes planus); e. Muscle hypotonia, Delayed gross motor development; f. Easy bruising; g. Manifestations of tissue extensibility and fragility (e.g., hiatal hernia, anal prolapse in childhood, cervical insufficiency); h. Surgical complications (postoperative hernias); i. Positive family history.

vEDS Major: D. Thin translucent skin; E. Intestinal/ Arterial/ Uterine fragility and/or rupture; F. Extensive bruising; G. Characteristic Facial appearance.

vEDS Minor: j. Acrogeria; k. Hypermobility of small joints; l. Tendon and muscle rupture; m. Talipes equinovarus (clubfoot); n. Early-onset varicose veins; o. Arteriovenous, carotid-cavernous sinus fistula; Positive family history, sudden death in (a) close relative(s).p. Pneumothorax/pneumohemothorax; q. Gingival recession; r.

hEDS Major: H. Generalised Joint Hypermobility; I. skin involvement.

hEDS Minor: s. Recurring joint dislocations; t. Chronic joint/limb pain; u. Positive family history.

kEDS Major: J. GJH; K. Severe muscle hypotonia at birth; L. Scoliosis at birth progressive; M. Scleral fragility and rupture of the ocular globe.

kEDS Minor: v. Tissue fragility, including atrophic scars; w. Easy bruising; x. Arterial rupture; y. Marfanoid habitus; z. Microcornea; aa. Radiologically considerable osteopenia; bb. Family history, i.e., affected sibs.

Abbreviations (alphabetical order): Abdominal Aortic aneurysm (AAA), Aortic aneurysm – NOS (AA-NOS), Aortic root dilatation (AoR), Blue sclera (BS), Bruising (Bru), Camptodactyly (Camp), Congenital bilateral hip dislocation (CHD), Constipation (Con), Deafness (D), Disproportionate Tall stature (TS), Fatigue (Ftg), Gastroesophageal reflux (GORD), Hallux valgus (HV), Hip dysplasia (HD), Hyperextensible skin (HS), Intracranial aneurysm (ICA), Kyphosis (Kyph), Mitral Valve Prolapse (MVP), Mitral Valve Regurgitation (MVR), Myopia (My), Osteopenia (OP), Pectus excavatum (PE), Pelvic girdle muscle weakness (PGMW), Periodontitis (Pd), Pes planus (PP), Premature osteoarthritis (Poa), Retinal Detachment (RD), Scoliosis (Sco), Soft velvety skin (SS), Striae (Str), Thin Skin (TS), Thoracic Aortic aneurysm (TAA), Urinary incontinence (UI), Joint Hypermobility (JHM), Varicose veins (VV)

Supplementary Table 2. Phenotypic data for vEDS Patients.

Patient ID	Age	Sex	Beighton score	cEDS Major criteria Minor criteria	vEDS Major criteria Minor criteria	hEDS Major criteria Minor criteria	kEDS Major criteria Minor criteria	Vascular/cardiac complications	Family History
44	30-39	F	5	C a, d	G q	H, I s, u	– –	–	**Mother:** GJH, OP
372	40-49	F	–	B f	D, F j, n	– t	– –	VV	**Father:** TS, D **Sister:** D **Brother:** D.
482	20-29	F	6	C d, g, h, i	D –	H, I t, u	– –	–	**Mother** GJH **Father** GJH, SS **Full Sister:** GJH **Full brother:** GJH, HS **Half-sister (mother's side):** GJH, TS **Half sister (father's side**), GJH, HS **Half brother (father's side):** GJH, HS **Maternal aunt:** Subarachnoid haemorrhage
798	20-29	F	5	C d, f, i	D, E k	H u	– –	Cavernous hemangioma	**Father:** GJH, Soft Skin **Brother:** GJH **Paternal aunts:** GJH **Paternal uncle:** GJH
1346	30-39	F	4	A, C d	D, E, G j	H, I t	– –	Scoliosis	**FHx (paternal side)**: ventricular tachycardia, Atrial fibrillation

EDS Diagnostic Criteria and Abbreviations as per lists in Supplementary Table 1.

Supplementary Table 3. Phenotypic data for hEDS Patients.

Patient ID	Age	Sex	Beighton score	cEDS Major criteria Minor criteria	vEDS Major criteria Minor criteria	hEDS Major criteria Minor criteria	kEDS Major criteria Minor criteria	Other features	Vascular/ cardiac complications	GI Symp	Dys-Autonomia	Family History
61	30-39	F	–	C –	– –	H –	– –	–	–	–	–	**Son:** hEDS **Sister:** hEDS, COL3A1:VUS
65	60-69	F	3	C –	E r	H –	– –		Aneurysm, NOS	–	+	**Mother:** ICA **Paternal grandmother:** Cerebral Hemorrhage **Paternal uncle:** Cerebral Hemorrhage
70	10-19	M	4	C d, f, i	– –	H t, u	– –	ejection systolic click	–	–	–	**Mother:** hEDS **Maternal grandmother:** OA, GJH, Umbilical hernia
74	50-59	F	–	C	– q, r	H t	– –	Str	–	–		**Brother:** PXE **Mother:** AA-NOS, Bru,VV
100	50-59	F	7	A, C i	E	H, I u	– –	–	ICA	–	–	**Brother:** GJH **Daughter:** GJH
107	40-49	M	4	– –	E r	H, I u	– –	–	–	–	–	**Paternal grandfather:** AA-NOS **Sister:** hEDS **Paternal cousin 1**: TAD. **Paternal cousin 2**: AoR.
191	30-39	F	3	C d	– n	H, I t, u	– –	MVP	–	–	–	**Mother:** GJH **Daughter:** GJH **Son:** GJH, GORD
374	50-59	M	-	C d	– –	H t	– –	MVP aortic valve surgery	-	-	-	**N/A**
385	30-39	F	–	C f	– r	H, I –	– –	MVP	–	–	+	**Father:** ICA **Paternal grandmother:** ICA, AAA
395	50-59	M	–	A, C a, i	– –	H, I u	– –	–	–	–	–	**Daughter:** MVP, GJH, SS, HS, BS
397	20-29	F	–	A, C a, d	– –	H, I u	– –	MVP	–	+	–	**Mother:** cEDS/ hEDS overlap **Father:** hEDS
402	30-39	M	6	A, C d, i	– –	H, I u	– –	–	–	–	–	**Father:** GJH, TS **Sister:** Knee dislocation, GJH, Heart murmur
404	40-49	M	9	A, C a, d, f, i	– –	H, I u	– –	–	–	–	–	**Mother**: GJH **Father:** GJH **Paternal grandmother:** AA-NOS **Paternal grandfather:** AA-NOS **Daughter:** hEDS
428	60-69	F	–	B, C a, f, g	D, F q	H, I u	– v, w	Poa		–	–	**Daughter:** hEDS
475	30-39	F	7	– a, d, g, i	– –	H, I u	– –		–	–	+	**Daughter:** PP, GJH **Son 1:** GJH, IF **Son 2:** GJH, Ftg
495	40-49	F	6	C d, g, i	– –	H s, t, u	– –	PGMW OP Bradycardia	–	–	–	**Daughter:** PGMW, UI **Mother:** PGMW, UI **Sister 1:** PGMW **Sister 2:** PGMW, VV. **Sister's 2 children:** GJH **Maternal aunt:** PGMW, UI, VV
536	40-49	M	1	A, B d, i	D p. r	I u	– –	Dilated cardiomyopath		–	–	–
560	20-29	F	5	A, C d, i	– –	H, I u	– –	–	–	+	–	**Mother:** hEDS **Sister:** hEDS, Filamin A gene mutation in exon 48 (de novo) **Maternal Grandmother:** GJH
566	60-69	M	4	A, C	E	H, I –	J x, y, aa	OP VV	–	–	–	**Father:** TS, My **Mother:** My
584	20-29	F	–	A, C i	– –	H, I u	– –	PE	–	–	–	**Son 1:** hEDS, TS, pneumothorax **Son 2:** GJH, Hyperextensible skin, PE
612	30-39	F	7	C i	– –	H u	– –	–	–	–	–	**Daughter:** hEDS
621	20-29	F	6	A, B i	– –	H, I t, u	– –	Palpitations	–	+	–	**Mother:** GJH, Maternal aunt: GJH, Sister (identical twin): GJH
630	30-39	F	7	C d, f, g	– –	H, I t, u	– y	PGMW MVR, Aortic regurgitation; Tricuspid regurgitation	–	+	–	**Father:** GJH, TS **Paternal grandfather:** GJH, TS **Paternal great grandfather:** GJH, TS
638	40-49	F	–	C d, i	– –	H, I s, t, u	– –	tall stature	–	–	+	**Sister:** hEDS **Father:** TS
650	30-39	F	7	C	– o	H –	– –	Livedo reticularis		+	–	**FHx of GJH** **Maternal aunt:** Pulmonary artery atresia

669	20-29	F	7	A, C d, g, i	E –	H, I s, u	– –	PMGW	–	–	–	**Mother:** PGMW **Sister:** GJH, PGMW **Daughter:** hEDS
670	30-39	F	8	B, C d, e, f, g, h, i	D	H, I u	– –	PMGW	–	–	–	**Mother:** PGMW, GJH **Father:** SS, Dupuytren's contracture **Daughter:** Goldenhaar syndrome, GJH **Son:** GJH, Cleft palate
673	50-59	M	3	C g	D	H u	– –	–	AoR	–	–	**Son:** GJH **Sister:** GJH
681	50-59	F	–	A, C –	– –	H, I t, u	J, L v, y	TS PGMW	–	+	–	**Mother:** GJH **Father:** Aortic aneurysm
682	40-49	F	6	A, C g, i	E q	H, I t, u		Pd	–	+	–	**Mother:** GJH, Pd **Father:** GJH, Pd **Brother:** GJH, Pd **Sister:** GJH, Pd **Maternal aunt:** GJH, Pd
703	10-19	F	–	C –	– –	H t, u	– –	–	–	–	–	–
755	40-49	F	4	A, C d, e	– –	H, I u	J, K –	–	–	+	–	**Father:** TS **2 daughters:** GJH, CHD
761	20-29	M	6	B, C d, f	– –	H, I t, u	J v	tall stature tibial bowing Sco	–	–	+	**Mother:** GJH, **Maternal cousins:** GJH
769	20-29	F	3	C d, g	– –	H s, t, u		brachydactyly	–	+	–	**Mother:** GJH **Maternal mother:** GJH **Maternal grandmother:** GJH **Maternal great grandmother:** GJH **Maternal aunt:** GJH **Father:** GJH, brachydactyly **Paternal grandmother:** OA, OP
778	20-29	F	7	A, C d, i	– –	H, I s, t, u		Palpitations	–	+	+	**Mother:** GJH, Cerebral Hemorrhage **Maternal grandmother:** GJH **Children:** GJH
781	40-49	F	5	A, C f	E –	H, I t, u	– –	–	ICA	+	–	**Father:** GJH, Hyperextensible skin **Paternal grandmother:** GJH, Hyperextensible skin **Children:** GJH **Grandson:** GJH
884	10-19	M	9	C e, f	– –	H, I u	J w	–	–	+	+	**Mother:** hEDS, BS Half-sister: hEDS, BS **Maternal grandmother:** hEDS, BS, IF **Uncle:** hEDS, BS
886	30-39	F	6	C –	– –	H u	– –	–	–	–	–	**Son:** hEDS, BS, GORD **Daughter:** hEDS, BS **Mother:** hEDS, BS, IF **Brother:** hEDS, BS
922	30-39	F	6	A, C f	E k	H, I u	– –	–	–	–	–	**Brother:** GJH, TS, PE
967	10-19	F	8	C a, d, f, i	– –	H, I s, u	– –	–	–	+	–	**Mother:** GJH, PGMW **Maternal grandmother:** PGMW **Maternal aunt:** GJH, PGMW
1263	30-39	F	5	C d, f	D n, r	H, I u	– –	-	-	-	-	-
1289	10-19	F	9	A, C a, d	D	H, I s, u	J y	–	–	–	Raynaud disease_OMI	**Mother:** GJH **Maternal cousin:** GJH
1337	40-49	F	5	C d	E –	H u	– –	–	Carotid artery dissection	+	–	**Mother:** GJH **Sister:** GJH, CHD
1341	30-39	F	8	C d, i	D	H s, t, u	– –	–	–	–	–	**Father:** Shoulder subluxation **Brother:** Shoulder subluxation **Sister:** Shoulder subluxation **Maternal grandfather:** VV **Maternal uncle:** GJH, VV, MVP **Maternal aunt:** VV
1344	40-49	F	-	A, C a, d, h, i	– –	H, I s	– –	OP	-	-	-	**Father:** GJH
1393	0-9	F	5	C d, e, i	– –	H, I s, t, u	– –	-	+	-	-	**Mother:** JHM, HS **Father:** JHM, TS, marfanoid **Brother:** JHM, SS. Multiple maternal relatives with GJH
1397	0-9	F	5	C –	– –	H u	– –	–	–	–	–	**Mother:** hEDS **Brother:** hEDS
1399	30-39	F	4	C d	– –	H s, u	– –	–	–	+	–	**Son**: hEDS **Daughter:** hEDS

1403	40-49	M	7	C a, d	E –	H, I u	J x, y	–	SaH AoR	–	–	**Brothers:** TS **Maternal uncle:** PE **Son:** PE
1421	10-19	M	7	C a	– –	H, I u	– –	–	–	–	–	**Mother:** hEDS **Maternal grandfather:** Abnormality of bladder, GJH
1422	40-49	F	–	A, C f	– –	H, I u	J w	Sco	–	+	–	**Father:** Abnormality of bladder, GJH **Son:** hEDS
1424	0-9	F	9	A, C e	– –	H, I u	– –	PE	-	+	-	**Mother**: GJH **Father:** GJH
1425	20-29	F	-	C –	– –	H	– –	-	-	+	+	**Mother**: GJH **Father:** GJH
1431	30-39	F	3	A, C d, f, g	– r	H, I s, u	– –	CHD	renal pelvis bleed	–	+	**Father:** TS, Kyph, My, RD **Paternal uncle:** My, RD **Paternal aunt:** My, RD **Brother:** My, RD **Paternal cousin:** Sudden cardiac death **Paternal relative:** Sudden cardiac death, GJH
1437	40-49	F	8	A, C –	E –c	H, I u	– –	-	-	+	-	**Father:** GJH **Son:** GJH
1438	10-19	M	5	A, C f	– –	H, I u	J w, y, bb	TS	–	Con	–	**Mother:** GJH, Arthralgia, Dysautonomia **Brother:** hEDS
1439	10-19	M	7	A, C f, g	– –	H, I u	J w, bb	–	–	–	–	**Mother:** GJH, Arthralgia, Dysautonomia **Brother:** hEDS
1443	20-29	F	6	C d, e	– –	H t	– –	-	-	+	+	**Paternal grandmother:** AAA **Maternal grandfather:** ICA
1444	30-39	F	6	– –	– –	H –	– –	-	-	+	+	**Cousin:** GJH
1450	30-39	F	–	B, C a	– –	H, I t, u, PROM	– –	Str	–	–	–	**Mother:** GJH, recurrent miscarriage **Sister:** GJH
1455	50-59	M	6	A, C	– –	H, I u	– –	tall stature OP aortic ejection click	VV	–	–	**Daughter:** GJH, TS
1461	30-39	F	5	C d	– r	H t	– –	–	–	–	+	**Maternal grandfather:** AAA; TS **Nieces from both paternal and maternal side:** GJH
1462	20-29	F	8	C a, d, f	– –	H t	J w, aa	PE OP	–	+	+	**Mother:** GJH, PP, Dysautonomia **Sister:** Arthralgia
1464	70-79	F	–	C	– –	H	– –	–	–	–	–	–
1477	20-29	M	7	C d, i	– –	H, I s, t, u	– –	-	-	+	-	**Brother:** GJH
1482	50-59	F	5	A, C d	D r	H, I s, t, u		tall stature	–	–	–	**Father:** HTAD age 69 **Mother:** GJH, Raynaud disease **Daughter:** GJH **Paternal uncle's daughter:** Knee dislocation
1484	50-59	F	4	C d, h	– –	H s, t, u	– –	–	VV	–	–	**Mother:** VV **Father:** VV **Sisters:** VV **Sons:** pain susceptibility, GJH **Daughter:** pain susceptibility
1491	20-29	F	6	C d, f	– –	H t	– y	–	–	–	–	–
1495	20-29	F	8	C d	– –	H, I t, u	– –	flexion contractures	–	–	+	**Father:** spina bifida **Mother:** GJH
1498	40-49	M	-	A, C i	– –	H, I u	J y, bb	tall stature	-	-	-	**Mother**: GJH **Daughter:** hEDS
1499	10-19	F	5	A, C i	– –	H, I t, u	J, L y, bb	-	-	+	+	**Father:** GJH, Sco
1500	20-29	F	4	B, C d, e, f	E –	H u	– –	-	SaH	-	-	**Mother**: GJH
1502	10-19	F	8	A, C d, e, f	– r	H, I s, t, u	– –	umbilical hernia	Epistaxis	–	+	**Mother:** Epistaxis, GJH, PGMW **Maternal aunt:** Epistaxis **Maternal great-grandmother:** Cerebral Hemorrhage **Father:** TS, Hyperextensible skin **Brother:** GJH
1507	30-39	M	–	B, C a, f, g	– –	H, I u	– –	MVP TS OP Sco	–	–	–	**Mother:** GJH **Sister:** GJH

1511	10-19	M	6	A, C d, i	– –	H, I u	– –	–	–	,	–	**Mother:** GJH **Maternal grandfather:** GJH **Brother:** hEDS **Sister:** GJH
1526	30-39	F	3	C f, g	– –	H u	– –	–	–	,	–	**Mother:** VV, PGMW **Brother:** GJH **Son:** hEDS **Cousins (maternal side):** hEDS
1527	10-19	M	3	C d, f	– –	H u	– –	–	–	–	+	**Mother:** hEDS **Maternal grandmother:** VV, PGMW **Maternal uncle:** GJH
1530	10-19	F	6	– g	– –	H, I u	– –	tall stature	–	–	–	**Mother:** Str **Father:** Str, GJH **Brother:** Str
1579	50-59	F	6	C d, f	– –	H s, t, u	– –	PGMW	–	+	–	**Father:** AAA **Son:** ,
1580	30-39	F	–	C d	– –	H s, t, u	– –	–	–	–	–	**Mother:** GJH
1581	40-49	F	7	C f	– –	H u	– –	–	–	–	–	–
1582	50-59	F	7	C d, e, f	– –	H, I t, u	– –	–	–	–	+	**Son:** hEDS
1595	10-19	F	7	C a	– –	H, I u	– –	–	–	–	–	**Mother:** hEDS **Sister:** GJH **Maternal aunt:** GJH
1596	50-59	F	–	C –	– –	H t, u	– –	–	–	+	+	**Sister:** GJH, Hyperextensible skin **Daughters:** hEDS
1600	20-29	F	8	C d, f	– –	H t, u	– –	PP Sco	–	,	+	**Father:** GJH **Sister:** GJH **Paternal grandfather:** GJH **Paternal uncles:** GJH **Paternal cousin:** GJH
1603	30-39	F	6	C f	– –	H t, u	– –	–	–	+	+	**Paternal grandmother:** GJH
1605	30-39	F	4	– –	– –	H t	– –	-	-	-	+	**N/A**
1607	40-49	F	6	C d, f	– –	H, I t, u	– –	–	–	+	–	**Son:** hEDS
1609	30-39	F	8	C –	– –	H t	– –	–	–	+, Crohn's disease	–	–
1613	50-59	F	5	C a, d	– –	H, I s, t	– –	PP	-	-	-	-
1616	20-29	F	7	C d	– –	H, I s, t	– –	PP	-	-	-	-
1618	30-39	F	8	C d, g	– –	H t	– –	-	-	-	-	-
1620	20-29	M	6	C d, f	– –	H, I t, u	– –					
1626	10-19	F	8	C d	– –	H u	– –	–	–	+	–	–
1629	30-39	F	5	C d, f	– n	H, I s, t, u	– –	PGMW Str	–	,	,	**Sister:** hEDS **Son:** GJH
1630	30-39	F	8	C a, d	– –	H, I t	– –	–	–	+	–	–
1641	30-39	F	7	C d	– –	H u	– –	PP	-	-	-	-
1642	20-29	F	–	C	– –	H t	– –	–	–	–	–	–
1656	20-29	F	7	C d, f	– –	H –	– –	–	–	–	+	–
1665	30-39	F	8	C a, d, f	–	H, I s, t, u	– –	Sco	–	+	+	**Maternal grandmother:** GJH **Nice:** GJH
1666	10-19	F	8	C –	– –	H t	– –	-	-	+		-
1669	30-39	F	8	C d	– –	H, I s, t	– –	PP	-	-	+	-
1681	40-49	F	7	C a, d, f	– –	H, I t	– –	-	-	+	+	-
1682	30-39	F	8	C d	– –	H t	– –	-	-	+	+	-
1695	20-29	F	8	C f	– –	H, I u	– –	-	-	+	+	**Mother**: GJH
1714	40-49	F	5	C –	– –	H t	– –	CHD	-	+	+	-
1717	40-49	F	7	C d	– –	H t	– –	Palpitations	–	,	–	–
1743	20-29	F	7	C d, f	– –	H, I s	– –	Kyph	-	+	-	-

EDS Diagnostic Criteria and Abbreviations as per lists in Supplementary Table 1.

Supplementary Table 4. Phenotypic data for kEDS Patients.

Patient ID	Age	Sex	Beighton score	cEDS Major criteria Minor criteria	vEDS Major criteria Minor criteria	hEDS Major criteria Minor criteria	kEDS Major criteria Minor criteria	Other features	Vascular/cardiac complications	Family History
821	0-9	M	–	C e	– –	H –	J, K, L bb	pectus carinatum	–	**Brother:** Kyphosis, GJH, gross motor delay
1396	0-9	M	7	C e, f	– –	H u	J w	umbilical hernia cutis laxa talipes valgus	–	**Mother, Sister**: hEDS

EDS Diagnostic Criteria and Abbreviations as per lists in Supplementary Table 1.

Supplementary Table 5. Phenotypic data for HDCT Patients.

Patient ID	Age	Sex	Beighton score	cEDS Major criteria Minor criteria	vEDS Major criteria Minor criteria	hEDS Major criteria Minor criteria	kEDS Major criteria Minor criteria	Other features	Vascular Complications	Family History
33	40-49	F	9	A, C a, d, f	E n	H, I s, u	J w, x, y	–	Carotid artery dissection	**Son:** GJH **Father:** GJH **Paternal grandmother:** GJH **Maternal aunt:** Cerebral Hemorrhage
34	30-39	F	3	A, C, d, i	E	H, I	– –	–	Carotid dissection	**Mother:** HS **Maternal grandfather:** HS **Father:** HV **Paternal grandmother:** HV, GJH
35	30-39	F	-	B, C a, f	D, E k, n, r	H –	– –	IF	–	**Mother**: peizogenic papules **Maternal grandfather:** peizogenic papules, Cerebral Hemorrhage
45	50-59	F	5	C –	E –	H u	– –	Pectus, Kyph	Carotid artery dissection	**Mother:** GJH **Brother**: GJH **2 children:** GJH
60	40-49	M	0	A –	E –	I u	– –	–	Carotid artery dissection	**Son:** GJH
72	50-59	M	-	A, C	E j, r	– –	– –	PP, Str Aplasia/Hypoplasia of fingers	–	**Brother:** HTAD **Father:** AAA (in his late 90s) **Mother:** HTAD (in her early 70s)
73	10-19	M	5	A, C f	D, j, r	H, I u	J w, bb	–	Carotid artery stenosis	
79	40-49	M	7	– e, i	– –	– –	– –	PGMW, OP, HV	Aneurysm	**Father:** GJH **Paternal grandmother:** GJH
99	60-69	M	0	A a, d	E	I	– –	Bru, Kyph	Carotid artery dissection	–
422	0-9	F	6	C f	D, F r	H, I u	J –	–	–	**Mother:** GJH **Father:** Str **Brother 1:** JHM, Camp **Brother 2:** JHM, Camp, TS, Bru, Inguinal hernia **Paternal grandfather:** AAA
423	0-9	M	8	A, C a, d	q, r	H, I u	J v, bb			**Mother:** GJH **Father**: Str **Sister: GJH**, TS, AoR, Camp **Brother:** GJH, Camp, Bru, Inguinal hernia **Paternal grandfather:** Aortic aneurysm; TS
446	40-49	M	4	A, C d, i	E f	I u	– –	–	Carotid artery dissection	**Daughter 1:** GJH **Daughter 2:** GJH
453	40-49	F	4	C a	E	– –	– –	OP	Carotid artery dissection	**Mother:** Bru
474	60-69	F	0	– d, f	D, E n	– –	– –	Triangular face, Microretrognathia, High-	Epidural haemorrhage, VV	–
479	20-29	F	6	A, C e, f, g	– –	H, I t	J, K w	PGMW	–	**Mother:** POA **Maternal grandmother:** MVR **Maternal great-grandmother:** Cerebral Hemorrhage

505	10-19	F	–	– g, i	– –	H u	– –	Non-epidermolytic palmoplantar keratoderma	–	**Mother:** hEDS, PGMW **Maternal grandmother, Maternal aunt 1:** PGMW **Maternal aunt 2:** PGMW, VV **Maternal aunt's 2 children,** GJH
526	50-59	F	7	C a, d	– –	H, I –	– –	Lumbar scoliosis, Spondylolithesis, HV, Inflammatory arthropathy	–	**Daughter:** GJH, MVP **Maternal grandmother:** Abnormal heart valve **Maternal cousin:** urinary incontinence **Sister's daughter:** Urinary incontinence, GJH
531	60-69	F	-	C i	– r	– –	– –	–	–	**Father:** GJH, Non-epidermolytic palmoplantar keratoderma **Sister** GJH, Non-epidermolytic palmoplantar keratoderma, Dissecting aortic aneurysm **Daughter:** Non-epidermolytic palmoplantar keratoderma **Maternal grandmother:** GJH
532	40-49	M	2	– –	E –	– –	– –	–	HTAD	–
538	30-39	F	8	C a, d	– –	H, I s, t, u,	– –	FLNA de novo mutn	HTAD	**Mother:** GJH **Sister (pt 560):** GJH
564	20-29	M	8	A, C a, d, g	– –	H, I u	– –	–	AoR	**Father:** hypertrophic obstructive cardiomyopathy **Mother:** GJH **Brother:** hypertrophic obstructive cardiomyopathy
567	50-59	M	4	B –	E –	I –	– –	OP	Aneurysm; (ilio-femoral artery)	–
620	20-29	F	5	C a, d, e, f, i	– –	H, I s, t, u	J, K w, y, bb	Sco, High-arched palate;	–	**Mother:** GJH **Brother:** Occipital horn syndrome, GJH, Kyph
635	40-49	F	7	C a, i	– –	H, I u	– –	Kyph, CHD, High-arched palate	–	**Daughter:** GJH, Spastic diplegia Bru, TS **Son:** GJH, Bru
651	20-29	F	-	C d	D n, r	H, I u	– –	–	VV	**Mother:** Carotid artery aneurysm; VV, TS, GJH
707	10-19	M	1	– a, d, e	– i	I s, t, u	– –	Poa	AoR	**Sister:** GJH,SS **grandmother:** GJH **Paternal aunt:** GJH **Paternal grandmother:** Bru
768	50-59	M	3	C –	E –	– –	– –	Micrognathia, High-arched palate; Kyp, PP	Aortic dissection, (infrarenal), Aneurysm (iliac artery)	–
777	20-29	F	7	C –	D r	– t, u	– –	OP	–	**Mother:** Cerebral aneurysm **Maternal great-grandmother:** Cerebral aneurysm
800	60-69	F	8	C d, g	E n	H –	– –	PE, Hypodontia	–	–

810	10-19	M	8	A, C d, i	D n	H, I s, u	J, K y, aa	HV	–	**Mother:** GJH **Father:** GJH **Brother:** GJH **Brother's daughters:** GJH
814	30-39	F	8	B, C d	D n, r	H s, t, u	J v	PP, HP. PGMW, HD	–	**Father:** Pectus carinatum, GJH **Paternal grandmother:** GJH **Mother:** GJH **Maternal aunt:** GJH **Sister 1:** HTAD **Sister 2:** GJH, TS, Bru, Sco
1387	50-59	M	–	A –	E –	I –	– –	OP	–	**Mother:** Cerebral Haemorrhage, Fibromuscular dysplasia
1394	20-29	M	4	A, B, C g, i	– m	H, I u	– –	Talipes Increased armspan to height ratio	–	**Mother:** hEDS **Father**: GJH, TS **Sister:** GJH,SS **Maternal grandmother:** GJH **Maternal grandfather:** GJH
1420	0-9	M	-	C d	– –	H s, t	– –		–	–
1503	0-9	F	8	C e, f	D r	H t, u	– –	–	–	**Mother:** GJH, SS **Maternal grandmother:** GJH, Subarachnoid haemorrhage **Maternal uncle:** GJH **Brother:** GJH
1504	40-49	F	-	C a, f	D, F n	H, I u	– –	–	–	**Sister:** GJH **Children:** GJH
1625	60-69	F	-	– g	– r	– t	– –	–	AoR	–
1688	30-39	F	6	C d, f	E g	H, I s, t, u	– –	–	Subarachnoid haemorrhage	**Brother:** hEDS
1744	30-39	F	7	– d	– –	– –	– –	Osteochondritis dessicans of ankles	_	**Father:** GJH **Mother,** **Maternal grandmother:** SaH

EDS Diagnostic Criteria and Abbreviations as per lists in Supplementary Table 1.

Supplementary Table 6. Pathogenic and Likely Pathogenic variants in this cohort with detailed phenotypes and ACMG classification and criteria.

Patient ID	Variant ID	Age	Sex	Clinical Diagnosis	Beighton score	Villefranche Criteria Major Minor	Aortic & Other Vascular involvement	Auto. Dom Family History	Skin Biopsy	Gene NM	Protein	Rs ID ClinVar ID (classification)	gnomAD allele frequency	CADD DANN	ACMG classification (See footnote) ACMG criteria
33	1	40-49	F	HDCT	9	A, C, E, H, I, J a, d, f, n, s, u, w, x, y	MVR Carotid dissection	+	normal	TGFB3 NM_003239.4 c.463C>T	p.Arg155Trp	rs868258653 543955 (LP/VUS)	0	33 0.999	LP PM2, PP5 PP3 (Supp)
34	2	30-39	F	HDCT	3	A, C, E, H, I d,i	Carotid artery dissection	+	normal	COL5A1 NM_000093.4 c.4068G>A	Splice	– 1000751 (VUS)	0	14.8 0.808	LP PM2, PP5 PP3 (Supp)
34	3	30-39	F	HDCT	3	A, C, E, H, I d,i	Carotid artery dissection	+	normal	ITGB3 NM_000212.3 c.565C>T	p.Pro189Ser	rs958609406 812735 (P)	0.0000119	28.9 0.999	P PP1, PS3 PS4, PP5 PP3 (S) PM2, PP2
402	4	30-39	M	hEDS Marfanoid	6	A, C, H, I d, i, u	–	+	normal	COL12A1 NM_004370.6 c.5097+1G>A	Splice	–	0.0000119	25.2 0.992	LP PVS1, PM2
479	8	20-29	F	HDCT	6	A, C, H, I, J, K e, f, g, t, w	–	+	normal	SMAD2 NM_001003652.3 c.842A>T	p.Glu281Val	– –	0	33 0.994	LP PM2, PP2 PP3 (S)
564	9	20-29	M	HDCT	8	A, C, H, I a, d, g, u	Aortic dilatation	Biparental	abnormal packing	TGFB2 NM_001135599.3 c.989G>A	p.Arg330His	rs1553303213 440982 (LP)	0	34 0.999	P PM2, PM5 PM1, PP5
755	10	40-49	F	hEDS	4	A, C, H, I, J, K d, e	–	+	normal	COL12A1 NM_004370.6 c.8321G>A	p.Gly2774Glu	–	0	25.7 0.997	P PM2, PP3 (S)
814	14	30-39	F	HDCT	8	B, C, D, H, J d, n, r, s, t, u, v	–	Biparental	abnormal packing	TGFBR2 NM_001024847.2 c.1613T>C	p.Val538Ala	– –	0	26.3 0.998	LP PM1, PM2 PP2 PS3 (ref 16)
1420	17	0-9	M	HDCT	–	C, H d, s, t	–	–	–	ALPL NM_000478.6 c.394G>A	p.Ala132Thr	rs757771793 –	0.000004	33 0.999	P PM1, PP2 PM2, PM5 PP3 (Sup) PP5
1484	18	50-59	F	hEDS	4	C, H d, h, s, t, u			–	COMP NM_000095.3 c.2048G>T	p.Arg683Leu	rs565459602	0.0000239	34 0.999	LP PM2, PP2 PP3 (S)
1528	19	30-39	M	cEDS	–	A, C, H, I d, f, g, k q, s, u	–	–	–	COL5A1 NM_001278074.1 c.3397C>T	p.Arg1133Ter	rs886042045 280931 (P)	0	41 0.998	P PVS1, PP5 PM2

Supplemental Table 6, 7 Keys:
Clinical Diagnosis: expert clinical diagnosis based on history and examination, prior to any diagnostic genetic testing.
Vascular involvement: as stated: – = no known vascular aneurysm/ dissection or aortic root dilatation.
Autosomal Dominant Family History: + = one or more affected individual on either side of the family, biparental = family history of GJH or related phenotypes in both sides of the family.
Skin Biopsy: 3mm punch biopsies were taken from the upper inner arm, with expert review of light microscopy (H&E and elastin van Geisen) and ultrastructural analysis (FMP and Prof. David Ferguson, Univ. of Oxford).

EDS Diagnostic Criteria as per list in Supplementary Table 1.

ACMG criteria as per Richards *et al.* (9): P = pathogenic, LP = likely pathogenic, VUS/LP = variant of uncertain significance close to criteria for LP classification, VUS = variant of uncertain significance, LB = likely benign, B = benign. Individual criteria ((9), Table 3)

VUS* are defined here as including VUS that according to ACGS criteria are "hot", "warm" or "tepid" Variants of Uncertain Significance (Figure 6 of https://www.acgs.uk.com/media/11631/uk-practice-guidelines-for-variant-classification-v4-01-2020.pdf). Segregation analysis, re-evaluation for specific phenotypic features and/or further functional analysis may enable variant reclassification, using ACMG criteria.

Supplementary Table 7. Variants of uncertain significance (CADD> 15) in EDS/LDS/HTAD and syndromic genes in this cohort which are close to Likely Pathogenic classification (VUS*).

Patient ID	Variant ID	Age	Sex	Clinical Diagnosis	Beighton score	Villefranche Criteria Major Minor	Aortic & Other Vascular involvement	Auto. Dom. Family History	Skin Biopsy	Gene. NM	Protein	Rs ID ClinVar ID	gnomAD allele frequency	CADD DANN	ACMG classification (See footnote) ACMG criteria
45	20	50-59	F	HDCT	5	C, E, H u	Carotid dissection	+	abnormal packing	VCAN ENST00000265077.3 c.10063+2dup	?	– –	0	25.2 –	VUS* PM2 PVS1 (M)
72	21	50-59	M	HDCT	–	A, C, E j, r finger aplasia	Femoral artery aneurysm, FHx HTAD	+	–	WNT10A NM_025216.3 c.443C>T	p.Ala148Val	rs373695499 899013 (VUS)	0.0000199	29.9 0.999	VUS* PM2 PP3 (M)
107	22	40-49	M	hEDS	4	E, H, I r, u	FHx Aneurysm	+	normal	KCNH1 NM_172362.3 c.1036A>G	p.Ile346Val (exomiser)	– –	0	– 0.998	VUS* PM2, PP2 PP3 (Supp)
107	23	40-49	M	hEDS	4	E, H, I r, u	FHx aneurysm	+	normal	ULK4 NM_017886.4 c.2979-1G>T	?	– –	0	26.7 0.994	VUS* PM2
474	24	60-69	F	HDCT	0	D, E n	Epidural haemorrhage	–	abnormal	NEDD4L NM_001144967.3 c.2425G>A	p.Asp809Asn HECT domain	rs868820698 956262 (VUS)		26.3 0.998	VUS* PM2 PP3 (Supp) PP2
475	25	30-39	F	hEDS	7	H, I a, d, g, i, u,	–	+	normal	PIEZO2 NM_022068.3 c.713T>G	p.Leu238Trp	rs927091191 427172 (VUS)	0.000142	27.4 0.834	VUS* PM2 PP2
479	26	20-29	F	HDCT	6	A, C, H, I, J, K e, f, g, t, w	–	+	normal	PIEZO1 ENST00000301015.9 c.2492C>T	p.Ser831Leu Transmembrane domain (helical)	rs1471934686 829803 (VUS/LP)	0.000013	32 0.999	VUS* PM2 PP5 (S)
482	27	20-29	F	vEDS	6	C, D, H, I d, g, h, i, t, u	–	Biparental	normal	SCN9A NM_002977.3 c.3930C>G	p.Ile1310Met	rs200947663 –	0	26.2 0.998	VUS* PM2 PP3 (M)
583	29	10-19	F	cEDS	8	A, B, C, H, I, J d, f, g, i, s, t, u	–	+	Small number Cauliflower fibrils	COL5A1 NM_001278074.1c.5130dupG	p.Ser1711ValfsTer67 (exomiser)	rs779189580 –	0.0000166	– 0.957	VUS* PVS1 (Exon 64) PM2
595	31	30-39	M	cEDS	6	A, C, H, I a, d, g, k, q	MVR	+	–	TGFB3 NM_003239.4 c.128T>C	p.Ile43Thr	rs765490133 –	0.00000398	25 0.998	VUS* PM2 PP3 (Supp)
806	35	10-19	M	cEDS	–	B, C, H, J e, I, u	–	+	normal	COL5A1 NM_000093.5 c.5136+151_5136+164del	?	rs762698019 –	0	– 0.957	VUS* (Intron 64) PM2
967	36	10-19	F	hEDS	8	C, H, I a, d, f, i, s, u	–	+	–	FLCN NM_144997.7 c.716G>A	p.Arg239His	rs753948488 253233 (VUS)	0.0000278	34 0.999	VUS* PM2, PM5 PP3 (M)
1002	37	50-59	F	cEDS	7	A, C, H, I d, i, s, u	–	+	Irregular collagen fibrils	MAP3K7 NM_145331.3 c.820C>T	p.Arg274Cys	– –	0	35 0.999	VUS* PM2 PP3 (Supp) PP5
1421	39	10-19	M	hEDS	7	C, H, I a, u	–	+	–	PIEZO2 NM_022068.3 c.6053A>G	p.Tyr2018Cys	rs772793550 –	0.000284	23.1 0.927	VUS* PM2 PP2 PP3 (Supp)
1451	40	10-19	F	cEDS	9	A, C, H, I d, g, i, t	fhx aneurysm	+	–	COL9A3 NM_001853.4 c.130G>A	p.Gly44Ser	rs770649938 –	0.0000495	23.5 0.976	VUS* PM2 (m) PP3 (M)
1495	42	20-29	F	hEDS	7	C, H, I d, t, u	–	+	–	PCNT NM_006031.6 c.8182C>T	p.Arg2728Cys	rs762890408 –	0.0000399	35 0.999	VUS* PM2 PP5
1498	43	40-49	M	hEDS	–	A, C, H, I, J i, u, y, bb	–	+	–	COL6A3 NM_004369.3 c.2042T>G	p.Val681Gly	rs753741086 938432 (VUS)	0.00000398	22.9 0.998	VUS* PM2 PP3 (Supp)

1530	45	10-19	F	hEDS	6	H, I g, u	–	Biparental	–	UPF3B NM_080632.3 c.263+2delT	?	rs118945278 –	0.0000593	25.2 –	VUS* PVS1 (VS)
1607	47	40-49	F	hEDS	6	C, H, I d, f, t, u GI dysfunction	–	+	–	SPTLC1 NM_006415.4 c.287del	p.Asn96MetfsTer6	– –	0	32 –	VUS* PM2
1620	48	20-29	M	hEDS	6	C, H, I d, f, t, u	–	+	–	PIEZO2 NM_022068.3 c.716C>T	p.Pro239Leu	rs776926434 1050407 (VUS)	0.0000071	34 0.973	VUS* PM2 PP2 PP3 (M)
1714	49	40-49	F	hEDS	5	C, H t,	–	–	–	MAT2A NM_005911.6 c.553A>G	p.Thr185Ala	– –	0	25 0.998	VUS* PM2 PP3 (M) PP2

ACMG criteria as per Richards *et al.* (9): P = pathogenic, LP = likely pathogenic, VUS/LP = variant of uncertain significance close to criteria for LP classification, VUS = variant of uncertain significance, LB = likely benign, B = benign. Individual criteria ((9), Table 3)

VUS* are defined here as including VUS that according to ACGS criteria are "hot", "warm" or "tepid" Variants of Uncertain Significance (Figure 6 of https://www.acgs.uk.com/media/11631/uk-practice-guidelines-for-variant-classification-v4-01-2020.pdf). Segregation analysis, re-evaluation for specific phenotypic features and/or further functional analysis may enable variant reclassification, using ACMG criteria.
EDS Diagnostic Criteria and Abbreviations as per lists in Supplementary Table 1.

Supplementary Table 8. Rare variants, (CADD> 15), in genes associated with familial intracranial aneurysm and loci associated with an increased risk of intracranial aneurysm in genome wide association studies (23, 24).

Patient ID	Clinical Diagnosis	Gene NM	Protein	CADD	gnomAD allele frequency	Exon or intron number / total number of exons	ClinVar ID (classification)	Rs ID	ACMG classification (See footnote)	Intracranial Aneurysm	Other vascular Involvement
34	HDCT	TMEM132B NM_052907.3 c.767G>A	p.Arg256Gln	23.3	0.000104	2/9	–	rs377588294	VUS PM2	–	–
54	hEDS	DNAH9 NM_001372.4 c.11678C>T	p.Ser3893Leu	24	0	61/69	–	rs761550523	VUS PM2	+	+
65	hEDS	ANGPTL6 NM_031917.2 c.1208G>A	p.Arg403Gln Fibrinogen like	28.7	0	5/6	–	–	VUS PM2	FHx ICA	–
65	hEDS	HSPG2 NM_005529.7 c.2633G>A	p.Arg878His	26.2	0.000236	21/97	875716 (VUS)	rs149479865	VUS PM2	ICA + FHx ICA	–
70, 884	hEDS	ARHGEF17 NM_014786.4 c.5651G>C	p.Cys1884Ser	22.6	0.000127	19/21	–	rs199726713	VUS PM2	–	–
79	HDCT	DNAH9 NM_001372.4 c.5644G>A	p.Asp1882Asn	31	0.0000398	27/69	–	rs371105048	VUS PM2	–	Aneurysm, NOS
99	HDCT	ARHGEF17 NM_014786.4 c.626G>A	p.Arg209His	28.1	0	1/21	–	–	VUS PM2 BP4 (Supp)	–	carotid dissection
100	hEDS	STARD13 NM_178006.4 c.2888C>A	p.Pro963His	28.2	0	12/14	–	rs1261673521	VUS PM2	+	–
422, 423	HDCT	ADAMTS15 NM_139055.3 c.263T>A	p.Leu88His	17.1	0	1/8	–	–	VUS PM2	–	FHx sudden death
453	HDCT	RNF213 NM_001256071.3 c.9178T>A	p.Phe3060Ile	23.3	0	29/68	–	–	VUS PM2	–	carotid dissection
755	hEDS	TMEM132B NM_052907.3 c.1862C>A	p.Thr621Asn	25.4	0.0000121	7/9	875716 (VUS)	rs776596875	VUS PM2 BP4 (Supp)	–	–
777	HDCT	ARHGEF11 NM_198236.3 c.1019C>T	p.Pro340Leu	22.7	0.00000796	12/14	–	rs1391083996	VUS PM2	ICA	–
1002, 1003	cEDS	RNF213 NM_001256071.3 c.1669G>T	p.Glu557Ter	35	0.00000398	9/68	–	rs755262916	VUS PM2	–	–
1424	hEDS	THSD1 NM_018676.4 c.1858C>T	p.Pro620Ser	22.7	0.00000398	5/5	–	rs1188780320	VUS PM2 BP4 (Supp)	FHx (SDR)	–
1665	hEDS	RNF213 NM_001256071.3 c.12496G>A	p.Asp4166Asn	25.9	0.00033	47/68	–	rs148157068	VUS PM2, BP2	–	–

ACMG criteria as per Richards *et al.* (9): P = pathogenic, LP = likely pathogenic, VUS/LP = variant of uncertain significance close to criteria for LP classification, VUS = variant of uncertain significance, LB = likely benign, B = benign. Individual criteria ((9), Table 3)

VUS* are defined here as including VUS that according to ACGS criteria are "hot", "warm" or "tepid" Variants of Uncertain Significance (Figure 6 of https://www.acgs.uk.com/media/11631/uk-practice-guidelines-for-variant-classification-v4-01-2020.pdf).

Segregation analysis, re-evaluation for specific phenotypic features and/or further functional analysis may enable variant reclassification, using ACMG criteria.

Supplementary Table 9. Rare variants of uncertain significance, (CADD> 15), in genes associated with EDS (1), as per gene list in Supplementary Methods.

Patient ID	Clinical Diagnosis	Gene NM	Protein	CADD	gnomAD allele frequency	Exon or intron number / total number of exons	ClinVar (Classification)	Rs ID	DANN	ACMG Classification (See footnote)
60	HDCT	COL6A1 NM_001848.2 c.2821C>T	p.Leu941Phe	23.5	0.000133	35/35	196948 (VUS/ LB)	rs147882179	0.994	VUS PM2, BP6
73	HDCT	COL6A1 NM_001848.2 c.1315C>T	p.Arg439Trp	29.8	0.0000309	19/35	662422 (VUS)	rs368239109	0.991	VUS PM2, BP6
372	vEDS	COL6A1 NM_001848.2 c.2873C>A	p.Ala958Asp	24.4	0.0000931	35/35	284877 (LB/ VUS)	rs763228065	0.997	VUS PM2, BP6
385	hEDS	C1R NM_001733.7 c.1286G>A	p.Cys377Tyr	–	0	8/9	–	–	0.999	VUS PM2
428	hEDS	COL6A3 NM_004369.3 c.3878A>G	p.Asp1293Gly	22.6	0	9/44	–	rs1222267030	0.998	VUS PM2
482	vEDS	COL6A3 NM_004369.3 c.3923G>A	p.Arg1308Gln	15.42	0.995	9/44	199093 (VUS)	rs774461787	0.995	VUS PM2, BP6
495	hEDS	COL5A1 NM_000093.5 c.3852+5G>T	Splice	–	0	48 / 65	–	rs763999542	0.733	VUS PM2 PP3 (Supp)
536	hEDS	COL12A1 NM_004370.6 c.1906A>G	p.Lys636Glu	14.72	0.0000163	11/66	–	rs754916465	0.991	VUS PM2 BP4 (Supp)
566	hEDS	COL6A2 NM_001849.3 c.2558G>T	p.Arg853Leu	22.1	0	28/28	–	–	0.961	VUS PM2
620	HDCT	COL12A1 NM_004370.6 c.6724+5G>A	Splice	20.1	0.00000405	41/65	–	rs746208956	0.966	VUS PM2 PP3 (Supp)
635	HDCT	COL6A1 NM_001848.2 c.3053A>G	p.His1018Arg	17.8	0.00000402	35/35	–	rs1310931207	0.967	VUS PM2
651	HDCT	COL6A3 NM_004369.3 c.8377G>A	p.Val2793Ile	19.41	0.0000159	38/44	500364 (VUS)	rs569907876	0.937	VUS PM2, BP6
768	HDCT	COL6A3 NM_004369.3 c.8377G>A	p.Val2793Ile	19.41	0.0000159	38/44	500364 (VUS)	rs569907876	0.937	VUS PM2, BP6
803	cEDS	COL6A2 NM_001849.3 c.1829G>A	p.Arg610His	23	0.0000519	25/28	896443 (LB/ VUS)	rs758550765	0.996	VUS PM2, BP6
806	cEDS	COL6A3 NM_004369.3 c.3754C>T	p.Arg1252Cys	24.6	0.000124	9/44	285636 (VUS)	rs563530370	0.999	VUS PM2, BP6 PP3 (M)
821	kEDS	COL6A3 NM_004369.3 c.4510C>T	p.Arg1504Trp	24.2	0.000434	9/43	166943 (VUS)	rs144223596	0.997	VUS PM2, BP6

1397	hEDS	COL1A1 NM_000088.4 c.3754C>T	p.Arg1252Cys	26.3	0.000012	48/51	1037654 (VUS)	rs781614679	0.998	VUS PM2 PP2 PP3 (Supp) BP6
1421	hEDS	C1R NM_001733.7 c.419C>T	p.Ala140Val	29.5	0.000135	3/11	–	rs200539827	0.999	VUS PM2 PP3 (Supp)
1451	cEDS	COL5A1 NM_000093.5 c.3013A>G	p.Thr1005Ala	18.24	0	39/66	212954 (VUS)	–	0.943	VUS PM2
1451	cEDS	COL5A1 NM_000093.5 c.3874G>A	p.Glu1292Lys	21.7	0	49/66	955996 (VUS)	–	0.993	VUS PM2
1502	hEDS	C1R NM_001733.7 c.158G>T	p.Gly52Val	32	0.00000408	2/11	–	rs1181587267	0.998	VUS PM2
1528	cEDS	COL1A1 NM_000088.4 c.1200+5G>A	Splice	21	0.00004501	18/50	566740 (VUS)	rs374322003	0.98	VUS PM2 PP3 (Supp)
1581	hEDS	COL5A2 NM_000393.5 c.4085A>G	p.Tyr1362Cys	24	0.0000279	52/54	573793 (VUS)	rs141206016	0.989	VUS PM2 PP3 (Supp)
1600	hEDS	COL6A3 NM_004369.3 c.7133C>G	p.Ala2378Gly	15.19	0	34/44	–	–	0.843	VUS PM2
1604	hEDS	COL6A2 NM_001849.4 c.1336G>A	p.Asp446Asn	24.8	0.000418	16/28	194621 (B/LB/VUS)	rs535007570	0.993	VUS BP6
1642	hEDS	COL6A3 NM_004369.3 c.7670T>A	p.Ile2557Asn	22.1	0.0000239	41/44	577635 (VUS)	–	0.932	VUS PM2

Key: ACMG criteria as per Richards et al. ref 9: P = pathogenic, LP = likely pathogenic, VUS/LP = variant of uncertain significance close to criteria for LP classification, VUS = variant of uncertain significance, LB = likely benign, B = benign. Individual criteria ((9), Table 3)

VUS* are defined here as including VUS that according to ACGS criteria are "hot", "warm" or "tepid" Variants of Uncertain Significance (Figure 6 of https://www.acgs.uk.com/media/11631/uk-practice-guidelines-for-variant-classification-v4-01-2020.pdf).

Segregation analysis, re-evaluation for specific phenotypic features and/or further functional analysis may enable variant reclassification, using ACMG criteria.

Supplementary Table 10. Rare variants of uncertain significance (CADD> 15) in genes associated with HTAD as per gene list in Supplementary Methods.

Patient ID	Clinical Diagnosis	Gene NM	Protein	CADD	gnomAD allele frequency	Exon or intron number / total number of exons	ClinVar ID. classification	Rs ID	DANN	ACMG classification (See footnote)	Vascular Involvement
65	hEDS	ROBO4 NM_019055.6 c.1475G>A	p.Arg492Gln	29.8	0.0000243	9/18	–	rs777639467	0.999	VUS PM2	femoral artery aneurysm
72	HDCT	ROBO4 NM_019055.6 c.713T>C	p.Leu238Pro	18.22	0.00000398	5/18	–	rs1446614640	0.966	VUS PM2	FHx HTAD
372	vEDS	SMAD3 NM_005902.4 c.207-3C>A	Splice	17.52	0.0000119	Int 1/8	580639 (VUS)	rs757772685	0.967	VUS PM2 PP3 (Supp)	N
428	hEDS	FBN2 NM_001999.4 c.3686C>A	p.Pro1229His	–	0.00000796	26/65	–	rs151192448	0.993	VUS PM2	N
453	HDCT	PRKG1 NM_006258.4 c.1427_1428insTACTAACACTTTGTATCAACGTTTAAGTTAGACAATACTTGTGCAAACTCT	p.Arg477ThrfsTer31	35	0	13/18	–	–	–	VUS	carotid artery dissection
475	hEDS	TGFBR1 NM_004612.4 c.214A>T	p.Ile72Leu	12.24	0.000199	2/9	178136 (VUS/LB)	rs111513627	0.976	VUS PM2, PP2 BP6	N
534	cEDS	FBN2 NM_001999.4 c.2536G>A	p.Glu846Lys	28.8	0.000135	25/71	213392 (LB/VUS)	rs375666281	–	VUS PM2, BP6	N
538	hEDS	FLNA NM_001110556.2 c.7813del	p.Leu2605TrpfsTer2	35	0	48/48	– –	–	–	P, reported PMID: **23032111**	AoR
560, 538	HDCT (538), hEDS (560)	PRKG1 NM_006258.4 c.980C>A	p.Thr327Asn	22.8	0.0000279	8/18	520129 (VUS)	rs138485549	0.989	VUS PM2	N
611	cEDS	FBN2 NM_001999.4 c.4328A>T	p.Asp1443Val	34	0.0000875	39/71	411817 (VUS/LB)	rs751400994	0.999	VUS PM2, PP3 (M) BP6	N
638	hEDS	NOTCH1 NM_017617.5 c.2935C>T	p.His979Tyr	24.1	0.00000402	18/37	–	rs1380298048	0.997	VUS PM2, PP2 BP6	N
651	HDCT	MYLK NM_053025.3 c.571C>G	p.Gln191Glu	19.02	0	7/34	198605 (VUS)	rs794727880	0.59	VUS PM2 BP4 (Supp)	fhx AoR
681	hEDS	TGFBR2 NM_003242.6 c.95-7T>C	?	–	0.0000083	Int 1/6	–	rs1386890539	0.873	VUS PM2 BP4 (Supp)	fhx aneurysm
755	hEDS	NOTCH1 NM_017617.5 c.1843G>A	p.Gly615Arg	28.4	0.00000818	11/34	576931 (VUS/LB)	rs764942073	0.999	VUS PM2, PP3 (M) PP2, BP6	N
798	vEDS	MYLK NM_053025.3 c.5477C>T	p.Ala1826Val	26.9	0.000291	33/34	252775 (LB/VUS)	rs147187907	0.999	VUS PM2, BP6	cavernoma
1393	hEDS	BGN NM_001711.6 c.1000G>A	p.Gly334Ser	33	0	8/8	–	rs1209725855	0.999	VUS PM2	AoR

1399 &1397	hEDS	ELN NM_000501.4 c.1543G>A	p.Val515Met	16.95	0.0000437	11/33	1008316 (VUS)	rs376258672	0.946	VUS PM2 BP4 (Supp)	N
1403	hEDS	TGFB2 NM_001135599.3 c.727G>T	p.Asp243Tyr	29.3	0	4/8	–	–	0.996	VUS PM2 PP3 (Supp)	AoR ICA
1421	hEDS	MFAP5 NM_002403.4 c.383G>A	p.Arg128His	32	0.00000796	8/9	–	rs373562256	0.999	VUS PM2 (M)	N
1443	hEDS	SMAD6 NM_005585.5 c.872T>C	p.Leu291Pro splice –3.	24.9	0.00000398	2/4	–	rs768096418	0.999	VUS PM2	fhx aneurysm
1600	hEDS	MYH11 NM_001040114.1 c.3895G>A	p.Val1299Ile	25.4	0.0000358	30/42	547546 (VUS/LB)	rs151058774	0.996	VUS PM2, BP6	N
1607	hEDS	FBN1 NM_000138.4 c.6819G>A	p.Met2273Ile	21.8	0.0000279	56/66	450683 (LB/VUS)	rs778027769	0.975	VUS PM2, PP2 BP6	N
1629	hEDS	SMAD6 NM_005585.5 c.475C>A	p.Arg159Ser MH1 domain	14.29	–	1/4	–	–	0.995	VUS PM2	N

Key: ACMG criteria as per Richards et al. ref 9: P = pathogenic, LP = likely pathogenic, VUS/LP = variant of uncertain significance close to criteria for LP classification, VUS = variant of uncertain significance, LB = likely benign, B = benign. Individual criteria ((9), Table 3)

VUS* are defined here as including VUS that according to ACGS criteria are "hot", "warm" or "tepid" Variants of Uncertain Significance (Figure 6 of https://www.acgs.uk.com/media/11631/uk-practice-guidelines-for-variant-classification-v4-01-2020.pdf).

Segregation analysis, re-evaluation for specific phenotypic features and/or further functional analysis may enable variant reclassification, using ACMG criteria.

Supplementary Table 11. Rare variants, (CADD> 15), in genes associated with syndromes with EDS associated features and Mendelian disorders with EDS symptomatology.

Patient ID	Clinical Diagnosis	Gene NM	Protein	CADD	gnomAD allele frequency	Exon or intron number / total number of exons	ClinVar ID classification	Rs ID	DANN	Vascular Involvement	ACMG classification (See footnote)
75	cEDS	PIEZO2 NM_022068.3 c.3236A>G	p.Tyr1079Cys	26.2	0.00027	22/52	430213 (VUS)	rs192225494	0.980	–	VUS PM2, PP2
79	HDCT	EMILIN NM_007046.3 c.82G>A	p.Gly28Ser	25.6	0	1/8	–	rs1174686741	0.998	aneurysm	VUS PM2
107	hEDS	IFIH1 NM_022168.4 c.2242G>A	p.Gly748Arg	–	0.0000119	11/16	1428095 (VUS)	rs764553894	0.999	fhx aneurysm	VUS PM2
385	hEDS	LAMA5 NM_005560.6 c.2623C>A	p.Arg875Ser Domain 4b	28.9	0.00000416	22/80	–	rs371962250	0.997		VUS PM2 BP4 (Supp)
396	cEDS	SCN9A NM_002977.3 c.2102C>G	p.Pro701Arg	23.5	0.00000485	14/27	376819 (VUS)	rs867106113	0.995	subclavian artery	VUS PM2 PP3 (Supp)
396	cEDS	ATP7A NM_000052.7 c.3790A>G	p.Ile1264Val	19.5	0	19/23	573762 (VUS)	rs782323741	0.996	subclavian artery	VUS PM2
397	hEDS	KCNH1 NM_172362.3 c.2762C>A	p.Thr921Lys	16.5	0	11/11	–	–	0.97	–	VUS PM2, PP2
422	HDCT	MED12 NM_005120.3 c.6201_6227del	p.Gln2068–Gln 2076del In frame Deletion	19.11	0	42/45	–	–	–	–	VUS PM2, BP3
475	hEDS	SYNE1 NM_182961.4 c.18193C>T	p.Arg6065Trp	35	0.0000398	96/146	284767 VUS	rs200209279	0.999	–	VUS PM2, BP6
505	HDCT	EMILIN NM_007046.4 c.1877T>A	p.Leu626Gln	26.2	0	4/8	–	–	0.996	–	VUS PM2
526	HDCT	IFIH1 NM_022168.4 c.2962G>A	p.Val988Ile	31	0	16/16	574103 (VUS)	rs74162090	0.998	fhx MVP, aortic valve dis.	VUS PM2
620	HDCT	SDSL NM_138342.4 c.626C>T Homozygous	p.Ala209Val	23	0.001 (0 homozy)	7/9	–	rs144688002	0.998	–	VUS PM2
635	HDCT	SYNE1 NM_182961.4 c.19730G>A	p.Arg6577Gln	32	0.000346	107/146	288606 (LB/VUS)	rs150387338	0.999	–	VUS/ LB BS2, BP6
718	cEDS	EMILIN NM_007046.4 c.2116C>T	p.Arg706Cys	26.2	0.0000119	4/8	–	rs747249536	0.999	–	VUS PM2
768	HDCT	IFIH1 NM_022168.4 c.1783C>T	p.Arg595Cys	26.6	0.0000165	10/16	–	rs191839015	0.997	infrarenal aortic dissection	VUS PM2
777	HDCT	MYH2 NM_001100112.1 c.1115G>A	p.Arg372His	35	0.0000119	12/40	–	rs750569547	0.999	FHx ICA	VUS PM2, PP3 (M)
806	cEDS	ACAN NM_013227.3 c.7204C>T	p.Arg2402Cys	34	0.0000161	17/19	1493820 (VUS)	rs751606366	0.999	–	VUS PM2
1464, 1620	hEDS	LAMA5 NM_005560.6 c.3964G>A	p.Gly1322Ser Domain 4b	32	0.000324	31/80	–	rs150741810	0.999	–	VUS PM2

1526	hEDS	WNK1 NM_213655.4 c.3188C>T	p.Ser1063Leu	16.8	0	9/28	–	–	0.996	–	VUS PM2 (m) BP4 (Supp)
1528	cEDS	WNK1 NM_001184985.1 c.3815G>T	p.Gly1272Val	23.5	0.00000795	12/28	–	rs750516612	0.697	–	VUS PM2, BP6
1530	hEDS	KIT NM_000222.3 c.867G>A	p.Met289Ile	22.1	0	5/21	–	–	0.993	–	VUS PM2 BP4 (Supp)
1596	hEDS	SYNE1 NM_182961.4 c.18679C>T	p.Arg6227Trp	34	0.0000517	99/146	284132 (VUS)	rs201873107	0.999	–	VUS PM2, BP6
1605	hEDS	LAMA5 NM_005560.6 c.2248G>A	p.Val750Met laminin EGF like 9 & disulfide	27.6	0.000112	18/80	2077900 (VUS)	rs201119098	0.999	–	VUS PM2

ACMG criteria as per Richards et al. ref 9: P = pathogenic, LP = likely pathogenic, VUS/LP = variant of uncertain significance close to criteria for LP classification, VUS = variant of uncertain significance, LB = likely benign, B = benign.

VUS* are defined here as including VUS that according to ACGS criteria are "hot", "warm" or "tepid" Variants of Uncertain Significance (Figure 6 of https://www.acgs.uk.com/media/11631/uk-practice-guidelines-for-variant-classification-v4-01-2020.pdf).

Segregation analysis, re-evaluation for specific phenotypic features and/or further functional analysis may enable variant reclassification, using ACMG criteria.

Supplementary Table 12. Rare variants, (CADD>15), in genes in linked regions for hEDS (Syx et al. ref 26).

Patient ID	Clinical Diagnosis	Rs ID	CADD DANN	Current Gene annotation	Gene	Exon or intron number / total number of exons	HGVSc	HGVSp	gnomAD allele frequency	ACMG classification (See footnote)
60	HDCT	rs376054888	25.5 0.997	a)	FGL1	6/10	ENST00000398056.2c.284G>C	ENSP00000381p.Gly95Ala	0.00007318	
65	hEDS	rs150106411	21.5 0.983	a)	POLR3D	6/8	ENST00000397802.4c.671G>A	ENSP00000380904.3 p.Arg224Gln	0	
65	hEDS	rs150161793	15 0.989	b)	BMP1	18/20	ENST00000306385.5c.2446C>G	ENSP00000305714.5 p.Pro816Ala	0.0001382	VUS PM2
73	HDCT	–	26.6	a)	CCAR2	17/20	ENST00000308511.4c.2220+1G>A	splice variant	0	
74	hEDS	rs760116990	34	a)	NPM2	5/9	ENST00000397940.1c.302_303del	ENSP00000381032.1 p.Pro101Argfs Ter21 pLi = 0	0.00006498	
107	hEDS	–	23.6 0.996	a)	PCM1	9/39	ENST00000325083.8c.1268A>G	ENSP00000327077.8 p.Gln423Arg	0	
136	cEDS	rs61756237	14.37 0.975	c)	TNFRSF10B	9/9	ENST00000276431.4c.1127C>T	ENSP00000276431.4 p.Ala376Val	0.0001584	VUS PM2
191	hEDS	rs35294054	34 0.999	a)	PDGFRL	4/7	ENST00000541323.1c.370C>T	ENSP00000444211.1 p.Arg124Cys	0.0002507	
383	cEDS	–	29.9 0.998	a)	PCM1	31/39	ENST00000325083.8c.5012A>G	ENSP00000327077.8 p.Asp1671Gly	0	
396	cEDS	–	24.6 0.998	a)	ADAM7	10/22	ENST00000175238.6c.905G>C	ENSP00000175238.5 p.Gly302Ala	0	
397	hEDS	–	24.6 0.998	a)	ADAM7	10/22	ENST00000175238.6c.905G>C	ENSP00000175238.5 p.Gly302Ala	0	
564	HDCT	–	29.4 0.984	a)	PCM1	27/39	ENST00000325083.8c.4523A>C	ENSP00000327077.8 p.Asp1508Ala	0	
583	cEDS	–	14.82 0.818	a)	DOCK5	2/52	ENST00000276440.7c.58A>G	ENSP00000276440.7 p.Asn20Asp	0	
583	cEDS	rs762023686	34 0.999	a)	SORBS3	18/21	ENST00000240123.7c.1496C>T	ENSP00000240123.7 p.Thr499Met	0.00001229	
595	cEDS	rs201363003	20.7 0.998	a)	CCAR2	13/21	ENST00000308511.4c.1535G>A	ENSP00000310670.4 p.Arg512His	0.00004874	
650	hEDS	rs748585448	33 0.996	a)	PDLIM2	3/10	ENST00000308354.7c.979C>T	ENSP00000312634.7 p.Arg327Trp	0.00003242	
673	hEDS	rs376663203	28.2 0.998	a)	DOCK5	7/52	ENST00000276440.7c.485A>G	ENSP00000276440.7 p.Asp162Gly	0.00007929	
703	hEDS	rs150225368	22.8 0.997	a)	LZTS1	4/4	ENST00000381569.1c.1483G>A	ENSP00000370981.1 p.Glu495Lys	0.0005212	
707	HDCT	rs769203969	16.53 0.956	a)	PCM1	3/39	ENST00000325083.8c.32G>T	ENSP00000327077.8 p.Gly11Val	0.00002043	

718	cEDS	rs143724214	14.58 0.892	b), c)	SLC39A14	3/9	ENST0000035 9741.5c.395C> T	ENSP0000035 2779.5 p.Ser132Leu	0.00013	VUS PM2 BP4 (Supp)
769	hEDS	–	24.5 0.999	a)	ADAM28	9/23	ENST0000026 5769.4c.737A >G	ENSP0000026 5769.4 p.Asn246Ser	0	
798	vEDS	rs746383239	24.7 0.996	b)	CSGALNACT1	5/10	ENST0000045 4498.2c.845A >C	ENSP0000041 1816.2 p.Asn282Thr	0.00002437	VUS PM2
821	kEDS	–	14.77 0.826	c)	SFTPC	4/6	ENST0000031 8561.3c.426C> A	ENSP0000031 6152.3 p.His142Gln	0	VUS PM2
1346	vEDS	rs760460873	17.35 0.995	a)	DOCK5	8/52	ENST0000027 6440.7c.649A >G	ENSP0000027 6440.7 p.Ser217Gly	0.000008135	
1464	hEDS	rs369514263	17.1 0.987	a)	FGL1	5/10	ENST0000039 8056.2c.82C> G	ENSP0000038 1133.2 p.Gln28Glu	0.00002849	
1484	hEDS	–	26.3 0.997	a)	FGF17	3/5	ENST0000035 9441.3c.211C> T	ENSP0000035 2414.3 p.Arg71Cys	0	
1498	hEDS	rs758593640	35 0.999	a)	CCAR2	18/21	ENST0000030 8511.4c.2269C >T	ENSP0000031 0670.4 p.Arg757Trp	0.000008122	
1499	hEDS	rs758593640	35 0.999	a)	CCAR2	18/21	ENST0000030 8511.4c.2269C >T	ENSP0000031 0670.4 p.Arg757Trp	0.000008122	
1504	HDCT	rs771448146	18.04 0.968	a)	PCM1	31/39	ENST0000032 5083.8c.5132C >A	ENSP0000032 7077.8 p.Thr1711Asn	0	
1524	cEDS	rs774318933	25.5 0.998	a)	PDGFRL	7/7	ENST0000054 1323.1c.1004C >T	ENSP0000044 4211.1 p.Thr335Met	0.00001219	
1528	cEDS	rs749514722	14.15 0.915	a)	ADAM7	12/22	ENST0000017 5238.6c.1156 A>C	ENSP0000017 5238.5 p.Lys386Gln	0.000004076	
1582	hEDS	rs374187681	17.51 0.998	c)	ASAH1	10/14	ENST0000038 1733.4: c.766A>C	ENSP0000037 1152.4 p.Ile256Leu	0.00006906	VUS PM2 PP2
1582	hEDS	rs145928227	23.5 0.994	a)	CCAR2	12/21	ENST0000030 8511.4c.1235 A>T	ENSP0000031 0670.4 p.Gln412Leu	0.00002847	
1616	hEDS	–	13.44 0.991	b)	CSGALNACT1	10/10	ENST0000045 4498.2:c.1548 A>G	ENSP0000041 1816.2 p.Ile516Met	0.00001218	VUS PM2
1630	hEDS	rs78484373	15.81 0.891	a)	FGL1	5/10	ENST0000039 8056.2c.113G >A	ENSP0000038 1133.2 p.Arg38His	0.00003658	
1665	hEDS	rs149782492	27.4 0.999	a)	SORBS3	18/21	ENST0000024 0123.7c.1549C >T	ENSP0000024 0123.7 p.Arg517Trp	0.00006939	

Current gene annotation:
a) Germline variants in this gene not currently associated with Mendelian disorder
b) Germline variants in this gene associated with disorder of bone metabolism or skeletal dysplasia
c) Germline variants in this gene associated with non-EDS / HTAD phenotype

ACMG classification as per Richards et al. (9): P = pathogenic, LP = likely pathogenic, = variant of uncertain significance close to criteria for LP classification, VUS = variant of uncertain significance, LB = likely benign, B = benign.

VUS* are defined here as including VUS that according to ACGS criteria are "hot", "warm" or "tepid" Variants of Uncertain Significance (Figure 6 of https://www.acgs.uk.com/media/11631/uk-practice-guidelines-for-variant-classification-v4-01-2020.pdf).

Segregation analysis, re-evaluation for specific phenotypic features and/or further functional analysis may enable variant reclassification, using ACMG criteria.

Supplementary Table 13. Rare germline variants (CADD> 15) in genes previously published in a linkage study (29) and genome wide association studies associated with, (p < 5 x 10-8), pelvic organ prolapse (PMID: 32184442), knee pain and rotator cuff injury (https://www.ebi.ac.uk/gwas/)

Patient ID	Clinical Diagnosis	Current Gene annotation	Gene	HGVSc	HGVSp	CADD	Rs ID	Exon or Intron / Total no. exons	gnomAD allele frequency	ACMG Classification (See footnote)
79	HDCT	c)	LAMC2	ENST00000264144.4 c.1669T>C	ENSP00000264144.4 p.Tyr557His	24	–	11/23	0	VUS PM2 PP3 (Supp)
100	hEDS	a)	HAS1	ENST00000222115.1 c.874G>A	ENSP00000222115.1 p.Glu292Lys	33	–	3/5	0	
136	cEDS	c)	TBX5	ENST00000310346.4 c.1203G>T	ENSP00000309913.4 p.Trp401Cys	33	rs377649723	9/9	0.00001221	VUS PM2
383	cEDS	a)	HAS1	ENST00000222115.1 c.1679G>A	ENSP00000222115.1 p.Trp560Ter	40	rs200444967	5/5	0.0001912	
428	hEDS	c)	FAT4	ENST00000394329.3 c.11147G>A	ENSP00000377862.3 p.Arg3716His	21.9	rs139635339	9/17	0.00013	VUS PM2
474	HDCT	c)	LAMC2	ENST00000264144.4 c.1105C>T	ENSP00000264144.4 p.Arg369Cys	34	rs552102778	9/23	0.0000008122	VUS PM2
495, 505	hEDS (495), HDCT (505)	c)	ROBO2	ENST00000487694.3 c.2066G>A	ENSP00000417335.2 p.Arg689His	34	rs376737394	15/27	0.0001099	VUS PM2 PP3 (Supp)
560	hEDS	c)	LAMC3	ENST00000361069.4 c.236C>T	ENSP00000354360.4 p.Ala79Val	27.2	rs186188737;rs772194826	1/28	0.00009384	VUS PM2
566	hEDS	c)	TBX5	ENST00000310346.4 c.330C>G	ENSP00000309913.4 p.Asp110Glu	24.5	–	4/9	0	VUS PM2 PP3 (Supp)
630	hEDS	c)	LAMC3	ENST00000361069.4 c.449G>A	ENSP00000354360.4 p.Arg150His	31	rs774775769	2/28	0.00001224	VUS PM2 PP3 (M)
967	hEDS	c)	FAT4	ENST00000394329.3 c.10063A>G	ENSP00000377862.3 p.Ile3355Val	22.5	–	9/17	0	VUS PM2
1263	hEDS	c)	SALL1	ENST00000251020.4 c.2920T>C	ENSP00000251020.4 p.Ser974Pro	20.6	rs144429956	2/3	0.00002034	VUS PM2 PP3 (Supp)
1393	hEDS	c)	LAMC3	ENST00000361069.4 c.1682C>T	ENSP00000354360.4 p.Thr561Ile	22.1	rs199701268	10/28	0	VUS PM2 BP4 (Supp)
1403	hEDS	c)	LAMC2	ENST00000264144.4 c.1079T>C	ENSP00000264144.4 p.Ile360Thr	25.7	–	9/23	0	VUS PM2
1421	hEDS	a)	HOOK3	ENST00000307602.4 c.1945A>T	ENSP00000305699.3 p.Lys649Ter	48	–	21/22	0	
1450	hEDS	a)	HAS1	ENST00000222115.1 c.1679G>A	ENSP00000222115.1 p.Trp560Ter	40	rs200444967	5/5	0.0001912	

1495	hEDS	c)	TBX5	ENST00000310346.4 c.113C>G	ENSP00000309913.4 p.Ser38Cys	25.6	–	2/9	0	VUS PM2
1626	hEDS	c)	SALL1	ENST00000251020.4 c.1673C>T	ENSP00000251020.4 p.Pro558Leu	20.2	–	2/3	0	VUS PM2 BP4 (Supp)
1642	hEDS	a)	LAMC1	ENST00000258341.4 c.4729C>T	ENSP00000258341.3 p.Arg1577Ter	37	rs1031794706	28/28	0	
1642	hEDS	a)	ADAM33	ENST00000356518.2 c.706C>T	ENSP00000348912.2 p.Arg236Cys	34	rs750423431	8/22	0.000004061	

Current gene annotation:

a) Germline variants in this gene not currently associated with Mendelian disorder

b) Germline variants in this gene associated with disorder of bone metabolism or skeletal dysplasia

c) Germline variants in this gene associated with non-EDS / HTAD phenotype

ACMG classification as per Richards et al. (9): P = pathogenic, LP = likely pathogenic, = variant of uncertain significance close to criteria for LP classification, VUS = variant of uncertain significance, LB = likely benign, B = benign.

VUS* are defined here as including VUS that according to ACGS criteria are "hot", "warm" or "tepid" Variants of Uncertain Significance (Figure 6 of https://www.acgs.uk.com/media/11631/uk-practice-guidelines-for-variant-classification-v4-01-2020.pdf).

Segregation analysis, re-evaluation for specific phenotypic features and/or further functional analysis may enable variant reclassification, using ACMG criteria.

Supplementary Table 14. Rare germline variants (CADD> 15) in genes previously published as abnormally expressed in skin fibroblasts from hEDS patients (15), list of genes in supplementary methods.

Patient ID	Clinical Diagnosis	Rs ID	CADD/ DANN	Current Gene annotation	Gene	Exon or intron / total number of exons	HGVSc	HGVSp Domain	gnomAD allele frequency	ACMG classification (See footnote)
34	HDCT	rs752525603	10.24 0.868	c)	ITGB3	1/15	ENST00000559488.1 c.16C>T	ENSP00000452786.1 p.Arg6Trp Signal Peptide	0.0002439	VUS PM2 PP2 BP4 (Supp)
45	HDCT	rs781077349	22.5 0.995	a)	ILKAP	7/12	ENST00000254654.3 c.571C>A	ENSP00000254654.3 p.Leu191Ile Metal ion binding, pLi=0.98	0.00002437	
61	hEDS	rs370293437	27 0.999	a)	C1QTNF9B	1/3	ENST00000382137.3 c.139G>A	ENSP00000371572.3 p.Gly47Arg Collagen like	0.00001629	
75	cEDS	rs140610274	29.5 0.998	c)	TNFAIP3	8/9	ENST00000237289.4 c.2036T>C	ENSP00000237289.4 p.Ile679Thr NFKB regulator	0.00009745	VUS PM2
385	hEDS	rs150777320	23.1 0.989	b)	TNFRSF11B	2/5	ENST00000297350.4 c.104C>A	ENSP00000297350.4 p.Thr35Asn Repeat region	0.0001422	VUS PM2 BS2
395	hEDS	rs747279227	21.3 0.991	a)	TNFRSF10A	4/10	ENST00000221132.3 c.614G>T	ENSP00000221132.3 p.Arg205Leu Repeat region	0.00002031	
395	hEDS	rs747279227	21.3 0.991	a)	TNFRSF10A	4/10	ENST00000221132.3 c.614G>T	ENSP00000221132.3 p.Arg205Leu, Repeat region	0.00002031	
397	hEDS	rs747279227	21.3 0.991	a)	TNFRSF10A	4/10	ENST00000221132.3 c.614G>T	ENSP00000221132.3 p.Arg205Leu Repeat region	0.00002031	
428	hEDS	rs773639782	24.6 0.999	a)	TNFAIP8L3	3/3	ENST00000327536.5 c.347C>T	ENSP00000328016. 5p.Ala116Val phosphoinositide binding	0.00004613	
431	cEDS	–	14.65 0.986	a)	TNFSF10	1/5	ENST00000241261.2 c.89G>A	ENSP00000241261.2 p.Cys30Tyr helical	0	
534	cEDS	–	27.7 0.998	c)	NFKB1	16/24	ENST00000226574.4 c.1678G>A	ENSP00000226574.4 p.Val560Met ANK1 CFLAR	0	VUS PM2 PP2 (Supp)
564	HDCT	rs202134968	25.2 0.998	a)	GSK3B	2/12	ENST00000316626.5 c.233C>T	ENSP00000324806.5 p.Ser78Leu Kinase	0.00001659	
768	HDCT	–	25.5 0.998	a)	SNAI3	3/3	ENST00000332281.5 c.764A>G	ENSP00000327968.5 p.His255Arg Zinc Finger	0	
769	hEDS	rs755736608	32 0.999	a)	TNFAIP8	2/2	ENST00000504771.2 c.133G>A	ENSP00000422245.1 p.Asp45Asn	0.00001308	
777	HDCT	rs766761788	14.59 0.970	a)	C1QTNF2	2/3	ENST00000393975.3 c.359G>A	ENSP00000377545.3 p.Arg120Gln collagen like	0.00004914	
798	vEDS	–	24	a)	TNFRSF25	7/10	ENST00000377782.3 c.720del	ENSP00000367013.3 p.Lys240Asnfs Ter14	0	

1002	cEDS	rs373918716	23.5 0.978	a)	TNFAIP8L3	3/3	ENST0000032 7536.5 c.613A>C	ENSP0000032 8016.5 p.Met205Leu phosphoinositi de binding	0.00003657	
1341	hEDS	–	27.1 0.996	a)	C1QTNF4	2/2	ENST0000030 2514.3 c.886G>T	ENSP0000030 2274.3 p.Ala296Ser C1Q2 domain	0.00001374	
1344	hEDS	–	27.1 0.996	a)	C1QTNF4 –	2/2	ENST0000030 2514.3 c.886G>T	ENSP0000030 2274.3 p.Ala296Ser C1Q domain	0.00001374	
1346	vEDS	rs756818049	26.5 0.993	a)	C1QTNF2	2/3	ENST0000039 3975.3 c.271G>A	ENSP0000037 7545.3 p.Gly91Ser helical	0.00001315	
1397	hEDS	–	24.9 0.996	a)	ITGBL1	2/11	ENST0000037 6180.3 c.154C>G	ENSP0000036 5351.3 p.Arg52Gly Repeat region	0	
1498	hEDS	rs766972313	24.9 0.992	c)	C1QTNF5 LORD	14/15	NM_00127843 1.2 c.6G>C	ENSP0000040 2389.2 p.Arg2Ser signal peptide	0.000007461	VUS PM2
1502	hEDS	rs139306246	22.7 0.996	a)	ILKAP	12/12	ENST0000025 4654.3 c.1166G>A	ENSP0000025 4654.3 p. Arg3 89Gln	0.00004088	
1511	hEDS	–	24.4 0.998	b)	TNFRSF11B	3/5	ENST0000029 7350.4 c.401G>C	ENSP0000029 7350.4 p.Gly134Ala, ? LOEUF = 0.5	0	VUS PM2 PP3 (Supp)
1527	hEDS	rs781311887	24.7 0.999	a)	AKTIP	6/10	ENST0000039 4657.7 c.415C>T	ENSP0000037 8152.6 p.Arg139Cys, ADA 0.992	0.00002851	
1527	hEDS	rs781311887	24.7 0.999	a)	AKTIP	6/10	ENST0000039 4657.7 c.415C>T	ENSP0000037 8152.6 p.Arg139Cys, ADA 0.992	0.00002851	
1603	hEDS	rs376335031	23.8	a)	TNFAIP8	2/2	ENST0000050 4771.2 c.107A>G	ENSP0000042 2245.1 p.Lys36Arg	0	
1603	hEDS	rs376335031	23.8 0.999	a)	TNFAIP8 –	2/2	ENST0000050 4771.2 c.107A>G	ENSP0000042 2245.1 p.Lys36Arg,	0.0001135	
1609	hEDS	–	23.1 0.998	c)	AKT3	4/14	ENST0000036 6539.1 c.259T>C	ENSP0000035 5497.1 p.Phe87Leu PH	0	VUS PM2 PP3 (Supp)
1629	hEDS	–	18.38	a)	TNFRSF10A	6/10	ENST0000022 1132.3 c.742_743del	ENSP0000022 1132.3 p.Leu248Glyfs Ter44 pLi=0, LOEUF = 1.6	0	
1669	hEDS	rs377409471	24.9 0.999	a)	PARVG	11/14	ENST0000044 4313.3 c.677G>A	ENSP0000039 1583.2 p.Arg226His CH2	0.000004061	
1682	hEDS	rs143172535	17.17 0.928	a)	TNFRSF25	7/10	ENST0000037 7782.3 c.626T>C	ENSP0000036 7013.3 p.Val209Ala Helical transmembran e domain, LOEUF = 0.6	0.00002969	

Current gene annotation:
a) Germline variants in this gene not currently associated with Mendelian disorder
b) Germline variants in this gene associated with disorder of bone metabolism or skeletal dysplasia
c) Germline variants in this gene associated with non-EDS / HTAD phenotype

ACMG classification as per Richards et al. (9): P = pathogenic, LP = likely pathogenic, = variant of uncertain significance close to criteria for LP classification, VUS = variant of uncertain significance, LB = likely benign, B = benign.

VUS* are defined here as including VUS that according to ACGS criteria are "hot", "warm" or "tepid" Variants of Uncertain Significance (Figure 6 of https://www.acgs.uk.com/media/11631/uk-practice-guidelines-for-variant-classification-v4-01-2020.pdf).

Segregation analysis, re-evaluation for specific phenotypic features and/or further functional analysis may enable variant reclassification, using ACMG criteria.

Supplementary Table 15. Rare germline variants (CADD> 15) in genes previously published as abnormally expressed in skin fibroblasts from vEDS patients (31), list of genes in supplementary methods.

Patient ID	Clinical Diagnosis	Rs ID	CADD/ DANN	Current Gene annotation	Gene	Exon or Intron / Total no. exons	HGVSc	HGVSp	gnomAD allele frequency	ACMG classification (See footnote)
65	hEDS	rs149479865	26.2 0.999	b)	HSPG2	21/97	ENST00000374695.3 c.2633G>A	ENSP00000363827.3 p.Arg878His	0.0002409	VUS PM2
536	hEDS	rs145474376	22.9 0.996	b)	HSPG2	46/97	ENST00000374695.3 c.5815G>A	ENSP00000363827.3 p.Ala1939Thr	0.00007685	VUS
650	hEDS	rs201421233	18.55 0.988	a)	P4HA3	7/13	ENST00000331597.4 c.934C>T	ENSP00000332170.4 p.Pro312Ser, ?	0.00007753	
1002	cEDS	rs150109595	19.84 0.989	b)	HSPG2	74/97	ENST00000374695.3 c.9908C>T	ENSP00000363827.3 p.Thr3303Met	0.00005578	VUS PM2 BP4 (Supp)
1263	hEDS	rs773364995	28.5 0.997	b)	HSPG2	61/97	ENST00000374695.3 c.7903G>A	ENSP00000363827.3 p.Glu2635Lys	0.00001221	VUS PM2
1438	hEDS	rs771862177	26.7 0.985	b)	HSPG2	88/97	ENST00000374695.3 c.12040C>A	ENSP00000363827.3 p.His4014Asn	0	VUS PM2
1439	hEDS	rs771862177	26.7 0.985	b)	HSPG2	88/97	ENST00000374695.3 c.12040C>A	ENSP00000363827.3 p.His4014Asn	0	VUS PM2
1580	hEDS	–	20.8 0.98	c)	TMEM130	5/8	ENST00000416379.2 c.722C>A	ENSP00000413163.2 p.Thr241Asn	0	VUS PM2 BP4 (Supp)
1607	hEDS	–	34 0.998	a)	HIST1H4L	1/1	NM_003546.3 c.259G>A	ENSP00000348258.2 p.Val87Met	0.000004061	
1629	hEDS	rs747291083	18.56 0.996	b)	HSPG2	16/97	ENST00000374695.3 c.2110A>G	ENSP00000363827.3 p.Ser704Gly	0.00002442	VUS PM2
1641	hEDS	rs773796176	22.1 0.998	b)	HSPG2	4/97	ENST00000374695.3 c.326G>A	ENSP00000363827.3 p.Arg109Gln	0.000004061	VUS PM2 BP4 (Supp)
1688	HDCT	rs770843975	33 0.999	a)	MMP24	4/9	ENST00000246186.6 c.794C>T	ENSP00000246186.6 p.Thr265Met	0.00004088	
1695	hEDS	rs774712031	28.6 0.998	a)	LRRFIP1	2/11	ENST00000392000.4 c.112C>T	ENSP00000375857.4 p.Arg38Cys	0.00001741	
1714	hEDS	rs75564013	21.8 0.990	a)	MMP24	9/9	ENST00000246186.6 c.1730G>C	ENSP00000246186.6 p.Arg577Pro	0.00008123	

ACMG criteria as per Richards *et al.* (9): P = pathogenic, LP = likely pathogenic, VUS/LP = variant of uncertain significance close to criteria for LP classification, VUS = variant of uncertain significance, LB = likely benign, B = benign. Individual criteria ((9), Table 3)

VUS* are defined here as including VUS that according to ACGS criteria are "hot", "warm" or "tepid" Variants of Uncertain Significance (Figure 6 of https://www.acgs.uk.com/media/11631/uk-practice-guidelines-for-variant-classification-v4-01-2020.pdf).

Segregation analysis, re-evaluation for specific phenotypic features and/or further functional analysis may enable variant reclassification, using ACMG criteria.

Supplementary Table 16. Rare germline variants (CADD> 15) in genes previously published as abnormally expressed in skin fibroblasts from cEDS patients (Ref 30), list of genes in supplementary methods.

Patient ID	Clinical Diagnosis	Rs ID	CADD DANN	Current Gene annotation	Gene	Exon or Intron / Total no. exons	HGVSc	HGVSp	gnomAD allele frequency	ACMG classification (See footnote) criteria
395	hEDS	–	22.5 0.998	a)	DTL	14/15	ENST00000366 c.1993G>A	ENSP00000355 p.Ala665Thr	0.0001178	
534	cEDS	–	29.4 0.999	a)	POSTN	9/23	ENST00000379 c.1160T>C	ENSP00000369 p.Leu387Pro	0	
967	hEDS	rs755934955	25.7 0.999	a)	EDIL3	9/11	ENST00000296 c.994G>A	ENSP00000296 p.Asp332Asn	0.00002033	
1289	hEDS	–	27.5 0.998	c)	KIF4A	8/31	ENST00000374 c.836A>G	ENSP00000363 p.Asp279Gly	0	VUS PM2 PP3 (Supp)
1421	hEDS	rs768395830	28.3 0.998	c)	CSPP1	12/29	ENST00000262 c.1576A>G	ENSP00000262 p.Asn526Asp	0.000008126	VUS PM2
1464	hEDS	rs142868256	23.5 0.985	c)	C3	37/41	ENST00000245 c.4535G>A	ENSP00000245 p.Arg1512His	0.0001178	VUS PM2 PP5 BP6
1642	hEDS	–	23.3 0.995	a)	POSTN	7/23	ENST00000379 c.766A>T	ENSP00000369 p.Thr256Ser	0	
1681	hEDS	rs142868256	23.5 0.985	c)	C3	37/41	ENST00000245 c.4535G>A	ENSP00000245 p.Arg1512His	0.0001178	VUS PM2 PM5 BP6
1717	hEDS	rs759948962	24.4 0.998	c)	C3	9/41	ENST00000245 c.910C>T	ENSP00000245 p.Arg304Trp	0.000004067	VUS PM2
1717	hEDS	rs141915646	26.7 0.998	a)	MKI67	8/15	ENST00000368 c.1513C>T	ENSP00000357 p.Arg505Cys	0.00003249	

Current gene annotation:
a) Germline variants in this gene not currently associated with Mendelian disorder
b) Germline variants in this gene associated with disorder of bone metabolism or skeletal dysplasia
c) Germline variants in this gene associated with non-EDS / HTAD phenotype

ACMG classification as per Richards et al. (9): P = pathogenic, LP = likely pathogenic, = variant of uncertain significance close to criteria for LP classification, VUS = variant of uncertain significance, LB = likely benign, B = benign.

VUS* are defined here as including VUS that according to ACGS criteria are "hot", "warm" or "tepid" Variants of Uncertain Significance (Figure 6 of https://www.acgs.uk.com/media/11631/uk-practice-guidelines-for-variant-classification-v4-01-2020.pdf).

Segregation analysis, re-evaluation for specific phenotypic features and/or further functional analysis may enable variant reclassification, using ACMG criteria.

Supplementary Table 17. Rare germline variants (CADD> 15) in genes previously published in genome wide association studies, associated with, ($p < 5 \times 10^{-8}$), self-assessed Beighton Score > 5 (6), list of genes in supplementary methods.

Patient ID (Beighton Score)	Clinical Diagnosis	Rs ID	CADD DANN	Current Gene annotation	Gene	Exon or Intron / Total no. exons	HGVSc	HGVSp Domain	gnomAD allele frequency	ACMG classification (See footnote)
44 (5)	vEDS	–	28.6 0.999	c)	PIEZO1	25/51	ENST00000301015.9 c.3575C>T	ENSP00000301 p.Ala1192Val Transmembran	0	VUS PM2
44 (5)	vEDS	–	23.7 0.972	b)	COL27A1	34/61	ENST00000356083.3 c.3481C>G	ENSP00000348 p.Pro1161Ala Collagen like 9	0	VUS PM2
45 (5)	HDCT	rs200031013	23 0.975	c)	PIEZO1	39/51	ENST00000301015.9 c.5647C>T	ENSP00000301 p.Arg1883Trp none	0.0002472	VUS PM2
60 (0)	HDCT	rs752193524	29.2 0.998	b)	COL27A1	26/61	ENST00000356083.3 c.3040C>T	ENSP00000348 p.Arg1014Cys Collagen like 7	0.000004063	VUS* PM2 PP3 (M)
61 (n/a)	hEDS	–	26 0.994	c)	PIEZO1	42/51	ENST00000301015.9 c.5978C>T	ENSP00000301 p.Ser1993Phe Helical transme	0	VUS PM2
61 (n/a)	hEDS	rs758079877	23.5 0.996	b)	COL27A1	60/61	ENST00000356083.3 c.5413G>A	ENSP00000348 p.Glu1805Lys C terminal prop	0.00001221	VUS PM2
99 (0)	HDCT	rs924560632 rs755738951	18.1 0.945	c)	PIEZO1	39/51	ENST00000301015.9 c.5602C>T	ENSP00000301 p.Arg1868Cys none	0.00006886	VUS PM2
385 (n/a)	hEDS	rs753059506	26.6 0.998	b)	COL27A1	50/61	ENST00000356083.3 c.4597G>A	ENSP00000348 p.Glu1533Lys Triple helical	0.00001218	VUS PM2
395, 397 (n/a, n/a)	hEDS	rs766146854	24 0.991	a)	NEDD4L	15/31	ENST00000400345.3 c.1370C>T	ENSP00000383 p.Pro457Leu Neighbouring p	0.000008.195	VUS PM2, PP2 BP6 (S)
422 (6)	HDCT	rs756716936	21.5 –	a)	STON1	1/3	NM_006873.4 c.773dup	ENSP00000310 p.Asn258Lysfs* LoF z = 1.08	0.0001535	
428 (n/a)	hEDS	rs750927939	27.5 0.994	c)	PIEZO1	51/51	ENST00000301015.9 c.7415C>T	ENSP00000301 p.Pro2472Leu None	0.00001323	VUS PM2

453 (4)	HDCT	rs756716936	21.5 –	a)	STON1	1/3	NM_006873.4 c.773dup	ENSP00000310 p.Asn258Lysfs LoF z = 1.08	0.0001535	
475 (7)	hEDS	–	24.5 0.995	c)	PIEZO1	47/51	ENST0000030 1015.9 c.6795C>G	ENSP00000301 p.Ile2265Met None	0	VUS PM2
479 (6)	HDCT	rs781648726	19.6 0.936	a)	NEDD4	1/22	ENST0000033 8963.2 c.1006G>A	ENSP00000345 p.Gly336Arg None	0.00002443	
526 (7)	HDCT	rs763621682	17.2 0.631	b)	COL27A1	27/61	ENST0000035 6083.3 c.3136C>T	ENSP00000348 Pro1046Ser Collagen like 7	0.00001633	VUS PM2
532 (2)	HDCT	rs150886795	18.24 0.990	a)	NEDD4	1/22	ENST0000033 8963.2 c.385G>A	ENSP00000345 p.Asp129Asn none	0.0003058	
635 (7)	HDCT	rs775232854	16.72 0.967	c)	VCAN	8/15	ENST0000026 5077.3 c.4380A>C	ENSP00000265 p.Glu1460Asp	0.000008149	VUS PM2 BP4 (Supp)
650 (7)	hEDS	–	34 –	a)	NOTCH4	27/30	ENST0000037 5023.3 c.4772del	ENSP00000364 p.Leu1591Argf LOEUF=0.74	0.000008257	
670 (8)	hEDS	rs532112751	24.4 0.996	c)	PIEZO1	27/51	ENST0000030 1015.9 c.3922C>G	ENSP00000301 p.Leu1308Val None	0.0001946	VUS PM2
673 (3)	hEDS	–	23.9 0.998	a)	NEDD4	15/22	ENST0000033 8963.2 c.3103A>G	ENSP00000345 p.Ile1035Val HECT	0.0000398	
769 (3)	hEDS	rs781127798	24.1 0.995	a)	MAB21L4	1/5	ENST0000038 8934.4 c.94C>T	ENSP00000373 p.Arg32Cys	0.00002893	
777 (7)	HDCT	rs778125678	22.6 0.996	a)	STON1	1/3	NM_006873.4 c.702A>C	ENSP00000310 p.Glu234Asp None	0.000005414	
778 (7)	hEDS	–	16.91 0.986	c)	PIEZO1	17/51	ENST0000030 1015.9 c.2279A>T	ENSP00000301 p.Asp760Val Neighbouring p	0	VUS PM2
814 (8)	HDCT	–	31 0.997	c)	NEDD4L	31/31	ENST0000040 0345.3 c.2893G>T	ENSP00000383 p.Val965Leu HECT	0	VUS PM2 PP2

884 (9)	hEDS	rs781001928	35 0.999	a)	ARHGAP44	19/21	ENST00000379672.5 c.1933C>T	ENSP00000368 p.Arg645Trp none	0.00002056	
1002 (7)	cEDS	rs568280615	24.3 0.997	c)	PIEZO1	22/51	ENST00000301015.9 c.3000C>A	ENSP00000301 p.Phe1000Leu Transmembran	0.0002875	VUS PM2
1396 (7)	kEDS	rs144412674	17.1 0.998	a)	STON1	1/3	NM_006873.4 c.1258G>A	ENSP00000310 p.Val420Met MHD	0.00004111	
1399 (4)	hEDS	rs144412674	17.1 0.998	a)	STON1	1/3	NM_006873.4 c.1258G>A	ENSP00000310 p.Val420Met MHD	0.00004111	
1420 (n/a)	HDCT	rs777936815	19.92	b)	COL27A1	12/61	ENST00000356083.3 c.2365_2367dup inframe insertion	ENSP00000348 p.Pro789dup LOUEF = 0.3	0.000008122	VUS PM2 PM4
1421 (7)	hEDS	rs754511035	16.14 0.955	b)	COL27A1	3/61	ENST00000356083.3 c.409G>A	ENSP00000348 p.Val137Ile N terminal prop	0.000004189	VUS PM2 BP4 (Supp)
1511 (7)	hEDS	rs767968797	23.9 0.999	a)	ABI3BP	3/35	ENST00000284322.5 c.311G>A	ENSP00000284 p.Arg104Gln None	0.00002849	
1527 (3)	hEDS	–	24.2 0.997	a)	XKR6	2/3	ENST00000416569.2 c.844T>C	ENSP00000416 p.Tyr282His	0	
1616 (8)	hEDS	rs141525894	24.3 0.996	a)	NOTCH4	30/30	ENST00000375023.3 c.5764G>A	ENSP00000364 p.Gly1922Arg none	0.000133	
1626 (8)	hEDS	rs773623130	16.31 –	a)	ABI3BP	intron 9/67	NM_001375547.2 c.910+5_910+6insA	? LOEUF = 0.56	0.0001247	
1666 (8)	hEDS	rs191960195	17.07 0.963	a)	ABI3BP	7/35	ENST00000284322.5 c.722C>T	ENSP00000284 p.Ala241Val None	0.0001058	
1695 (8)	hEDS	rs765636311	22.4 0.994	a)	NOTCH4	20/30	ENST00000375023.3 c.3203C>A	ENSP00000364 p.Pro1068His multiple	0	

Current gene annotation:

a) Germline variants in this gene not currently associated with Mendelian disorder

b) Germline variants in this gene associated with disorder of bone metabolism or skeletal dysplasia

c) Germline variants in this gene associated with non-EDS / HTAD phenotype

ACMG classification as per Richards et al. (9): P = pathogenic, LP = likely pathogenic, = variant of uncertain significance close to criteria for LP classification, VUS = variant of uncertain significance, LB = likely benign, B = benign.

VUS* are defined here as including VUS that according to ACGS criteria are "hot", "warm" or "tepid" Variants of Uncertain Significance (Figure 6 of https://www.acgs.uk.com/media/11631/uk-practice-guidelines-for-variant-classification-v4-01-2020.pdf).

Segregation analysis, re-evaluation for specific phenotypic features and/or further functional analysis may enable variant reclassification, using ACMG criteria.

Supplementary Table 18. Rare variants (CADD > 20) identified in EDS patients of differing clinical EDS subtypes, in genes not currently associated with human disease or variants in genes not currently associated with an EDS phenotype. These variants have high in silico pathogenicity scores and some published evidence of biological plausibility.

Patient ID	Variant ID	Age	Sex	Clinical Diagnosis	Beighton score	Villefranche Major/ Minor	Aortic & Other Vascular involvent	Auto. Dom. Family History	Skin Biopsy	Gene NM	Current Gene annotation	Protein Domain	Rs ID ClinVar	gnomAD allele frequency	CADD DANN	ACMG classification (See footnote) criteria
34	50	30-39	F	HDCT	3	A, C, E, H, I d,i	Carotid artery dissection	–	normal	PTGER4 NM_000958.3 c.644G>T	a)	p.Arg215Leu helical transmembrane (3AA).	– –	0	29.2 0.998	
404	51	40-49	M	hEDS	9	A, C, H, I a, d, f, i, u	–	+	Occasional irregular collagen fibril	MMP25 NM_022468.5 c.580C>T	a)	p.His194Tyr	rs1004972120 –	0	28.9 –	
446	52	40-49	M	HDCT	4	A, C, E, I d, i, f, u	Carotid artery dissection	+	irregular collagen fibril size	ADAMTS5 NM_007038.5 c.2314A>G	a)	p.Thr772Ala spacer domain	– –	0	22.6 0.998	
446	53	40-49	M	HDCT	4	A, C, E, I d, i, f, u	Carotid artery dissection	+	irregular collagen fibril size	ADAMTS16 NM_139056.4 c.2459G>A	a)	p.Arg820Gln spacer domain	rs748937514 –	0.0000281	32 0.999	
446	54	40-49	M	HDCT	4	A, C, E, I d, i, f, u	Carotid artery dissection	+	irregular collagen fibril size	NFAT5 NM_138713.4 c.3446T>A	a)	p.Val1149Asp	– –	0	25.8 0.981	
505	55	10-19	F	HDCT	–	H g, l, u	–	+	–	ROBO2 NM_002942.5 c.2018G>A	c)	p.Arg673His Fibronectin III2	rs376737394 346696 (LB)	0.000121	34 0.999	VUS PM2, PP3 (Supp) BP6 (S)
566	56	60-69	M	hEDS	5	A, C, E, H, I , J x, y, aa	–	biparental	Collagen fibril size variability	SYAP1 NM_032796.4 c.37C>T	a)	p.Gln13Ter	– –	0	36 0.998	
703	57	10-19	F	hEDS	–	C, H t, u	–	–	–	LZTS1 NM_021020.5 c.1483G>A	a)	p.Glu495Lys	rs150225368 –	0.0005212	22.8 0.997	
761	58	20-29	M	hEDS	6	B, C, H, I, J d, f, t, u, v		+	–	C9 NM_001737.5 c.1052C>G	c)	p.Ser351Cys Transmembrane	rs1999424520 –	0.0000318	25.5 0.991	VUS PM2
1396	59	0-9	M	kEDS	7	C, H, J e, f, u, w	–	+	–	INO80D NM_017759.5 c.1822–1823delAC	a)	p.Thr608Ter	– –	0	35 –	
1450	60	30-39	F	hEDS	–	B, C, H, I a, t, u premature rupture of membranes	–	+	Collagen fibril size variability	MMP8 NM_002424.3 c.679C>T	a)	p.His227Tyr	rs769627751 –	0.00000518	23.6 0.995	
1491	61	20-29	F	hEDS	6	C, H d, f, t, y	–	–	–	FBN3 NM_032447.5 c.6988C>T	a)	p.Arg2330Trp TB 9 domain	rs372443838 –	0.0000678	34 0.999	
1620	62	20-29	M	hEDS	6	C, H, I d, f, t, u	–	+	–	ITGA2 NM_002203.4 c.1027A>G	c)	p.Asn343Asp	– –	0	28.4 0.998	VUS PM2
1625	63	60-69	F	HDCT	–	– g, r, t megacolon	AoR	–	–	TGFB1/1, NM001042454.3 c.199C>T	a)	p.Arg67Trp Nr Phosphoserine	– –	0	35 0.999	
1695	64	20-29	F	hEDS	8	C, H, I f, u	–	+	–	NOTCH4 NM_004557.4 c.3203C>A	a)	p.Pro1068His	rs765636311 –	0	22.4 0.994	
1717	65	40-49	F	hEDS	7	C, H d, t	–	–	–	C3 NM_000064.3 c.910C>T	c)	p.Arg304Trp Neighbours phosphoserine	rs1189452748 –	0.00000399	24.4 0.999	VUS PM2

ACMG criteria as per Richards *et al.* (9): P = pathogenic, LP = likely pathogenic, VUS/LP = variant of uncertain significance close to criteria for LP classification, VUS = variant of uncertain significance, LB = likely benign, B = benign. Individual criteria ((9), Table 3)

VUS* are defined here as including VUS that according to ACGS criteria are "hot", "warm" or "tepid" Variants of Uncertain Significance (Figure 6 of https://www.acgs.uk.com/media/11631/uk-practice-guidelines-for-variant-classification-v4-01-2020.pdf). Segregation analysis, re-evaluation for specific phenotypic features and/or further functional analysis may enable variant reclassification, using ACMG criteria.
EDS Diagnostic Criteria as per list in Supplementary Table 1.

Supplementary Table 19. Variants identified in EDS patients of differing clinical EDS subtypes with a 'candidate gene' approach based on reported Marfan mouse models, EDS mechanisms, Skeletal dysplasia, Matrisome, Myopathies, Integrins, Dedicator of cytokinesis (DOCK), circadian rhythm genes, Ephrins, Tetraspanins (TSPANs) and serine proteases.

Patient ID	Clinical Diagnosis	Current Gene annotation	Gene	HGVSc	HGVSp	CADD	Rs ID	Exon	gnomAD allele frequency	ACMG Classification (See footnote) criteria
Marfan Mouse Model genes										
61	hEDS	c)	IRF7	ENST00000397566.1 c.1424T>C	ENSP00000380697.1 p.Leu475Pro	20.4	rs376761232	9/9	0.00002048	VUS PM2 PP3 (Supp)
75	cEDS	a)	TMEM176B	ENST00000447204.2 c.16G>A	ENSP00000410269.2 p.Val6Met	22.5	–	2/7	0	
404	hEDS	a)	MMP25	ENST00000336577.4 c.580C>T	ENSP00000337816.4 p.His194Tyr	28.9	–	4/10	0	
474	HDCT	c)	SCUBE3	NM_152753.4 c.2578G>A	p.Val860Ile CUB domain	24.8	rs76742237	19/22	0..0000159	VUS PM2
567	HDCT	c)	IRF7	ENST00000397566.1c.1180G>T	ENSP00000380697.1p.Gly394Cys	27.5	rs368953784	7/9	0.00001254	VUS PM2 PP3 (Supp)
653	cEDS	a)	MMP25	ENST00000336577.4 c.85_86insGCGCGTCGCCGCACCGTTAAAAATCACGTCCTGCATACTCTCGCCGCGAAGC	ENSP00000337816.4 p.Val29GlyfsTer7	28.6	–	1/10	0	
922	hEDS	a)	NFAT5	NM_138713.4 c.1165G>A	p.Gly389Ser RH domain	23.3	rs753948488	6/15	0.0000244	
1387	HDCT	a)	TMBIM1	NM_022152.6 c.847G>A	p.Glu283Lys	34	rs76243510	12/12	0.0004781	
1444	hEDS	c)	SCUBE3	NM_152753.4 c.2518C>T	p.Arg840Cys	35	rs1464548360	19/22	0.00000398	VUS PM2
1451	cEDS	a)	IGFBP2	ENST00000233809.4 c.221C>T	ENSP00000233809.4 p.Pro74Leu	23.1	–	1/4	0	
1500	hEDS	a)	TMBIM1	NM_022152.6 c.817C>G	p.Leu273Val	23.3	–	12/12	0	
1524	cEDS	a)	TMBIM1	NM_022152.6 c.412del	p.Tyr138ThrfsTer12 LOEUF = 1.11	35	rs775344685	5/12	0.0000159	
1595	hEDS	a)	NFAT5	NM_138713.4 c.2907G>C	p.Gln969His	22.8	rs759928002	13/15	0.0000398	

EDS candidate Genes										
107	hEDS	a)	COL5A3	ENST00000264828.3 c.1307G>A	ENSP00000264828.3 p.Arg436Gln	24.8	rs773225571	12/67	0.00001642	
534	cEDS	a)	FBN3	NM_032447.5 c.6661C>T	p.Arg2221Trp EGF like 36 & cysteine disulfide domains	27.3	rs202020932	54/64	0.0000123	
538, 560	HDCT (538), hEDS (560)	c)	C2	ENST00000299367.5 c.1716G>C	ENSP00000299367.5 p.Lys572Asn	23.9	rs376278843	13/18	0.0001411	VUS PM2
584	hEDS	a)	CR1L	NM_175710.2 c.382C>T	p.Arg128Ter LOEUF = 1.6 Splice + 5	36	rs199942497	04/12	0.000223	
769	hEDS	a)	ADAM28	ENST00000265769.4 c.737A>G	ENSP00000265769.4 p.Asn246Ser	24.5	–	9/23	0	
798	vEDS	a)	COL5A3	ENST00000264828.3 c.361G>A	ENSP00000264828.3 p.Ala121Thr	24.1	rs199691548	3/67	0.00006152	
810	HDCT	a)	COL5A3	ENST00000264828.3 c.2260C>T	ENSP00000264828.3 p.Pro754Ser	15.55	–	30/67	0	
1346	vEDS	a)	ADAMTS20	ENST00000389420.3 c.1957C>T	ENSP00000374071.3 p.Arg653Cys	32	rs79065113	14/39	0.00004138	
1387	HDCT	a)	ADAM23	ENST00000264377.3 c.1369G>A	ENSP00000264377.3 p.Gly457Ser	18.3	rs759614751	14/26	0.00001219	
1450	hEDS	a)	MMP8	ENST00000236826.3 c.679C>T	ENSP00000236826.3 p.His227Tyr	23.6	rs769627751	5/10	0.00005286	
1484	hEDS	c)	C8A	ENST00000361249.3 c.1528C>T	ENSP00000354458.3 p.Leu510Phe	27.9	rs200018561	10/11	0.00008122	VUS PM2
1630	hEDS	a)	FBN3	NM_032447.5 c.4886C>T	p.Thr1629Ile EGF like 25 domain	28.5	rs376299515	39/64	0.000203	
1641	hEDS	a)	ADAMTS20	ENST00000389420.3 c.4781_4782dup	ENSP00000374071.3 p.Ala1595GlnfsTer39	36	–	31/39	0	
1642	hEDS	a)	ADAM33	ENST00000356518.2 c.706C>T	ENSP00000348912.2 p.Arg236Cys	34	rs750423431	8/22	0.00000406	
1681	hEDS	a)	MMP8	ENST00000236826.3 c.782A>C	ENSP00000236826.3 p.Tyr261Ser	27.6	–	5/10	0.00001669	
1688	HDCT	a)	ADAMTS4	ENST00000367996.5 c.1700G>A	ENSP00000356975.4 p.Arg567His	33	rs139714128	6/9	0.00006548	
1688	HDCT	a)	MMP24	ENST00000246186.6 c.794C>T	ENSP00000246186.6 p.Thr265Met	33	rs770843975	4/9	0.00004088	

Skeletal Dysplasia										
1450	hEDS	b)	TRPV4	NM_021625.5 c.1634T>C	p.Ile545Thr	20.7	rs757630049	10/16	0	VUS PM2 PM1
Matrisome										
383	cEDS	a)	DSEL	ENST00000310045.7 c.2788C>T	ENSP00000310565.7 p.Arg930Ter	42	–	2/2	0	
595	cEDS	a)	ROCK1	ENST00000399799.2 c.1208G>A	ENSP00000382697.1 p.Arg403His	22.9	rs374052961	10/33	0.00008004	
635	HDCT	c)	CHSY1	ENST00000254190.3 c.278C>G	ENSP00000254190.3 p.Thr93Ser	22.7	rs142148989	1/3	0.0002626	VUS PM2
1289	hEDS	a)	CHPF	ENST00000243776.6 c.2026G>A	ENSP00000243776.6 p.Glu676Lys	34	–	4/4	0	
1443	hEDS	a)	CHPF2	ENST00000035307.2 c.1375C>T	ENSP00000035307.2 p.Arg459Trp	32	rs749772535	4/4	0.00004971	
1443	hEDS	a)	DSEL	ENST00000310045.7 c.607A>T	p.Arg203Ter	35	rs143469336	2/2	0.00000796	
1665	hEDS	a)	DSEL	N_032160.3 c.1061A>C	p.Asn354Thr	24.3	rs374976853	2/2	0.0000159	
1669	hEDS	a)	CHSY3	ENST00000305031.4 c.1013C>T	ENSP00000302629.4 p.Thr338Met	34	rs761257284	2/3	0.000004061	
Myopathy										
703	17	d)	MYH2	ENST00000245503.5 c.5540G>A	ENSP00000245503.5 p.Arg1847His	33	rs748605415	38/40	0.0001462	VUS PM2 BS2
777	HDCT	d)	MYH2	ENST00000245503.5c.1115G>A	ENSP00000245503.5p.Arg372His	35	rs750569547	12/40	0.00001218	VUS* PM2 PP3 (M)
1477	hEDS	a)	ABLIM2	ENST00000447017.2 c.1768G>A	ENSP00000393511.2 p.Val590Ile	23.9	rs200508979	20/21	0.0002302	
1620	hEDS	a)	ABLIM2	ENST00000447017.2 c.337C>T	ENSP00000393511.2 p.Arg113Trp	31	–	3/21	0	
Integrins										
44	vEDS	a)	ITGA10	ENST00000369304.3 c.1655C>T	ENSP00000358310.3 p.Ala552Val	33	–	14/30	0	
383	cEDS	a)	ITGA10	ENST00000369304.3 c.2592G>T	ENSP00000358310.3 p.Lys864Asn	24.2	–	21/30	0	
475	hEDS	a)	ITGA10	ENST00000369304.3 c.2071C>T	ENSP00000358310.3 p.Arg691Cys	28.2	rs782455269	16/30	0.00002031	
612	hEDS	a)	ITGA10	ENST00000369304.3 c.790C>T	ENSP00000358310.3 p.Arg264Ter	36	rs782338989	8/30	0.00002872	
673	hEDS	a)	ITGA2	ENST00000296585.5 c.757T>A	ENSP00000296585.5 p.Phe253Ile	33	–	7/30	0	

673	hEDS	a)	ITGA2	ENST00000296585.5 c.764C>T	ENSP00000296585.5 p.Ala255Val	34	–	7/30	0	
718	cEDS	a)	ITGA2	ENST00000296585.5 c.85G>A	ENSP00000296585.5 p.Ala29Thr	31	rs374701439	2/30	0.00005286	
1504	HDCT	a)	ITGA2	ENST00000296585.5 c.2474T>G	ENSP00000296585.5 p.Phe825Cys	27.5	rs759539816	20/30	0.00003259	
1504	HDCT	a)	ITGA2	ENST00000296585.5 c.1790G>A	ENSP00000296585.5 p.Arg597His	23.4	rs770216834	14/30	0.00004895	
1620	hEDS	a)	ITGA2	ENST00000296585.5 c.1027A>G	ENSP00000296585.5 p.Asn343Asp	28.4	–	9/30	0	
1681	hEDS	a)	ITGA10	ENST00000369304.3 c.1562G>A	ENSP00000358310.3 p.Arg521His	29	–	13/30	0	
1743	hEDS	c)	ITGA2B	ENST00000262407.5 c.2902T>C	ENSP00000262407.5 p.Tyr968His	24.3	rs5914	28/30	0	VUS PM2 PP2
DOCK										
73	HDCT	c)	DOCK6	ENST00000294618.7 c.1631A>G	ENSP00000294618.6 p.His544Arg	23	–	14/48	0	VUS PM2
74	hEDS	c)	DOCK6	ENST00000294618.7 c.4445G>A	ENSP00000294618.6 p.Ser1482Asn	23.8	–	35/48	0	VUS PM2
385	hEDS	c)	DOCK6	NM_020812.4 c.484G>A	p..Glu162Lys	20	rs766200535	5/48	0.00000971	VUS PM2 BP4 (Supp)
385	hEDS	a)	DOCK9	ENST00000376460.1 c.4223C>T	ENSP00000365643.1 p.Ser1408Phe	28.3	–	39/57	0	
1424	hEDS	c)	DOCK2	NM_004946.3 c.4090C>T	ENSP00000256935.8 p.Arg1364Cys	35	rs536724336	41/52	0.00002033	VUS PM2 PP2
1450	hEDS	c)	DOCK6	ENST00000294618.7 c.4641C>A	ENSP00000294618.6 p.Phe1547Leu	22.8	–	36/48	0	VUS PM2
1491	hEDS	c)	DOCK6	NM_020812.4 c.2629C>T	p.Arg877Cys		rs199553475	22/48	0.000181	VUS PM2
1503	HDCT	c)	DOCK6	NM_020812.4 c.3811C>T	p.Arg1271Cys	24.4	rs376724815	30/48	0.0000563	VUS PM2 BP4 (Supp)
1613	hEDS	a)	DOCK9	ENST00000376460.1 c.2438C>T	ENSP00000365643.1 p.Ser813Phe	29.9	rs778275450	22/57	0.000008204	
1630	hEDS	c)	DOCK6	NM_020812.4 c.3310C>T	p.Arg1104Trp	35	rs767376510	27/48	0.0000377	VUS PM2
1656	hEDS	c)	DOCK3	ENST00000266037.9c.1490T>C	ENSP00000266037.8 p.Ile497Thr	26.8	rs748558159	16/53	0.00002032	VUS PM2 PP2

Circadian Genes										
446	HDCT	c)	PER2	ENST00000254657.3 c.2434G>A	ENSP00000254657.3 p.Gly812Arg	22.6	rs201525818	19/23	0.0002591	VUS PM2 BP4 (Supp)
526	HDCT	a)	ZFHX3	ENST00000268489.5 c.2443G>A	ENSP00000268489.5 p.Val815Met	24	–	2/10	0	
564	HDCT	c)	PER1	ENST00000317276.4 c.3223T>C	ENSP00000314420. 4p.Ser1075Pro	26.8	–	20/23	0	VUS PM2
635	HDCT	a)	ZFHX3	ENST00000268489.5 c.9872T>C	ENSP00000268489.5 p.Leu3291Pro	19.21	–	10/10	0	
671	HDCT	a)	SEC61B	ENST00000223641.4 c.137G>A	ENSP00000223641.4 p.Arg46His	34	–	03/04	0.0000131	
821	kEDS	c)	PER1	ENST00000317276.4 c.3583C>G	ENSP00000314420.4 p.Arg1195Gly	24.1	rs200744636	22/23	0.0000004	VUS PM2
1443	hEDS	a)	ZFHX3	ENST00000268489.5 c.2213A>G	ENSP00000268489.5 p.Lys738Arg	22	rs755685914	2/10	0.000028	
1528	cEDS	a)	ZFHX3	ENST00000268489.5 c.7561G>A	ENSP00000268489.5 p.Ala2521Thr	21.4	rs140414544	9/10	0.0000077	
1717	hEDS	a)	ZFHX3	ENST00000268489.5 c.5821A>G	ENSP00000268489.5 p.Arg1941Gly	22.6	rs760103457	9/10	0.000012	
Ephrins										
372	vEDS	a)	EPHA8	NM_020526.5 c.2635C>T	p.Arg879Trp protein kinase domain	33	rs147803148	15/17	0.0000325	
409	cEDS	a)	EPHA8	NM_020526.5 c.2753G>A	p.Arg918Gln	25.5	rs141279306	16/17	0.000121	
777	HDCT	a)	EFNA1	NM_004428.3 c.556C>T	p.Arg186Cys	35	rs760306344	5/5	0.0000119	
TSPANs										
75	cEDS	c)	TSPAN12	NM_012338.4 c.184G>A	p.Val64Met	29.9	–	04/08	0	VUS PM2
99	HDCT	a)	TSPAN14	NM_030927.4 c.20C>G	p.Ser7Cys	26.1	–	02/09	0	
136	cEDS	a)	TSPAN2	NM_005725.6 c.626T>C	p.Val209Ala	24.9	rs34749181	8/8	0.000171	
396	cEDS	a)	TSPAN9	NM_00116832(c.620C>T	p.Thr207Met	33	rs141218062	07/08	0.0000723	
564	HDCT	a)	TSPAN17	NM_130465.5 c.355G>T	p.Asp119Tyr	31	rs367611196	4/9	0.0000066	
595	cEDS	a)	TSPAN3	NM_005724.6 c.380A>G	p.Asn127Ser	21.2	rs370307435	04/07	0.000013	
1387	HDCT	a)	TSPAN15	NM_012339.5 c.649C>T	p.Arg.217Trp	33	rs200107830	07/08	0.000131	
1462	hEDS	a)	TSPAN17	NM_130465.5 c.620G>C	p.Arg207Pro	33	–	06/09	0	
1681	hEDS	a)	TSPAN32	NM_139022.3 c.913A>T	p.Arg305Ter	35	–	10/10	0	

1656	hEDS	a)	TSPAN9	NM_00116832(c.661G>A	p.Ala221Thr	23.3	rs149866702	08/08	0.000046	
1665	hEDS	a)	TSPAN1	NM_005727.4 c.643G>A	p.Val215Met	24.7	rs149302587	09/09	0.000125	
Serine proteases										
60	HDCT	c)	TMPRSS5	NM_030770.4 c.702C>G	p.Ser234Arg	22	–	8/13	0	
99	HDCT	c)	TMPRSS5	NM_030770.4 c.1216G>A c.1216G>A	p.Gly406Arg	25.8	–	12/13	0.0000197	
396	cEDS	a)	PRSS36	NM_173502.5 c.2371G>T	p.Glu791Ter	39	rs201757658	15/15	0.0000591	
396	cEDS	a)	TMPRSS15	NM_002772.3 c.687T>G	p.Phe229Leu	27	rs138300762	7/25	0.00000657	
397	hEDS	a)	PRSS36	NM_173502.5 c.2371G>T	p.Glu791Ter	39	rs201757658	15/15	0.000591	
423	HDCT	a)	PRSS35	NM_153362.3 c.410G>A	p.Arg137Met	22.9	rs148479497	02/02	0.000177	
475	hEDS	a)	TMPRSS9	NM_182973.3 c.1253C>T	p.Pro418Leu	24.3	rs150970765	9/17	0.000131	
567	HDCT	a)	PRSS50	NM_013270.5 c.115G>T	p.Gly39Cys	23.1	rs151210292	7/11	0.0000197	
922	hEDS	a)	PRSS53	NM_00103950 c.91C>T	p.Arg31Cys	34	rs377044450	03/11	0.0000197	
1424	hEDS	c)	TMPRSS6	NM_00137450 c.290G>A	p.Arg97Gln	24.6	rs531422898	03/18	0.0000197	VUS PM2 BP4 (Supp)
1461	hEDS	a)	PRSS22	NM_022119.4 c.433G>A	p.Val145Met	24.4	–	04/06	0	
1462	hEDS	c)	PRSS12	NM_003619.12 c.419G>T	p.Ser140Ile	25.2	rs775377995	01/13	0.000046	VUS PM2
1462	hEDS	a)	TMPRSS9	NM_182973.3 c.682del	p.Cys228Valfs Ter71	33	–	07/18	0	
1484	hEDS	c)	PRSS12	NM_003619.4 c.1640C>A	p.Ala547Asp	33	rs201005601	09/13	0.0000855	VUS PM2
1579	hEDS	a)	TMPRSS12	NM_182559.3 c.805G>A	p.Gly269Arg	32	rs369598424	05/05	0.000105	

Current gene annotation:

a) Germline variants in this gene not currently associated with Mendelian disorder

b) Germline variants in this gene associated with disorder of bone metabolism or skeletal dysplasia

c) Germline variants in this gene associated with non-EDS / HTAD phenotype

d) Germline variants in this gene associated with a myopathy phenotype

ACMG classification as per Richards et al. (9): P = pathogenic, LP = likely pathogenic, = variant of uncertain significance close to criteria for LP classification, VUS = variant of uncertain significance, LB = likely benign, B = benign.

VUS* are defined here as including VUS that according to ACGS criteria are "hot", "warm" or "tepid" Variants of Uncertain Significance (Figure 6 of https://www.acgs.uk.com/media/11631/uk-practice-guidelines-for-variant-classification-v4-01-2020.pdf).

Segregation analysis, re-evaluation for specific phenotypic features and/or further functional analysis may enable variant reclassification, using ACMG criteria.