Genetic complexity of diagnostically unresolved Ehlers-Danlos syndrome

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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 ALDH118A1 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.9 GJH is a common population trait: 5% of 14 year olds had a Beighton score >6 in the ALSPAC cohort. 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. 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 Number 04/Q0108/44). Patients were 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), kyphoscoliotic EDS (kEDS) (2 male), and hypermobile EDS (hEDS) (22 male/87 female). Patients reported previously offered. Patients with confirmed molecular diagnoses of monogenic EDS, with arterial aneurysm(s) in proband and/or family member(s) and complex and/or severe symptoms. Patients with confirmed diagnoses of mono- or polygenic HTAD or EDS were excluded. Patients reported previously offered. Patients with confirmed molecular diagnoses of mono- or polygenic HTAD or EDS were excluded.

**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 unrelate d EDS cases of Caucasian ancestry together with whole-genome sequence data (2-fold to 20-fold coverage) from 248 ALSPAC controls11 sequenced as part of the UK10K study. The software package TASER13 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 were identified in cases presenting with arterial aneurysm(s) and hEDS or kEDS features (table 1). Two novel heterozygous pathogenic COL12A1 variants were identified in cases presenting with arterial aneurysm(s) and hEDS or kEDS features (table 1).

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

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Variant ID</th>
<th>Age (years)</th>
<th>Gender</th>
<th>Clinical diagnosis</th>
<th>Gene/NM</th>
<th>Protein</th>
<th>ACMG classification</th>
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<tr>
<td>33</td>
<td>1</td>
<td>40–49</td>
<td>F</td>
<td>HDCT</td>
<td>TGF83</td>
<td>p.Arg1557Tp</td>
<td>LP</td>
</tr>
<tr>
<td>34</td>
<td>2</td>
<td>30–39</td>
<td>F</td>
<td>HDCT</td>
<td>COL5A1</td>
<td>NM_000093.4</td>
<td>Splice</td>
</tr>
<tr>
<td>402</td>
<td>4</td>
<td>30–39</td>
<td>M</td>
<td>HEDS</td>
<td>COL12A1</td>
<td>NM_004370.6</td>
<td>Splice</td>
</tr>
<tr>
<td>479</td>
<td>8</td>
<td>20–29</td>
<td>M</td>
<td>HDCT</td>
<td>SMAD2</td>
<td>NM_001003652.3</td>
<td>c.842A&gt;T</td>
</tr>
<tr>
<td>564</td>
<td>9</td>
<td>20–29</td>
<td>M</td>
<td>HDCT</td>
<td>TGF82</td>
<td>NM_00135599.3</td>
<td>c.989G&gt;A</td>
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<tr>
<td>755</td>
<td>10</td>
<td>40–49</td>
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<td>HEDS</td>
<td>COL12A1</td>
<td>NM_004370.6</td>
<td>c.8321G&gt;A</td>
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<tr>
<td>814</td>
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<td>30–39</td>
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</tbody>
</table>

Additional variant annotation is given in online supplemental tables 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 Lammitt et al. 2023; Vandersteen AM, et al. J Med Genet 2023:0:1–7. doi:10.1136/jmg-2023-109329)

hEDS patient 107 carried a novel cysteine substitution (variant 37) in MAPK7 (cardiospondylarocopalcial syndrome, MIM 137800) 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 WNT110A (variant 21, CADD=30, odontoonychoedermal 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.

## 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 1495 who was not known to have a personal or familial 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

Additional 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 Meeste-Loeys. 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 IFIH1 (CADD=31), in the helicase domain (MIM 606951, Singleton-Merten syndrome, acroosteolysis and aortic valve calcification). HDCT patient 79 carried EMILIN1 VUS at amino acid residue 28, close to residue 22, thought to affect N terminal signal peptide cleavage.15 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). A single patient with hEDS in our cohort (patient 703) had a LZTS1 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.26 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.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 endomyosal 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); perisin 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 GWAs

We identified multiple rare variants with CADD >15 in genes associated (p<5×10^-8) with self-measured Beighton score >5 in a published GWAS. These included the PIEZO Type Mechano-sensitive Ion Channel Component 1 (PIEZO1) and NEDD4 E3 ubiquitin protein ligase (NEDD4) (online supplemental table 17). PIEZO1 is a mechanotransducer 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 aeurysmal 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 betal signalling; mice lacking its homologue, hic5, show deficient smooth muscle cell response to vascular injury (MIM 602353).17 This variant at TGFB1/1 Arg 67, neighbours phosphoserine 68, hence may disturb signal transduction. hEDS 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, IGBP2 and TMEM176B and MIM2.40 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 NOUTCH4, (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 aeurysms (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 (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. We also noted multiple heterozygous VUS in autosomal recessive skeletal dysplasia genes, CANT1, TONSIL, OSTM1 (data not shown).

Biallelic pathogenic variants in ADAMTS22 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, we found multiple rare variants in other (non-annotated) serine proteases (online supplemental table 19).

Integrins, ephrin, ciliopathy, TSPANs, 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 ITGB1BPI) (online supplemental table 19). Integrin-collagen interactions are integral to wound healing, inflammation, innate immunity and via TGFBeta signalling and other pathways. 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 cathecolamine synthesis. Ciliopathies are generally associated with complex phenotypes; however, variants in IFT88 and NEATC3 were recently reported with bicuspid aortic valve. We identified two novel variants in these genes. Wound healing is known to be under circadian rhythm control through local and central mechanisms. 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. We also identified multiple variants in various TSPANs. TSPAN2 regulates TGFβ1/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 (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 UCSC 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 proteoglycone) 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 muscular 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.

**DISCUSSION**

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

(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.

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), erythropoiesis (n = 1) with compelling supporting clinical and in silico criteria for pathogenicity, according to ACOS 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, INORD, SYAP1), with biological plausibility based on published data. Animal models of vascular disease and EDS phenotypes. A large number of rare variants with CADD > 15 were identified in genes not previously reported as associated with a Mendelian phenotype. The 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.

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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.

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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, CC, MB) and at the UCH hypermobility clinic (HR, 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, DIT-M and AM, phenotype summary and review by AMV, CK, RAW, DIT-M, FMP and FSoD; TASER analysis by RD and HJC. The paper was written by AMV and TJA. TJA acts as guarantor.

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Competing interests

TA is co-founder and director of the company BioCaptcha. 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, Cambridge62 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.

Supplemental material

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