Genetic Testing for Connective Tissue Disorders

Policy Number: AHS – M2144 – Genetic Testing for Connective Tissue Disorders	Policy Revision Date: 07/01/2025 Initial Policy Effective Date: 12/01/2024

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I. Policy Description

More than 200 heritable connective tissue disorders exist and include Marfan Syndrome (MFS), Ehlers-Danlos syndrome (EDS), epidermolysis bullosa (EB), and Loeys-Dietz syndrome (LDS).[1] Every disorder impacts connective tissue differently, including several with vascular implications, and clinical severity varies within each disorder.

Terms such as male and female are used when necessary to refer to sex assigned at birth.

II. Related Policies

Policy Number	Policy Title
AHS-M2083	Genetic Testing for Ophthalmologic Conditions
AHS-M2145	General Genetic Testing, Germline Disorders

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For individuals who have consulted with a cardiology specialist prior to genetic testing, *FBN1* variant testing for Marfan Syndrome **MEETS COVERAGE CRITERIA** in the following situations:
   a) When Marfan syndrome is suspected based on clinical features, but a definitive diagnosis cannot be made using established clinical diagnostic criteria (see Note 1).
   b) For an asymptomatic individual who has an affected first-degree blood relative (see Note 2) with a known pathogenic or likely pathogenic variant.
   c) For the prenatal diagnosis or preimplantation genetic diagnosis (PGD) of Marfan syndrome in the offspring of patients with known pathogenic or likely pathogenic variants.
2) Genetic testing for Loeys-Dietz Syndrome (*TGFBR1* or *TGFBR2* variant) **MEETS COVERAGE CRITERIA** in the following situations:
   a) To confirm or establish a diagnosis of LDS in an individual with vascular characteristics of LDS (see Note 3).

b) For an asymptomatic individual who has an affected first-degree blood relative (see Note 2) with a known pathogenic or likely pathogenic variant.

c) For individuals who meet other clinical diagnostic criteria for Marfan syndrome but who do not have the clinical feature of ectopia lentis.

d) For individuals who are clinically suspected of having Marfan Syndrome but who have tested negative for *FBN1*.

3) For individuals with characteristics of vascular Ehlers-Danlos Syndrome (vEDS) (see Note 4), genetic panel testing for *COL3A1* and *COL1A1* variants to confirm or establish a diagnosis of vEDS **MEETS COVERAGE CRITERIA.**

4) The following genetic testing for heritable thoracic aortic disease (HTAD) (see Note 5) **MEETS COVERAGE CRITERIA:**

   a) Multigene panel testing (see Note 6, Note 7) in the following situations:

      i) For individuals with thoracic aortic disease (TAD) **and** syndromic features of Marfan syndrome, Loeys-Dietz syndrome, **or** vascular Ehlers-Danlos syndrome.

      ii) For individuals presenting with TAD before 60 years of age.

      iii) For individuals with a family history of either TAD or peripheral/intracranial aneurysms in a first- or second-degree relative (see Note 2).

      iv) For individuals with a first- or second-degree relative (see Note 2) who had an unexplained sudden death at a relatively young age.

   b) Testing restricted to the known variant for individuals with a close blood relative (see Note 2) with a known pathogenic or likely pathogenic variant.

5) The following genetic testing for epidermolysis bullosa (EB) **MEETS COVERAGE CRITERIA**:

   a) Single gene or multigene panel testing (see Note 6, Note 8) for individuals for whom there is a clinical suspicion of EB.

   b) Testing restricted to the known pathogenic or likely pathogenic variant in the following situations:

      i) For the biological parents of an individual who has been identified to have a pathogenic or likely pathogenic variant for EB.

      ii) For the prenatal diagnosis or PGD of EB in the offspring of individuals with known pathogenic or likely pathogenic variants.

      iii) *COL7A1* testing for individuals being considered for beremagene geperpavec.

6) For individuals with characteristics of hypermobile Ehlers-Danlos syndrome (hEDS) (see Note 9), genetic testing to confirm or establish a diagnosis **DOES NOT MEET COVERAGE CRITERIA.**

7) All other gene testing for Marfan Syndrome or other connective tissue disorders, including Ehlers-Danlos Syndrome, **DOES NOT MEET COVERAGE CRITERIA**.

Sentara Health Plans

NOTES:

Note 1: Clinical Diagnostic Criteria for Marfan Syndrome is as follows:

Revised Ghent nosology — The 2010 revised Ghent nosology puts greater weight on aortic root dilatation/dissection and ectopia lentis as the cardinal clinical features of MFS and on testing for mutations in FBN1.[2,3]

- In the absence of family history of MFS, the presence of one of any of the following criteria is diagnostic for MFS:
  - Aortic criterion (aortic diameter Z ≥2 or aortic root dissection) and ectopia lentis*
  - Aortic criterion (aortic diameter Z ≥2 or aortic root dissection) and a causal *FBN1* mutation
  - Aortic criterion (aortic diameter Z ≥2 or aortic root dissection) and a systemic score ≥7 points*
  - Ectopia lentis and a causal *FBN1* mutation that has been identified in an individual with aortic aneurysm
- In the presence of family history of MFS (as defined by the above criteria), the presence of one of any of the following criteria is diagnostic for MFS:
  - Ectopia lentis
  - Systemic score ≥7 points*
  - Aortic criterion (aortic diameter Z ≥2 above 20 years old, Z ≥3 below 20 years, or aortic root dissection) *

For criteria with an asterisk (*), the diagnosis of MFS can be made only in the absence of discriminating features of Shprintzen-Goldberg syndrome, Loeys-Dietz syndrome, or vascular Ehlers-Danlos syndrome and after *TGFBR1/2*, collagen biochemistry, or *COL3A1* testing if indicated.

Systemic score — The revised Ghent nosology includes the following scoring system for systemic features:[2,3]

- Wrist AND thumb sign: 3 points
- Wrist OR thumb sign: 1 point
- Pectus carinatum deformity: 2 points
- Pectus excavatum or chest asymmetry: 1 point
- Hindfoot deformity: 2 points
- Plain pes planus: 1 point
- Pneumothorax: 2 points
- Dural ectasia: 2 points
- Protrusio acetabuli: 2 points
- Reduced upper segment/lower segment ratio AND increased arm span/height AND no severe scoliosis: 1 point
- Scoliosis or thoracolumbar kyphosis: 1 point
- Reduced elbow extension (≤170 degrees with full extension): 1 point
- Facial features (at least three of the following five features: dolichocephaly, malar hypoplasia, enophthalmos, downslanting palpebral fissures, retrognathia): 1 point
- Skin striae: 1 point
- Myopia >3 diopters: 1 point
- Mitral valve prolapse: 1 point

Note 2: Close blood relatives include first-degree relatives (e.g., parents, siblings, and children), second-degree relatives (e.g., grandparents, aunts, uncles, nieces, nephews, grandchildren, and half-siblings), and third-degree relatives (great-grandparents, great-aunts, great-uncles, great-grandchildren, and first cousins).

Note 3: Clinical features of Loeys-Dietz Syndrome: aortic/arterial aneurysms/tortuosity, arachnodactyly, bicuspid aortic valve and patent ductus arteriosus, blue sclerae, camptodactyly, cerebral, thoracic or abdominal arterial aneurysms and/or dissections, cleft palate/bifid uvula, club feet, craniosynostosis, easy bruising, joint hypermobility, ocular hypertelorism, pectus carinatum or pectus excavatum, scoliosis, talipes equinovarus, thin skin with atrophic scars, velvety and translucent skin, widely spaced eyes.[4]

Note 4: Clinical features of vascular EDS (vEDS):[5]

- Inheritance: Autosomal dominant
- Major criteria
    1. Family history of vEDS with documented causative variant in COL3A1
    2. Arterial rupture at a young age
    3. Spontaneous sigmoid colon perforation in the absence of known diverticular disease or other bowel pathology
    4. Uterine rupture during the third trimester in the absence of previous C-section and/or severe peripartum perineum tears
    5. Carotid-cavernous sinus fistula (CCSF) formation in the absence of trauma
- Minor criteria
    6. Bruising unrelated to identified trauma and/or in unusual sites such as cheeks and back
    7. Thin, translucent skin with increased venous visibility
    8. Characteristic facial appearance
    9. Spontaneous pneumothorax
    10. Acrogeria
    11. Talipes equinovarus
    12. Congenital hip dislocation
    13. Hypermobility of small joints
    14. Tendon and muscle rupture
    15. Keratoconus
    16. Gingival recession and gingival fragility
    17. Early onset varicose veins (under age 30 and nulliparous if female)
- Minimal criteria suggestive for vEDS:
  A family history of the disorder, arterial rupture or dissection in individuals less than 40 years of age, unexplained sigmoid colon rupture, or spontaneous pneumothorax in the presence of other features consistent with vEDS should all lead to diagnostic studies to determine if the individual has vEDS. Testing for vEDS should also be considered in the presence of a combination of the other "minor" clinical features listed above.

Note 5: Heritable thoracic aortic disease (HTAD) usually involves the aortic root, ascending aorta, or both; however, it may also present with distal aortic disease and aortic dissection.[6]

Note 6: For two or more gene tests being run on the same platform, please refer to AHS-R2162 Reimbursement Policy.

Note 7: When multigene panel testing is performed in individuals with suspected heritable thoracic aortic disease (HTAD), the panel must include the following genes: *ACTA2, COL3A1, FBN1, LOX, MYH11, MYLK, PRKG1, SMAD3, TGFB2, TGFBR1,* and *TGFBR2.*[6]

Note 8: When multigene panel testing is performed in individuals with suspected EB, the panel must include the following genes: *CD151, COL17A1, COL7A1, DSP, DST, EXPH5, FERMT1, ITGA3, ITGA6, ITGB4, JUP, KLHL24, KRT14, KRT5, LAMA3, LAMA3A, LAMB3, LAMC2, PKP1, PLEC,* and *TGM5.*[7]

Note 9: Clinical features of Hypermobile EDS (hEDS):[5]

- Inheritance: Autosomal dominant
- Molecular basis: Unknown
- Clinical diagnosis
  : Clinical spectrum ranging from asymptomatic joint hypermobility, through "non-syndromic" hypermobility with secondary manifestations, to hEDS.

The clinical diagnosis of hEDS needs the simultaneous presence of criteria 1 AND 2 AND 3:

Criterion 1: Generalized Joint Hypermobility (GJH)
Criterion 2: Two or More Among the Following Features (A–C) MUST Be Present (for Example: A and B; A and C; B and C; A and B and C):

Feature A: systemic manifestations of a more generalized connective tissue disorder (a total of five must be present):

1. Unusually soft or velvety skin
2. Mild skin hyperextensibility
3. Unexplained striae such as striae distensae or rubrae at the back, groins, thighs, breasts and/or abdomen in adolescents, men or prepubertal women without a history of significant gain or loss of body fat or weight
4. Bilateral piezogenic papules of the heel
5. Recurrent or multiple abdominal hernia(s) (e.g., umbilical, inguinal, crural)
6. Atrophic scarring involving at least two sites and without the formation of truly papyraceous and/or hemosideric scars as seen in classical EDS
7. Pelvic floor, rectal, and/or uterine prolapse in children, men, or nulliparous women without a history of morbid obesity or other known predisposing medical condition
8. Dental crowding and high or narrow palate
9. Arachnodactyly, as defined in one or more of the following: (i) positive wrist sign (Steinberg sign) on both sides; (ii) positive thumb sign (Walker sign) on both sides
10. Arm span-to-height ≥1.05
11. Mitral valve prolapse (MVP) mild or greater based on strict echocardiographic criteria
12. Aortic root dilatation with Z-score > +2

Feature B: positive family history, with one or more first degree relatives independently meeting the current diagnostic criteria for hEDS.

Feature C: musculoskeletal complications (must have at least one):

1. Musculoskeletal pain in two or more limbs, recurring daily for at least 3 months
2. Chronic, widespread pain for ≥3 months
3. Recurrent joint dislocations or frank joint instability, in the absence of trauma (a or b)

a. Three or more atraumatic dislocations in the same joint or two or more atraumatic dislocations in two different joints occurring at different times
b. Medical confirmation of joint instability at two or more sites not related to trauma

Criterion 3: All the Following Prerequisites MUST Be Met:

1. Absence of unusual skin fragility, which should prompt consideration of other types of EDS
2. Exclusion of other heritable and acquired connective tissue disorders, including autoimmune rheumatologic conditions. In patients with an acquired connective tissue disorder (e.g., lupus, rheumatoid arthritis, etc.), additional diagnosis of hEDS requires meeting both Features A and B of Criterion 2. Feature C of Criterion 2 (chronic pain and/or instability) cannot be counted towards a diagnosis of hEDS in this situation.
3. Exclusion of alternative diagnoses that may also include joint hypermobility by means of hypotonia and/or connective tissue laxity. Alternative diagnoses and diagnostic categories include, but are not limited to, neuromuscular disorders (e.g., myopathic EDS, Bethlem myopathy), other HCTD (e.g., other types of EDS, Loeys–Dietz syndrome, Marfan syndrome), and skeletal dysplasias (e.g., OI). Exclusion of these considerations may be based upon history, physical examination, and/or molecular genetic testing, as indicated.

IV. Table of Terminology

Term	Definition
AAP	American Academy of Pediatrics
AATS	American Association for Thoracic Surgery
ACC	American College of Cardiology
ACCF	American College of Cardiology Foundation
ACMG	American College of Medical Genetics
ACR	American College of Radiology
ACTA2	*Actin alpha 2, smooth muscle gene*
AD	Autosomal dominant
ADAMTS2	*ADAM metallopeptidase with thrombospondin type 1 motif 2 gene*
aEDS	Arthrochalasia Ehlers-Danlos syndrome
AHA	American Heart Association
AngII	Angiotensin II
AR	Autosomal recessive
ARBs	Angiotensin receptor blockers
ASA	American Stroke Association
ATR1	Angiotensin II receptor type 1
B3GALT6	*Beta-1,3-galactosyltransferase 6* gene
B4GALT7	*Beta-1,4-galactosyltransferase 7* gene
BAV	Bicuspid aortic valve
BCS	Brittle cornea syndrome
C	Carboxy
C1R	Complement C1r
C1S	Complement C1s
CCS	Canadian Cardiovascular Society
CCSF	Carotid-cavernous sinus fistula
cEDS	Classical Ehlers-Danlos syndrome

CHST14	*Carbohydrate sulfotransferase 14* gene
clEDS	Classical-like Ehlers-Danlos syndrome
CLIA ’88	Clinical Laboratory Improvement Amendments Of 1988
CMS	Centers For Medicare and Medicaid Services
CNV	Copy number variant
COL12A1	*Collagen type XII alpha 1 chain* gene
COL1A1	*Collagen type I alpha 1 chain* gene
COL1A2	*Collagen type I alpha 2 chain* gene
COL1A2 NMD	*Collagen type I alpha 2* gene nonsense-mediated mRNA decay
COL3A1	*Collagen type III alpha 1 chain* gene
CPD	Clinical provisional diagnosis
CT	Computerized tomography
cvEDS	Cardiac-valvular Ehlers-Danlos syndrome
CVS	Chorionic villus sampling
D4ST1	Dermatan 4-sulfotransferase-1 protein
DDEB	Dominant dystrophic epidermolysis bullosa
dEDS	Dermatosparaxis Ehlers-Danlos syndrome
DNA	Deoxyribonucleic acid
DSE	*Dermatan sulfate epimerase* gene
EB	Epidermolysis bullosa
EDS	Ehlers-Danlos syndrome
EFEMP2	*EGF containing fibulin extracellular matrix protein 2* gene
EGF	*Epidermal growth factor*
ELN	*Elastin gene*
EM	Electron microscopy
FBN1	*Fibrillin-1* gene
FBN2	*Fibrillin-2* gene
FDA	Food and Drug Administration
FKBP14	*FKBP (FK506 binding protein) prolyl isomerase 14* gene
FKBP22	*FKBP (FK506 binding protein) prolyl isomerase 22* gene
FLNA	*Filamin A* gene
GAG	Glycosaminoglycan
GJH	Generalized joint hypermobility
HCTD	Heritable connective tissue disorders
hEDS	Hypermobile Ehlers-Danlos syndrome
HP	Hydroxylysyl-pyridinoline
HPLC	High-performance liquid chromatography
IFM	Immunofluorescence mapping
kEDS	Kyphoscoliotic
KRT14	*Keratin 14* gene
KRT5	*Keratin 5* gene
LDS	Loeys-Dietz syndrome
LDTs	Laboratory-developed tests
LH1	Lysyl hydroxylase 1
LOX	Lysyl oxidase gene

LP	Lysyl-pyridinoline
MCC	Meets coverage criteria
mcEDS	Musculocontractural Ehlers-Danlos syndrome
mEDS	Myopathic Ehlers-Danlos syndrome
MFS	Marfan syndrome
MLPA	Multiplex ligation-dependent probe amplification
MRI	Magnetic resonance imaging
MVP	Mitral valve prolapse
MYH11	*Myosin heavy chain 11* gene
MYLK	*Myosin light chain kinase* gene
NGS	Next-generation sequencing
NORD	National Organization for Rare Disorders
OI	Osteogenesis imperfecta
PCR	Polymerase chain reaction
pEDS	Periodontal Ehlers-Danlos syndrome
PGD	Preimplantation genetic diagnosis
PGT-M	Preimplantation genetic testing for monogenic diseases
PLEC	*Plectin gene*
PLOD1	*Procollagen-Lysine,2-Oxoglutarate 5-Dioxygenase 1* gene
PRDM5	*PR/SET Domain 5* gene
PRKG1	*Protein kinase cGMP-dependent 1* gene
qPCR	quantitative polymerase chain reaction
RCT	Randomized controlled trial
RDEB	Recessive dystrophic epidermolysis bullosa
RNA	Ribonucleic acid
SCA	Society of Cardiovascular Anesthesiologists
SCAI	Society for Cardiovascular Angiography and Interventions
SDS-PAGE	Sodium dodecyl sulphate–polyacrylamide gel electrophoresis
SIR	Society of Interventional Radiology
SKI	SKI proto-oncogene
SLC2A10	*Solute Carrier Family 2 Member 10* gene
SLC39A13	*Solute Carrier Family 39 Member 13* gene
SMAD3	*Mothers against decapentaplegic homolog 3* gene
SMAD4	*Mothers against decapentaplegic homolog 4* gene
SMC	Smooth muscle cell
spEDS	Spondylodysplastic Ehlers-Danlos syndrome
STS	Society of Thoracic Surgeons
SVM	Society for Vascular Medicine
TAD	Thoracic aortic disease
TAAD	Thoracic aortic aneurysm and dissection
TEM	Transmission electron microscopy
TGFB	*Transforming growth factor beta gene*
TGFB2	*Transforming Growth Factor B 2 Ligand* gene
TGFB3	*Transforming Growth Factor B 3 Ligand* gene
TGFBR	*Transforming growth factor beta receptor* gene

TGFBR1	*Transforming Growth Factor B Receptor I* gene
TGFBR2	*Transforming Growth Factor B Receptor II* gene
TGF-β	Transforming Growth Factor-B
TNXA	*Tenascin XA* (Pseudogene)
TNXB	*Tenascin XB* gene
vEDS	Vascular Ehlers-Danlos Syndrome
VUS	Variant of unknown significance
WES	Whole exome sequencing
WGS	Whole genome sequencing
ZIP13	Zrt- and Irt-like protein 13
ZNF469	*Zinc finger protein 469* gene

V. Scientific Background

Connective tissue helps to bind and support other types of tissue in the body. Unfortunately, many types of connective tissue afflictions exist, including more than 200 heritable connective tissue disorders such as Marfan Syndrome (MFS), Ehlers-Danlos syndrome (EDS), epidermolysis bullosa (EB), and Loeys-Dietz syndrome (LDS). Each disorder affects connective tissue in a different manner. Symptoms may include joint issues, bone growth problems, blood vessel damage, cranial structural problems, skin problems, and height issues.[8]

Connective tissue disorders share pathophysiology and a genetic overlap with TAAD disorders, which are often secondary to underlying connective tissue disorders, and arise from abnormalities in the structural integrity of the connective tissue of the aortic wall.

Thoracic Aortic Aneurysm and Dissection (TAAD) disorders

TAAD, or Thoracic Aortic Aneurysm and Dissection (TAAD) disorders is a term for conditions that contain mutations that lead to aortic aneurysms. TAAD encompasses a group of connective tissue conditions characterized by structural abnormalities and weakness in the aortic wall, predisposing affected individuals to aneurysm formation and the need for subsequent dissection. Historically, while syndromic conditions such as Marfan syndrome and vascular EDS were recognized, TAAD was intended to capture the "non-syndromic" group using a progressive study of single genes, candidate genes and ultimately exomes and genomes. The loci were mapped by traditional genetic tools; a rise in the use and expediency of next generation sequencing accelerated the identification of key genes that make up TAAD disorders

TAAD disorders can have both genetic and sporadic origins, with hereditary forms linked to mutations in genes encoding structural proteins or signaling pathways that maintain vascular integrity. These include mutations in *FBN1* (associated with Marfan syndrome), *CAOL3A1* (linked to vascular Ehlers-Danlos syndrome), and *TGFBR1/TGFBR2* (involved in Loeys-Dietz syndrome). Pathogenic variants disrupt the extracellular matrix or signaling mechanisms, resulting in faulty aortic walls and reduced resistance to reduced resistance to hemodynamic stress.[6,9]

Several labs offer panel testing to identify mutations in individuals with syndromic and non-syndromic forms of aneurysms. Ambry Genetics offers the TAADNext panel, analyzing 35 genes associated with thoracic aortic aneurysms and related disorders.[10] Prevention Genetics offers the "Marfan syndrome and related Aortopathies Panel," testing for syndromic and non-syndromic causes of TAAD.[11] GeneDx

offers the Marfan/TAAD panel, which includes genes related to Marfan syndrome, Loeys-Dietz syndrome, and other related disorders.[12] Invitae offers the Aortopathy Comprehensive panel, analyzing genes associated with inherited aortopathy and related conditions.[13] LabCorp offers the GeneSeq Cardio: Familial Aortopathy panel and Fulgent genetics provides the “Marfan Syndrome and Thoracic Aortic Aneurysm and Dissection NGS Panel,” testing for genes associated with TAAD.[14,15]

Marfan syndrome

Marfan Syndrome (MFS) was first described more than 100 years ago by a Parisian professor of pediatrics, Antoine-Bernard Marfan. He was the first to report the association of long slender digits with other skeletal abnormalities in a 5-year-old girl.[16] MFS is a fairly common condition with an incidence of about 1 in 3000 to 5000 individuals. MFS is a systemic disorder of connective tissue with significant clinical variability across a broad phenotypic continuum, ranging from mild isolated features to severe and rapidly progressive neonatal multiorgan disease.[17] Ocular findings include myopia, ectopia lentis, and an increased risk for retinal detachment, glaucoma, and early cataracts. Skeletal system symptoms include “bone overgrowth and joint laxity, disproportionately long extremities for the size of the trunk, overgrowth of the ribs, and scoliosis.” The major cause of death in MFS results from cardiovascular system problems, including aortic root dilatation and rupture, mitral or tricuspid valve prolapse, and enlargement of the proximal pulmonary artery. Severe and prolonged regurgitation of the mitral or aortic valve can lead to left ventricular dysfunction and heart failure. Patients presenting with one isolated symptom are rare. However, with careful management, life expectancy approximates that of the general population.[3,18,19]

MFS primarily affects connective tissue, particularly the fibrillin component of the extracellular matrix. Fibrillins are large glycoproteins that form extracellular microfibrils that provide elasticity and structural support to tissues, modulate elastic fiber biogenesis and homeostasis, and regulate the bioavailability and activity of different growth factors.[20,21] Fibrillin-1 is an important matrix component of both elastic and nonelastic tissues.[3] Mutations can lead to impaired fibrillin-1 protein function, causing extracellular matrix integrity to fail.[20] These fibrillin-1 problems also cause smooth muscle cell (SMC) contractile dysfunction and dysregulation of the tensile strength of aortic tissue, which is a common finding in many cardiovascular conditions.[22] Recent studies indicate a role for SMC phenotype in the pathogenesis of MFS. Early phenotypic switch resulting from *FBN1* mutation appears to be associated with initiation of important metabolic changes in SMCs that contribute to subsequent pathology.[23] Mutation in *FBN1* has been shown to dysregulate the transforming growth factor-β (TGF-β) pathway, as matrix sequestration of cytokines is crucial to their regulated activation and signaling.[24,25]

Ehlers Danlos syndrome (EDS)

Ehlers Danlos syndrome is a term that encompasses several rare genetic connective tissue disorders. Each disorder is characterized by specific features, including “skin hyperextensibility, joint hypermobility, and tissue fragility,” and affects approximately 1 in 5000 individuals.[26] EDS hypermobile type (hEDS) is the most common type of EDS. Unfortunately, the genetic basis for hEDS is still unknown, meaning that a genetic test to confirm diagnosis is not available for this subtype. As of 2017, an international forum has classified EDS into 13 different subtypes. The table below has been modified from Malfait, et al. (2017) and lists all EDS types:

Clinical EDS Subtype	Abbreviation	Inheritance Pattern	Genetic Bases	Protein

Classical EDS	cEDS	AD (autosomal dominant)	Major: COL5A1, COL5A1 Rare: COL1A1	Type V collagen Type I collagen
Classical-like EDS	clEDS	AR (autosomal recessive)	TNXB	Tenascin XB
Cardiac-valvula	cvEDS	AR	COL1A2 (biallelic mutations that lead to COL1A2 NMD and absence of pro α2(I) collagen chains)	Type I collagen
Vascular EDS	vEDS	AD	Major: COL3A1 Rare: COL1A1	Type III collagen Type I collagen
Hypermobile EDS	hEDS	AD	Unknown	Unknown
Arthrochalasia EDS	aEDS	AD	COL1A1, COL1A2	Type I collagen
Dermatosparaxis EDS	dEDS	AR	ADAMTS2	ADAMTS-2
Kyphoscoliotic EDS	kEDS	AR	PLOD1 FKBP14	LH1 FKBP22
Brittle Cornea syndrome	BCS	AR	ZNF469 PRDM5	ZNF469 PRDM5
Spondylodysplastic EDS	spEDS	AR	B4GALT7 B3GALT6 SLC39A13	β4GalT7 β3GalT6 ZIP13
Musculocontractural EDS	mcEDS	AR	CHST14 DSE	D4ST1 DSE
Myopathic EDS	mEDS	AD or AR	COL12A1	Type XII collagen
Periodontal EDS	pEDS	AD	C1R C1S	C1r C1s

This naming convention has also been adopted by The Ehlers Danlos Society (EDS, 2017), who previously used Villefranche nosology to classify EDS types. Unfortunately, no cure for EDS currently exists, and treatments may include physical therapy, braces, counseling, and pain medication.[26]

Vascular EDS (vEDS) is characterized by "arterial aneurysm, dissection and rupture, bowel rupture, and rupture of the gravid uterus" and affects 1 in 50,000 to 200,000 individuals.[27] These arterial aneurysms may be life threatening. As noted in the table above, this disorder is due to mutations in the *COL3A1* or *COL1A1* genes, with a sequence analysis of *COL3A1* thought to identify approximately 98% of vEDS cases.[5] A diagnosis depends on clinical features, including family history. Aneurysms occur in other types of EDS, including classical EDS (cEDS), due to vascular fragility.[28] Johansen, et al. (2020) published a recent cross-sectional study with data collected from 18 patients with genetically verified vEDS and 34 patients with genetically verified LDS. The median age at diagnosis was 34 years. "Most respondents (87%) had cardiovascular surveillance visits, 58% yearly or more often, and still 29% had no antihypertensive medications."[29]

Loeys-Dietz syndrome

Loeys-Dietz syndrome (LDS) was first described in 2005 and is now considered an autosomal dominant connective tissue disorder characterized by "aortic aneurysms and generalized arterial tortuosity, hypertelorism, and bifid/broad uvula or cleft palate."[30] LDS was initially characterized by mutations in the transforming growth factor β receptor I (*TGFBR1*) and transforming growth factor β receptor II (*TGFBR2*) genes; however, additional genes have been identified, including the mothers against decapentaplegic homolog 3 (*SMAD3*) gene, the transforming growth factor β 2 ligand (*TGFB2*) gene, and the transforming growth factor β 3 ligand (*TGFB3*) gene.[3,30] If a mutation is identified in all three genes, transforming growth factor-β (TGF-β) signaling is affected and patients typically exhibit similar craniofacial, cutaneous, cardiovascular, and skeletal features. Vascular involvement in LDS has recently been studied by Jud and Hafner (2019) who published a case study which followed a woman with a history of ectasias of the aortic arch, abdominal aorta, carotid bulbs, and common femoral arteries, as well as an asymptomatic aneurysm in superior mesenteric artery. In comparing surgical outcomes between those with LDS versus MFS, it was found that LDS patients had a greater likelihood of reoperation for aortic arch aneurysms than MFS patients, and that those with mutations in *TGFBR1* had higher rates of reoperation than those with *TGFBR2* mutations.[32]

Epidermolysis bullosa

Epidermolysis bullosa (EB) is a group of hereditary diseases characterized by mucosa and skin fragility due to mutations that affect skin structural proteins, causing the skin to easily blister. Four major types of EB have been identified and include EB simplex, junctional EB, dystrophic EB, and Kindler syndrome.[33] Unfortunately, there is currently no effective therapeutic option for this disorder, and treatment largely focuses on wound management. All of the major EB types may result from mutations in the keratin 5 (*KRT5*) or keratin 14 (*KRT14*) gene.[34,35] These two genes work together to encourage strength in the epidermis. Mutations prevent the keratin from assembling in necessary networks, leading to fragility. Further, a rare type of EB, known as Ogna, has been associated with mutations in the *PLEC* gene, leading to issues in the attachment of the epidermis to other layers of the skin.[35] Ryan, et al. (2016) note that ventricular dysfunction and aortic dilation have been identified in patients with recessive dystrophic EB.

Clinical Utility and Validity

More than 90% of patients with the typical Marfan phenotype have mutations involving the gene encoding the connective tissue protein fibrillin-1 (*FBN1*). Out of a sample of 93 patients with MFS, 85 (91%) were found to have a *FBN1* mutation. The eight remaining patients did not display any drastically different clinical features or family history, and the authors suggest that *FBN1* mutations that go undetected are due to technical limitations.[37] Most patients have a family history of MFS, but up to 25% have a mutation *de novo*. Mutations are in one of five categories: nonsense, frameshift (deletion, insertion), splicing errors, a missense mutation that substitutes or creates cysteine residues, or a missense mutation affecting a conserved *EGF* sequence. Although the phenotypic variability is wide, mutations involving exon skipping tend to result in more severe disease. Genetic findings have importance in the diagnosis, risk stratification, and clinical management of patients, as well as identifying potentially affected relatives.[3]

Becerra-Munoz, et al. (2018) conducted a prospective cohort study to summarize variants in *FBN1* and establish a genotype-phenotype correlation. Genotype-phenotype correlations have identified that patients with MFS and truncating variants in *FBN1* presented a higher proportion of aortic events

compared to a more benign course in patients with missense mutations. A total of 84 patients fulfilled the Ghent diagnostic criteria, and of these 84, 44 had missense mutations and 35 had truncating mutations. However, of the 44 with missense mutations, only six had suffered an aortic event (such as aortic aneurysm) whereas 20 of the 35 with a truncating mutation had suffered an aortic event.[38] Up to 10% of patients with the Marfan phenotype have no identifiable mutation in the *FBN1* gene. Rather, mutations are identified in TGF-beta receptor 1 (*TGFBR1*) and *TGFBR2* genes. It has been proposed that patients with the Marfan phenotype and *TGFBR1* or *TGFBR2* mutations be classified as having LDS to properly address the potential for more aggressive vascular disease than seen in MFS.[3]

The diagnosis of MFS is now established by an *FBN1* pathogenic variant known to be associated with Marfan syndrome AND one of the following: aortic root enlargement (Z-score ≥2.0), ectopia lentis, demonstration of aortic root enlargement (Z-score ≥2.0) and ectopia lentis OR a defined combination of features throughout the body yielding a systemic score ≥7.[18] These features are summarized in the 2010 Ghent nosology, which is slightly altered for patients under 20 years old.[3] Due to the identification of *FBN1* as the genetic basis for MFS and .its subsequent effects, the understanding of MFS as a structural disorder has become one of a developmental abnormality with broad effects on the morphogenesis and function of multiple organ systems. Importantly, this also introduced new biological targets for treatment strategies in MFS.[39,40]

Current clinical studies have elucidated a medical regimen for patients with MFS to help control the progression of cardiovascular manifestations and resulting mortality. The standard of care for medical management includes the use of β-blockers with supplementation or replacement by angiotensin receptor blockers (ARBs). However, the best course of treatment is a subject of ongoing research.[25,41] However, a Cochrane review concluded, “Based on only one, low-quality RCT comparing long-term propranolol to no treatment in people with Marfan Syndrome, we could draw no definitive conclusions for clinical practice.” The authors concluded that further, high-quality, randomized trials were needed to evaluate the long-term efficacy of beta-blocker treatment in people with Marfan syndrome.[42] Sellers, et al. (2018) recently reported, “Despite promising preclinical and pilot clinical data, a recent large-scale study using antihypertensive angiotensin II (AngII) receptor type 1 (ATR1) blocker losartan has failed to meet expectations at preventing MFS-associated aortic root dilation, casting doubts about optimal therapy.” Their mouse study suggested that “increased protective endothelial function, rather than ATR1 inhibition or blood pressure lowering, might be of therapeutic significance in preventing aortic root disease in MFS.”[43]

Johansen, et al. (2020),Shalhub, et al. (2020),Ritelli, et al. (2020) analyzed vEDS data from 11 institutions between the year 2000 and 2015. Data used for this study included family history, clinical features, diagnostic criteria, demographics, and molecular testing results. A total of 173 individuals were identified for the purposes of this study, with 11 excluded because pathogenic *COL3A1* variants were not identified. Of the remaining individuals, 86 had been diagnosed with a pathogenic *COL3A1* variants, and 76 were diagnosed with only clinical criteria. “Compared with the cohort with pathogenic *COL3A1* variants, the clinical diagnosis only cohort had a higher number of females (80.3% vs 52.3%; P < .001), mitral valve prolapse (10.5% vs 1.2%; P = .009), and joint hypermobility (68.4% vs 40.7%; P < .001). Additionally, they had a lower frequency of easy bruising (23.7% vs 64%; P < .001), thin translucent skin (17.1% vs 48.8%; P < .001), intestinal perforation (3.9% vs 16.3%; P = .01), spontaneous pneumothorax/hemothorax (3.9% vs 14%, P.03), and arterial rupture (9.2% vs 17.4%; P = .13).”[44] This study highlights the importance of genetic testing for a vEDS diagnosis as the symptoms of vEDS overlap with many other disorders and a correct diagnosis is necessary for efficient disease treatment. Further,

not all *COL3A1* variants are pathogenic, meaning that genetic results must be interpreted by a trained professional.

Using a next-generation sequencing (NGS) multigene panel, Mariath, et al. (2019) identified 11 disease-causing variants of EB in a Brazilian population with an efficiency of 94.3%. Other studies that they have included have calculated efficiencies of 83.5% for a panel with 21 genes, 90% with 49 genes, and 97.7% in 21 genes, where all identified mutations were only in five genes. This conveys the clinical utility of gene variants in EB that could be translated to other connective tissue disorder mutations. In a study done with children with inherited EB, the accuracy of several diagnostic techniques, which included electron microscopy (EM), immunofluorescence mapping (IFM), and clinical provisional diagnosis (CPD) was evaluated. It was found that IFM, EM, and CPD yielded an accuracy of 75%, 75%, and 81.5%, respectively.[47] All genetic components, tissue specimen, and clinical history are all necessary for a confirmed EB diagnosis.

Li, et al. (2021) conducted a study in northwestern China to determine the genotype-phenotype correlation for thoracic aortic aneurysm and dissection via NGS. They screened 15 genes from 212 patients to find that 67 (31.60%) patients in this cohort had a (likely) pathogenic variant, “42 (19.81%) had a variant of uncertain significance (VUS), and 103 (48.58%) had no variant (likely benign/benign/negative),” with 135 reportable variants. With *FBN1*, a gene implicated in MFS, they found that “patients with truncating and splicing mutations are more prone to developing severe aortic dissection than those with missense mutations, especially frameshift mutations (82.76% vs. 42.86%),” and “the positive rate of genetic testing was higher in TAAD [thoracic aortic aneurysm and dissection] patients with family history than in those without (76.74% vs. 18.94%)”.

Chen, et al. (2021) investigated how genetic testing could aid in avoiding the occurrence of MFS among Chinese families. Using data from 11 families, as well as variant classification and interpretation through pedigree analysis, the researchers were able to support two families who agreed to pre-implantation genetic testing for monogenic diseases (PGT-M) as part of the *in vitro* fertilization process. They were able to identify 11 potential-disease causing *FBN1* variants and found that “nine variants were classified as likely pathogenic/pathogenic variants. Among 11 variants, eight variants were missense and seven of them were located in the Ca-binding *EGF*-like motifs. Moreover, half of them substituted conserved Cysteine residues.” They also found one splice site variant, one frameshift variant, one synonymous variant, and two *de novo* variants. All variants were detected by polymerase chain reaction (PCR). Ultimately, the two MFS families were able to give birth to a baby without the *FBN1* mutation, as the healthy embryo was selected using haplotype analysis “to deduce the embryo’s genotype by using single nucleotide polymorphisms.” This demonstrated the tangible benefits of genetic testing for eliminating MFS and the development of comorbid conditions among future generations.

Damseh, et al. (2022) conducted a retrospective study using the 2017 EDS classification criteria on 72 pediatric patients who were referred for evaluation of EDS. From this initial cohort, 18 patients met the clinical criteria for an EDS subtype diagnosis, and 15 were confirmed molecularly. 75% (n=54) of the patients also had clinical features that belonged to EDS and other joint hypermobility syndromes, but not a complete qualification of EDS clinical criteria. From those 54 patients, it was discovered that 12 patients (22%) had a molecular genetic diagnosis of EDS. An EDS genetic panel, microarray, whole exome sequencing, single gene sequencing, familial variant testing, and other genetic panels were utilized to confirm genetic based diagnoses of EDS. Of the 15 patients who met clinical criteria and had a positive molecular diagnosis and 12 that did not meet clinical criteria but had a positive molecular diagnosis, 41% had classical EDS, 26% had arthrochalasia EDS, 11% had kyphoscoliotic EDS, and 22% had vascular EDS.

The researchers ultimately "observed a correlation between generalized joint hypermobility, poor healing, easy bruising, atrophic scars, skin hyperextensibility, and developmental dysplasia of the hip with a positive molecular result." This study aided in expanding the scope of the 2017 EDS classifications into the pediatric population and effecting changes to clinical decision making and treatment.

Veatch, et al. (2022) utilized clinical exam data and genetic testing results to understand the phenotypic and genotypic correlation for hereditary connective tissue diseases from 2016-2020. From a cohort of 100 unrelated individuals, the researchers isolated six likely pathogenic, and 35 classified "potentially pathogenic variants of unknown clinical significance." They found that those with potentially pathogenic variants and pathogenic/likely pathogenic variants of the same genes exhibited similar symptoms, as those with "connective tissue symptoms had suggestive evidence of increased odds of having skin (odds ratio 2.18, 95% confidence interval 1.12 to 4.24) and eye symptoms (odds ratio 1.89, 95% confidence interval 0.98 to 3.66) requiring further studies." Ultimately, the symptoms were broken up into classes of minimal skeletal symptoms (e.g., limb asymmetry, scoliosis, pes planus), more skeletal than connective tissue (e.g., joint hypermobility, dental defects, repeated ligament and cartilage disease), nervous, or gastrointestinal (e.g., irritable bowel syndrome, food intolerance) symptoms, and more nervous system (e.g., migraines, neuropathy) symptoms. Comprehending the spectrum of phenotypic heterogeneity could guide consequential clinical decision making for surveilling and counseling patients with hereditary connective tissue disorders and their current and future families.[51]

VI. Guidelines and Recommendations

American College of Cardiology (ACC)/American Heart Association (AHA)

The ACC released 2022 guidelines on thoracic aortic disease jointly with the American Heart Association:

Regarding heritable TAD:

- In patients with aortic root/ascending aortic aneurysms or aortic dissection and risk factors for heritable TAD, it is recommended that genetic testing be used to identify pathogenic/likely pathogenic variants.
- In patients with heritable TAD who have a pathogenic/likely pathogenic variant, genetic testing of at-risk biological relatives is recommended.

In the evaluation and genetic testing protocol for patients suspected of having TAD, the authors note these risk factors:

- "Features of MFS, LDS, or vEDS
- Family history of TAD, intracranial or peripheral aneurysm
- TAD < 60 y of age"

The following risk factors for familial TAD are identified in the guideline:

- "TAD and syndromic features of Marfan syndrome, Loeys-Dietz syndrome, or vascular Ehlers-Danlos syndrome.
- TAD presenting at age < 60 y
- A family history of either TAD or peripheral/intracranial aneurysms in a first- or second-degree relative.
- A history of unexplained sudden death at a relatively young age in a first- or second-degree relative."[6]

Regarding multigene pane testing, the ACC/AHA recommends, "Although the recommendations focus on individuals with a high risk for a single gene mutation, genetic testing may have a role in many TAD patients. A multigene panel comprising all genes suspected to cause HTAD is the most cost-effective and clinically useful approach to testing. Only pathogenic or likely pathogenic variants are disease-causing and should be used for cascade genetic testing all relatives at risk for inheriting the disease-causing variant."

Genetic testing panels for heritable thoracic aortic disease may include 11 genes that are confirmed to have a highly penetrant risk for TAD: *FBN1, LOX, COL3A1, TGFBR1, TGFBR2, SMAD3, TGFB2, ACTA2, MYH11, MYLK, and PRKG1*. These panels also include genes that increase the risk for genetically triggered thoracic aortic disease and/or lead to features related to Marfan syndrome, Loeys-Dietz syndrome, or vascular Ehlers-Danlos syndrome. Lastly, in patients who meet clinical diagnostic criteria for Marfan syndrome but do not have the clinical feature of a dislocated lens, genetic testing should be considered to exclude an alternative diagnosis of Loeys-Dietz syndrome.

American Heart Association

The AHA published a guideline regarding genetic testing for inherited cardiovascular diseases. The AHA notes that genetic testing plays a major role in diagnosing both Loeys-Dietz Syndrome and Marfan Syndrome, as well as confirming diagnoses of familial thoracic aortic aneurysm and dissection. A confirmed diagnosis may then affect timing of treatment or extent of screening for family members of the proband.

The AHA cites an ACMG list of "Genes Associated With Cardiovascular Disorders in Which Secondary/Incidental Findings Are Reportable." *COL3A1* is listed for Ehlers-Danlos Syndrome and *FBN1, TGFBR1, TGFBR2, SMAD3, ACTA2, MYH11* are listed for Marfan syndrome, Loeys-Dietz syndromes, and familial thoracic aortic aneurysms and dissections.

The AHA then lists another ACMG list of "Lists of Genes to Be Considered for Testing From Guidelines and Statements." Regarding heritable thoracic aortic aneurysm(s) or dissection(s), the genes *ACTA2, COL3A1, FBN1, MYH11, SMAD3, TGFB2, TGFBR1, TGFBR2, MYLK, LOX, PRKG1* are listed as having "definitive or strong evidence", and the genes *EFEMP2, ELN, FBN2, FLNA, NOTCH1, SLC2A10, SMAD4, SKI*, are considered as "potentially diagnostic."[52]

American College of Medical Genetics (ACMG)

The ACMG recommends the following diagnostic evaluations for a MFS diagnosis: a physical exam, family history, echocardiogram, dilated eye exam, CT or MRI, and the consideration of *FBN1* gene sequencing.[53] The ACMG notes that, since *FBN1* mutations may cause conditions other than MFS (such as EDS and LDS), clinical features must be used to diagnose MFS properly. The ACMG further notes *SMAD3*, *ACTA2*, and *MYH11* as potential genes of interest in identifying MFS, in addition to *FBN1*, *TGFBR1*, and *TGFBR2.*[53]

Regarding LDS, the ACMG notes that "LDS strongly resembles the vascular form of Ehlers–Danlos syndrome, especially in terms of thin skin."[53] Further, a diagnostic evaluation of LDS includes the following: a "physical exam, family history, echocardiogram, dilated eye exam (to exclude MFS), magnetic resonance angiography of the head, neck thorax, abdomen and pelvis, and TGFBR1 and *TGFBR2* gene sequencing."[53] Specifically, the ACMG states that "In a patient found to have consistent

facial features, bifid uvula, and arterial tortuosity, the diagnosis [of LDS] can be confirmed with *TGFBR* testing."[53]

Regarding EDS hypermobile type, the ACMG recommends the following diagnostic evaluation: a physical exam, family history, echocardiogram and dilated eye exam (to exclude MFS). The guidelines also specifically state that "Diagnosis is based on clinical evaluation and family history. A small subset of individuals with the hypermobile form of EDS have an insertion or deletion in the *TNXB* gene."[53]

ACMG also published a statement titled "Recommendations for reporting of secondary findings in clinical exome and genome sequencing." In it, *COL3A1* is listed for Ehlers-Danlos Syndrome, vascular type, and *FBN1, TGFBR1, TGFBR2, SMAD3, ACTA2,* and *MYH11* were listed as relevant genes for aortopathies.[54,55]

American Academy of Pediatrics (AAP)

The AAP released an updated guideline in 2023 on the medical management of children with MFS:

- When clinical features suggest Marfan syndrome, but do not fully meet diagnostic criteria, genetic testing for *FBN1* mutations is recommended to clarify the diagnosis.
- Genetic testing of *FBN1* should be considered for those children suspected of having Marfan syndrome (but who may not meet full clinical criteria) after physical, cardiac, and ophthalmic evaluation.
- Asymptomatic individuals with a first-degree relative diagnosed with MS should consider genetic testing to determine their risk and guide surveillance strategies.
- Additionally, patients who "fit clinical criteria for Marfan syndrome in whom no pathogenic variant is found in the *FBN1* gene should continue to be followed…In addition, broader genomic testing should be considered in these individuals."[56]

The Marfan Foundation

The Marfan Foundation has released recommendations on certain aspects of testing for MFS. The Foundation mentions several situations in which genetic testing may be useful, such as patients with features of multiple disorders, patients with a clinical symptom characteristic of MFS (such as ectopia lentis), children of parents affected by MFS, or adults with MFS that are considering having children. Prenatal testing may be performed, either a chorionic villus sampling (CVS) at 10-11 weeks or amniocentesis at 16-18 weeks. However, the parent's mutation must be confirmed before proceeding with either prenatal test.[57]

Screening of first-degree relatives of patients with MFS is also warranted. Aortic imaging may be performed if the mutation has not been identified.[58]

The Ehlers Danlos Society and the International Consortium on the Ehlers-Danlos Syndromes

These guidelines state that Ehlers-Danlos syndrome "Molecular diagnostic strategies should rely on NGS technologies, which offer the potential for parallel sequencing of multiple genes. Targeted resequencing of a panel of genes, for example, *COL5A1*, *COL5A2*, *COL1A1* and *COL1A2*, is a time- and cost-effective approach for the molecular diagnosis of the genetically heterogeneous EDS. When no mutation (or in case of an autosomal recessive condition only one mutation) is identified, this approach should be complemented with a copy number variant (CNV) detection strategy to identify large deletions or

duplications, for example Multiplex Ligation-dependent Probe Amplification (MLPA), qPCR, or targeted array analysis. Alternatively, or in a second phase, whole exome sequencing (WES) or whole genome sequencing (WGS) and RNA sequencing techniques can be used, with data-analysis initially focusing on the genes of interest for a given EDS subtype. In absence of the identification of a causal mutation, this approach allows to expand the analysis to other genes within the genome. This is particularly interesting in view of the clinical overlap between EDS subtypes and with other HCTDs, and the observation that in an important proportion of EDS-patients, no pathogenic variants are identified in any of the known EDS-associated genes."[5]

For cEDS, the following guidelines were given:

- "Molecular screening by means of targeted resequencing of a gene panel that includes at least the *COL5A1, COL5A2, COL1A1*, and *COL1A2* genes, or by WES or WGS, is indicated. When no mutation is identified, this approach should be complemented with a CNV detection strategy to identify large deletions or duplications.
  Absence of these confirmatory findings does not exclude the diagnosis, as specific types of mutations (e.g., deep intronic mutations) may go undetected by standard diagnostic molecular techniques; however, alternative diagnoses should be considered in the absence of (a) *COL5A1, COL5A2, COL1A1*, or *COL1A2* mutation(s)."[5]

For classical-like EDS (clEDS), the following guidelines were given:

- "Molecular analysis of the *TNXB* gene should be used as the standard confirmatory test. Difficulties in DNA testing are related to the presence of a pseudogene (*TNXA*), which is more than 97% identical to the 3' end of *TNXB* (exons 32–44). With the only exception of exon 35, which partially shows a TNXB-specific sequence, exon and intron sequences in this region are identical or almost identical in both the gene and the pseudogene. This has implications both for sequencing and deletion/duplication analysis.
- For sequence analysis of *TNXB*, two approaches are recommended.
  - Sanger sequencing of the entire *TNXB* gene.
  - Next-generation sequencing of *TNXB* + Sanger sequencing of the pseudogene region."
- If no or only one causative mutation is identified by classic sequencing, additional methods that allow detection of large deletions/duplications should be added. So far no method is able to specifically detect TNXB CNVs in the highly homologous exons 32–34 and 36–44. CNV analysis of exon 35 is currently used to detect deletions in this region, including the 30 kb deletion
- Absence of these confirmatory findings does not exclude the diagnosis, as specific types of mutations (e.g., deep intronic mutations) may go undetected by standard diagnostic molecular techniques; however, alternative diagnoses should be considered in the absence of a TNXB mutation."[5]

For cardiac-valvular EDS (cvEDS), the following recommendations were given:

- "Molecular screening by Sanger sequencing of *COL1A2*, or targeted resequencing of a gene panel that includes *COL1A2* is indicated. When no mutation is identified, this approach should be complemented with a CNV detection strategy to identify large deletions or duplications.
- In case of unavailability of genetic testing, SDS PAGE demonstrates total absence of (pro-) α2(I) collagen chains.

- Whereas absence of these confirmatory biochemical findings allows to exclude the diagnosis of cvEDS, absence of these confirmatory genetic findings does not exclude the diagnosis, as specific types of mutations (e.g., deep intronic mutations) may go undetected by standard diagnostic molecular techniques."[5]

For vEDS, the following guidelines were given:

- "Molecular screening by Sanger sequencing of *COL3A1*, or targeted resequencing of a gene panel that includes *COL3A1* and *COL1A1* (the latter to identify the above-listed arginine-to-cysteine substitution mutations) is indicated. When no mutation is identified, this approach should be complemented with a CNV detection strategy to identify large deletions or duplications.
- Absence of these confirmatory findings does not exclude the diagnosis, as specific types of mutations (e.g., deep intronic mutations) may go undetected by standard diagnostic molecular techniques; however, alternative diagnoses should be considered in the absence of a *COL3A1* or *COL1A1* mutation."[5]

For hypermobile EDS (hEDS), the following guidelines were given:

- "The diagnosis of hEDS remains clinical as there is yet no reliable or appreciable genetic etiology to test for in the vast majority of patients."[5]

For arthrochalasia EDS (aEDS), the following guideline were given:

- "Molecular screening by Sanger sequencing of *COL1A1* and *COL1A2*, or targeted resequencing of a gene panel that includes these genes, is indicated. When no mutation is identified, this approach should be complemented with a CNV detection strategy to identify large deletions or duplications.
- In case of unavailability of genetic testing, SDS PAGE of the pepsin-digested collagen in the medium or cell layer of cultured dermal fibroblasts demonstrates the presence of a mutant pNα1(I) or pNα2(I) chain (precursor procollagen chains in which the carboxy (C)-but not the amino (N)-propetide is cleaved off).
- TEM of skin specimens shows loosely and randomly organized collagen fibrils with a smaller and more variable diameter, and an irregular outline. These findings may support the diagnosis, but cannot confirm it.
- Absence of a causative mutation in *COL1A1* or *COL1A2* that leads to complete or partial deletion of the exon 6 of either gene excludes the diagnosis of aEDS."[5]

For dermatosparaxis EDS (dEDS), the following guidelines were given:

- "Molecular screening by Sanger sequencing of targeted resequencing of a gene panel that includes *ADAMTS2* is indicated. When no, or only one, causative mutation is identified, this approach should be complemented with a CNV detection strategy to identify large deletions or duplications.
- In case of unavailability of genetic testing, SDS, PAGE demonstrates presence of pNα1(I) and pNα2(I) chains of type I procollagen extracted from dermis in the presence of protease inhibitors or detected in fibroblast cultures.
- TEM shows collagen fibrils in affected skin specimens with a hieroglyphic pattern. These ultrastructural findings are usually typical but may be almost indistinguishable from those observed in aEDS. As such, they are not sufficient to confirm the diagnosis.

- Absence of these confirmatory findings does not exclude the diagnosis of dEDS, as specific types of mutations (e.g., deep intronic mutations) may go undetected by standard diagnostic molecular techniques; however, alternative diagnoses should be considered in the absence of ADAMTS2 mutations."[5]

For kyphoscoliotic (kEDS), the following recommendations were given:

- Laboratory confirmation of kEDS should start with the quantification of deoxypyridinoline (Dpyr or LP for lysyl-pyridinoline) and pyridinoline (Pyr or HP for hydroxylysyl-pyridinoline) cross-links in urine quantitated by means of high-performance liquid chromatography (HPLC). An increased Dpyr/Pyr ratio is a highly sensitive and specific test for kEDS caused by biallelic PLOD1 mutations (kEDS-PLOD1), but is normal for biallelic FKBP14 mutations (kEDS-FKBP14).
- The normal ratio of Dpyr/Pyr cross-links is approximately 0.2, whereas in kEDS-PLOD1 the ratio is significantly increased (approximately 10–40 times increase, range 2–9). This method is fast and cost-effective and it can also be used to determine the pathogenic status of a VUS in PLOD1.
- SDS–PAGE may detect faster migration of underhydroxylated collagen chains and their derivatives in kEDS-*PLOD1* but not in kEDS-*FKBP14*. However, abnormalities in migration can be subtle.
- Molecular analysis for kEDS-*PLOD1* may start with MLPA analysis of *PLOD1*, for the evaluation of the common intragenic duplication in PLOD1 caused by an Alu-Alu recombination between introns 9 and 16 (the most common mutant allele).
- Molecular screening by means of targeted resequencing of a gene panel that includes *PLOD1* and *FKBP14*, is indicated when MLPA of *PLOD1* fails to identify the common duplication. Such a gene panel my also include other genes associated with phenotypes that clinically overlap with kEDS, such as *ZNF469, PRDM5, B4GALT7, B3GALT6, SLC39A13, CHST14* and *DSE*. Alternatively, WES may be performed. When no, or only one, causative mutation is identified, this approach should be complemented with a CNV detection strategy to identify large deletions or duplications in these genes.
- TEM on skin specimens has shown variable diameters and abnormal contours of the collagen fibrils and irregular interfibrillar space, but these abnormalities are not unique to this condition. As such, whereas TEM on a skin biopsy can support diagnosis, it cannot confirm it.
- Whereas absence of an abnormal urinary LP/HP ratio excludes the diagnosis of kEDS-PLOD1, absence of the confirmatory genetic findings does not exclude the diagnosis of kEDS, as specific types of mutations (e.g., deep intronic mutations) may go undetected by standard diagnostic molecular techniques and/or other, yet to be discovered, genes, may be associated with this phenotype; however, alternative diagnoses should be considered in the absence of *PLOD1* or *FKBP14* mutations."[5]

For brittle cornea syndrome (BCS), the following guidelines were given:

- "Molecular screening by means of targeted resequencing of a gene panel that includes ZNF469 and PRDM5 is indicated. Such a gene panel my also include other genes associated with phenotypes that clinically overlap with BCS, such as *PLOD1, FKBP14, B4GALT7, B3GALT6, SLC39A13, CHST14*, and *DSE*. Alternatively, WES may be performed. When no, or only one, causative mutation is identified, this approach should be complemented with a CNV detection strategy to identify large deletions or duplications in these genes.

- Absence of these confirmatory findings does not exclude the diagnosis, as specific types of mutations (e.g., deep intronic mutations) may go undetected by standard diagnostic molecular techniques, and other, yet unknown genes, might be associated with BCS."[5]

For spondylodysplastic EDS (spEDS), the following guidelines were given:

- Molecular screening by means of targeted resequencing of a gene panel that includes *B4GALT7, B3GALT6*, and *SLC39A13* is indicated. Such a gene panel my also include other genes associated with phenotypes that clinically overlap with spEDS, such as *PLOD1, FKBP14, ZNF469, PRDM5, CHST14,* and *DSE*. Alternatively, WES may be performed. When no, or only one, causative mutation is identified, this approach should be complemented with a CNV detection strategy to identify large deletions or duplications in these genes.
- For definite proof of GAG deficiency (*B4GALT7* and *B3GALT6* mutations), biochemical methods to assess GAG synthesis in patients' cultured fibroblasts are currently available in many specialized laboratories.
- The laboratory measurement of urinary pyridinolines, lysyl-pyridinoline (LP) and hydroxylysyl-pyridinoline (HP) quantitated by HPLC allows the detection of an increased ratio LP/HP to approximately 1, (compared to a normal value of approximately 0.2) in patients with mutations in *SLC39A13*. This fast and cost-effective method can also be used to determine the pathogenic status of a VUS (see also "verification of diagnosis" in kEDS-PLOD1).
- Absence of confirmatory genetic findings does not exclude the diagnosis of spEDS, as specific types of mutations (eg deep intronic mutations) may go undetected by standard diagnostic molecular techniques, and still other, yet to be discovered, genes may be associated with these phenotypes. In case no *B4GALT7*, *B3GALT6*, or *SCL39A13* mutations are identified, alternative diagnoses should however be considered."[5]

For musculocontractural EDS (mcEDS), the following guidelines were given:

- "Molecular screening by means of targeted resequencing of a gene panel that includes *CHST14* and *DSE* is indicated. Such a gene panel my also include other genes associated with phenotypes that clinically overlap with mcEDS, such as *PLOD1, FKBP14, ZNF469, PRDM5*, *B4GALT7, B3GALT6* and *SLC39A13*. Alternatively, WES may be performed. When no, or only one, causative mutation is identified, this approach should be complemented with a CNV detection strategy to identify large deletions or duplications in these genes.
- Absence of these confirmatory genetic findings does not exclude the diagnosis of mcEDS, as specific types of mutations (e.g., deep intronic mutations) may go undetected by standard diagnostic molecular techniques. In case no CHST14 or DSE mutations are identified, alternative diagnoses should be considered."[5]

For myopathic EDS (mEDS), the following guidelines were given:

- "Molecular screening by means of targeted resequencing of a gene panel that includes *COL12A1* is indicated. Such a gene panel my also include other genes associated with phenotypes that clinically overlap with mEDS, such as *COL6A1*, *COL6A2*, *COL6A3*. Alternatively, WES may be performed. When no, or only one, causative mutation is identified, this approach should be complemented with a CNV detection strategy to identify large deletions or duplications in these genes.

- Absence of these confirmatory findings does not exclude the diagnosis, as specific types of mutations (eg deep intronic mutations) may go undetected by standard diagnostic molecular techniques, and other, yet to be discovered, genes may be associated with this phenotype."[5]

For periodontal EDS (pEDS), the following guidelines were given:

- "Identification of known or compatible mutations by sequence analysis of C1R and C1S. Large deletions or null mutations that completely remove C1r or C1s protein function do not cause pEDS.
- At present it cannot be stated whether absence of a C1R or C1S mutations excludes the diagnosis because the experience with the molecular diagnosis is limited."[5]

Canadian Cardiovascular Society (CCS)

The CCS has published recommendations for MFS stating a strong recommendation for clinical and genetic screening for anyone with suspected MFS "to clarify the nature of the disease and provide a basis for individual genetic counseling."[59]

The CCS also published recommendations for non-Marfan genetic forms of aortic disease such as thoracic aortic disease (TAD). These guidelines state that "We recommend screening for TAD-associated genes in non-BAV aortopathy index cases to clarify the origin of disease and improve clinical and genetic counselling."[59] These guidelines also state that individuals with a known LDS mutation (such as *TGFBR1/2*, *TGFB*, *SMAD3*, *ACTA2*, or *MYH11*) should receive complete aortic imaging when diagnosed and six months after diagnosis.

International Group of Specialists with a Broad Aggregate Experience in the Care of Individuals with Vascular EDS

Recommendations made by this group of vEDS specialists recommend to "identify causative variants in *COL3A1* prior to [the] application of diagnosis" of vEDS.[27]

National Organization for Rare Disorders (NORD)

NORD has posted recommendations on EB stating that "When EB is suspected, a skin biopsy should be obtained and sent to an appropriate laboratory to confirm the diagnosis with transmission electron microscopy (TEM) and/or immunofluorescent antibody/antigen mapping. Molecular genetic testing for mutations in most of the genes known to be associated with the various types of EB is clinically available."[60]

On the diagnosis of EDS, the NORD has stated that diagnosis is generally made using patient histories and clinical findings, and that genetic testing can help in the diagnosis of some subtypes. Electron microscopic analysis could also aid in revealing the collagen abnormalities seen in EDS. "The clinical evaluation of individuals with suspected or diagnosed EDS typically includes assessments to detect and determine the extent of skin and joint hyperextensibility." The NORD also posted recommendations of utilizing computerized tomography scanning, magnetic resonance imaging (MRI), and echocardiography to observe any mitral valve prolapse and aortic dilatation. On kEDS, NORD has written of confirmatory tests using "either a urine sample and extrapolated ratio of deoxypyridinoline to pyridinoline cross-links, or on a skin biopsy sample and measurement of lysyl hydroxylase enzyme activity from skin fibroblast cells."[61]

Dystrophic Epidermolysis Bullosa Research Association (DEBRA) International

An international group was convened to develop a clinical practice guideline on laboratory testing for epidermolysis bullosa (EB). The following recommendations apply:

- A strong recommendation that EB lab diagnosis be performed with the first clinical suspicion of EB (Grade C).
- Early diagnosis by immunofluorescence antigen mapping (IFM) and genetic testing to provide prognosis and help aid in decision making in most cases (Grade B/C).
- A consideration that DNA-based prenatal diagnosis can be considered for all EB subtypes and be performed upon family request (Grade B).
- A strong recommendation that EB laboratory diagnosis be performed in accredited laboratories that have the necessary expertise (Grade D).
- The individual in question and the biological parents should be genetically tested to provide accurate genetic counseling and a risk assessment (Grade B).
- The methods listed for genetic testing include: NGS with a targeted EB gene panel, Whole Exome Sequencing, and Sanger Sequencing. Other methods can be considered in certain cases: SNP arrays for segregation analysis, MLPA, qPCR, and RNA-Seq, as well as homozygosity mapping in case of consanguinity. Hotspot and recurrent pathogenic variants can be tested in targeted situations (Grade B)
- IFM is recommended to help attain a rapid diagnosis and prognosis (Grade C)

The following prioritization strategies of EB laboratory diagnosis should be considered:

- "In neonates, IFM should be the first diagnostic step as it delivers rapid results. In parallel, genetic testing should always be performed."
- "In cases with characteristic clinical features, including localized dominant EBS or DEB, for which IFM will frequently not deliver a useful result, genetic testing by NGS or SS can deliver a final diagnosis."
- In EB (sub)types with genetic heterogeneity or in cases with uncharacteristic findings, without a clear candidate gene, genetic testing by NGS is recommended."[62]

Additionally, "In EB (sub)types with genetic heterogeneity, in cases with-out a clear candidate gene, where candidate genes have been ruled out, or in cases when SS was the first chosen method and did not identify the pathogenic variant, targeted NGS with the 21 known EB genes [*CD151, COL17A1, COL7A1, DSP, DST, EXPH5, FERMT1, ITGA3, ITGA6, ITGB4, JUP, KLHL24, KRT14, KRT5, LAMA3, LAMA3A, LAMB3, LAMC2, PKP1, PLEC,* and *TGM5*][7]or WES with targeted filtering for laboratory diagnosis of EB is recommended (level of evidence 2++, grade of recommendation B) Subsequently, confirmation of novel pathogenic variants found this way should be performed by SS (level of evidence 4, grade of recommendation D). Recent data showed that in clinically unaffected parents, mosaicism may be detected by NGS more often than expected (depending on the coverage of the NGS platform), which has important impacts on genetic counselling. The advantage of targeted EB gene panels is that they obviously have a much higher coverage per gene and base. However, current WES platforms should also provide sufficient coverage per gene and base to provide accurate results, but it is recommended to confirm this in individual laboratories."[62]

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, please visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). As an LDT, the U. S. Food and Drug Administration has not approved or cleared this test; however, FDA clearance or approval is not currently required for clinical use.

VYJUVEK (beremagene geperpavec) is an FDA approved therapy for certain individuals with epidermolysis bullosa. "Indications and usage: VYJUVEK is a herpes-simplex virus type 1 (HSV-1) vector-based gene therapy indicated for the treatment of wounds in patients 6 months of age and older with dystrophic epidermolysis bullosa with mutation(s) in the *collagen type VII alpha 1 chain (COL7A1)* gene. . . VYJUVEK is a biological suspension, mixed into excipient gel, for topical application."[63]

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81403	Molecular pathology procedure, Level 4 (eg, analysis of single exon by DNA sequence analysis, analysis of >10 amplicons using multiplex PCR in 2 or more independent reactions, mutation scanning or duplication/deletion variants of 2-5 exons)
81405	Molecular pathology procedure, Level 6 (eg, analysis of 6-10 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 11-25 exons, regionally targeted cytogenomic array analysis)
81408	Molecular pathology procedure, Level 9 (eg, analysis of >50 exons in a single gene by DNA sequence analysis)
81410	Aortic dysfunction or dilation (eg, Marfan syndrome, Loeys Dietz syndrome, Ehler Danlos syndrome type IV, arterial tortuosity syndrome); genomic sequence analysis panel, must include sequencing of at least 9 genes, including FBN1, TGFBR1, TGFBR2, COL3A1, MYH11, ACTA2, SLC2A10, SMAD3, and MYLK
81411	Aortic dysfunction or dilation (eg, Marfan syndrome, Loeys Dietz syndrome, Ehler Danlos syndrome type IV, arterial tortuosity syndrome); duplication/deletion analysis panel, must include analyses for TGFBR1, TGFBR2, MYH11, and COL3A1
81479	Unlisted molecular pathology procedure

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Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

Sentara Health Plans

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X. Review/Revision History

Effective Date	Summary
07/01/2025	Reviewed and Updated: Updated background, guidelines, and evidence-based scientific references. Literature review necessitated the following changes in coverage criteria: Replaced “mutation” with “pathogenic or likely pathogenic variant” in CC1.b., CC2.b., with “variant” in CC3. New CC4 and CC5: “4) The following genetic testing for heritable thoracic aortic disease (HTAD) (see Note 5) MEETS COVERAGE CRITERIA: a) Multigene panel testing (see Note 6, Note 7) in the following situations: i) For individuals with thoracic aortic disease (TAD) and syndromic features of Marfan syndrome, Loeys-Dietz syndrome, or vascular Ehlers-Danlos syndrome.

	ii) For individuals presenting with TAD before 60 years of age. iii) For individuals with a family history of either TAD or peripheral/intracranial aneurysms in a first- or second-degree relative (see Note 2). iv) For individuals with a first- or second-degree relative (see Note 2) who had an unexplained sudden death at a relatively young age. b) Testing restricted to the known pathogenic or likely pathogenic variant for individuals with a close blood relative (see Note 2) with a known pathogenic or likely pathogenic variant. 5) The following genetic testing for epidermolysis bullosa (EB) MEETS COVERAGE CRITERIA: a) Single gene or multigene panel testing (see Note 6, Note 8) for individuals for whom there is a clinical suspicion of EB. b) Testing restricted to the known pathogenic or likely pathogenic variant in the following situations: i) For the biological parents of an individual who has been identified to have a pathogenic or likely pathogenic variant for EB. ii) For the prenatal diagnosis or PGD of EB in the offspring of patients with known pathogenic or likely pathogenic variants. iii) COL7A1 testing for individuals being considered for beremagene geperpavec." New Note 2, defining first-degree relatives: "Note 2: First-degree relatives include parents, full siblings, and children of the individual." "(see Note 2)" replaces description of first-degree relatives in CC1.b. and CC2.b. New Note 5, 7, and 8, to define HTAD and EB and to define rules for multigene panel testing. Note 2-5 numbers shifted with addition of new notes, adjusted within criterion to align. Former Note 4, now Note 6, edited to change "5" to "two" to align with guidance in R2162: "Note 6: For two or more gene tests being run on the same platform, please refer to AHS-R2162-Reimbursement Policy." Added CPT code 81403, 81479
12/01/2024	Initial Policy Implementation