Corporate Medical Policy

Genetic Testing for Connective Tissue Disorders AHS – M2144

File Name: genetic_testing_for_connective_tissue_disorders
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Description of Procedure or Service

Definitions
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) (NIH, 2016). Every disorder impacts connective tissue differently, including several with vascular implications, and clinical severity varies within each disorder.

******Note: This Medical Policy is complex and technical. For questions concerning the technical language and/or specific clinical indications for its use, please consult your physician.***

Policy

BCBSNC will provide coverage for genetic testing for genetic testing for connective tissue disorders when it is determined the medical criteria or reimbursement guidelines below are met.

Benefits Application

This medical policy relates only to the services or supplies described herein. Please refer to the Member's Benefit Booklet for availability of benefits. Member's benefits may vary according to benefit design; therefore member benefit language should be reviewed before applying the terms of this medical policy.

When Genetic Testing for Connective Tissue Disorder is covered

1. For individuals who have consulted with a cardiology specialist prior to genetic testing, *FBN1* mutation testing for Marfan Syndrome is considered medically necessary 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 below).
    b. For an asymptomatic individual who has an affected first-degree blood relative (i.e., parent, sibling, child) with a known mutation.
    c. For the prenatal diagnosis or preimplantation genetic diagnosis (PGD) of Marfan syndrome in the offspring of patients with known disease-causing variants.

2. Genetic testing for Loeys-Dietz Syndrome (*TGFBR1* or *TGFBR2* mutation) is considered medically necessary in the following situations:

    a. To confirm or establish a diagnosis of LDS in an individual with vascular characteristics of LDS (see Note 2 below).

b. For an asymptomatic individual who has an affected first-degree blood relative (i.e., parent, sibling, child) with a known mutation.
c. For individuals suspected of having Marfan Syndrome who have tested negative for *FBN1*.

3. For individuals with characteristics of vascular Ehlers-Danlos Syndrome (vEDS) (see Note 3), genetic panel testing for *COL3A1* and *COL1A1* mutations to confirm or establish a diagnosis of (vEDS) is considered medically necessary.

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* (Loeys et al., 2010; Wright & Connolly, 2022).

- 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 (Loeys et al., 2010; Wright & Connolly, 2022):

- 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: 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 (Loeys & Dietz, 2018).

Note 3: Clinical features of Vascular EDS (vEDS) from The 2017 International Classification For The Ehlers-Danlos Syndromes (Malfait et al, 2017):

- 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 4: For 5 or more gene tests being run on the same platform, please refer to AHS-R2162 Laboratory Procedures Medical Policy.

When Genetic Testing for Connective Tissue Disorder is not covered

Reimbursement is not allowed for all other gene testing for Marfan syndrome or other connective tissue disorders, including Ehlers-Danlos Syndrome.

Reimbursement is not allowed for genetic testing to confirm or establish a diagnosis of hypermobile Ehlers-Danlos syndrome (hEDS) for individuals with characteristics of hEDS (see Note 5).

Note 5: Clinical features of Hypermobile EDS (hEDS) from The 2017 International Classification For The Ehlers-Danlos Syndromes (Malfait et al, 2017):

- Inheritance
  Autosomal dominant
- Molecular basis
  Unknown
- Clinical diagnosis
  Clinical spectrum ranging from asymptomatic joint hypermobility, through "non-syndromic" hypermobility with secondary manifestations, to hEDS (see "*A Framework for the Classification of Joint Hypermobility and Related Conditions*" *by Castori et al., this issue*)
  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.

Policy Guidelines

Table of Terminology

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
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
RCT	Randomized controlled trial
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 fisease
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

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 (NIH, 2016) 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 (NIH, 2016).

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 (Radke & Baumgartner, 2014). 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 (Faivre et al., 2007). 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 (Dietz, 2017; Pyeritz, 2017; Wright & Connolly, 2022).

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 (Davis & Summers, 2012; Grewal & Gittenberger-de Groot, 2018). Fibrillin-1 is an important matrix component of both elastic and nonelastic tissues (Wright & Connolly, 2022). Mutations can lead to impaired fibrillin-1 protein function, causing extracellular matrix integrity to fail (Grewal & Gittenberger-de Groot, 2018). 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 (Nataatmadja et al., 2003). 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 (Dale et al., 2017). 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 (Bin Mahmood et al., 2017; Neptune et al., 2003).

EDS 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 (Pauker & Stoler, 2022). 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-valvular	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 (Pauker & Stoler, 2022).

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 (Byers et al., 2017). 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 (Malfait et al., 2017). A diagnosis depends on clinical features, including family history. Aneurysms occur in other types of EDS, including classical EDS (cEDS), due to vascular fragility (Malfait, 2018). 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 (Johansen et al., 2020)."

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" (MacCarrick et al., 2014). 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 (MacCarrick et al., 2014; Wright & Connolly, 2022). 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 (Seike et al., 2020).

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 (Murrell, 2022). 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 (Coulombe et al., 1991; NIH, 2020). 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 (NIH, 2020). 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 (Loeys et al., 2004). 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 (Wright & Connolly, 2022).

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 (Becerra-Munoz et al., 2018). 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 (Wright & Connolly, 2022).

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 (Dietz, 2017). These features are summarized in the 2010 Ghent nosology, which is slightly altered for patients under 20 years old (Wright & Connolly, 2022). 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 (Dietz et al., 2005; Jensen & Handford, 2016).

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 (Bin Mahmood et al., 2017; Hiratzka et al., 2010). 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 (Koo et al., 2017). 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 (Sellers et al., 2018)."

Johansen et al. (2020); Ritelli et al. (2020); Shalhub 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) (Shalhub et al., 2020)." 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 (Saunderson et al., 2019). 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 (Veatch et al., 2022).

Guidelines and Recommendations

American College of Cardiology (ACC)

The ACC released guidelines on thoracic aortic disease jointly with the American Association for Thoracic Surgery, American College of Radiology, American Stroke Association, Society of Cardiovascular Anesthesiologists, Society for Cardiovascular Angiography and Interventions, Society of Interventional Radiology, Society of Thoracic Surgeons, and Society for Vascular Medicine. The MFS-specific guidelines are listed below:

- An echocardiogram is recommended at the time of diagnosis of Marfan syndrome to determine the aortic root and ascending aortic diameters and six months thereafter to determine the rate of enlargement of the aorta.
- Annual imaging is recommended for patients with Marfan syndrome if stability of the aortic diameter is documented. If the maximal aortic diameter

is 4.5 cm or greater, or if the aortic diameter shows significant growth from baseline, more frequent imaging should be considered.
- If a mutant gene (*FBN1*, *TGFBR1*, *TGFBR2*, *COL3A1*, *ACTA2*, *MYH11*) associated with aortic aneurysm and/or dissection is identified in a patient, first-degree relatives should undergo counseling and testing.
- Sequencing of other genes known to cause familial thoracic aortic aneurysms and/or dissection (*TGFBR1*, *TGFBR2*, *MYH11*) may be considered in patients with a family history and clinical features associated with mutations in these genes (Hiratzka et al., 2010).
- Aortic imaging is recommended in patients with LDS or a who have a confirmed genetic mutation known to predispose an individual to aortic aneurysms and aortic dissections (*TGFBR1, TGFBR2, FBN1, ACTA2*, or *MYH11*)

American Heart Association (AHA)

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" (Musunuru et al., 2020).

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 (Pyeritz, 2012). 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* (Pyeritz, 2012).

Regarding LDS, the ACMG notes that "LDS strongly resembles the vascular form of Ehlers–Danlos syndrome, especially in terms of thin skin" (Pyeritz, 2012). 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 (Pyeritz, 2012)." 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 (Pyeritz, 2012)."

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" (Pyeritz, 2012).

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 (Miller et al., 2022; Miller et al., 2021).

American Academy of Pediatrics (AAP)

The AAP has released guidelines on the management of supervision of children with MFS. However, they allude to genetic testing of *FBN1*, stating it is "best reserved" for patients with "strong clinical suspicion" of MFS. The AAP states that younger patients (18 and under) should be evaluated periodically instead of undergoing genetic testing (Tinkle & Saal, 2013).

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 (Marfan_Foundation, 2013).

Screening of first-degree relatives of patients with MFS is also warranted. Aortic imaging may be performed if the mutation has not been identified (Marfan_Foundation, 2015).

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 (Malfait et al., 2017)."

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)" (Malfait et al., 2017).

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 (Malfait et al., 2017)."

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 (Malfait et al., 2017)."

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 (Malfait et al., 2017)."

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 (Malfait et al., 2017)."

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 (Malfait et al., 2017)."

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 (Malfait et al., 2017)."

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) [Hautala et al., 1993].
- 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 (Malfait et al., 2017)."

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 (Malfait et al., 2017)."

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 (Malfait et al., 2017)."

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 (Malfait et al., 2017)."

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 (Malfait et al., 2017)."

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 (Malfait et al., 2017)."

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" (Boodhwani et al., 2014).

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 (Boodhwani et al., 2014)." 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 6 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 (Byers et al., 2017).

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" (NORD, 2013).

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 (CT) 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" (NORD, 2017)

State and Federal Regulations, as applicable

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.

Billing/Coding/Physician Documentation Information

This policy may apply to the following codes. Inclusion of a code in this section does not guarantee that it will be reimbursed. For further information on reimbursement guidelines, please see Administrative Policies on the Blue Cross Blue Shield of North Carolina web site at www.bcbsnc.com. They are listed in the Category Search on the Medical Policy search page.

Applicable service codes: 81405, 81408, 81410, 81411

BCBSNC may request medical records for determination of medical necessity. When medical records are requested, letters of support and/or explanation are often useful, but are not sufficient documentation unless all specific information needed to make a medical necessity determination is included.

Scientific Background and Reference Sources

For policy titled: Marfan Syndrome AHS – M2144

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Specialty Matched Consultant Advisory Panel review 3/2020

Medical Director review 3/2020

Specialty Matched Consultant Advisory Panel review 3/2021

Medical Director review 3/2021

Medical Director review 11/2022

Medical Director review 4/2023

Policy Implementation/Update Information

1/1/2019	BCBSNC will provide coverage for genetic testing for marfan syndrome when it is determined to be medically necessary because criteria and guidelines are met. Medical Director review 1/1/2019. Policy noticed 1/1/2019 for effective date 4/1/2019. (jd)
5/14/19	Reviewed by Avalon 1st Quarter 2019 CAB. Minor revisions to Description and When Covered sections; no change to policy intent. Policy guidelines extensively revised. Referenced updated. Medical Director review 5/2019. (jd)
10/29/19	Wording in the Policy, When Covered, and/or Not Covered section(s) changed from Medical Necessity to Reimbursement language, where needed.
5/12/20	Policy title changed from "Marfan Syndrome" to "Genetic Testing for Connective Tissue Disorders. Reviewed by Avalon 1st Quarter 2020 CAB. Added items #3 and #4 to the When Covered section. Under the When Not Covered section, added "All others" to the not medically necessary statement. Added Related Policies section. Description, policy guidelines, and references updated. Specialty Matched Consultant Advisory Panel review 3/2020. Medical Director review 3/2020. (jd)
4/20/21	Specialty Matched Consultant Advisory Panel review 3/2021. Medical Director review 3/2021. (jd)
5/4/21	Reviewed by Avalon 1st Quarter 2021 CAB. Added the following statement to item 1 under the When Covered section: "the individual needs to consult cardiology specialist prior to genetic testing" for clarity; removed the following from item 2 "If FBN1 mutation

testing is negative"; item 3 reworded about genetic testing (COL3A1 and COL1A1) for vascular Ehlers-Danlos syndrome (vEDS); reworded item 4 for clarity. Note 3 updated. Added 2nd statement to When Not Covered section: "Reimbursement is not allowed for genetic testing to confirm or establish a diagnosis of hypermobile Ehlers-Danlos syndrome (hEDS) in individuals with characteristics of hEDS (see Note 5).; and added Note 5. Description section, policy guidelines and references updated. Medical Director review 4/2021. (jd)

5/17/22 Reviewed by Avalon 1st Quarter 2022 CAB. Updated policy guidelines; adding Table of Terminology and references. Medical Director review 4/2022. (jd)

12/13/22 Off-cycle review by Avalon 3rd Quarter 2022 CAB. Policy Guidelines and References updated. When Covered section edited for clarity and consistency. Added coverage criteria 2c "For individuals suspected of having Marfan Syndrome who have tested negative for FBN1", removed coverage criteria 4 related to multi-gene testing outside of vEDS. Medical Director review 11/2022. (tm)

5/16/23 Reviewed by Avalon 1st Quarter 2023 CAB. Removed Related Policies section, edited Note 4 under When Covered section for clarity. Updated policy guidelines and References. No change to policy statement. Medical Director review 4/2023. (tm)