Genetic Testing for Connective Tissue Disorders AHS – M2144

**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.

**Related Policies**

General Genetic Testing, Germline Disorders AHS – M2145

***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. Genetic testing (FBN1 mutation) for Marfan syndrome is considered medically necessary for any of the following indications
   a. 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. Testing of an asymptomatic individual who has an affected first-degree blood relative (i.e., parent, sibling, child) with a known mutation.
   c. The prenatal diagnosis or PGD of Marfan syndrome in the offspring of patients with known disease-causing variants.

2. If FBN1 mutation testing is negative, genetic testing for Loeys-Dietz Syndrome (TGFBR1 or TGFBR2 mutation) is considered medically necessary for any of the following indications:
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a. To confirm or establish a diagnosis of LDS in an individual with characteristics of LDS (see Note 2 below)

b. Testing of an asymptomatic individual who has an affected first-degree blood relative (i.e. parent, sibling, child) with a known mutation.

3. Genetic testing (COL3A1 and COL1A1) for vascular Ehlers-Danlos Syndrome (Veds) is considered medically necessary for any of the following indications:
   a. To confirm or establish a diagnosis of LDS in an individual with characteristics of LDS (see Note 2 below)
   b. Testing of an asymptomatic individual who has an affected first-degree blood relative (i.e. parent, sibling, child) with a known mutation.

4. Panel gene testing connective tissue disorders* (See Note 4) is considered medically necessary when ALL of the following criteria are met:
   a. Signs and symptoms of a vascular connective tissue disorder; AND
   b. Family history of vascular issues, such as aneurysms

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 (B.L. Loeys et al., 2010; Wright & Connolly, 2018).

- 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 (B. L. Loeys et al., 2010; Wright & Connolly, 2018):

- Wrist AND thumb sign: 3 points
- Wrist OR thumb sign: 1 point
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- 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 (B. Loeys & Dietz, 2018).

Note 3: According to the Genetic and Rare Diseases Information Center (GARD) of the National Institutes of Health (NIH), vEDS (also known as ecchymotic type or Sack-Barabas type) is the most severe form of EDS. The common symptoms of vEDS can include rupture of tissues/fragile tissues; easy bruising; thin, translucent skin; small joint hypermobility; characteristic facial appearance, such as thin lips, small chin, thin nose, and/or large eyes; acrogeria; pneumothorax; joint dislocations and subluxations; congenital clubfoot; and receding gums (GARD, 2017).

Note 4: For 5 or more gene tests being run on the same platform, such as multi-gene panel next generation sequencing, please refer to AHS-R2162 Reimbursement Policy.

When Genetic Testing for Connective Tissue Disorder is not covered

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

Policy Guidelines

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 (H. Dietz, 2017; Pyeritz, 2017; Wright & Connolly, 2018).

MFS primarily affects connective tissue, particularly the fibrillin component of the extracellular matrix. Fibrillins are large glycoproteins that form extracellular microfibrils which 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, 2018). Mutations lead to impaired fibrillin-1 protein function, which causes 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, 2020). 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:

<table>
<thead>
<tr>
<th>Clinical EDS Subtype</th>
<th>Abbreviation</th>
<th>Inheritance Pattern</th>
<th>Genetic Bases</th>
<th>Protein</th>
</tr>
</thead>
</table>
| Classical EDS        | cEDS         | AD (autosomal dominant) | Major: *COL5A1*, *COL5A1*  
Rare: *COL1A1* | Type V collagen |
|                      |              |                     |               |         |
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<table>
<thead>
<tr>
<th>Classical-like EDS</th>
<th>cEDS</th>
<th>AR (autosomal recessive)</th>
<th>TNXB</th>
<th>Tenascin XB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac-valvular</td>
<td>cvEDS</td>
<td>AR</td>
<td>COL1A2 (biallelic mutations that lead to COL1A2 NMD and absence of pro α2(I) collagen chains)</td>
<td>Type I collagen</td>
</tr>
<tr>
<td>Vascular EDS</td>
<td>vEDS</td>
<td>AD</td>
<td>Major: COL3A1</td>
<td>Type III collagen</td>
</tr>
<tr>
<td>Hypermobile EDS</td>
<td>hEDS</td>
<td>AD</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>Arthrochalasia EDS</td>
<td>aEDS</td>
<td>AD</td>
<td>COL1A1, COL1A2</td>
<td>Type I collagen</td>
</tr>
<tr>
<td>Dermatosparaxis EDS</td>
<td>dEDS</td>
<td>AR</td>
<td>ADAMTS2</td>
<td>ADAMTS-2</td>
</tr>
<tr>
<td>Kyphoscoliotic EDS</td>
<td>kEDS</td>
<td>AR</td>
<td>PLOD1</td>
<td>LH1</td>
</tr>
<tr>
<td>Brittle Cornea syndrome EDS</td>
<td>BCS</td>
<td>AR</td>
<td>ZNF469</td>
<td>ZNF469</td>
</tr>
<tr>
<td>Spondylodysplastic EDS</td>
<td>spEDS</td>
<td>AR</td>
<td>B4GALT7</td>
<td>β4GalT7</td>
</tr>
<tr>
<td>Musculoskeletal EDS</td>
<td>mcEDS</td>
<td>AR</td>
<td>CHST14</td>
<td>D4ST1</td>
</tr>
<tr>
<td>Myopathic EDS</td>
<td>mEDS</td>
<td>AD or AR</td>
<td>COL12A1</td>
<td>Type XII collagen</td>
</tr>
<tr>
<td>Periodontal EDS</td>
<td>pEDS</td>
<td>AD</td>
<td>C1R</td>
<td>C1r</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C1S</td>
<td>C1s</td>
</tr>
</tbody>
</table>

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, 2020).

Vascular EDS (vEDS) is characterized by “arterial aneurysm, dissection and rupture, bowel rupture, and rupture of the gravid uterus” and affects 1/50,000 to 1/200,000 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, Velvin, and Lidal (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, 2018). 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.

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, 2019). 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 Validity and Utility

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 (B. 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 residue, 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, 2018).

Becerra-Munoz et al. (2018) conducted a prospective cohort study to summarize variants in FBN1 and establish the 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, they have mutations in TGF-beta receptor 1 (TGFBR1) and TGFBR2 genes.
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It has been proposed that patients with the Marfan phenotype and TGFBR1 or TGFBR2 mutations be classified as having Loeys-Dietz syndrome (LDS) to properly address the potential for more aggressive vascular disease than seen in MFS (Wright & Connolly, 2018).

The diagnosis of Marfan syndrome 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 (H. Dietz, 2017). These features are summarized in the 2010 Ghent nosology, which is slightly altered for patients under 20 years old (Wright & Connolly, 2018). 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. (H. C. Dietz, Loeys, Carta, & Ramirez, 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, Lawrence, & Musini, 2017).” Sellers et al 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)”.

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.

Applicable Federal Regulations
A search for “Marfan Syndrome” on 01/27/2020 yielded zero lab testing results. This is also true for additional searches containing the following terms: “Ehlers-Danlos syndrome,” “Epidermolysis bullosa,” and “Loeys-Dietz syndrome.” A search for any specific tests of the FBN1 gene also did not yield any lab testing results. Additionally, 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 (FDA, 2018).

Guidelines and Recommendations

American College of Cardiology (ACC) (Hiratzka et al., 2010)

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 6 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 College of Medical Genetics (ACMG) (Pyeritz, 2012)

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,
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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).”

American Academy of Pediatrics (AAP) (Tinkle & Saal, 2013)

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).

Marfan Foundation (Foundation, 2013a, 2013b)

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 (Foundation, 2013a).

Screening of first-degree relatives of patients with MFS is also warranted. Aortic imaging may be performed if the mutation has not been identified (Foundation, 2013b).

International Consortium on the Ehlers-Danlos Syndromes (Malfait et al., 2017)

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
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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 (cLEDSDS), 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 (TNX4), 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 guidelines 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
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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 may 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 may 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 may 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 values 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 (e.g. deep intronic mutations) may go undetected by standard diagnostic molecular techniques, and still other, yet to be
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 may 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 may 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 (e.g., 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) (Boodhwani et al., 2014)

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, TGFβ, SMAD3, ACTA2, or MYH11) should receive complete aortic imaging when diagnosed and 6 months after diagnosis.

The Ehlers Danlos Society (EDS, 2017)
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The Ehlers Danlos Society published the 2017 International EDS Classification document which includes required clinical criteria for an EDS diagnosis.

For cEDS, the Ehlers Danlos Society has stated that “Molecular analysis of COL5A1 and COL5A2 genes identifies a causal mutation in more than 90% of the patients and should be used as the standard confirmatory test. In case of unavailability of genetic testing, electron microscopy findings of collagen flowers on skin biopsy can support the clinical diagnosis (EDS, 2017).”

For vEDS, the Ehlers Danlos Society has stated that “The diagnosis of vEDS rests on the identification of a causative variant in one allele of COL3A1 (EDS, 2017).”

**International Group of Specialists with a Broad Aggregate Experience in the Care of Individuals with Vascular EDS (Byers et al., 2017)**

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, 2013)**

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).”

**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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Foundation, M. (2013b). OVERVIEW OF CARDIAC MANAGEMENT IN MARFAN SYNDROME.


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**For policy titled: Genetic Testing for Connective Tissue Disorder**

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Policy Implementation/Update Information

<table>
<thead>
<tr>
<th>Date</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>1/1/2019</td>
<td>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)</td>
</tr>
<tr>
<td>5/14/19</td>
<td>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)</td>
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<tr>
<td>10/29/19</td>
<td>Wording in the Policy, When Covered, and/or Not Covered section(s) changed from Medical Necessity to Reimbursement language, where needed.</td>
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Medical policy is not an authorization, certification, explanation of benefits or a contract. Benefits and eligibility are determined before medical guidelines and payment guidelines are applied. Benefits are determined by the group contract and subscriber certificate that is in effect at the time services are rendered. This document is solely provided for informational purposes only and is based on research of current medical literature and review of common medical practices in the treatment and diagnosis of disease. Medical practices and knowledge are constantly changing and BCBSNC reserves the right to review and revise its medical policies periodically.