GeneDx Review: 07/2011

Test Information Sheet

Genetic Testing for Marfan Syndrome (MFS), Loeys-Dietz Syndrome (LDS) and Related Disorders
FBN1, TGFBR1, TGFBR2 genes

Mendelian Inheritance in Man Number: 154700 (Marfan syndrome); 129600 (ectopia lentis syndrome); 604308 (MASS syndrome); 134797 (FBN1 gene); 609192, 610168 (Loeys-Dietz syndrome); 190181 (TGFBR1 gene); 190182 (TGFBR2 gene)

Clinical features:
Marfan syndrome (MFS) is a connective tissue disorder that can affect multiple organ systems including the skeletal, ocular, and cardiovascular systems and is caused by mutations in the FBN1 gene. A diagnosis is based on the presence of major and minor clinical criteria, as established by the Ghent nosology (de Paepe 1996, Loeys 2010). Skeletal features can include chest malformations (pectus carinatum/excavatum), tall stature, increased joint mobility, and scoliosis. Eye findings most commonly include lens dislocation (ectopia lentis) and myopia. The cardiovascular features are typically mitral valve prolapse and/or aortic root dilatation, which can progress to aortic dissection. Patient management and treatment is mainly focused on slowing the progression of aortic root dilation, the most common cause of morbidity and early mortality. Therefore, genetic testing is important for identifying presymptomatic family members who carry a FBN1 mutation, and at risk for developing features of Marfan syndrome, who will benefit from appropriate monitoring for aortic root dilatation.

Mutations in the FBN1 gene have also been observed in families with isolated ectopia lentis and MASS syndrome (myopia, mitral valve prolapse, borderline/non-progressive aortic root dilation, skeletal and skin findings). MASS is a connective tissue disorder related to Marfan syndrome but with milder cardiovascular findings.

Loeys-Dietz syndrome (LDS) is a systemic connective tissue disorder caused by mutations in the TGFBR1 or TGFBR2 genes. The skeletal features of Loeys-Dietz syndrome, such as joint laxity, arachnodactyly, pectus deformity, and scoliosis, can overlap with the Marfan phenotype, however most individuals with LDS have features in other organ systems not typical of Marfan syndrome (Loeys 2005). Patients with Loeys-Dietz syndrome can exhibit various craniofacial, neurodevelopmental, skeletal and skin abnormalities, however features specific to LDS include hypertelorism, cleft palate or bifid uvula, and arterial or aortic aneurysms and aterial tortuosity (Van Hemelrijk 2010). The primary risk for early mortality is due to ateral tortuosity and/or aortic root dilatation, which results in an increased risk of aterial or aortic aneurysm (Van Hemelrijk 2010). Since patients with LDS can develop potentially lethal arterial aneurysms that would not be detected by echocardiogram, differentiating between Loeys-Dietz and Marfan syndromes is necessary to determine the appropriate clinical management. Similar to Marfan syndrome, genetic testing is important for identifying presymptomatic family members at risk for developing Loeys-Dietz syndrome.

Mutations in the TGFBR1 gene have also been observed in families with multiple self-healing squamous epithelioma (MSSE), also known as Ferguson-Smith disease. MSSE is a skin cancer condition that presents with multiple, locally invasive skin tumors that normally regress, leaving scars. Loss-of-function mutations in the TGFBR1 gene have been reported in individuals with MSSE, while gain-of-function mutations are presumed to result in the Loeys-Dietz syndrome phenotype (Goudie 2011).

Inheritance pattern: Autosomal dominant with variable expressivity. Approximately 25% of Marfan cases and 75% of Loeys-Dietz cases result from de novo mutations.

Genetics:
The FBN1 gene is located on chromosome 15q21.1 and has 65 exons coding for the fibrillin-1 protein. Fibrillin 1, with fibrillin 2 and fibrillin 3, forms microfibrils that are present in elastic and non-elastic fibers contributing to the structure of multiple organ systems in the body. Fibrillin 1 also has been shown to aid in the regulation of growth factors, such as TGFβ. Mutations in FBN1 result in decreased formation of microfibrils and increased TGFβ signaling, ultimately resulting in the clinical features observed in Marfan syndrome.

The TGFBR1 gene is located on chromosome 9q22 and contains 9 exons coding the transforming growth factor-beta receptor, type 1. The TGFBR2 gene is located on chromosome 3p22, consists of 7 exons, and codes for the transforming growth factor-beta receptor, type 2. TGFBR1 and TGFBR2 are transmembrane serine/threonine receptor kinases involved in cell cycle regulation within the TGF-beta signaling pathway. While the mechanism of disease is not well defined, mutations in TGFBR1 or TGFBR2 appears to enhance the TGF-beta signaling pathway with the downstream effect of...
accumulated protein products causing fiber disorganization and increased collagen deposits thought to weaken the
vasculature (Van Hemelrijk 2010).

**Reasons for referral:**
- Confirmation of a clinical diagnosis
- Differentiation between Marfan syndrome, Loeys-Dietz syndrome and phenotypically related disorders.
- Presymptomatic testing; identification of family members at risk for Marfan syndrome or Loeys-Dietz syndrome to
  allow for appropriate screening and management
- Genetic counseling and recurrence risk assessment
- Prenatal diagnosis in families with a known mutation in the FBN1, TGFBR1/2 genes

**Test method:**
Mutation analysis of the FBN1, TGFBR1 and TGFBR2 genes is performed on genomic DNA from the submitted specimen
using bi-directional sequence analysis of the coding exons, and the corresponding intron/exon boundaries. If no mutation is
found by sequencing in the FBN1 gene, deletion/duplication analysis is available using ExonArrayDx, a targeted array
CGH with exon level resolution, to evaluate for a deletion or duplication in one or more exons in the FBN1 gene. Mutations
found in the first person of a family to be tested are confirmed by repeat analysis using sequencing, restriction fragment
analysis, or other appropriate method.

**Mutation spectrum:**
Missense, nonsense, splicing, and insertion/deletion mutations have been reported in the FBN1 gene. Missense mutations
account for approximately 60% of mutations identified and frequently affect cysteine residues involved in disulfide bonding
(Boileau 2005). Mutations resulting in protein truncation (nonsense and frameshift mutations) account for greater than 30%
of mutations identified (Arbustini 2005, Boileau 2005). In a minority of cases, large deletions of multiple exons have been
reported in the FBN1 gene (Matyas 2007). While the phenotypic spectrum is broad for FBN1 mutations, some generalized
genotype-phenotype correlations have been observed. A clustering of mutations in exons 24-32 has been reported in
neonatal Marfan syndrome and atypically severe Marfan syndrome (Faivre 2009). Missense mutations involving a cysteine
residue tend to occur more frequently in patients with ectopies lentis, while nonsense mutations have been reported to be
more prevalent in patients with major skeletal criteria (Arbustini 2005).

Missense mutations account for the majority of mutations in the TGFBR1 and TGFBR2 genes, though nonsense, splicing,
and insertion/deletion mutations have also been reported (Van Hemelrijk 2010). No genotype-phenotype correlations
between the TGFBR1 and TGFBR2 genes has been observed to date, as mutations in either gene can result in the same
combination of clinical features for Loeys-Dietz syndrome (Loeys 2006).

**Test sensitivity:**
Sequence analysis of all exons in the FBN1 gene is expected to identify a mutation in 72-93% of individuals with a clinical
suspicion of Marfan syndrome, with the mutation detection rate approaching 93% in individuals fulfilling a clinical
diagnosis of Marfan syndrome based on the Ghent nosology (Boileau 2005; Arbustini 2005; Stheneur 2009; Akutsu 2010).
The test sensitivity significantly decreases for individuals who do not meet Ghent criteria for Marfan syndrome (Stheneur
2009). Large deletions have been detected in approximately 2% of individuals who did not have a mutation identified by
sequencing (Matyas 2007).

The mutation detection rate for sequence analysis of all exons in the TGFBR1 and TGFBR2 genes in patients with Loeys-
Dietz syndrome has not been well established, but may be as high as 87% in patients with a strong clinical suspicion of LDS
(Stheneur 2008). Of patients with Loeys-Dietz syndrome with an identifiable mutation, 75% have a mutation in the
TGFBR2 gene and 25% have a mutation in the TGFBR1 gene.

**Specimen Requirements and Shipping/Handling:**
- **Blood:** A single tube with 1-5 mL whole blood in EDTA. Ship overnight at ambient temperature, using a cool pack
  in hot weather. Specimens may be refrigerated for 7 days prior to shipping.
- **Buccal Brushes:** Cannot be accepted.
- **Saliva:** Cannot be accepted.
- **Prenatal Diagnosis:** For testing of a known familial mutation, please submit 20 mL amniotic fluid, 20mg villi
  (minimum 15mg), or 2 T25 flasks of cultured amniocytes or cultured villi. Ship specimen overnight at
  ambient temperature, using a cool pack in hot weather.
Required Forms:
- Sample Submission (Requisition) Form – complete all pages – including
- Payment Options Form or Institutional Billing Instructions

Prices** and Turn-Around Time - Fees are subject to change without notice:

Test # 510: FBN1 and TGFBR1/2 sequencing in a new patient $3,375 Approx. 8 weeks
Test # 511: TGFBR1 and TGFBR2 sequencing in a new patient $1,550 Approx. 4-6 weeks
Test # 458: FBN1, TGFBR1/2 deletion/duplication testing (ExonArrayDx) $1000 Approx. 3 weeks
Test # 9011: Targeted testing of a relative for a known mutation (deletion)* $ 350 ($500) Approx. 2-3 weeks
Test # 902: Prenatal diagnosis for specific known mutation(s) * $2000 Approx. 2 weeks

* For CPT codes for carrier and prenatal testing, please see our website: http://www.genedx.com
** For contracted institutions

CPT codes for mutation detection in a new patient - All codes and units apply:

Test # 510 FBN1, TGFBR1, TGFBR2 sequencing
83891 x 1 unit
84311 x 1 unit
83892 x 2 units
83900 x 1 unit
83901 x 75 units
83904 x 77 units
83912 x 1 unit

Test # 511 TGFBR1 and TGFBR2 sequencing only
83891 x 1 unit
84311 x 1 unit
83892 x 2 units
83900 x 1 unit
83901 x 15 units
83904 x 34 units
83912 x 1 unit

Test# 458 FBN1, TGFBR1, TGFBR2 deletion/duplication testing (ExonArrayDx)
83891 x 1 unit
84311 x 1 unit
88271 x 42 units
88291 x 1 unit

Possible ICD9 Codes:
759.82 Marfan syndrome
754.32 Pectus excavatum
379.32 Subluxation of lens
737.43 Scoliosis
701.3 Striae
441.0 Dissection of aorta, unspecified site
441.5 Aortic aneurysm of unspecified site, without rupture
441.9 Aortic aneurysm of unspecified site, ruptured
512.8 Spontaneous Pneumothorax

References: