

## Assay Summary

### **Fibrillin-1 and Transforming Growth Factor $\beta$ Receptor Gene Mutation Analysis**

#### **Marfan Syndrome Type I and II,**

#### **Marfan-like Connective Tissue Disorder, Familial Ectopia Lentis and Familial Aortic Aneurysm**

#### *Synopsis*

Marfan syndrome (MS) is a systemic disorder of connective tissue with a high degree of clinical variability. Cardinal manifestations involve the cardiovascular, musculoskeletal, ocular, and central nervous systems. Of particular concern is the risk for the development of a life threatening, aortic aneurysm or dissection. The syndrome shows autosomal dominant inheritance (only one copy of a mutation is necessary for expression of disease) and complete penetrance, but is notable for variability in the age of onset, tissue distribution, and severity of clinical manifestations, both between and within affected families. Because mutations are heterogeneous and approximately 30% of MS cases are due to de novo mutations, direct testing often requires complete gene analysis. Mutations in fibrillin-1 (FBN1) have been firmly established as a cause of MS, as well as other conditions such as familial ectopia lentis, and in families with an inherited form of aortic aneurysms. Defects in the gene that encodes fibrillin-1, the main structural component of the elastin-associated microfibrils, are responsible for these disorders. Mutations in the FBN1 gene are detected in approximately 80% and 40% of patients that meet and do not meet Ghent diagnostic criteria for MS, respectively.

Recently, Mizuguchi et al. found a strong association between mutations in the Transforming Growth Factor  $\beta$  Receptor 2 gene (TGFB2) and MS<sup>4</sup>. In their study, they reported 4 mutations (1 splicing and 3 missense, which affected highly conserved amino acids). All mutations co-segregated with disease, and were not found in controls. Recent work by Loeys et al. examined ten families and described a new aortic aneurysm syndrome characterized by hypertelorism, bifid uvula/cleft palate, and generalized arterial tortuosity with ascending aortic aneurysm and dissection, now called Loeys-Dietz Syndrome (LDS). Mutations in the TGFB1 and TGFB2 genes were associated with disease<sup>5</sup>. While there are some overlapping features between MS and LDS, ectopia lentis seems to be a very rare finding in individuals with TGFB mutations, and LDS patients often have craniofacial abnormalities such as hypertelorism, and bifid uvula.

#### *Indications for testing*

Individuals with a clear diagnosis of Marfan syndrome, Loeys Dietz syndrome, or any Marfan-like phenotype including familial ectopic lentis, and familial aortic aneurysm. To help make a diagnosis in suspected Marfan syndrome, Loeys-Dietz syndrome, Marfan-like syndrome, familial ectopia lentis or familial aortic aneurysm. Once a specific mutation is known in a family, presymptomatic testing for appropriate family members can be performed.

#### *Methodology*

All coding exons and associated intron junctions of the each gene are analyzed by direct DNA sequence analysis using an automated fluorescent sequencer. When a mutation is detected, confirmation is carried out by sequencing in the opposite direction, in second independent PCR amplification. If no mutation is found sequence analysis is performed in both directions. At-risk family members can be offered DNA sequence analysis of only the region of the gene with the previously identified mutation.

FBN1 large deletions are found in 2% of MS<sup>7-11</sup>. We have incorporated the SALSA MLPA kit which is a rapid, high-throughput technique for copy number quantification, specifically testing for large deletions/duplications for the FBN1, TGFRB1/2 genes in Marfan syndrome, Loeys Dietz syndrome, or any Marfan-like phenotype. This assay should be considered for patients with any Marfan-like phenotype where full gene sequencing did not detect a mutation.

### ***Performance/Limitations***

Mutations in the FBN1 gene are detected in approximately 80% and 40% of patients that meet and do not meet Ghent diagnostic criteria for MS, respectively. For patients presenting with a diagnosis of Loeys-Dietz syndrome, the detection rates range from 100% to 30% for Type I and II respectively<sup>6</sup>. This method will not detect mutations located in regions of the genes that are not analyzed (non-coding exon sequences, intron sequences other than the splice junctions, and upstream and downstream sequences). The method also will not detect gross genetic alterations including inversions. Some sequence alterations that may be detected (such as those causing missense or synonymous changes) will be of unknown clinical significance. The MLPA method is designed to detect deletions of one or more exons of the FBN1 gene. However, mutations and/or polymorphisms very close to the probe ligation site may also result in a reduced relative peak area. Therefore, apparent deletions detected by a single probe will be confirmed by a second method whenever possible. Interpretation of test results should be in the context of the patient's ethnicity, clinical and family histories, and other laboratory test results.

### ***Specimen Requirements***

(a) Blood samples: 2 tubes with a total of 6 ccs in ACD (yellow top) or EDTA (lavender top) tubes. Keep at ambient temperature and ship by overnight courier. Samples must be received in our laboratory within 72 hours of draw.

**Note:**

- i) for infants, a minimum of 3 ccs is sufficient.
- ii) we accept DNA; at least 10 micrograms is required.

(b) Prenatal samples: 2 T25 flasks of confluent cells sent padded to arrive on M/Tu/W. A blood sample from the mother maybe required (2 tubes with a total of 6 ccs in ACD (yellow top) or EDTA (lavender top) tubes) for use as positive control. Maternal cell contamination studies are not done here but are required for autosomal disorders and dosage analysis on X-linked disorders. We would be happy to assist in coordinating sending out a specimen for this purpose.

### ***Test Request Form (TRF)***

- a) A completed MDL [TRF](#) is required for each specimen. Please submit the completed TRF with the specimen. Complete testing and billing information must be provided before the specimen is processed.
- b) [Marfan Patient Information Form](#): Include a completed Marfan Patient Information .

<b>Order Codes</b>	<b>CPT Codes</b>	<b>TAT</b>
FBN1-SEQ (FBN1 gene, full gene sequencing )	83890, 83898(x67), 83894, 83904(x67), 83912	4 wks
FBN1-CAS (FBN1 gene, targeted mutation analysis, known mutation)	83890, 83898, 83894, 83904, 83912	3 wks
FBN1-PD (FBN1 gene, known mutation detection, prenatal )	83890, 83898, 83894, 83904, 83912	2 wks
FBN1-DEL (FBN1 gene, MLPA analysis)	83890, 83896 (x55), 83909, 83912	3 wks
FBN1-DEL-CAS (FBN1 gene, MLPA analysis, known deletions/duplications)	83890, 83896 (x55), 83909, 83912	3 wks
FBN1-DEL -PD (FBN1 gene, MLPA analysis, prenatal)	83890, 83896 (x55), 83909, 83912	3 wks
TGFBR1-SEQ (TGFBR1 gene, full gene sequencing )	83890, 83898(x11), 83894, 83904(x11), 83912	4 wks
TGFBR1-CAS (TGFBR1 gene, targeted mutation analysis, known mutation )	83890, 83898, 83904, 83894, 83912	3 wks
TGFBR1-PD (TGFBR1 gene, known mutation detection, prenatal )	83890, 83898, 83904, 83894, 83912	2 wks
TGFBR1-DEL (TGFBR1 gene, MLPA analysis)	83890, 83896 (x11), 83909, 83912	3 wks
TGFBR1-DEL-CAS (TGFBR1 gene, MLPA analysis)	83890, 83896 (x11), 83909, 83912	3 wks
TGFBR1-DEL-PD (TGFBR1 gene, MLPA analysis, prenatal)	83890, 83896 (x11), 83909, 83912	3 wks
TGFBR2-SEQ (TGFBR2 gene, full gene sequencing )	83890, 83898(x8), 83894, 83904(x9), 83912	4 wks
TGFBR2-CAS (TGFBR2 gene, targeted mutation analysis, known mutation )	83890, 83898, 83904, 83894, 83912	3 wks
TGFBR2-PD (TGFBR2 gene, known mutation detection, prenatal )	83890, 83898, 83904, 83894, 83912	2 wks
TGFBR2-DEL (TGFBR2 gene, MLPA analysis)	83890, 83896 (x9), 83909, 83912	3 wks
TGFBR2-DEL-CAS (TGFBR2 gene, MLPA analysis)	83890, 83896 (x9), 83909, 83912	3 wks
TGFBR2-DEL-PD (TGFBR2 gene, known mutation detection, prenatal)	83890, 83896 (x9), 83909, 83912	3 wks

### **References**

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9. Blyth, G. et al. (2008) Am J Med Genet A.146A (10):1320-4
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11. HGMD, <http://www.hgmd.cf.ac.uk/ac/gene.php?gene = FBN1>

NOTE: This test is performed pursuant to a license agreement with Roche Molecular Systems, Inc