

<p><b>Matrix DNA Diagnostics – Tulane University</b>  <b>Patient Consent form – Fibrillin-1 Mutation assay</b>  <b>Detection of Mutations in the Fibrillin-1 Gene (FBN1) Causing Marfan syndrome and Related Disorders</b></p>
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**BACKGROUND:** Fibrillin 1 is a protein that plays an important role in the formation and stability of elastic fibers. Elastic fibers in turn are important structural components of major blood vessels such as the aorta, and certain parts of the eyes, skeletal system, skin and lungs. Mutations in the gene coding for fibrillin 1 (FBN1) may cause severe disorders involving these tissues such as Marfan syndrome. The DNA testing carried out in our laboratory will detect most but not all of the mutations in the FBN1 gene. Since the FBN1 gene is complex, a careful search for mutations requires multiple manipulations and analyses of the DNA. For this reason, a complete analysis may require two to four weeks, and may occasionally require samples from other family members.

**TEST LIMITATIONS:** The analysis may fail to detect some disease causing mutations. Currently we do not have an accurate estimate of how many mutations will be detected, but over 90% were found in our control studies. It may also be difficult to determine if some changes represent actual mutations or neutral changes. Additionally, mutations in unrelated genes causing similar diseases will not be detected. A negative test result does not exclude the presence of Marfan Syndrome. Because of these potential limitations, results should be interpreted with the assistance of a qualified physician or genetic counselor. We are confident that this test will detect mutations and provide definitive information in the majority of patients with a clinical diagnosis of Marfan syndrome.

**SAMPLE** The DNA test requires extraction of DNA from the patient's blood. The DNA can also be extracted from a tissue sample, such as a small piece of skin, but this is usually less convenient. Most commonly, blood is drawn by trained person designated by your physician. The blood is sent by overnight courier to the laboratory. There, the DNA is extracted and over several weeks the analysis is performed. The result is sent in written form to the physician who will relay it to the patient or relative designated to receive the result. The results are confidential and will not be released to any institution or individual without your written consent.

**STORAGE** In most instances not all the DNA is consumed in the analyses. The remaining DNA will be stored in this laboratory for two years, after which the DNA sample will be discarded without informing the patient or family members. The DNA will not be released to any institution or individual without your written consent. The DNA may be used at the discretion of the laboratory supervisor for research purposes, under an anonymous label. The results of the research studies will not be reported to the patient or his physician at any time, nor will the laboratory assume any responsibility of the research studies on these specimens.

**LABORATORY** The laboratory is an up-to-date molecular genetics facility and uses the most current techniques clinically practical to perform the analysis. The techniques used may change to become more sensitive or specific in the future. In such cases, at the request of your physician, the test may be rerun on your sample, if clinically indicated.

**LEGAL ISSUES** The laboratory assumes no responsibility for injury or illness resulting from the drawing of blood or removal of tissue samples from the patient. The laboratory also assumes no responsibility for liability or loss incurred as a result of the outcome of this test. The laboratory assumes no responsibility for the mislabeling or misidentification of submitted patient specimens and assumes all submitted patient data to be correct as listed in the submission form.

In signing this form, I indicate that I understand the information presented above and agree to these stipulations.

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<b>Patient Signature</b>	<b>Date</b>	<b>Witness Signature</b>	<b>Date</b>



**FORM 1- Instructions for submission of specimen for DNA testing**

**The patient should be fully informed about the test.**

**Nature of the test:** The test detects mutations in gene(s) involved in the synthesis of proteins of connective tissue. Extraction of DNA from blood or tissue of the affected individual is required.

**Test limitations:** Only the gene implicated in the disorder will be studied which your physician will determine. Mutations in other genes will not be detected. The rate of mutation detection varies with the disorder and the gene studied. Mutations in non-structural portions of the gene will generally not be detected.

**Test results:** The test results are reported to the physician in writing. The test generally takes about 2-4 weeks; we try to expedite the analysis for prenatal testing.

**Sample requirements**

•**Whole Blood:** 10 cc (adults) in purple top (EDTA) tube should be drawn by venipuncture. In the case of small children, 3-5cc of EDTA blood is generally sufficient amount to complete the analysis. There are no special dietary or blood drawing considerations. Patient must not have received a transfusion recently. Heparin inhibits the PCR reaction, therefore heparin tubes are not acceptable. DNA extracted from heparin-contaminated blood is not accepted

•**Fibroblasts, amniocytes, CVS:** Preferably 2-4 confluent T25-flasks of cultured cells. It is highly advisable to call prior to submitting cultured cells.

•**Tissues:** Please call the lab at 504-988-7706. We do not take whole tissue or paraffin blocks. We do accept extracted DNA from tissues, but not from paraffin blocks.

**Sample submission**

Sample should be shipped by **overnight courier, preferably Federal Express** (Use DHL/Airborne with caution as we have experienced significant delivery issues with them). Please send with a cold pack during summer months. If blood sample can't be shipped the same day, please store at +4C until shipment. All tubes/flasks should be labeled with patient name and date of birth, or other identifying criteria. Please do not send any samples on Friday. **Patient Information Form** (Form 2), **Payment Information Form** (Form 3) and an **OPTIONAL signed Patient Consent Form** should accompany the sample. If laboratory fee is paid by check that accompanies the sample, Form 3 is not needed. Check should be made out to **Tulane University**.

<b>Charges</b>	
<b>Osteogenesis Imperfecta:</b> Collagen I Gene (COL1A1 and COL1A2) Analysis	\$1800
<b>Chondrodysplasias:</b> Collagen II Gene (COL2A1) Analysis	\$1400
<b>Stickler/Marshall Syndrome:</b> Analysis of the COL2A1 and COL11A1 Genes	\$2300
<b>Marfan Syndrome:</b> Fibrillin -1 gene (FBN1) Analysis	\$1400
<b>Metaphyseal dysplasia type Schmid:</b> Collagen X gene (COL10A1) Analysis	\$460
<b>Known mutation (family member or prenatal)</b> in any of the above	\$350

We can assist with test description and related information for insurance companies.

<p><b>Sample and Paperwork should be sent to:</b> Please call (504) 988-7706 before submitting a sample for the first time <b>if you need assistance.</b></p>	<p>Matrix DNA Diagnostics          Attn: DNA Diagnostics Laboratory          Center for Gene Therapy          Tulane University Health Science Center          1430 Tulane Avenue, TB 28          Tidewater 2140          New Orleans, LA 70112</p>
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<b>Patient name:</b>	<b>Date of Birth:</b>
<b>Hospital Reference Number:</b>	<b>SSN:</b>
<b>Address:</b>	<b>Telephone:</b>

**Patient Diagnosis/Clinical information:**

**Required attachments:**

- copy of a driving license for the policy holder, if insurance bill
- copy of clinical history and physical examination

<b>Check for the test requested:</b>	<input type="checkbox"/>	COL1A1/COL1A2 gene analysis for Osteogenesis Imperfecta
	<input type="checkbox"/>	COL2A1 gene analysis for SED, ACGII, HCG, Kniest dysplasia
	<input type="checkbox"/>	COL2A1 /COL11A1 gene analysis for Stickler/Marshall Syndrome
	<input type="checkbox"/>	FBN1 gene analysis for Marfan Syndrome
	<input type="checkbox"/>	COL10A1 gene analysis for Schmid Metaphyseal dysplasia
Proband name must be provided in box above.	<input type="checkbox"/>	Known Mutation (family member or prenatal) in any of the above

<b>Type of specimen:</b>	<b>Is this a prenatal test YES NO</b>
<b>Collected by:</b>	<b>Collection date:</b>

<b>Referring Physician Name</b>	<b>Contact person</b>
<b>Telephone Fax</b>	<b>Address</b>
<b>Request Date</b>	<b>Physician's Authorized Signature</b>

**If this is a known family mutation, please provide the proband's name or the proband report.**

### Form 3: Payment Information/Invoice

**1. Payment by check.** Please make the check payable to **Tulane University** and send to:

Matrix DNA Diagnostics  
Center for Gene Therapy  
1430 Tulane Ave, SL-99  
New Orleans, LA 70112

<b>ICD-9 code:</b>	756.5	<b>Tax/Fed ID#:</b>	72-0423889	<b>CLIA#:</b>	39D0903989
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**2. Payment by Institution.**

Contact Person:	Phone number	Bill to- address
	Fax number	

**3. Payment by Insurance Company. ATTACH A READABLE PHOTOCOPY OF THE INSURANCE CARD (BOTH SIDES)! CARDHOLDER INFORMATION MUST ALSO BE INCLUDED (address and date of birth)!**

Name of Policy Holder:	SSN or member#:	Plan/Group#:
Insurance Provider's Name:	Phone Number:	Address:
Claim Dept's phone #:	Insurance Contact person:	Pre-approval number:

**DISCLAIMER:** DNA Diagnostics may not be an in-network provider with most insurance companies. We reserve the right to decline/refuse any insurance as a payment option. The patient accepts full responsibility for any payment not covered by insurance. **Patients may be required to provide alternate funding, upon request, in advance for any amount that may not be covered by insurance. WE DO NOT ACCEPT ANY MEDICAID.**

FBN1 for Marfan syndrome

CPT codes	Description	# Performed	Cost/test	Extended
83890	DNA isolation	1	\$10.00	\$10.00
83898	Polymerase chain reaction	30	\$10.00	\$300.00
83904	Nucleic acid probe w/ sequencing	27	\$40.00	\$1080.00
83912	Interpretation and report	1	\$10.00	\$10.00
<b>TOTAL COST</b>				<b>\$ 1400.00</b>

FBN1 analysis is a multistep test, which involves sequencing of a very large gene and, therefore, involves multiple CPT codes. For more information, please call the lab about the test (504-988-7706)

<b>In signing this form, I indicate that I understand the information presented above and agree to these stipulations.</b>	
Patient Signature	Date

## **ACCURACY AND SENSITIVITY OF DNA ASSAY (PCR-SEQUENCING) MUTATIONS CAUSING MARFAN SYNDROME (MFS)**

**Center for Gene Therapy - Tulane University Health Sciences Center**

### **The Assay:**

Our assay involves two steps:

- (a) **amplifying** DNA sequences from all 65 exons and exon flanking regions for the FBN1 gene for human fibrillin-1 using 66 specific primer pairs for the polymerase chain reaction (PCR);
- (b) nucleotide **sequencing** of all the PCR products

### **Sensitivity Assay:**

In preliminary tests of our assay, we asked the question: "Will the assay detect all the mutations that have been previously found in the genes for type I procollagen and similar genes?" We assayed genes that were previously shown to contain 78 different single base mutations. We detected 77 or 98.7%.

Our further data on the sensitivity of the assay came from analyses we have carried out on DNA samples (blood or fibroblasts) sent to us by clinical geneticist who made the diagnosis of Marfan syndrome or related disorder.

### **Interpretations of the Test Results:**

Most patients and families with Marfan syndrome are suspected to have "private mutations" in the sense that the same mutation is rarely found in more than one unrelated family. Hence the need for analysis of about 22,000 base pairs from each patient to detect a mutation. Fortunately, however, the base sequence of the FBN1 gene for fibrillin-1 is highly conserved and there are few neutral variations that change a codon for an amino acid or a consensus site for RNA splicing of an exon in normal individuals.

### **Predicting the Severity of the Disease:**

Most FBN1 gene mutations have proved to be "private" mutations, that is mutations that are unique to a family or individual. To date it has proved difficult to correlate specific mutations with the severity of phenotypic alterations. The exception appears to be in a condition termed neonatal MFS. These are rare cases presenting at birth with progressive features. They are felt to represent the severe end of the clinical spectrum. Point mutations have been identified in exons 24-26 and splicing errors have been identified in exons 31 or 32. These exons encode the initial 2 EGF-like domains in the longest group of repeated EGF-like domains. All told 47 tandemly repeated EGF-like domains are present. Most mutations causing MFS are missense mutations in one of these EGF-like-domains and lead to an alteration in an amino acid involved with calcium binding or in one of the six conserved cysteine residues. In addition to the splicing errors mentioned above nonsense mutations leading to FBN1 null allele or truncated fibrillin-1 molecules have also been identified. The best guide is the severity of the same disease in another member of the same family who also has the same mutation. Even this guide, however, can be misleading in that there are rare instances in which one member of the family with the mutation has a severe form of disease whereas the other member of the same family with the same mutation has a milder form.

### **Mutations Causing MFS that Cannot Be Detected by the DNA Assay:**

The assay will not detect mutations that alter promoter sequences, enhancer sequences, or repressor sequences of the FBN1 gene. Such sequences have not been accurately enough defined and they show large variations between one normal individual and another. However, mutations in such sequences appear to be a very rare cause of MFS. The test will also not detect mutations in genes other than the gene for fibrillin-1 that may cause the same or similar signs and symptoms as those seen in typical patients with MFS.