

**Matrix DNA Diagnostics – Tulane University**  
**Patient Consent form – COL2A1/COL11A1 Mutation assay**  
**For Patients with Stickler, Marshall or Stickler-like Syndromes**

**BACKGROUND** Collagen II is a tough, fibrous protein that provides a major part of the strength of the cartilage. It is also found in intervertebral disks and inner ear. Collagen XI is a quantitatively minor collagen associated with collagen II. Mutations in the COL2A1 gene can cause decreased synthesis of the protein or synthesis of defective protein. Defects in collagen II often result in structural or growth disorders frequently accompanied by eye abnormalities. Mutations in the COL11A1 gene can result in similar but milder phenotypes. Null allele mutations in the COL2A1 gene and more deleterious mutations (exon-skippings and glycine substitutions) in the COL11A1 gene can cause phenotypes of Stickler, Marshall or Stickler-like syndromes.

DNA testing carried out in our laboratory will detect most, but not all, of the mutations in the COL2A1 and COL11A1 genes. These genes are among the more complex of human genes. Therefore, complete analysis of the genes for the presence of mutations requires a large number of careful manipulations and analyses of the DNA. For this reason, completion of the test may take two to four weeks in order to establish a definitive result, and may occasionally require samples from other family members.

**TEST LIMITATIONS** The analyses may fail to detect a few mutations in patients with mutations in the COL2A1 and COL11A1 genes as a cause of the disease. We do not have an accurate estimate of how many mutations are missed, but our best estimate is that mutation is detected in about 70-80% of the patients with Stickler/Marshall syndromes. Also, a few changes in the structure of the genes that are detected by the test are difficult to interpret in terms of whether they in fact cause the disease or are more neutral changes. Mutations in other genes are not detected. Overall, in a majority of patients with Stickler/Marshall syndromes, the test will detect a mutation and provide definitive information that the mutation causes the disease.

**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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**Patient Signature**                      **Date**                      **Witness Signature**                      **Date**

For any questions, please do not hesitate to call us at (504) 988-7706



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

#### Charges

<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

**Sample and Paperwork should be sent to:** Please call (504) 988-7706 before submitting a sample for the first time **if you need assistance.**

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



<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 READABLEPHOTOCOPY 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.**

COL2A1 and COL11A1 for Stickler/Marshall syndromes

CPT codes	Description	# Performed	Cost/test	Extended
83890	DNA isolation	1	\$10.00	\$10.00
83898	Polymerase chain reaction	60	\$10.00	\$600.00
83904	Nucleic acid probe w/ sequencing	42	\$40.00	\$1680.00
83912	Interpretation and report	1	\$10.00	\$10.00
<b>TOTAL COST</b>				<b>\$ 2300.00</b>

\*Please note that the COL2A1/COL11A1 analysis is a multistep test which involves sequencing the entire coding region of two very large genes and, therefore, involves multiple tests to isolate the genes, identify the genes, and sequence all coding regions in every patient. 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>	
<b>Patient Signature</b>	<b>Date</b>

**MATRIX DNA Diagnostics - Tulane University Health Sciences Center**  
**Sensitivity of Mutation analysis by PCR - CSGE - DNA sequencing**

We would prefer to estimate the sensitivity, or accuracy, of our analyses based on the testing we have performed by using known sequence variations in the genes we are analyzing i.e. state that we can find X % of mutations in a given gene taken that the mutation is there. We feel that this would give the best description of our service i.e. when one orders an analysis, (s)he would know that we can detect a vast majority (>90%, maybe even >95%) of the genetic variations in the gene of interest.

However, this conflicts with the more commonly used disease-based approach where the sensitivity is stated as mutations found in X % of the patients with clinical diagnosis of, for example, achondrogenesis type II. This is more practical to clinical personnel, but from our point of view, it might be somewhat biased, especially with diseases such as mild Osteogenesis Imperfecta or Stickler/Marshall syndrome, that feature varying phenotypes and overlap with other conditions.

To combine these different views, we removed the portion of our report describing the sensitivity/accuracy of the test. Instead, we will now attach this “analysis log” presenting our results. I hope you can find the answer to your question from the tables below. If not, please don’t hesitate to contact the lab at (504) 988-7706 (phone) or 988-7704 (fax) or [ccrain@tulane.edu](mailto:ccrain@tulane.edu).

**Table 1. Testing of previously known nucleotide changes**

GENE	Known changes	Found	Comments
Collagens (COL1A1, -1A2, -2A1, -3A1, -7A1)	86	85	
M13 phage	10	10	
Factor IX	35	31	
<b>TOTAL</b>	<b>131</b>	<b>126 (96%)</b>	

Large deletions/insertions and homozygous mutations are mostly not detected. However, these mutations account for only 2-3% of reported mutations in these genes. We feel that we can easily detect > 90% of the mutations in the sequences analyzed.

**Table 2. Chondrodysplasias (clinical and research)**

Clinical diagnosis	# Studied	Defect Found	Comments
Stickler/Marshall Sdr	62	45 (73%)	COL2A1 and COL11A1 genes analyzed. Patients with no eye findings were excluded
SED/SEMD	36	23 (64%)	Very heterogeneous group
Kniest dysplasia	4	3 (75%)	
Hypo-/achondrogenesis II	23	21 (91%)	Excluded: 2 patients with diagnosis of ACGII, no mutation found. Later, based on histological and/or x-ray evaluation, dx changed to ACG IA and IB, respectively.
Schmid Metaphyseal dysplasia	12	6 (50%)	One inconclusive. The regions of interest both sequenced and scanned for mutations by CSGE

**Table 3. Osteogenesis imperfecta (OI), osteoporosis and related (clinical and research)**

Clinical diagnosis	# Studied	De fect Found	Comments
OI mild – “classic” type IA	47	44 (94%)	multiple fractures, blue sclerae, normal stature – other :hearing loss, easy bruising
OI IB or mild “atypical” OI	15	9 (60%)	very heterogeneous group, variation from one fracture + slightly gr ayish sclerae to multiple fractures with DI. Mutations less likely to be found in the mildest forms.
OI type II	30	26 (87%)	Mutation not found: 3/4 dx by us
OI type III	21	17 (81%)	Mutation not found: 2/3 dx by us
OI type IV	17	13 (76%)	Mutation not found: 1 from consang parents
Osteoporosis or frx w/o OI stigmata	52	2 (4%)	
OI vs abuse	25	2 (8%)	both patients with mutation had, in addition to fractures, other clinical findings related to osteogenesis imperfecta

Assigning some patients to above groups was quite difficult due to inconclusive referral diagnosis (such as OI or rule-out OI), limited amount of clinical data and/or overlapping phenotypes.