

## Matrix DNA Diagnostics - Tulane University

### Patient Consent form - COL2A1 Mutation assay

For Patients with type II collagenopathies

**BACKGROUND** Collagen II is a tough, fibrous protein that provides a major part of the strength of the cartilage. It is also an important component of the vitreous of the eye. Mutations in the gene that code for collagen II (COL2A1) cause either decreased synthesis of the protein or cause synthesis of defective forms of the protein. Defects in collagen II protein often result in structural or growth disorders that are frequently accompanied by eye abnormalities. Collagen II defects have been found in many chondrodysplasias such as achondrogenesis type II, hypochondrogenesis, spondyloepi(meta)physeal dysplasia, Kniest dysplasia and Stickler syndrome, and have been shown to be the cause of the disease in these patients.

DNA test carried out in our laboratory will detect most, but not all, of the mutations that can be found in the collagen II gene and that can cause different chondrodysplasias. The COL2A1 gene is among the more complex of human genes. Therefore, complete analysis of the gene for the presence of mutations requires a large number of careful manipulation and analyses of the DNA. For this reason, completion of the test may take four to six 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 who have mutations in the COL2A1 gene 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 >90% are in fact detected. 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 the vast majority of patients with osteogenesis imperfecta, 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 designate 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 a 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. The gene studied will be determined by the physician and in consultation with the laboratory. Mutations in other genes will not be detected. The rate of mutation detection varies with the disorder and the gene studied. Mutation in non-structural portions of gene will generally not be detected.

**Test results.** The test results are reported to the physician in writing. The test will generally take 4-8 weeks. For prenatal testing, we try to expedite the analysis.

### 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 a 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 4 confluent T25-flasks of 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**. Please send with a cold pack if possible. If blood sample can't be shipped the same day, please store at +4C until shipment. All tubes/flasks must be labeled with patient name and date of birth. Please do not send any samples on Friday. **Patient Information Form** (Form 2), **Payment Information Form** (Form 3) and **signed Patient Consent Form** must 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 upon request.

<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
<p><b>Sample and Paperwork should be sent to:</b> Please call (504) 988-7706 before submitting a sample for the first time.</p>	<p>Matrix DNA Diagnostics            Attn: Dr. Darwin Prockop            Center for Gene Therapy            Tulane University Health Science Center            1430 Tulane Avenue, TB 28            Tidewater 2140            New Orleans, LA 70112</p>



## FORM2 - TEST REQUISITION FORM

Patient name:

Date of Birth:

Hospital Reference Number:

SSN:

Address:

Telephone:

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

Check for the test requested:

COL1A1/COL1A2 gene analysis for Osteogenesis Imperfecta

COL2A1 gene analysis for SED, ACGII, HCG, Kniest dysplasia

COL2A1 /COL11A1 gene analysis for Stickler/Marshall Syndrome

FBN1 gene analysis for Marfan Syndrome

COL10A1 gene analysis for Schmid Metaphyseal dysplasia

Proband name must be provided in box above.

Known Mutation (family member or prenatal) in any of the above

Type of specimen:

Is this a prenatal test? YES NO

Collected by:

Collection date:

Referring Physician Name

Contact person

Telephone

Address

Fax

Request Date

Physician's Authorized Signature

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

**We do accept credit card payments and bank/wire transfers call (504) 988-7706 for details.**

ICD-9 code:	756.5	Tax/Fed ID#:	72-0423889	CLIA#:	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 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 a 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. **WE DO NOT ACCEPT ANY MEDICAID.**

COL2A1 mutation screening for Chondrodysplasias

CPT codes	Description	# Performed	Cost/test	Extended
83890	DNA isolation	1	\$10.00	\$10.00
83894	Electrophoresis	10	\$7.00	\$70.00
83898	Polymerase chain reaction	27	\$10.00	\$270.00
83903	Mutation scanning	46	\$20.00	\$920.00
83904	Nucleic acid probe w/ sequencing	3	\$40.00	\$120.00
<u>83912</u>	<u>Interpretation and report</u>	<u>1</u>	<u>\$10.00</u>	<u>\$10.00</u>
<b>TOTAL COST</b>				<b>\$ 1400.00</b>

\*Please note that COL2A1 mutation screening is a multi-step test which involves screening the entire coding sequence of a very large gene and involves multiple tests to isolate/identify/sequence all suspicious coding regions. 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

## 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 chapter 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:crcrain@tulane.edu). I’ll keep updating the data periodically – most of the numbers are still very small, which might compromise the significance of the data. All comments are greatly appreciated.

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

GENE	Known changes	Found	Comments
Fibrillar collagens (COL1A1, -1A2, -2A1, -3A1)	78	77	
COL7A1	8	8	
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 <u>no eye findings</u> were excluded
SED/SEMD	36	23 (64%)	Very heterogeneous group – it seems that mutations are more common in SED congenita and SEMD than in SED tarda
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 by Cedar-Sinai Med Ctr, dx changed to ACGIA and IB, respectively.

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

Clinical diagnosis	# Studied	Defect 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 grayish 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 without 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.