

Dear Colleague,

We are willing to perform a collagen study in your patients with Osteogenesis Imperfecta or Ehlers Danlos syndrome. For each of these diseases we need a skin fibroblast culture from the proband to perform a biochemical and molecular study of the respective collagens  $\alpha$ -chains/collagen genes, involved.

**Prior to starting the analysis** we need a detailed clinical report from the proband and the family. For patients with OI we appreciate to have a look to the X-rays while for patients with Ehlers-Danlos syndrome a detailed checklist, which you can find in attachment, has to be filled in rigorously. (Please notice there are each checklist consists of several excell sheets.)

Please find below the necessary information concerning prelevation, mailing of the biopsy and the costs of the analysis.

To perform the protein and gene studies, the prelevation of a skin biopsy from the proband is necessary. Where possible, we also welcome blood samples from the (unaffected) parents of the affected proband. The biopsy has to be taken under sterile conditions and be kept either in tissue culture medium or in any other sterile solution. If tissue culture facilities are not available to you, the skin biopsy can be sent directly to us, either in tissue culture or in sterile solution. In this case, it is essential that the biopsy reach the laboratory within 48 hours after prelevation. This means that it certainly has to be sent by special courier. If a tissue culture facility is available in your institute, we prefer you to grow the skin fibroblasts and store a frozen part of the fibroblast culture in case a problem arises with the transport. The remaining material can be sent to us. Ideally, we need two tissue culture flasks (T25 or T80), containing a confluent fibroblast culture. The flasks have to be filled completely with medium to avoid drying out of the cells. If antibiotics other than streptomycine/penicillin are used for the culture, it should be mentioned explicitly.

The culture has to be sent to the Department of Medical Genetics by courier in order to reach the lab as quickly as possible. Please specify the time of sample referral by fax and send them preferably in the beginning of the week. Arrival of the samples will be confirmed by fax to you.

#### **General approach to the biochemical and molecular diagnosis:**

A dermal fibroblast culture is used for a double purpose. Fibroblasts are metabolically labelled with  $^{14}\text{C}$  Proline and studied electrophoretically by SDS-PAGE. Another part of the culture is used for the isolation of mRNA that is converted to cDNA prior to analysis. Genomic DNA is isolated from leukocytes or from cultured fibroblasts, amniocytes or chorionic villi.

Each patient is first investigated at the **biochemical level** by the evaluation of radioactively labelled type I (pro)collagen molecules by SDS-PAGE

Based on the biochemical results, **molecular analysis** is performed:

- Patients with evidence for the presence of a structurally abnormal collagen (type I,III or V) are subjected to a cDNA mutation screening of the respective genes. (COL1A1 and COL1A2 for OI; COL3A1 for vascular EDS and COL5A1 and COL5A2 for classical EDS).
- In patient in which reduction is observed biochemically or with a history of , screening for the presence of a null-allele is the first step in the molecular investigations. If a null allele is present, a genomic mutation screening is performed. In patients with two transcripts, a mutation screening on cDNA is started.
- In patients with a normal collagen profile the clinical history is used to direct further molecular work i.e. in patients with clinically mild OI, the possibility of a COL1A1 null-allele is investigated prior to genomic DNA mutation screening while in patients with a more severe clinical phenotype, a cDNA based mutation analysis is performed. Genomic DNA analysis is performed only for specific indications.

An interval of 4-6 months must be taken into account before the results can be expected. This period is indeed necessary to perform the biochemical and molecular studies.

The cost for the analysis comprise €500 for the biochemical investigations and null-allele testing and €1000 for more extended mutation analysis.

With best regards,

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