

## **Multi-level molecular assessment based on laser microdissection of corneas to reveal biomarkers and therapeutic targets of keratoconus**

Keratoconus (KTCN) is an eye disorder, which results in the change of cornea's shape from spherical to conical and the formation of pathological corneal cone. Patients have impaired vision problems because the affected cornea no longer fulfills its function. KTCN is the common disorder and occurs in all ethnics group with probability of occurrence similar among male and female. This is a complex disease, both environmental and genetic factors influence the appearance of the disorder. The causes of the disease and the chain of events / subsequent pathological changes taking place in its course remain unclear.

The human cornea, despite its small size, consists of many layers. In the light of the development of new laboratory technics, it is necessary to examine these layers separately, not together as so far. For the first time we plan to separate patient's corneal layers to identify molecular changes in those layers. Then we will be able to learn more about the mechanisms of the disease.

We hypothesize that disease-connected morphological changes observed in particular parts of corneal tissue, so as the structure and shape of the cornea in KTCN are correlated with gene expression level in so far unidentified/unconfirmed genes and signaling pathways.

The goal of this project is to identify genes involved in pathologic KTCN cone formation process and disease development based on characteristics of corneal layers and regions of healthy and keratoconus corneas. The purpose will be achieved through detailed morphological examination and molecular characterization of the keratoconus corneal tissue. Such studies are the important step in the direction of improvements in medical diagnostics and treatment schemes. The obtained results will support the increase of knowledge about the KTCN pathogenesis and provide important insight into KTCN etiology.

We will examine corneas from patients, which undergo corneal transplantation due to really advanced state of the disease. Each derived cornea will be cut into four separate layers, as well as we will divide corneas towards designed by us three topographic regions. These regions will be the consecutive circles diverging from the top of the cone, i.e. a place with the most advanced lesions. In this way we may analyze how the gene expression changes in correlation with the disease phenotype taking into consideration proteins and metabolites identified in particular corneal layers and regions. As a necessary control we will also examine healthy corneas derived from a tissue bank. The DNA- and RNA-involving aspects as well as proteome and metabolome profiles will be assessed using laser microdissection, RNA sequencing, whole exome sequencing and techniques for proteome and metabolome assessments. The obtained data will answer the questions whether and in what manner the expression, the DNA and RNA profiles and well as proteome and metabolome profiles, differ between healthy and KCTN. We plan to detect and identify the molecular factors, the biomarkers, which will be useful in medical diagnostics and then reveal the potential therapeutic targets of keratoconus.