Keratoconus (KTCN) is an eye disease characterized by progressive thinning and conical protrusion of the cornea. The structural abnormalities in different layers of corneal tissue result in altered refractive powers, and a loss of visual function. The prevalence of KTCN in the general population is between one in 2,000 and one in 375 individuals. The first symptoms of KTCN usually appear during the second or early in the third decade of life. The management of KTCN depends on the disease stage and includes visual correction with contact lenses, corneal collagen cross-linking (CXL), or corneal transplant surgery. The environmental factors, such as eye rubbing or contact lens wear, influence disease development. However, genetic triggers also play an important role in KTCN.

The human cornea is consisted of five layers: epithelium, Bowman's layer, stroma, Descemet's membrane, and endothelium. Abnormalities in KTCN are observable in each layer of the cornea. Moreover, these alternations are different in particular regions/zones of each layer, which is the most visible in corneal epithelium where a pattern of the *epithelial doughnut* (thin cone center surrounded by thickened annulus) appears.

Numerous genes, *loci,* and sequence variants are postulated as involved in the pathogenesis of KTCN. However, based on our previous research we concluded that a molecular picture of the keratoconic cone is incomplete and should be supplemented with an additional element – comprehensive Whole Genome Methylation profiling.

The are many alternations in the gene activity that do not involve exclusively DNA sequence variation. DNA methylation is an epigenetic mechanism involving the presence of methyl groups at CpG dinucleotides through a modification of the C5 position of the cytosine to form 5-methylcytosine by adding a methyl group. Abnormal increases or decreases in DNA methylation are called hypermethylation or hypomethylation, respectively. High levels of DNA methylation at genes' promoters are generally negatively correlated with gene expression. During development, the profile of DNA methylation changes in respect of de novo DNA methylation and demethylation. Therefore, differentiated cells develop a unique DNA methylation profile regulating tissue-specific gene expression.

Here, we hypothesize that DNA methylation acts as the modulator of gene expression in the particular corneal layers and regions in KTCN corneas. As the correlation between the methylation and the KTCN phenotype remains unclear, a detailed investigation of the three *topographic* regions in the corneal epithelium, stroma, and endothelium will address the questions regarding the KTCN epigenetic indicators and mechanistic aspects of cone formation.

The goal of this project is to find differentially methylated sites in the whole genome sequence, and KTCN epigenetic indicators.

The study will be performed on human corneas, obtained from 22 patients with KTCN undergoing the corneal transplant, and 22 corneas from a tissue bank, derived from deceased control individuals. The laser capture microdissection will be implemented to identify and separate corneal layers and *topographic* regions. Assessment of methylome will be performed by Whole Genome Enzymatic Methyl-Seq. The bioinformatic analyses will be conducted in direction of the selection of differentially methylated sites, especially those located in CpG islands, promoters, UTRs, and enhancers. Additional integrative analyses will include collected transcriptomic (RNA-Seq, Spatial transcriptomics), proteomic (MALDI-MS), genomic (WGS, ES), and other epigenomic (ATAC-Seq) data derived from the same biological material. The results will be first verified using pyrosequencing, and functionally, using the dCas9 CRISPR epigenomic editing system.

The study will enable an understanding of the role of DNA methylation in KTCN pathogenesis. The comprehensive study design with a multi-omic approach, including the data integration analyses, enables the identification of the epigenetic indicators of KTCN.