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.

Numerous genes, *loci*, and sequence variants are postulated as involved in the pathogenesis of KTCN. Since none of these genes is responsible for KTCN in the general population, we hypothesize that additional elements, especially those mapped within the regulatory regions controlling gene function, might influence the disease phenotype. These elements include various types of sequence variation. The goal of this project is to assess the potential involvement of changes in chromatin accessibility and novel sequence variants in the regulatory regions in KTCN pathogenesis.

The study will be performed in human corneas, obtained from 20 patients with KTCN undergoing the corneal transplant, and 20 control corneas from a tissue bank, derived from deceased control individuals. Tissues will be morphologically assessed to identify altered corneal regions. Cell nuclei, DNA, and corneal cryosections (frozen tissue sections) will be prepared to perform genome-wide analyses of the human genome (DNA), transcriptome (RNA), proteome (proteins), and nucleosome availability (chromatin) to define the role of not yet investigated elements using state-of-the-art methods in molecular biology: transposase-accessible chromatin with sequencing (ATAC-Seq), whole-genome sequencing (WGS), spatial transcriptomics, and proteome assays.

Using ATAC-seq we will detect open chromatin regions, to which transcription factors can bind and regulate gene expression, in different topographic regions of the cornea. WGS will allow to identify KTCN-specific sequence variation. Using the spatial transcriptomics, we will point mainly to the differential expression of genes and their interactions in molecular pathways. Chosen variants, detected based on all mentioned procedures, will be further analyzed using the reporter assay analyses that allow to characterize their potential function in the genome.

The study will enable an understanding of the role of genome-wide genetic variation, with an emphasis on variability within regulatory elements, to further elucidate factors influencing the pathogenesis of KTCN. The project outcomes may inform the future treatment strategies in KTCN.