The role of DNA methylation in etiology of high myopia in Polish children

Research project objectives:

High myopia (HM), an eye disorder with a refractive error (RE) of -6.0 dioptres [D] or higher and axial length (AL) min 26.0 mm, is a major cause of blindness in the developed countries. HM has a multifactorial etiology, including genetic and environmental factors as near work, artificial light exposure, lack of physical activity outdoors, and a higher level of education. Thus far, number of candidate genes and sequence variants specific to populations and even families has been identified.

DNA methylation is an epigenetic process regulating the gene expression without changing the DNA sequence. The change in DNA methylation level might be the link between environmental factors and HM.

The project is an extension of our long-term studies in the field of genetic and epigenetic causes of HM. Recently, we performed the genome-wide methylation analysis on DNA of Polish children with HM and without HM as control group. The proposed project is a continuation of the research and PI's PhD project titled 'Characteristic of chosen (epi)-genetic aspects in high myopia in Polish patients'. PI performed analyses of the results of DNA methylation in whole genome and selected CpG dinucleotides with altered methylation, in HM children when compared to controls, for the analyses of the proposed project. Those are CpG dinucleotides in *GSTM1*, *PPP1R18*, *XRCC2*, *OXA1L*, *FARP2*, *ABHD13*, *SORBS2*, *SLC25A3P1*, *TANC1*, and *ATXN1* genes. **Preliminary results suggest that changes in methylation of these genes might cause changes in their expression level and this way contribute to HM phenotype in Polish children.** To test that hypothesis we plan to validate the results of DNA methylation of whole genome by the use of another technique (Aim 1); analyse the expression level of genes with altered methylation (Aim 2); assess the methylation and expression profile in ocular cell lines (Aim 3); and search for the sequence variants in differentially methylated genes (Aim 4).

Research project methodology:

Material and nucleic acids isolation: The study will be performed on DNA of 18 Polish children with HM (aged 4–12 years, RE −6.0 - −15.0 D in at least one eye, AL 26.22 - 27.85 mm), and 18 Polish children without HM (same age and gender) used previously in genome-wide DNA methylation analyses. DNA and RNA samples will be also isolated from newly recruited group of Polish children and commercially available cell lines derived from retinal cells.

Aim 1: Validation of the genome-wide methylation results using an alternative technique:
The methylation of selected gene fragments will be checked by another method in DNA of Polish children.

Aim 2: Expression analyses of differentially methylated genes:
The experiment is to check the relation of the methylation and expression of chosen genes.

Aim 3: Functional studies on retinal cell lines - assessment of methylation and expression profile:
The methylation and expression profile of the studied genes in the retinal cell lines will be determined, and compared with the results obtained from Polish children. In order to investigate if the differential methylation of studied genes influences the expression profile of the retinal cells, DNA demethylation will be performed.

Aim 4: Screening of sequence variants in differentially methylated genes:
Sequencing will be perform to check if the methylation itself is responsible for gene expression changes in HM or in combination with pathogenic sequence variants of the studied genes.

The impact of the project results:

The expected result of the project is a confirmation that differential methylation of studied genes is a cause of expression changes in human blood and retinal cells and this could contribute to HM in Polish children. Our methylation studies in HM are the only conducted in Polish population so the results will be of interest for ophthalmologists, researchers and students of medical and biological fields. Identification of new HM causes is a step towards a better quality of the molecular diagnostics, and potential treatment.