

The mechanism of puberty and the processes influencing its genetic basis in humans are still not fully explored. In view of technological progress and new possibilities for detecting changes in the human genome, a wide and comprehensive genetic analysis is planned in patients with isolated hypogonadotropic hypogonadism (IHH) to search for new genes containing mutations that underlie IHH. The genetic basis of more than 50% of the IHH is still unknown.

The overall aim of this project is to identify yet undiscovered genetic factors underlying organogenesis in the hypothalamus, pituitary gland and olfactory bulb, which are responsible for the development of isolated central hypogonadism. It is also planned to estimate the prevalence of causal mutations in the coding regions of genes involved in the pathogenesis of IHH in the study population and to define the so-called mutational sites "hot-spot" or potential defects characterized by an increased incidence in the population.

The molecular methods to be used, are currently considered to be the most modern and powerful tools for: (1) the study of the human genome and (2) the search for new genes involved in the pathogenesis of certain diseases. Such an analysis has not been performed in patients with IHH in the Polish population.

In the first stage of the study, we will be searching for mutations in known IHH genes using the *high-throughput* new generation sequencing platform (NGS), Benchtop Sequencer, owned by the Department of Endocrinology, Metabolism and Internal Medicine Laboratory in Poznań and a dedicated panel of known genes in patients with IHH. Innovative technology sequencing gives the ability to detect potentially pathogenic rare variants / mutations that were completely overlooked in associative studies, and enables the study of genetic variants associated with changes in the protein architecture. This applies particularly to small insertion/deletion (so-called. INDELs) associated with a frameshift and a significant change in protein composition.

Analysis of the resulting sequences will be performed using dedicated programs (e.g. IONReporter) and public bioinformatics tools (Ensembl, NCBI). Other genetic analysis will make use of dedicated software or other commonly used methods in published studies of this type. In silico analysis of the pathogenicity of detected variants will be implemented through software PolyPHEN and Sift (coding sequences) SpliceSite (intron sequences) or any other published tools designed for this purpose. Reference sequences of DNA, RNA and proteins, for comparison will be obtained from genetic public databases, e.g., GenBank, or sets of genomic HGMD.

This project aims to determine the importance of newly discovered and previously known genetic factors in the etiology of IHH in the study population. We hope that the use of the latest technologies and tools such as next-generation sequencing (NGS) for the genome study, will significantly expand our knowledge of neuroendocrinology and genetically conditioned ground IHH and will shed new light on congenital anomalies associated with the hypothalamic pituitary-gonadal axis.