Disorders of sex development, although they are not very common, constitute a serious problem for affected individuals. They consist of the appearance of incompatibilities between the patient's karyotype and phenotype. Disorder of male sexual differentiation may be due to Leydig cell hypoplasia - a rare disease entity with the occurrence estimated to be around 1:1,000,000 men. It is caused, inter alia, by inactivating mutations in the gene encoding the receptor for luteinizing hormone/human chorionic gonadotrophin (LHCGR). They result in partial or complete loss of receptor function. Leydig cell hypoplasia is characterized by a broad spectrum of symptoms that include the presence of female genitalia, hypospadias, cryptorchidism, lack of puberty and infertility. Depending on the degree of LHCGR receptor inactivation, we distinguish two types of Leydig cell hypoplasia - type 1 is used for completely inactivating mutations, while type 2 refers to partially inactivating mutations.

Nevertheless, mutations in the *LHCGR* gene may not only be inactivating but also activating. Activating mutations result in constitutive receptor activation. Under physiological conditions, receptor activation occurs due to stimulation of the receptor with a suitable ligand (i.e LH or hCG). In the case of constitutively-active mutations, the receptor remains active at all times which results in excessive activation of signalling pathways and thus in excessive production of steroids.

The main assumption of this project is to conduct a series of assays to characterize three novel mutations identified in patients. The first two mutations were discovered in a heterozygous male patient with female external genital organs and a female body structure, who was directed to endocrine clinics due to delayed sexual maturation. Based on the phenotype observed in this patient and the results of preliminary studies, we have formulated a hypothesis that mutations in both alleles of the *LHCGR* gene are of inactivating nature. The third mutation was identified in a female patient with severe hyperandrogenism resulting in disorder of ovulation, secondary amenorrhea and infertility. In this case, the mutation seems to be an activating mutation. In addition, sequencing results revealed that the patient was also heterozygous, but she was carrying one wild type allele and the second with mutation. Interestingly, this is the only activating mutation in the *LHCGR* gene found in a female patient so far. Because mutations have been identified in patients presenting extremely rare clinical symptoms, it is necessary to examine their impact on the functionality of the LHCGR receptor. The first part of the project will focus on the molecular characteristics of inactivating mutations, while the second part will focus around the activating mutation.

Our work on the LHCGR is important to understand the structure function of this receptor, which belongs to a very large family of receptors called G protein-coupled receptors (GPCRs). GPCRs are the largest family of receptors in humans and one of the main targets of commercial therapeutics. Thus, understanding the biology of very unusual receptors, and their functions, is important for us to understand human physiology and reproduction.