A novel mutation of the luteinizing hormone/chorionic gonadotrophin receptor gene leading to Leydig cell hypoplasia type 1

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Introduction: Proper functioning of the luteinizing hormone/chorionic gonadotrophin receptor (LHCGR) is essential for male sexual differentiation and maintenance of normal testosterone levels, as well as for ovarian function and fertility in females. Completely inactivating mutations of the LHCGR gene in 46,XY individuals lead to Leydig cell hypoplasia type 1, characterized by a female phenotype without signs of pubertal development, presence of testicular intra-abdominal remnants and absence of Mullerian duct derivatives. Due to their important role in reproduction, inactivating mutations of the LHCGR gene are very rarely encountered in clinical settings.

Aim of the work: To study a case of male pseudohermaphroditism caused by a novel LHCGR mutation and explain the mechanism by which this mutation leads to Leydig cell hypoplasia type 1.

Methods: Sequencing of the LHCGR gene was done. Functional studies were performed after transfection of HEK293 cells with the mutant and wild-type (WT) LHCGR gene. Generation of cAMP was measured under stimulation by agonists. This was also performed after preincubation with an allosteric LHCGR agonist known to facilitate proper folding of misfolded LHCGR mutants. Membrane localization of the mutant receptor was analyzed by flow cytometry and immunocytochemistry. Also, the capacity of the mutant receptor to be activated in the endoplasmic reticulum by co-expression of hCG was tested.

Results: A novel mutation of the LHCGR gene (1850delG) was identified in a homozygous state. The deletion causes a frame-shift mutation and a prematurely truncated protein.
cAMP generation was significantly reduced for the mutant receptor compared to WT. Also, despite preincubation with the allosteric agonist, cAMP generation did not improve in the 1850delG cells. Flow cytometry and immunocytochemistry revealed that the 1850delG mutant receptor was not found on the cell surface, unlike WT LHCGR. However, the mutant receptor was detected in permeabilised cells. Co-transfection of culture cells with the LHCGR and hCG genes did not lead to cAMP generation in the 1850delG cells.

Conclusions: We present a novel LHCGR inactivating mutation in a patient with female phenotype, but 46,XY karyotype. This is one of few frame-shift LHCGR mutations described. The functional studies found that the mutant receptor cannot reach the cell membrane and is incapable of signaling, most likely due to its profoundly altered tertiary structure.