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

Iulia Potorac[1], Adolfo Rivero-Müller [2,3,4], Axelle Pintiaux[1,6] , Adrian Daly[1], Albert Thiry[7], Catherine Rydlewski[8], Michelle Nisolle[6], Anne-Simone Parent[1,9], Ilpo Huhtaniemi [2,5], Albert Beckers [1]

[1] Department of Endocrinology, CHU de Liège, Université de Liège, Domaine Universitaire du Sart-Tilman, 4000 Liège, Belgium

[2] Department of Physiology, Institute for Biomedicine, University of Turku, Turku, Finland

[3] Faculty of Natural Sciences and Technology, Åbo Akademi University, Turku, Finland

[4] Department of Biochemistry and Molecular Biology, Medical University of Lublin, 20-093, Lublin, Poland

[5] Department of Surgery and Cancer, Institute of Reproductive and Developmental Biology, Hammersmith Campus, Imperial College London, London, United Kingdom

[6] Department of Gynecology, CHU de Liège, Université de Liège, Domaine Universitaire du Sart-Tilman, 4000 Liège, Belgium

[7] Department of Anatomopathology, CHU de Liège, Université de Liège, Domaine Universitaire du Sart-Tilman, 4000 Liège, Belgium

[8] Department of Medical Genetics, Erasme Hospital, Brussels, Belgium

[9] Department of Pediatrics, CHU de Liège, Université de Liège, Domaine Universitaire du Sart-Tilman, 4000 Liège, Belgium

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.