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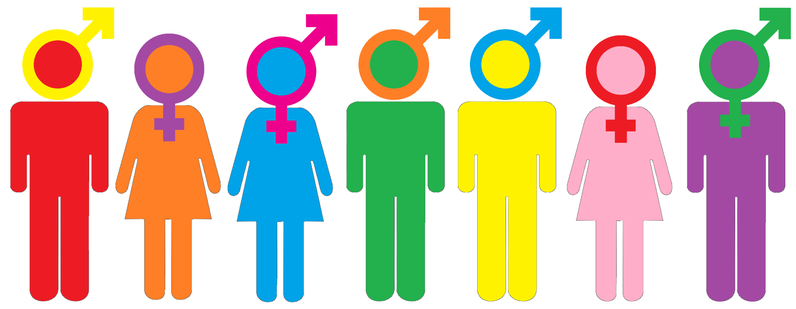

Functional studies of a new mutation in the LH/CG receptor gene identified in 2 sisters with 46,XY DSD

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Introduction and Objectives

Disorders (or Differences) of **S**ex **D**evelopment (DSD) are rare congenital conditions in which the development of chromosomal, gonadal, or anatomical sex is atypical.

The luteinizing hormone/chorionic gonadotropin receptor (*LHCGR*) is important for male sex development. Autosomal recessive mutations in *LHCGR* lead to a disturbance of the hypothalamic-pituitary-testicular axis (HPTA) and disruption of testosterone synthesis. The appearance ranges from male, an ambiguous to a completely female sex.

We found two compound heterozygous mutations in the *LHCGR* via exome sequencing, a new p.F138S mutation in combination with the previously described c.580A>G mutation in exon 6A (Kossack et al. 2008) in two sisters with 46,XY DSD and complete inconspicuous female appearance. Deleterious effect of the p.F138S mutation was assessed by functional analysis.

Methods

Allelic distribution of mutations was determined by cloning and sequencing of long range PCR fragments containing exon 5 to cryptic exon 6A.

Expression vectors containing LHCGR mutation were generated for functional assays. Cyclic AMP production of the LHCGR mutation p.F138S was analyzed by direct (Radioimmunoassay - RIA) and indirect (cAMP-responsive element containing reporter genes - pCRE-Luc and cAMP binding luciferases - GloSensor) cAMP measurements.

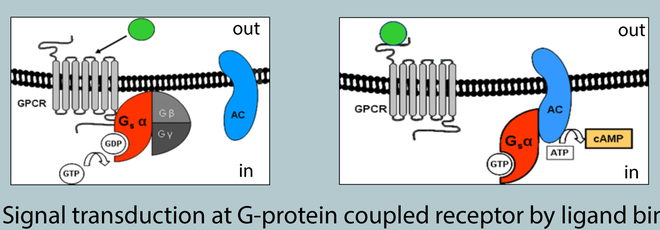

Fig. 1: Signal transduction at G-protein coupled receptor by ligand binding
GPCR G-protein coupled receptor
AC adenylate cyclase
cAMP cyclic adenosine-3',5'-monophosphate
ATP adenosine triphosphate

Intracellular localization of the receptor was analyzed by immunoimaging. The antibody binds to the N-terminal part of the receptor. If the cells aren't permeabilized only the receptor on the outside of the plasma membrane will be detected. Glycosylation was studied by glycosidase F treatment and immunoblot.

References

Kossack, N., et al. (2008). "Mutations in a novel, cryptic exon of the luteinizing hormone/chorionic gonadotropin receptor gene cause male pseudohermaphroditism." PLoS Med **5**(4): e88.

Results and Conclusion

Complete loss of function of the p.F138S mutation was demonstrated by three different cAMP assays (Fig.2) Immunoimaging showed that the mutant receptor is expressed internally, but did not reach the membrane surface. (Fig.3)

Treatment with glycosidase F and subsequent immunoblot revealed an incomplete glycosylation of the receptor. (Fig.4) Compound heterozygosity was proven by long range PCR and subcloning of the fragment containing both mutants. (Tab. 1)

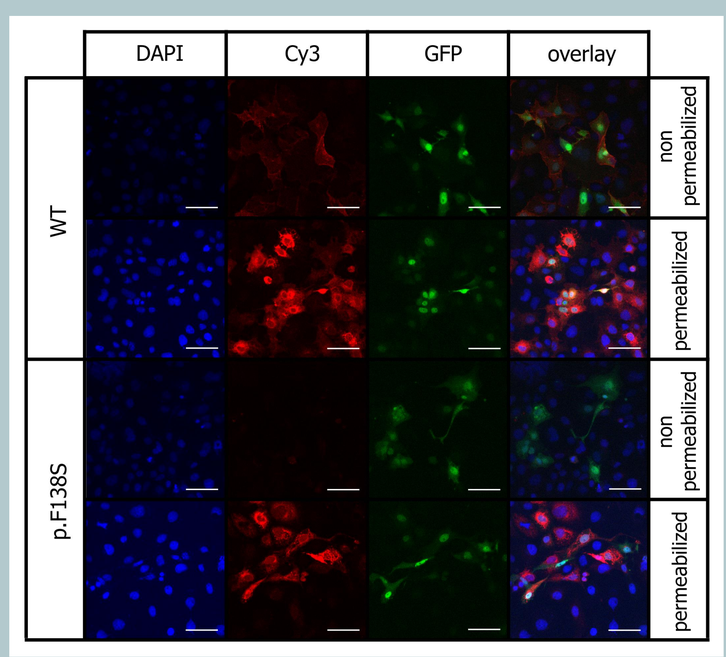

Fig. 3: Immunoimaging of LHCGR (red) and GFP (green) expressed from pTracer in COS7 cells.
Staining of non permeabilized cells detect only the HA tagged N-terminal part of LHCGR at the surface of the plasma membrane.
In permeabilized cells also the internal localized LHCGR were detected.
GFP is produced by an human elongation factor 1 (EF-1α) promotor from the same plasmid.
DAPI (blue) nucleus of all cells, Cy3 (red) LHCGR, GFP (green) all transfected cells, bar indicated 50 µM

Tab. 1.: Long range PCR, subcloning and sequencing revealed a compound heterozygous status of the F138S and c.580A>G mutation.

K1	F138 (WT)	c.580A>G	c.599T>C, rs4637173	A>G, rs4490239	rs68073206 WT (A)
K2	F138 (WT)	c.580A>G	c.599T>C, rs4637173	A>G, rs4490239	rs68073206 WT (A)
K4	S138 (Mut)	c.580A, WT	c.599T, WT rs4637173	rs4490239 WT (A)	rs68073206 A>C
K7	S138 (Mut)	c.580A, WT	c.599T, WT rs4637173	rs4490239 WT (A)	rs68073206 A>C

Chromosomal Position GRCh37/hg19 Assembly and frequency of identified variants:
p.F138S = Chr2:48,950,806 (not in ExAc Browser, coverage ~121000 exomes;
c.580A>G = Chr2:48,948,875 (not in ExAc Browser, coverage ~16580 exomes);
rs4637173 = Chr2:48,948,856 (ExAc MAF= 0.205)
rs4490239 = Chr2:48,948,802 (ExAc MAF=0.2026);
rs68073206 = Chr2:48,948,707 (1000g MAF=0.2658)

The mutation p.F138S in Exon 5 leads to a loss of function of LHCGR. Together with the second previously described mutation in cryptic exon 6A these compound heterozygous mutations explain the autosomal recessive disorder. The functional data fully support the observed clinical phenotype. This example shows that next to the chromosomes the hormones take an important influence for the sex development. In addition these patients clarified the important role of the cryptic exon 6a for genetic analyses.

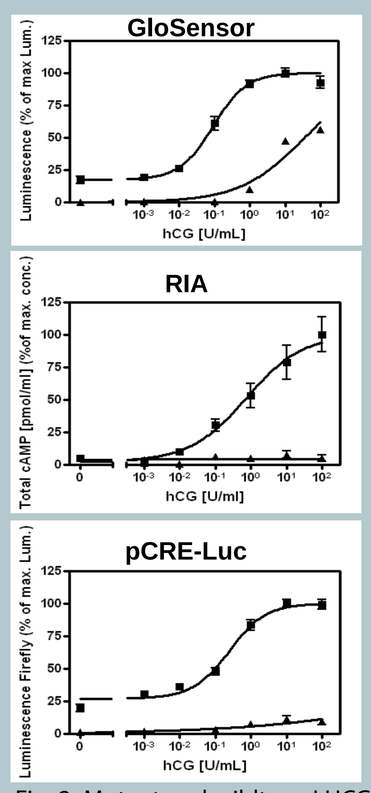

Fig. 2: Mutant and wildtype LHCGR were expressed for 24h in COS7 cells. The production of cAMP was stimulated by different concentrations of human chorion gonadotropin. The resulting cAMP levels were measured directly (RIA) and indirectly (GloSensor and pCRE-Luc).
■wildtype, ▲mutation p.F138S

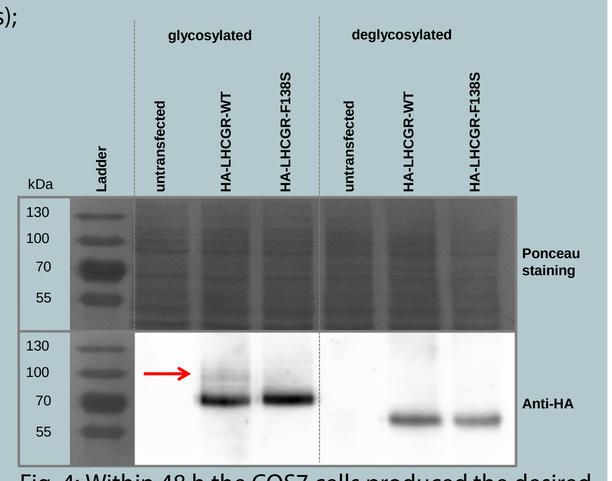

Fig. 4: Within 48 h the COS7 cells produced the desired proteins. Then the cells were lysated and the protein treated with N-glycosidase F. Both treated and untreated protein were separated by size, transferred on membrane and visualize by antibody staining. The red arrow shows the complete glycosylation of the wildtype.

Gonads & DSD

The authors have nothing to disclose.

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