**Introduction and Objectives**

Disorders (or Differences) of Sex Development (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 incontinent 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 exons 6A. Expression vectors containing LHCGR mutation were generated for functional assays. Cyclic AMP production of the LHGRG 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.

Intracellular localization of the receptor was analyzed by immunofluorometry. 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 treatment and immunoblots.

**References**


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**Results and Conclusion**

Complete loss of function of the p.F138S mutation was demonstrated by three different cAMP assays (Fig. 2). Immunoimagining 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)

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

<table>
<thead>
<tr>
<th>Chromosomal Position</th>
<th>Accession number</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>p.F138S</td>
<td>c.580A&gt;G</td>
<td>K1</td>
</tr>
<tr>
<td>c.5997C</td>
<td>A&gt;G</td>
<td>K2</td>
</tr>
<tr>
<td>c.6373T</td>
<td>A&gt;G</td>
<td>K3</td>
</tr>
<tr>
<td>c.6373T, c.580A&gt;G</td>
<td>A&gt;G</td>
<td>K4</td>
</tr>
<tr>
<td>c.5997C, c.6373T</td>
<td>A&gt;G</td>
<td>K5</td>
</tr>
</tbody>
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The mutation p.F138S in Exon 5 leads to a loss of function of LHGRG. 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.

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**Gonads & DSD**

The authors have nothing to disclose.