Mutations at the SF-1 ligand-binding domain can lead to different effects on DNA binding: report of two novel mutations

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Introduction:
Steroidogenic factor-1 (SF-1), encoded by NR5A1 gene, is a key regulator of steroidogenesis and reproductive development1. NR5A1 mutations found in 46,XY patients with disorder of sex development (DSD), are associated with a range of conditions within the spectrum of phenotypes, including testicular dysgenesis, hypospadias and male infertility2.

Methods:
Two mutations (p.C247T and p.K396Rfs*34), identified in the SF-1 ligand domain (LBD) have been analyzed to estimate their functional influence on SF-1 transcriptional activity. Luciferase reporter gene expression was reduced for both p.C247T and p.K396Rfs*34 when tested on AHAH and STAR promoters. Whereas the transcription activity for p.K396Rfs*34 was completely null, p.C247T retained a very low activity. Western blot showed that normal and mutant proteins were expressed in similar amounts. EMSA was also performed to analyze if those mutations would disturb SF-1 DNA binding ability. Results showed that the mutation p.K396Rfs*34 abolished the ability to bind DNA, whereas the formation of a protein-DNA complex was still observed for p.C247T. Clinical data of the patients are detailed in Table 1.

Results:

Table 1: Clinical data of the patients with NR5A1 mutations.

<table>
<thead>
<tr>
<th>NR5A1 gene mutation</th>
<th>Karyotype/ Aminated sex</th>
<th>Age at first visit</th>
<th>Urethral opening</th>
<th>Gonadal location (R/L)</th>
<th>Basal gonadotropins</th>
<th>Basal steroidogenesis</th>
<th>Response to hCG</th>
<th>Gonadal histology (R/L)</th>
<th>Family</th>
</tr>
</thead>
<tbody>
<tr>
<td>p.C247T</td>
<td>46,XY/Female to male</td>
<td>20 years</td>
<td>Penducal</td>
<td>IC / LSF</td>
<td>Elevated FSH</td>
<td>Normal</td>
<td>No response</td>
<td>Dysraphic, testes bilaterally</td>
<td>M-N</td>
</tr>
<tr>
<td>p.K396Rfs*34</td>
<td>46,XY/Male</td>
<td>7.8 years</td>
<td>Penducal</td>
<td>LSF / LSF</td>
<td>Prepubertal</td>
<td>Prepubertal</td>
<td>No response</td>
<td>N/A</td>
<td>N/P</td>
</tr>
</tbody>
</table>

References:

Conclusion:
It is already known that, mutations at SF-1 LBD, may result in variable effects depending on their location and alterations in the ligand specificity/recognition4. This was also observed here, once both mutations localized in the LBD had completely different effects on DNA binding. However, both patients present partial gonadal dysgenesis, suggesting that the genotype-phenotype correlation, especially for mutations within the LBD, remains elusive. SF-1 function/ regulation is very complex and must be increasingly studied, mainly because the number of different phenotypes correlated with mutations on this gene has been constantly increased.

DISCLOSURE STATEMENT: The authors have nothing to disclose.

Fig. 1: Structure of human SF-1 is characterized by a DNA-binding domain (DBD) containing two zinc fingers, an accessory hinge region, a ligand-binding domain (LBD), and two functional activation domains, AF-1 and AF-2, located at the hinge region and at the C-terminal in LBD, respectively.

Fig. 2: A) Part of electrophoregram sequences showing the mutations c.T41C-A in exon 5 and c.T187delA within exon 7. B) Transactivation of AHAH and STAR Promoters in HeLa cells, showing no activity of the mutant p.K396Rfs*34 and low activity of p.C247T. C) Expression levels of SF-1 protein in the Western Blot. D) EMSA assays revealed that only the SF-1 mutant p.C247T was able to bind to an SF-1 specific DNA sequence, whereas the p.K396Rfs*34, lost this ability.