Structural analysis of the impact of a novel androgen receptor gene mutation in two unrelated adult patients with mild androgen insensitivity syndrome

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Context
Androgen insensitivity syndrome (AIS)
- Rare X-linked recessive disorder
- Caused by androgen receptor (AR) gene mutations
- Spectrum of androgen dysfunction
  - gynecomastia and/or infertility in mild AIS (MAIS)
- Ambiguous or undermasculinized genitalia in partial AIS
- Complete testicular feminization in complete AIS
- More than 800 different mutations in the AR gene identified

Objective
- To report a novel mutation in the AR gene associated with MAIS in two unrelated adult patients presenting for infertility and a decrease in physical athletic performance.
- To characterize the functional impact of this mutation using 3D modeling studies

Patients and Methods
- **Patient 1** was referred at the age of 38 years for infertility.
  - He had gynecomastia, bilateral testicular hypotrophy and mild gynecomastia.
  - His semen analysis showed oligoasthenoteratospermia.
  - Lab tests revealed decreased testosterone levels, increased FSH, and decreased androgen sensitivity index (ASI) suggesting AIS.
  - The couple underwent successful in vitro fertilization and intracytoplasmic sperm injection resulting in a twin pregnancy.
- **Patient 2** was referred at the age of 45 years for evaluation of a fatigue and a decrease in physical athletic performance.
  - He had a history of gynecomastia, surgically treated during adolescence but normal external genitalia.
  - He also presented with oligoasthenoteratospermia, decreased testosterone plasma levels and an increased ASI.
  - Despite his impaired semen analysis, he fathered two children without assisted reproductive technology.
  - Because of his persistent fatigue, the patient was offered a trial of high dose dlydrotestosterone therapy which improved his symptoms and his quality of life.
- Family history for infertility or gynecomastia was negative in both patients.
- AR gene analysis was performed from peripheral blood by Sanger sequencing.
- Structural analysis and molecular 3D modeling of the mutated AR was performed

Hormone levels in the two patients with MAIS

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Normal range for men</th>
<th>Patient 1</th>
<th>Patient 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal FSH (IU/L)</td>
<td>1.5-12.4</td>
<td>7.6</td>
<td>1.6</td>
</tr>
<tr>
<td>Basal LH (IU/L)</td>
<td>1.7-8.6</td>
<td>14.6</td>
<td>7.2</td>
</tr>
<tr>
<td>Total Testosterone (nmol/L)</td>
<td>11.8-34.5</td>
<td>50</td>
<td>38.5</td>
</tr>
<tr>
<td>SHBG (nmol/L)</td>
<td>10-50</td>
<td>59</td>
<td>122.7</td>
</tr>
<tr>
<td>Inhibine B</td>
<td>80-270</td>
<td>92</td>
<td>NA</td>
</tr>
<tr>
<td>AMH (ng/mL)</td>
<td>2.13</td>
<td>5.8</td>
<td>NA</td>
</tr>
<tr>
<td>Estradiol (pM/L)</td>
<td>36-220</td>
<td>132</td>
<td>139</td>
</tr>
<tr>
<td>ASI (IU x nmol/L)</td>
<td>6.7-138.7</td>
<td>730</td>
<td>477</td>
</tr>
</tbody>
</table>

Semen analysis results in the two patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>WHO 2010 criteria</th>
<th>Patient 1</th>
<th>Patient 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Semen volume</td>
<td>&gt;1.5 ml</td>
<td>10</td>
<td>2.5</td>
</tr>
<tr>
<td>Total sperm count</td>
<td>&gt;39 million</td>
<td>5.2</td>
<td>22</td>
</tr>
<tr>
<td>Sperm concentration</td>
<td>&gt;15 million/ml</td>
<td>0.52</td>
<td>8.8</td>
</tr>
<tr>
<td>Total Motility</td>
<td>&gt;40 %</td>
<td>35</td>
<td>20</td>
</tr>
<tr>
<td>Progressive Motility</td>
<td>&gt;32 %</td>
<td>5</td>
<td>NA</td>
</tr>
<tr>
<td>Vitality</td>
<td>&gt;50 % of vital sperm</td>
<td>48</td>
<td>NA</td>
</tr>
<tr>
<td>Sperm Morphology</td>
<td>&gt;4 % of normal sperm</td>
<td>36</td>
<td>12</td>
</tr>
</tbody>
</table>

AR gene sequencing in the two patients revealed a common novel missense mutation, Ala699Thr, in exon 4 within the ligand binding domain

Conclusions
- The structural modifications induced by the p.Ala699Thr mutation very likely account for the mild androgen insensitivity syndrome seen in these two patients.
- Complementary functional studies will be required to confirm the pathogenicity of this mutation.
- This study highlights the usefulness of structural studies in providing a greater understanding of the functional consequences of a mutation and expands the database of AR gene mutations.
- The proper diagnosis of adult patients with MAIS may be helpful for the adequate counseling of infertile male patient undergoing assisted reproductive techniques.

The authors declare no conflict of interest