Next-generation Sequencing (NGS) as a Rapid Molecular Diagnosis in Patients with 46,XY Disorder of Sex Development (DSD)

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Conflict of interest: None

Introduction:

- 46,XY DSD occurs as a result of testicular developmental disorders, defect in androgen synthesis or action.
- It is of critical importance to make a fast and accurate diagnosis in terms of sex determination and management of patients.
- The diagnosis of DSD is quite costly and it takes a considerable amount of time due to lengthy hormonal and genetic analysis.

Aim: The use of targeted Next-generation sequencing of all known genes associated with 46 XY DSD for a fast molecular genetic diagnosis in patients in whom underlying defect of DSD was not previously diagnosed.

Materials and Methods:

- 20 pediatric patients with 46,XY DSD were recruited which suspected testicular developmental disorders and defect in androgen synthesis.
- Androgen receptor (AR) and 5-alpha reductase (SRD5A2) gene mutations were excluded by Sanger sequencing.
- The forty six genes that have been shown to be related to 46,XY DSD were sequenced by Illumina MiSeq Next Generation Sequencing System and the Illumina TruSight "Exome Kit.

Results:

- The parents of 14 (66.7%) cases were consanguineous.
- Nine (45%) mutations in 4 different genes were identified in 20 patients (Figure 1)
- Six mutations found in unrelated individuals were novel.
- Mutations in the HSD17B3 gene were observed in 6 patients (30%).
- Table 1 shows clinical and molecular characteristics of patients.

![Figure 1. Nine (45%) mutations were found in 20 patients with DSD by NGS](image)

Table 1: Clinical and molecular characteristics of the identified variants in the study

<table>
<thead>
<tr>
<th>Patient/age (years)</th>
<th>Assigned sex</th>
<th>Gene</th>
<th>Transcript ID</th>
<th>cDNA</th>
<th>Protein</th>
<th>Mutation Type</th>
<th>MT</th>
<th>Polyphen2 score</th>
<th>SIFT</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>4/0.6</td>
<td>M</td>
<td>SRY</td>
<td>NM_003140.2</td>
<td>c.535C&gt;T/c.535C&gt;T</td>
<td>p.R178X/p.R178X</td>
<td>NS/NS</td>
<td>DC</td>
<td>NA</td>
<td>NA</td>
<td>Novel</td>
</tr>
<tr>
<td>6/7</td>
<td>M</td>
<td>WT1</td>
<td>NM_024426.4</td>
<td>c.1187C&gt;T/wt</td>
<td>p.P396R/wt</td>
<td>M</td>
<td>DC</td>
<td>PD</td>
<td>D</td>
<td>Novel</td>
</tr>
<tr>
<td>8/18</td>
<td>F</td>
<td>HSD17B3</td>
<td>NM_000197.1</td>
<td>c.524G&gt;C/c.524G&gt;C</td>
<td>p.R175T/p.R175T</td>
<td>M/M</td>
<td>DC</td>
<td>PD</td>
<td>D</td>
<td>Novel</td>
</tr>
<tr>
<td>9/7</td>
<td>F</td>
<td>HSD17B3</td>
<td>NM_000197.1</td>
<td>c.239G&gt;A/c.239G&gt;A</td>
<td>p.R80Q/p.R80Q</td>
<td>M/M</td>
<td>DC</td>
<td>PD</td>
<td>D</td>
<td>Known</td>
</tr>
</tbody>
</table>

M: Missense, NS: Nonsense, MT: MutationTaster, DC: Disease causing, PD: Probably Damaging, D: Damaging, T: Tolerated, NA: Not available, wt: Wild Type

Conclusions:

- Targeted next-generation sequencing is an efficient, rapid and cost-effective technique for the mutation detection in genetically heterogeneous diseases such as 46,XYDSD.
- HSD17B3 gene mutations may be one of the most common causes of 46,XY DSD in societies having high rate of consanguineous marriages.
- To identify the genetic etiology of 46, XY DSD in individuals without any mutation, whole exome sequencing would be useful.