Background

DSDs (conditions with atypical development of chromosomal, gonadal or anatomic sex) are classified into 3 groups: sex chromosome DSD, 46,XY DSD and 46,XX DSD. Around 1 newborn in 5000 presents ambiguous genitalia with a major challenge for male or female assignment. The identification of a genetic cause can contribute to a correct diagnosis and to optimize both management and genetic counselling (1).

Objective and hypotheses

To describe the results of the diagnostic activity on a large cohort of cases (chromosomal DSD excluded), mostly (80%) from the Nord-Est Italian Regions referring to our centre in the period 1991-2016.

Methods

Genomic DNA of patient and parents was isolated from peripheral blood leukocytes by differential lysis, recently by the MagNA Pure system (Roche) and QiAmp DNA Blood Mini Kit. The coding exons and relative flanking regions of the genes listed in Tab 1 were analysed by Sanger sequencing initially with the CEQ8800 sequencer system (Beckman-Coulter) then with the ABI PRISM 3730. The search for CNVs in candidate genes/regions was performed by MLPA analysis using the following SALSA MLPA kits (MRD-Holland): P185-Intersor (NR0B1, WNT4, SOX9, NR5A1, SRY) P134-1 A1 Gonadal Development Disorder (SRSR2A, HSD17B3, DMRT1, CYP17A1) for gonadal dysgenesis 46,XY and 46,XX and for defects of androgen synthesis/ action; P050-C1 (AR); for CYP11B1 home made specific probes and P0002a reference probes. The raw data were analyzed by Coffyscaler software.

Patients

- 46, XY DSD: 194 cases
- 46, XX DSD: 114 cases

Analysed by Sanger sequencing and/or MLPA for the major candidate genes/regions for the specific DSD condition.

RESULTS

A GENETIC CAUSE WAS IDENTIFIED IN 217/308 CASES (70%)

<table>
<thead>
<tr>
<th>GONADAL DYSG</th>
<th>PMDS</th>
<th>DEFECTS OF ANDROGEN SYNTHESIS OR ACTION</th>
<th>ISOLATED HYPOSPADIAS</th>
<th>TDS/DOTDS+</th>
<th>ANDROGEN EXCESS CLASSICAL FORMS</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRY</td>
<td>5</td>
<td>AR</td>
<td>AMH</td>
<td>SRY+</td>
<td>CYP21A2</td>
</tr>
<tr>
<td>NRSA1</td>
<td>2</td>
<td>AMH2</td>
<td>SRD5A2</td>
<td>AR</td>
<td>CYP11B1</td>
</tr>
<tr>
<td>DUP DAX1</td>
<td>2</td>
<td>HSD1B3</td>
<td>SRY</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>DEL 9p</td>
<td>1</td>
<td>HSD1B3</td>
<td>AMH2</td>
<td>1</td>
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</tr>
<tr>
<td>DEL ENH SOX9</td>
<td>2</td>
<td>CYP17A1</td>
<td>SRY</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>WT1</td>
<td>1</td>
<td>HSD3B2</td>
<td>SRY</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>MD* 14/17 = 52%</td>
<td></td>
<td>MD* 11/14 = 78.6%</td>
<td>MD* 85/133 = 63.9%</td>
<td>MD* 1/20 = 5%</td>
<td>MD* 4/7 = 51.7%</td>
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<td></td>
<td></td>
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<td>MD* 107/107 = 100%</td>
</tr>
</tbody>
</table>

*Mutation detection rate

KEY POINTS & COMMENTS

- Both the group of Gonadal Dysgenesis and Isolated Hypospadias are the less characterized, thus confirming their highly genetic heterogeneity and, in particular for the latter, the possible multifactorial origin.
- A panel of a limited n. of genes (AR, SRD5A2, NR5A1, HSD1B3) is sufficient to obtain a good mutation detection (62.4%) for the group of defects of androgen synthesis/action.

The total mutation detection is 57.2% (111/194), 63.2 (110/174) if Isolated Hypospadias were excluded.

In the non characterized cases we can not exclude dysmorphisms, mosaicism, micro-rearrangements, digenic inheritance, somatic mutations or mutations in the regulatory regions, as well as steroidogenic defects (adrenal/gonadal) non easily detectable in the adult, by post or by traditional hormonal tests.

IN THE NEW CASES (CAH excluded) It is therefore important (in agreement with 1,2,3) to perform:
- aCGH (CAH excluded) before other tests in order to find microdeletions/duplications associated to several DSD phenotypes.
- URINARY STEROID PROFILES (before any gonadal intervention) and/or COMPLETE STEROID PROFILE BY LC/MS/MS.
- analysis (in the aCGH negatives) of a panel of ~10 “BASIC” GENES (MD+ 50%), followed in the negative cases by a panel of 25-30 “RARE” GENES, possibly by advanced technologies like NGS to permit more rapid analysis.

IN OUR NOT CHARACTERIZED CASES (STILL FOLLOWED UP) WE ARE PERFORMING: aCGH and NGS WITH A “DSD RARE” PANEL OF 30 GENES.

ACKNOWLEDGEMENTS

We are very grateful to all the Clinicians for their collaboration to study some very interesting cases and for their enthusiasm to discuss the results.

REFERENCES