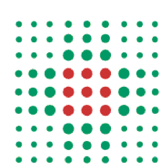

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GENOTYPING PATIENTS WITH DIFFERENCES OF SEX DEVELOPMENT (DSD): 25 YEARS OF INVESTIGATION OF AN ITALIAN POPULATION OF 308 CASES (194 46,XY AND 114 46,XX)

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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).

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.

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 leucocytes by different tools, 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 CEQ8000 sequencer system (BeckmanCoulter) 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 (MRC-Holland):
P185-Intersex (NR0B1, WNT4, SOX9, NR5A1, SRY) P334-A1 Gonadal Development Disorder (SRD5A2, HSD17B3, DMRT1, CYP17A1) for gonadal dysgenesis 46,XY and 46,XX and for defects of androgen synthesis/action; P050-C1 CAH; for CYP11B1 home made specific probes and P300A2 reference probes.
The raw data were analyzed by Coffalyser software.

Tab1 TESTED GENES

GENE	LOCUS
AMH	19p13.3
AMHR2	12q13.13
AR	Xq12
CBX2	17q25.3
CYP11B1	15q24.1
CYP17A1	10q24.32
CYP21A2	6p21.33
DHH	12q13.12
HSD3B2	1p12
HSD17B3	9q22.32
MAMLD1	Xq28
NR0B1 (DUP)	Xp21.2
NR5A1	9q33.3
POR	7q11.23
SOX9 (DEL ENH)	17q24.3
SRD5A2	2p23.1
SRY	Yp11.2
WT1	11p13

RESULTS

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

46,XY GONADAL DYSG		46,XY PMDS		46,XY DEFECTS OF ANDROGEN SYNTHESIS OR ACTION		46,XY ISOLATED HYPOSPADIAS		46,XX TDSD/OTDSD♦		46,XX ANDROGEN EXCESS CLASSICAL FORMS	
SRY	5	AMH	9	AR	50	MAMLD1	1	SRY +	4	CYP21A2	101
NR5A1	2	AMHR2	2	SRD5A2	15					CYP11B1	6
DUP DAX1	2			NR5A1	12						
DEL 9p	1			HSD17B3	6						
DEL ENH SOX9	2			CYP17A1	1						
WT1	1			HSD3B2	1						
MD*	14/27 = 52%	MD*	11/14 = 78.6%	MD*	85/133 = 63.9%	MD*	1/20 = 5%	MD*	4/7 = 57.1%	MD*	107/107 = 100%

*Mutation Detection rate

KEY POINTS & COMMENTS

46, XY DSD

- **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, HSD17B3) 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.

46, XX DSD

- **The large prevalence of defects of adrenal steroidogenesis is confirmed and this group reached the 100% of mutation detection if Sanger sequencing is combined with MLPA analysis for both the main genes: CYP21A2 and CYP11B1.**
- **Among the testicular/ovotesticular DSD♦, only the TDSD** (SRY +) **were characterized, whereas the 3 OTDSD cases** (analysed elsewhere also for RSPO1 and WNT4) **are still waiting,** thus confirming the genetic heterogeneity of this condition.

The total mutation detection is 93.9% (107/114)

In the non characterized cases we can not esclude dysmorphisms, mosaicisms, 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 (CAIS escluded) **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.**

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