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Combining clinical and genetic approaches in diagnosing a large Brazilian cohort of patients with 46,XY Differences of Sex Development (DSD)

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Introduction

Diagnosing patients with 46,XY differences of sex development (DSD) is quite challenging. All individuals with a suspected DSD need a thorough clinical, biochemical, genetic and imaging evaluation. Most 46,XY DSD studies have been focused only on molecular data, with no attention to the biochemical work-up. However, the biochemical work-up is equally important and large scale data analysis that combines hormonal data and molecular genetic investigations is lacking.

Objective

To analyze the clinical and genetic findings of a large cohort of patients with 46,XY DSD from a single center.

Patients and Methods

- ✓ We studied 247 non-syndromic individuals with 46,XY DSD including 159 sporadic and 88 familial cases from 39 unrelated families.
- ✓ Data regarding the age of first evaluation, external genitalia appearance and hormone profile were collected from medical files. Hormonal profile included measurement of LH, FSH, testosterone (T), androstenedione (A) and dihydrotestosterone (DHT) at basal and/or after hCG stimulation test.
- ✓ LH, FSH and T levels were measured by immunoassays; androstenedione was measured by immunoradiometric and more recently by LCMS ; DHT was measured by immunoradiometric purified by column chromatography and more recently by LCMS.
- ✓ The presence of Mullerian derivatives was evaluated by a pelvic ultrasound and/or cystourethrogram.
- ✓ The patients were classified into groups, accordingly to their phenotype (Table 1).
- ✓ For molecular diagnosis, most patients (84%) were screened by Sanger. All patients without a previous molecular diagnosis by Sanger or who entered in the study after the year of 2015 were studied by massively parallel sequencing. The sporadic cases were analyzed by a DSD target panel, composed of 63 DSD-related genes and the familial cases by whole exome sequencing (WES). For exome sequencing, we included the probands, their first-degree relatives and other affected members of their families (if available).
- ✓ The variants were classified according to the ACMG criteria. Pathogenic or likely pathogenic variants were considered diagnostic.

Results

- ✓ The median age of the patients at first visit was: 14 (range from 0.1 to 50) years.
- ✓ The patients' classification as well as the summary of the molecular results are depicted in Table 1.
- ✓ A total of 103 candidate variants were identified; 87 variants were classified as pathogenic or likely pathogenic. Molecular diagnosis was possible in 73 (46%) sporadic cases and in 31 (77.5%) familial cases
- ✓ Pathogenic or likely pathogenic variants were identified in *NR5A1* and in *DHX37* in 12 and 8 probands, which corresponds to 21% and 14% of all gonadal dysgenesis (GD) cases, respectively, being the most frequent cause of GD.
- ✓ The patients with *NR5A1* variants had variable phenotypes, ranging from severe external genitalia undervirilization to isolated perineal hypospadias. None of these patients had adrenal insufficiency.
- ✓ Five patients (41%) with *NR5A1* variants had preserved gonadal function and absence of uterus. All of them were firstly classified as having a DSD of unknown etiology.
- ✓ *DHX37* was the only gene associated with embryonic testicular regression syndrome (ETRS), accounting for 30% of the índex cases with this phenotype.[2]
- ✓ A high percentage of diagnosis was obtained in patients with AIS phenotype (100% of the patients with CAIS and 81% of the patients with PAIS) after the identification of two deleterious synonymous variants[3] in the *AR* gene and one variant located in the promoter region of this gene[4].

Table 1: Summary of the clinical classification and molecular results of the Brazilian cohort of patients with 46,XY DSD

46,XY DSD group	Number of patients		% of Patients with pathogenic/ likely pathogenic variants
	Sporadic	Familial	
1) Gonadal dysgenesis (GD); n=64		(n of families)	
Complete GD; n=23	17	6 (3)	15% (3/20)
Partial GD; n=26	23	3 (2)	60% (14/25)
ETRS; n=15	10	5 (3)	30% (4/13)
			36% (21/57)
2) Defects in T production (DTP); n=73			
5 α-reductase deficiency; n=34	16	18 (6)	95% (21/22)
17β- HSD type 3 deficiency; n=15	7	8 (4)	100% (11/11)
17α-OH deficiency; n= 11	3	8 (4)	100% (7/7)
3β-HSD type 3 deficiency; n= 4	2	2 (1)	100% (3/3)
Leydig cell hypoplasia; n= 9	0	9 (5)	80% (4/5)
			96% (46/48)
3) Defects in testosterone action (AIS; n= 44)			
Complete AIS; n=26	13	13 (5)	100% (18/18)
Partial AIS; n=18	7	11 (4)	90% (10/11)
			96.5% (28/29)
4) Persistent Mullerian duct Syndrome (PMDS; n=8)	8	0	**85.7% (7/8)**
5) DSD of unknown etiology (UD; n= 59)	53	6 (3)	**26.7% (15/56)**
Previous gonadectomy; n=11			
Prepubertal age; n=18			
Other; n=30			
TOTAL	159	88 (39)	**58.5% (116/198)**

- ✓ A T/DHT ratio at basal and after hCG was available in 46 and 39 patients, respectively, including 12 patients under 6 months of age, 23 patients at prepubertal/intrapubertal age and 34 after pubertal age.
- ✓ Analysis of sensitivity and specificity of the T/DHT ratio was perfomed by a ROC curve using MedCalc software 8.1.1.0. The molecular diagnosis was considered the gold standard for diagnosing 5-alpha reductase tipe 2 deficiency. The results are depicted in the following table:

T/DHT basal	Sensitivity (%)	Specificity (%)
13	100	71.4
47.7	33	100
T/DHT after hCG	**Sensitivity (%)**	**Specificity (%)**
11.9	100	62
24	86.7	95.9
41	60	100

- ✓ A T/A ratio < 0.8 was observed in 13 of 16 patients (81%) with molecular diagnosis of HSD17B3 deficiency. Among these three patients, the androstenedione was measured by LCMS in two of them.
- ✓ A T/A ratio < 0.8 was also observed in 1 out 49 patients, who had a diagnosis of SRD5A2 deficiency.

Discussion/Conclusion

The molecular diagnosis was established in 58.5% of the 46,XY DSD individuals, mainly in patients with AIS and DTP, with a lower diagnostic yield in GD patients. *NR5A1* defects are the most common cause of GD, but *DHX37* emerges as an important cause, especially for ETRS. Clinical classification matched with the genetic analysis in most cases, probably because there were few newborns in the cohort, whose DSD diagnosis can be more difficult and missleading. However, six GD cases (all of them with *NR5A1* variants), two adults with SRD5A2 deficiency and one adult with HSD17B3 deficiency would have been missed by clinical assessment, reinforcing the importance of the molecular studies for diagnosing patients with 46,XY DSD of any age. In the other hand, the correct clinical classification of the patients with AIS phenotype was of an unprecedented value because it allowed us not to restrict the molecular analysis to the non-synonymous variants of the exonic regions of the *AR* gene, leading to the identification of a higher percentage of molecular diagnosis in comparison to other cohorts, (80% and 30% of the patients with CAIS and PAIS, respectively)[5]. There are still one patient with AIS and one family of patients with Leydig cell hypoplasia who remain without a molecular diagnosis, indicating that other mechanisms are involved in these steroid resistances.

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