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Molecular diagnosis of patients with 46,XY differences in sex development in a single tertiary center.

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INTRODUCTION

Differences/disorders of sex development (DSD) comprise a group of congenital conditions, affecting human sex determination and/or differentiation. Patients with DSD are classified in: sex chromosome DSD; 46,XY DSD and 46,XX DSD.

46,XY DSD include defects in androgen synthesis or action, or disorders of gonadal development with complete (CGD)/partial (PGD) gonadal dysgenesis.

OBJECTIVE

The aim of this study was to characterize the molecular genetic diagnosis of individuals with 46,XY DSD followed at Garrahan Pediatric Hospital.

CLINICAL MATERIAL AND METHODS

- Medical records of 140 patients (P) followed at the Endocrinology Department because of 46,XY DSD were reviewed. DNA samples were obtained in 87/140.
- Subjects were divided into 3 groups (G1, G2, G3) based on clinical characteristics, hormonal measurements, gonad histology and ultrasound/laparoscopic findings: **G1: defects in androgen synthesis (n=8), G2: defects in androgen action (n=39) and G3: disorders of gonadal development CGD/PGD (n=40).**

	Candidate gene sequencing	Copy number variations[1]	Targeted gene panel[2,3]	Whole exome sequencing[3]
G1 (n=8)	*StAR, CYP17A1, HSD3B2, POR* or SRD*5A2*			
G2 (n=39)	*AR*			
G3 (n=40)	*SRY, NR5A1* and/or *WT1*	*SRY, SOX9, NR0B1, NR5A1* and *WNT4* by MLPA. Whole genome CGH in 4 P	*AKR1C2, AKR1C4, AMH, AMHR2, AR, ARX, ATRX, CBX1, CBX2, DHCR7, DHH, DMRT1, DMRT2, EMX2, FGF9, GATA4, HHAT, LHCGR, MAMLD1, MAP3K1, NR0B1, NR5A1, ROCK1, RSPO1, SOX10, SOX3, SOX8, SOX9, SRY, STARD8, TSPYL1, VAMP7, WNT4, WT1, WWOX, ZFPM2.*	In the remaining undiagnosed individuals

[1] **CNVs**. Assessed by MLPA (Intersex P185 C2 MRC Holland) both in DNA from peripheral blood leukocytes and, when available, gonadal tissue. In 4 P whole genome CGH (Agilent Sureprint G3 Human CGH Microarray 4X180K) was performed.

[2] **TGP**. 35 genes known to cause XY DSD or known to play a role in gonadal differentiation and genitourinary tract development.

[3] Every clinically significant variant was confirmed by Sanger sequencing in proband and parents to elucidate inheritance pattern.

RESULTS

Candidate gene sequencing

G1	G2	G3
StAR [1] IVS1-2G>A	***AR*** [2] p.Trp399Valfs*95 p.Leu822Pro p.Arg832* p.Pro767Ser p.[Cys602=/Cys602Phe] p.[Glu804=/Glu804*] p.Ile899Phe p.[His730=/His730Glnfs*38] p.Met750Val p.Arg608Gln p.Val890Met p.Asp691del p.Phe726Cys p.Arg856Cys p.Gln658Argfs*3 p.[Gln98=/Gln98Hisfs*8] c.1617_2763del p.Arg841Cys p.(Ser889=) p.Ala597Thr p.Asn611Ile p.Glu707Asp p.His886Tyr	***SRY*** p.Met64Val ***NR5A1***[3,4] p.Tyr183* p.Trp279* p.Gly77Glu p.Arg313His p.Arg69His p.Ser303Arg p.Arg241Trp ***WT1*** p.Arg462Trp p.Tyr261* IVS7+1G>T IVS9+4C>T p.Arg434His IVS9+5G>A IVS9+1G>A
CYP17A1 p.[Arg358Gln];c.[1434-1437dupCATC]		
HSD3B2 p.[Val228Met];[Val228Met] p.[Arg249*];[Arg249*]		
POR p.[Ser331Cysfs*24];[Pro399_Glu401dup] p.[Gly88Ser];[Gly88Ser]		
SRD5A2 p.[Gly183Ser];[Gly196Asp] p.[Gln56Arg];[Gly183Ser])		

[1]Baquedano MS. *et al.* Unique dominant negative mutation in the N-terminal mitochondrial targeting sequence of StAR, causing a variant form of congenital lipoid adrenal hyperplasia. J Clin Endocrinol Metab. 2013;98(1):E153-61. [2]Touzon MS *et al*. Androgen Insensitivity Syndrome: Clinical Phenotype and Molecular Analysis in a Single Tertiary Center Cohort. J Clin Res Pediatr Endocrinol. 2019; 11(1): 24–33. [3]Warman DM *et al*. Three New SF-1 (NR5A1) Gene Mutations in Two Unrelated Families with Multiple Affected Members: Within-Family Variability in 46,XY Subjects and Low Ovarian Reserve in Fertile 46,XX Subjects. Horm Res Paediatr 2011;75:70–77. [4]Perez Garrido N *et al.* Functional Characterization of Two Mutations Located in the Ligand Binding Domain in the SF1. Int J Endocrinol Metab Disord 1(4).
Underlined mutations have not been reported in the literature.

Targeted gene panel

G3	
ZFPM2	***NR5A1***
p.[Val763Ile];[Glu30Gly]	p.Glu395del
p.Gln889Glu	
p.Tyr992Phe	
p.Leu564Ile	

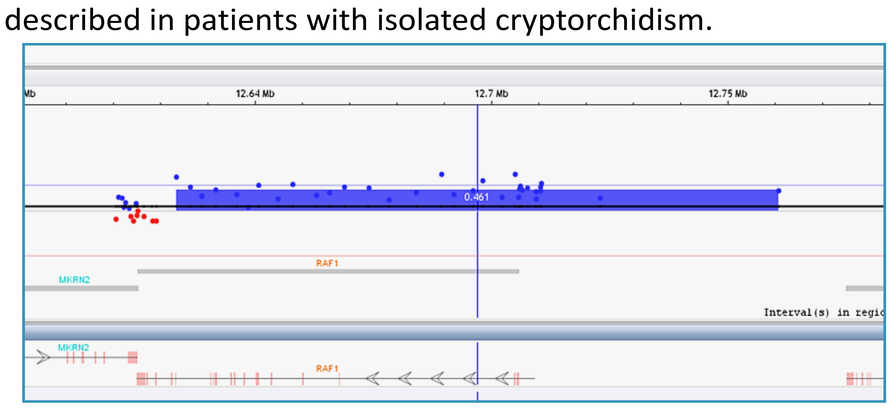
Whole exome sequencing

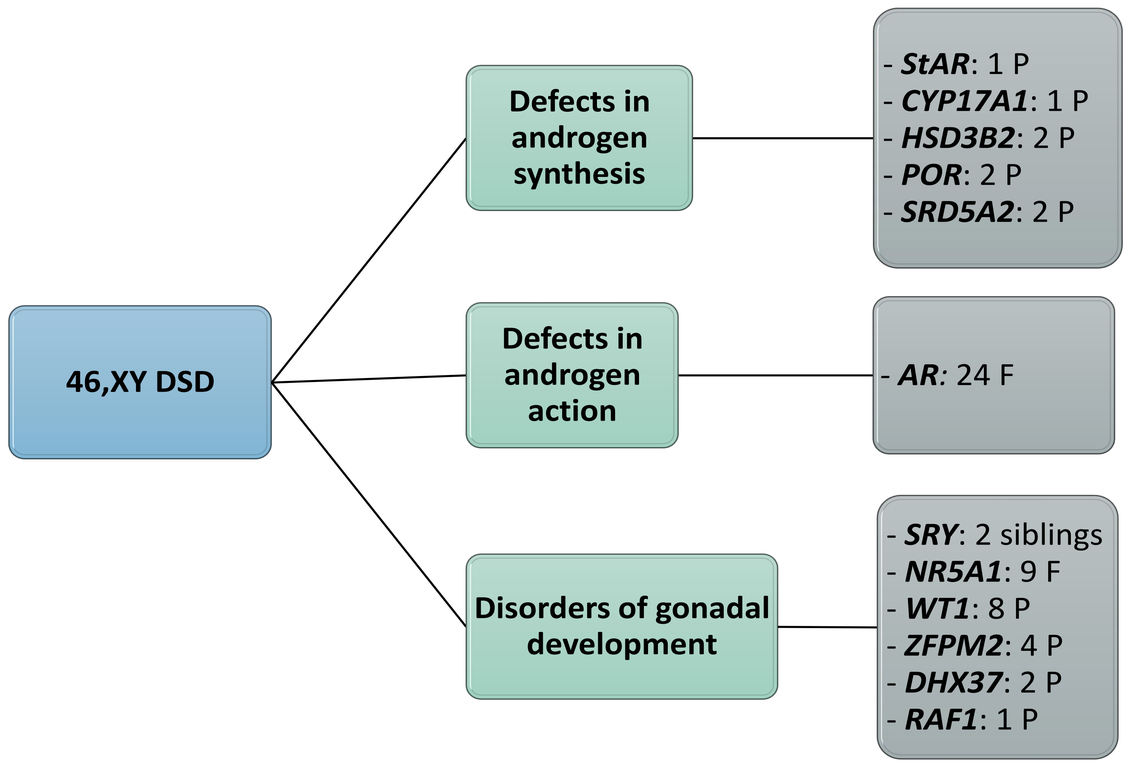
G3	
DHX37	
p.Arg308Gln	
NR5A1	
p.Glu320fs	

Whole genome CGH

G3

• 127kb duplication in 3p25.2 implicating 11 of the 16 coding exons of ***RAF1*** gene, previously described in patients with isolated cryptorchidism.





CONCLUSIONS

- **In this cohort, excluding enzymatic defects, molecular characterization was reached in approximately 63% (50/79).**
- **Diagnosis in 46,XY DSD can be challenging due to overlapping clinical characteristics or poor genotype/phenotype correlation. Thus, candidate gene sequencing strategy might not be adequate in all cases.**
- **NGS can be a better approach to reach an etiologic diagnosis reducing time and medical interventions.**
- **Other etiologies should be considered: non coding genomic regions, oligo/multigenic inheritance, epigenetic pathways or environmental factors.**