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 fourty 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)

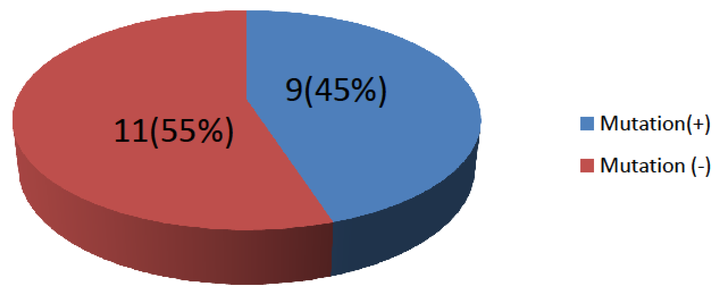

Figure 1. Nine (45%)mutations were found in 20 patients with DSD by NGS

- 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.

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

Patient/age (years)	Assigned sex	Gene	Transcript ID	cDNA	Protein	Mutation Type	MT	Polyphen2 score	SIFT	Reference
1/10	F	HSD17B3	NM_000197.1	c.160A>G/ c.160A>G	p.T54A/p.T54A	M/M	DC	PD	D	Novel
2/13	F	HSD17B3	NM_000197.1	c.861C>A/c.861C>A	p.Y287X/p.Y287X	NS/NS	DC	NA	NA	Known
3/17	F	HSD17B3	NM_000197.1	c.239G>A/c.277G>A	p.R80Q/p.E93K	M/M	DC/DC	PD/PD	D/D	Known/Novel
4/0.6	M	SRY	NM_003140.2	c.535C>T/c.535C>T	p.Q178X/p.Q178X	NS/NS	DC	NA	NA	Novel
5/7	M	LHCGR	NM_000233.3	c.1448C>A/c.1448C>A	p.A483D/p.A483D	M/M	DC	PD	D	Novel
6/7	M	WT1	NM_024426.4	c.1187C>T/wt	p.P396R/wt	M	DC	PD	D	Novel
7/15	M	HSD17B3	NM_000197.1	c.861C>A/c.861C>A	p.Y287X/p.Y287X	NS/NS	DC	NA	NA	Known
8/18	F	HSD17B3	NM_000197.1	c.524G>C/c.524G>C	p.R175T/p.R175T	M/M	DC	PD	D	Novel
9/7	F	HSD17B3	NM_000197.1	c.239G>A/c.239G>A	p.R80Q/p.R80Q	M/M	DC	PD	D	Known

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

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Sex Differentiation

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