Evaluation of Genetic Etiology in Patients with 46,XY Disorders of Sex Development:One Center Experience

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Background: Disorders of sex development (DSD) are a heterogeneous group of disorders related to sex determination and differentiation. Although several genetic abnormalities have been discovered through genetic analyses, the underlying genetic causes of 30–40% of the 46,XY DSD cases are not yet known.

Aims and Objectives: To identify genetic defects in patients with 46,XY DSD.

Methods:
Seventy-six patients with 46,XY DSD were studied.

As a First Step

56 patients suspected to have androgen insensitivity syndrome or 5 alfa reductase deficiency according to their hormonal results are screened for *SRD5A2* and *AR* gene mutations via Sanger sequencing.

Next Step

- 22 patients who do not carry mutations in these genes
- 20 patients suspected to have gonadal dysgenesis or androgen synthesis defects are enrolled into the next step

31 DSD associated genes

- Sequenced using in-house-designed next generation sequencing (NGS) targeted gene panel, using an Ion Torrent platform
- Analyzed for gross deletion/duplication with MLPA
- Segregation analysis was performed for family members for novel missense alterations suspected to be causative.

Genes in DSD Panel: *ATF3* (1q32.3, NM_001674.3); *AR* (Xq11.2-q12, NM_000044.3); *AMHR2* (12q13, NM_020547.2); *BNC2* (9p22.2, NM_017637.5); *BMP4*(14q22-q23, NM_001202.3); *CYP11A1* (15q23-q24, NM_000781.2); *CBX2* (17q25.3, NM_005189.2); *CYB5A* (18q23, NM_001914.3); *DMRT1* (9p24.3, NM_021951.2); *DHH* (12q12-q13.1, NM_021044.2); *DAX1*(Xp21.3-p21.2, NM_000475.4), *GATA4* (8p23.1-p22, NM_002052.3); *HHAT* (1q32,NM_018194.5); *HSD17B3* (9q22, NM_000197.1); *HOXA4*(7p15-p14, NM_002141.4);*HOXB4*(17q21.32, NM_024015); *HOXB6*(17q21.3, NM_018952.4); *LHCGR*(2p21, NM_000233.3); *MAP3K1* (5q11.2, NM_005921.1);*MAMLD1*(Xq28, NM_005491); *NR5A1* (9q33, NM_004959.4); *POR* (7q11.2, NM_000941.2); *RSPO1* (1p34.3, NM_001038633.3); *SRD5A2* (2p23; NM_000348.3); *STAR*(8p11.2, NM_000349.2); *SOX9* (17q24.3-q25.1, NM_000346.3); *SOX3* (Xq27.1, NM_005634.2); *SRY* (Yp11.3, NM_003140.2); *WNT4* (1p36.23-p35.1, NM_030761.4); *WT1* (11p13, NM_024426.4); *ZFPM2* (8q23, NM_012082.3)

Results:

- In the first group, *SRD5A2* and *AR* gene mutations are detected 60.7% of cases.
- In the second group, six previously described and 13 possible DSD associated rare variants was identified in eight different genes within a total of 17 cases, reaching a diagnostic rate of 38.6 % for second group.
- Highest rate of pathogenic alterations is identified in *HSD17B3* gene (16.7%) which is followed by *DHH, NR5A1*, *LHCGR, POR, HOXA4, WT1* and *ZFMP2*.

Table 1. Molecular characteristics of the identified variants in 46,XY DSD patients with gonadal dysgenesis and androgen synthesis defect

Patient no	Assigned sex	Gene	Zygosity	Location/ nucleotide	Protein	Mutation type	Mutation taster	Polyphen 2 score	SIFT	Reference
1	male	DHH	Homozygous Homozygous	Exon 1 c.71G>C Exon 3 c.1063C>T	p.Gly24Ala p.Arg355Cys	Missense Missense	Disease causing Disease causing	Probably damaging Probably damaging	Damaging Tolerated	Novel Novel
2	male	DHH	Homozygous	Exon 3 c.1146G>A	p.Trp382*	Nonsense	Disease causing	-	-	Novel
3	Male	WT1	Heterozygous	Exon 9 c.1385G>T	p.Arg462Leu	Missense	-	-	-	Known
4	Male	ZFPM2	Heterozygous	Exon 3 c.286G>T	p.Asp96Tyr	Missense	Disease causing	Benign	Damaging	Novel
5	Female	NR5A1	Heterozygous	Exon 3 c.151G>T	p.Glu51*	Nonsense	Disease causing	-	-	Novel
6	Male	NR5A1	Heterozygous	Exon 4 c.247G>T	p.Val83Leu	Missense	Disease causing	Possibly damaging	Damaging	Novel
7	Male	HOXA4	Heterozygous	Exon 1 c.9G>T	p.Met3Ile	Missense	Disease causing	Probably damaging	Damaging	Novel
8	Male	HSD17B3	Homozygous	[Exon 1]; [Exon 10] c.[139A>G]; [704T>C]	p. [Met47Val]; [Met235Thr]	[Missense]; [Missense]	Disease causing	Probably damaging	Damaging	Known Novel
9	Female	HSD17B3	Homozygous	Intron 10 c.607-1G>A	-	Splice site	-	-	-	Known
10	Female	HSD17B3	Homozygous	Exon 2 c.182G>A	p.Gly61Glu	Missense	Disease causing	Probably damaging	Damaging	Novel
11	Female	HSD17B3	Homozygous	Exon 2 c.167C>T	p.Ala56Val	Missense	Disease causing	Probably damaging	Damaging	Novel
12	Female	HSD17B3	Homozygous	Intron 3 c.277+4A>T	-	Splice site	-	-	-	Known
13	Female	HSD17B3	Homozygous	Exon 3 c.277G>A	p.Glu93Lys	Missense				Known
14	Female	HSD17B3	Homozygous	Exon 9 c.639_640insA	p.Glu214fs*4	Frame shift	Disease causing	-	-	Novel
15	Female	POR	Homozygous	Exon 11 c.1196_1204dupCCTCGGAGC	p.Pro399_Gln401dup	In frame duplication	-	-	-	Novel
16	Female	LHCGR	Homozygous	Exon 11 c.1435C>T	p.R479*	Nonsense	-	-	-	Known
17	Male	LHCGR	Homozygous	Exon 2 c.203C>T	p.Ala68Val	Missense	Disease causing	Probably damaging	Tolerated	Novel

Conclusions:

- Genetic analyses following clinical and hormonal evaluation is essential for the management of patients with 46,XY DSD with a great phenotypic and genetic heterogeneity.
- NGS targeted gene panel seems powerful tool to detection mutations in DSD.