

Development of a next generation sequencing panel for Disorders of Sex Development (DSDs)

Graham Fewes (1), Lowri Hughes (1), Kirsten McKay Bounford (1), Trevor Cole (1), Nils Krone (2),
Fiona MacDonald (1), Stephanie Allen (1),

(1) West Midlands Regional Genetics Service, Birmingham Women's NHS Foundation Trust, Birmingham, B15 2TG

(2) School of Clinical and Experimental Medicine, University of Birmingham, Birmingham, B15 2TT

Background

Disorders of sex development (DSDs) refer to congenital disorders where the chromosomal, gonadal or anatomical sex is atypical. Patients typically present neonatally with ambiguous genitalia preventing immediate gender assignment or during adolescence where atypical sexual development becomes apparent. Genetic testing is key in establishing a diagnosis, allowing for personalised patient management and may significantly reduce the period of uncertainty for families regarding the sex of rearing of their child. Cytogenetics may provide guidance on possible causes and where further investigation is indicated, however a definitive molecular diagnosis is only made in around 20% of cases. Current DSD molecular testing strategies are not ideal, as tests for only a few of the many associated genes are currently available and require sequential testing. The development of next generation sequencing (NGS) strategies allows for multiple genes to be investigated simultaneously at a reduced cost compared to the Sanger sequencing strategies. Such tests also reduce long waiting times caused by sequential gene testing.

Gene Panel

A TruSeq custom amplicon panel has been designed covering 32 genes associated with 46,XX and 46,XY DSD.

46, XX DSD		46, XY DSD	
Disorders of Ovarian Development	Androgen Excess	Disorders of Testicular Development	Disorders of Hormone synthesis or action
SRY (Yp11.3)	HSD3B2 (1p13)	WT1 (11p13)	DHCR7 (11q12-q13)
SOX9 (17q24)	POR (7q11.2)	CBX2(17q25)	LHCGR (2p21)
RSPO1 (1p34.3)	CYP11B1 (8q21-q22)	NR5A1/SF1 (9q33)	StAR (8p11.2)
WNT4 (1p35)	CYP19A1 (15q21)	SRY (YP11.3)	CYP11A1 (15q23-24)
	Glucocorticoid receptor (5q31)	SOX9 (17q24-q25)	HSD3B2 (1p13.1)
		DHH (12q13.1)	CYP17A1 (10q24.3)
		ARX (Xp22.13)	POR (7q11.2)
		TSPYL1 (6q22-23)	CYB5A (18q23)
		MAMLD1/CXORF6 (Xq28)	HSD17B3 (9q22)
		DMRT1 (9p24.3)	SRD5A2 (2p23)
		ATRX (Xq13.3)	AR (Xq11-q12)
		NR0B1/DAX1 (Xp21.3)	AMH (19p13.3-p13.2)
		WNT4 (1p35)	AMH receptor (12q13)

Methods and Validation

40 validation samples and 11 patient samples were run on an Illumina MiSeq. Preliminary analysis was performed using MiSeq reporter software and BAM files viewed within NextGENs v.2.3.3 software to generate variant call lists.

Following variant comparison variants were classified as likely artefact, likely SNP, Variant and no result at a 15% mutation threshold and minimum 15 read depth. All pathogenic variants are confirmed by Sanger sequencing.

Validation confirmed the presence of 26 known mutations and identified one previously unknown mutation (Case 1). Within and between run comparison showed no significant variant call differences.

Pathogenic mutations were identified in 3/11 (27%) of patient samples (Cases 2-4).

Case 1: A 46,XY female heterozygous for a familial p.Cys268Tyr mutation in 17βHSD was shown to have a pathogenic frame shift mutation within AR; c.2407dupC; p.Gln803Pro*27.

Case 2: A 46,XY female was shown to carry a homozygous mutation c.695C>T; pSer232Leu within HSD17β3.

Case 3: A 46,XY male with Mullerian structures was shown to carry a homozygous mutation c.289C>T p.Arg97* within AMHR2.

Case 4: A 46,XY female was shown to carry a heterozygous c.69C>T p.Tyr23* within NR5A1 (SF1).

Conclusions and Discussion

A targeted NGS panel for 32 genes associated with Disorders of Sexual Development has been developed and shown to be effective and efficient in the detection of causative mutations in this complex group of disorders.

Providing early accurate diagnosis enables informed decisions and discussions with parents regarding the likely natural history and the immediate, medium and long term patient management. In turn, these studies will enhance the efficient use of NHS resources, avoid unnecessary investigations, surgery and prevent inappropriate gonadectomy. The molecular basis of the disorders also provides an accurate risk of recurrence for families further aiding clinical management.

This 32 gene panel for Disorders of Sexual Development and reflex testing is now available as a diagnostic service from the West Midlands Regional Genetics Laboratory.



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