WES analysis of a cohort of 94 patients presenting with 46,XY and 46,XX DSD

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Introduction

Differences of Sex Development (DSD) are diagnosed in approximately one out of 4'500 newborns. Currently, due to the lack of knowledge on the complete gene and protein pathways involved in sex development and DSD, causative genetic variants can only be identified in about 50% of the affected patients. We used whole exome sequencing (WES) on a group of 94 Patients presenting with 46,XY and 46,XX DSD, in order to identify causative variants and potential new DSD genes.

Methods

A. Current data

71 patients diagnosed with 46,XY DSD

23 patients diagnosed with 46,XX DSD

Whole Exome Sequencing (WES)

B. Variants in genes related to DSD

ADAMTS16	C2ORF80	CYP26B1	ESR2	FMR1	HOXA4	LEPR	MKS1	PBX1	SETBP1	TACR3
AKAP2	CBX2	DAX1	ESPN	FOG2	HOXA13	LGR5	MSH5	PIP5K1B	SMOC2	TBX15
AKR1C1	CDKN1C	DHCR24	ETV4	FOXL2	HOXB6	LGR8	MYBL1	POLR3A	SOX2	TCTN3
AKR1C2	CEP41	DHCR7	FAM58A	FRAS1	HOXD	LHB	NLGN4X	POR	SOX3	TDRD7
AKR1C4	CHD7	DHH	FAM189A2	FREM2	HS6ST1	LHCGR	NEK1	PRKACG	SOX8	TOX2
AMH	CNGA1	DIAPH2	FAT4	FSHR	HSD17B1	LHFPL5	NELF	PROK2	SOX9	TSPYL
AMHR2	CTNNB1	DMRT1	FBLN2	FSHβ	HSD17B3	LHX1	NIPAL1	PROKR2	SOX10	TSPYL1
AR	CREBBP	DNMT3B	FEZF1	GATA4	HSD17B4	LHX3	NKD2	PROP1	SPECC1L	TUBB3
ARX	CUL4B	DUSP6	FGF8	GNRH1	HSD3B2	LHX4	NLGN4X	PSMC3IP	SPRY4	UBR1
ATF3	CYB5	DUSP15	FGF9	GnRHR	ICK	LHX9	NMT2	PTK2B	SRD5A1	WDR11
ATRX	CYB5A	DYNC2H1	FGF17	GPR54	IL17RD	LHβ	NOBOX	PTPN11	SRD5A2	WDR60
B3GALTL	CYP11A1	EAP1	FGFR1	GRIP1	INSL3	LMNA	NR5A1	RIPK4	SRY	WNT4
BCOR	CYP11B1	EMX1	FGFR2	HCCS	IRF6	MAMLD1	NR5A2	ROR2	STAG3	WNT7A
BMP4	CYP17A1	EMX2	FIGLA	HDAC8	KAL1	MAP3K1	NR0B1	RSPO1	STAR	WT1
BMP7	CYP19A1	ERCC6	FIG4	HESX1	KISS1	MCM8	NSMF	SALL1	SUPT3H	WWOX
BMP15	CYP1B2	ESCO2	FKBP4	HFM1	KISS1R	MID1	OPHN1	SCARF2	SYCE1	ZFPM2
BNC2	CYP21A2	ESR1	FLRT3	HHAT	LEP	MKKS	PAX2	SEMA3A	TAC3	DHX37

Table modified from Baetens, D. et al[1]

Rare (MAF < 0.5%) variants in known DSD genes

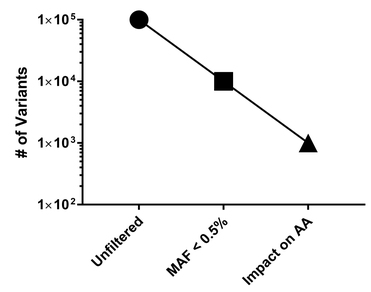
Diagnosis	# Patients	Genes
46,XX DSD	12 / 23	CYP19A1, HSD17B4, CYP21A2, STAR, CYP17A1
46,XY DSD	21 / 71	AR, SF1, SOX9, STAR, CBX2, DAX1, CYP17A1, HS6ST1

Unknown variants
functional analysis

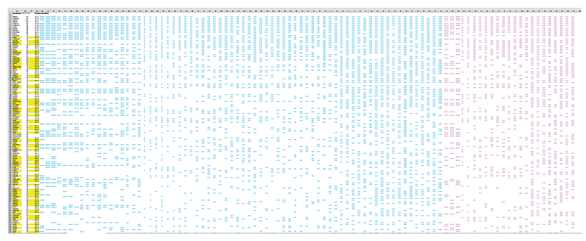
Known variants
no further action

B. Identification of potential new DSD candidate genes

I. Filtering of variants

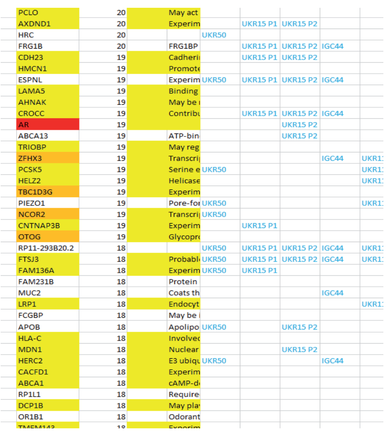


II. Comparison between patients



8505 affected genes common between at least two patients

III. Discover potential DSD candidates



Expression pattern

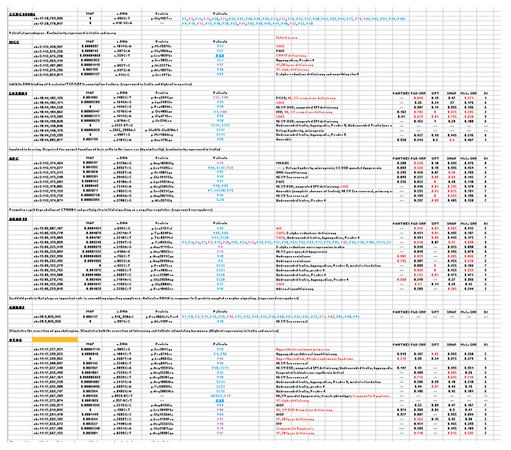
Known functions

Involvement in sex development related pathways

# of patients with variants

65 genes selected for further analysis

IV. Further analysis

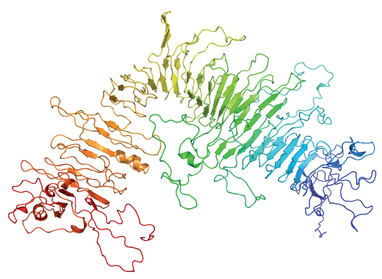


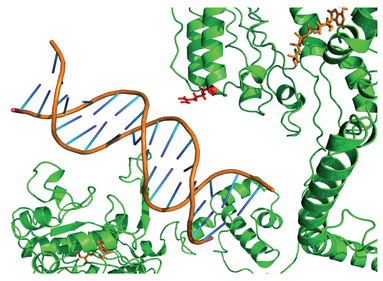
Similarity of phenotype and XX/XY specificity

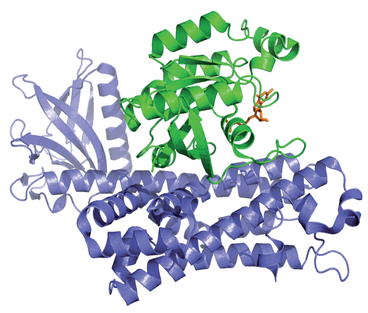
Multiple patients with same variant

Variant type and exact MAF

3D modelling of WT and variant protein







V. Selection of potential candidates

Gene	Function	# P	Patient Phenotype
AKAP13	Scaffold protein known to activate RHOA. Reduced fertility observed in mouse model.[2]	8	Undescended testes and hypospadias (4P), AIS (2P), adrenal insufficiency (1p), 46,XX gonadal dysgenesis (1P)
CCDC88C	Negative regulator of Wnt signaling pathway, inhibits CTNNB1 stabilization.[3]	6	Adrenal insufficiency (1P), undescended testes and hypospadias (3P), 46,XY gonadal dysgenesis (1P), delayed puberty and azoospermia (1P)
NPAP1	May be involved in spermatogenesis. Specific to primate species.[4]	8	46,XY gonadal dysgenesis (6P), AIS (1P), hypospadias (1P), undescended testes and azoospermia (1P)
NWD1	May play a role in AR protein steady-state levels. Expression significantly upregulated under SRY and SOX9 expression in HEK.[5]	8	46,XY gonadal dysgenesis (6P), undescended testes and hypospadias (1P), 46,XX gonadal dysgenesis (1P)
PDZD2	PDZD2 is cleaved to produce a secreted peptide and contains a nuclear localization sequence.[6]	12	Undescended testes and hypospadias (3P), hypospadias (2P), delayed puberty (1P), Anorchy (1P), AIS (1P), 46,XY gonadal dysgenesis (1P), aromatase deficiency (2P), hypogonadotropic hypogonadism (1P)

Conclusions

WES is a powerful tool for diagnostics that allowed us to identify potential causative variants in 21 of 71 46,XY DSD and in 12 of 23 46,XX DSD patients. The diagnosis of patients with already known variants can directly be confirmed, while unknown variants first need to be further analyzed. Comparison of all rare variants shared between patients and further filtering steps led to the identification of the five new potential DSD genes: AKAP13, CCDC88C, NPAP1, NWD1 and PDZD2. Further experiments in vivo (mouse/fly) and/or in vitro (appropriate human cell models) are needed to confirm their influence in sex development and its differences.

References:

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