Level of Uncertainty in Diagnostic Evaluation of Boys With XY Disorders of Sex Development

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Background

Although the availability of next generation sequencing and detailed endocrine tests may have increased the likelihood of reaching a diagnosis in boys with XY DSD, it has also led to challenges in interpretation of results.

Objectives

To examine the range of endocrine and molecular genetic variation in a group of boys undergoing evaluation for XY DSD.

Methods

Boys with XY DSD who were evaluated and discussed at the DSD Diagnostic Board in Glasgow from 2016 to 2019 were included. Sequence variants were classified according to ACMG guidelines and Class 3 variants of uncertain clinical significance (VUS) were divided into 3A and 3B, depending on whether the phenotype was consistent or not, respectively.

Results

N=124	Median (Range) or N (%)
Age (years)	0.87 (0,17.95)
External Masculinization Score (EMS)	8.25 (2, 12)
Positive Family History of DSD	34 (27)
Parental Consanguinity	8 (7)
Associated Malformations	80 (65)
Recognised Genetic Syndrome	13 (11)

Table 1. Clinical Characteristics

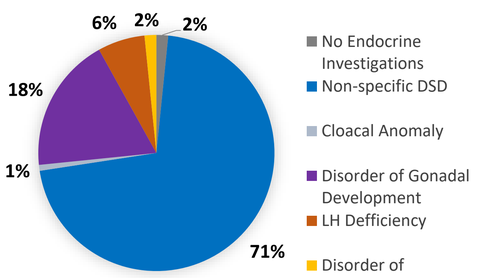

Figure 1. Endocrine assessment results

N=122	Yes/No
GnRH stimulation test	79/43
HCG stimulation test	94/28
Anti-Mullerian Hormone	114/8
Urine Steroid Profile	21/101

Table 2. Range of endocrine investigations

Endocrine assessment revealed an abnormality in 27% of cases

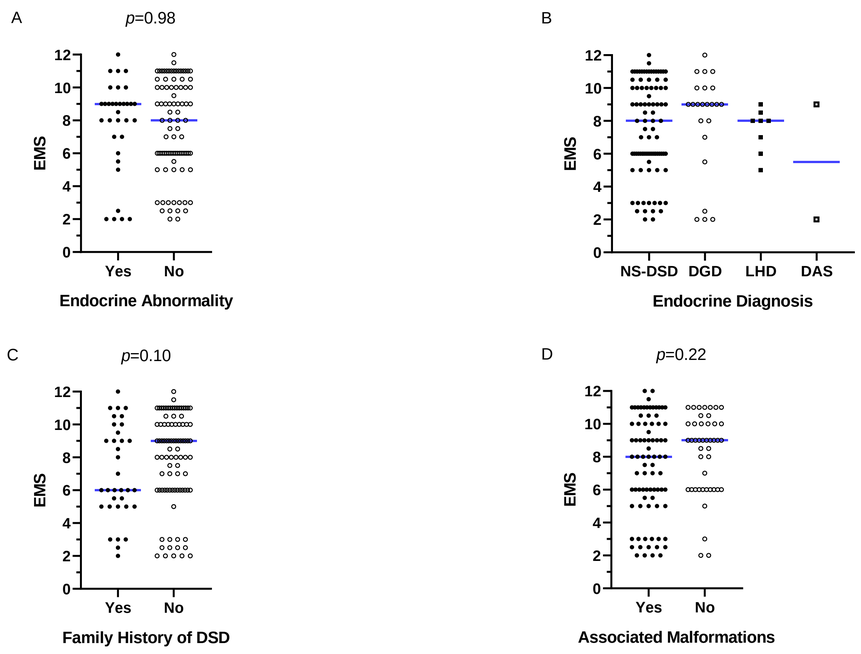

Figure 2. Comparison of phenotypes of XY DSD boys with or without family history of DSD, associated malformations, endocrine abnormalities and between subgroups of endocrine abnormalities identified

No significant differences in phenotypes between those in whom family history of DSD and associated malformations were present or not, between boys with normal and abnormal endocrine investigations were found

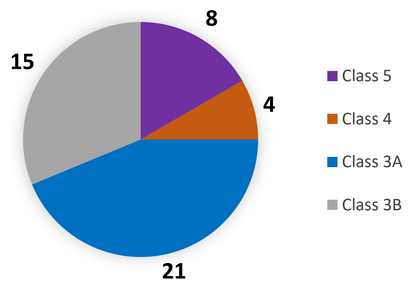

Figure 3. Gene variants identified

N=124	Yes/No	Genetic Variant
7 Gene Analysis	55/69	8
21 Gene Analysis	2/122	-
56 Gene Analysis	57/67	25
Array-CGH	83/41	18

Table 3. Range of genetic investigations

Of the 80 boys who had molecular genetic analysis by a combination of methods, variants were found in 8/55 (15%) by Sanger sequencing of seven genes and 25/57 (44%) by NGS of fifty-six genes

Results continued

Figure 4. Likelihood of detecting gene variant based on EMS and endocrine investigations

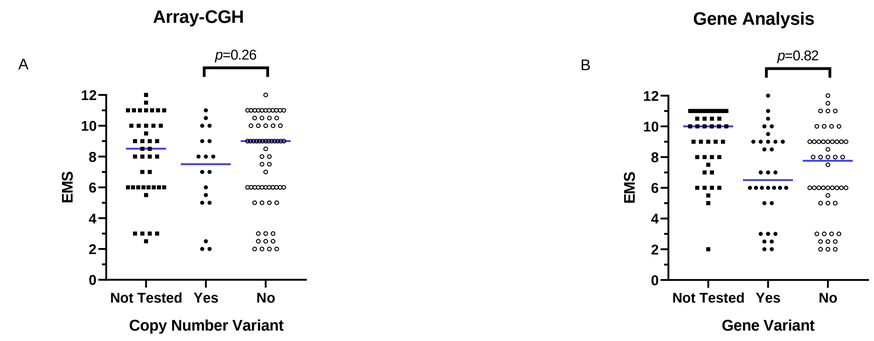

Figure 5. Comparison of phenotypes of XY DSD boys with or without genetic abnormality identified by array-CGH and gene analysis

The appearance of external genitalia seems to be unrelated to the presence of genetic abnormality

DSD gene	DNA change	Protein change	Inheritance/ Zygosity	Class (ACMG, 2015)*	Endocrine results	EMS	Clinical features
Disorders of gonadal development							
GATA4	c.942G>T	p.(Glu314Asp)	AD/heterozygous	3A	NA	11.0	DH
NR5A1	c.1379A>T	p.(Gln460Leu)	AD/heterozygous	3A	NS-DSD	9.5	MH, UUDT
NR5A1	c.116G>T	p.(Arg39Leu)	AD/heterozygous	3A	NS-DSD	6.0	M, PH
NR5A1[k]	c.1379A>T	p.(Gln460Leu)	AD/heterozygous	3A	NS-DSD	2.0	BS, M, PH, BUDT
MAP3K1[h]	2431A>G	p.(Met811Val)	AD/heterozygous	3B	NS-DSD	5.0	M, PH, BUDT
SPRY4[h]	841G>A	p.(Val281Met)	AD/heterozygous	3B	NS-DSD	5.0	M, PH, BUDT
Disorders of androgen synthesis or action							
HSD3B2[b]	c.518T>G	p.(Leu173Arg)	AR/heterozygous	5	NS-DSD	7.0	M, DH
HSD3B2[h]	c.745C>T	p.(Arg249*)	AR/heterozygous	5	NS-DSD	5.0	M, PH, BUDT
HSD3B2[a]	c.15C>A	p.(Cys5*)	AR/heterozygous	5	DAS	2.0	BS, M, PH, BUDT
HSD3B2[a]	c.244G>A	p.(Ala82Thr)	AR/heterozygous	5	DAS	2.0	BS, M, PH, BUDT
HSD17B3	c.645A>T	p.(Glu215Asp)	AR/heterozygous	5	NS-DSD	2.5	BS, M, PH, UUDT
HSD17B3[d]	c.133C>T	p.(Arg45Trp)	AR/heterozygous	4	NS-DSD	6.0	M, PH
HSD17B3[c]	c.277+4A>T	-	AR/heterozygous	4	NS-DSD	3.0	BS, M, PH
HSD17B3 [d]	c.645A>T	p.(Glu215Asp)	AR/heterozygous	3A	NS-DSD	6.0	M, PH
HSD17B3 [c]	c.133C>T	p.(Arg45Trp)	AR/heterozygous	3A	NS-DSD	3.0	BS, M, PH
HSD17B3[i]	c.133C>T	p.(Arg45Trp)	AR/heterozygous	3B	NS-DSD	9.0	PH
AR	c.6A>G	p.(Glu2=)	XLR/hemizygous	3A	NS-DSD	12.0	Gynaecomastia
AR[f]	c.2095G>A	p.(Ala699Thr)	XLR/hemizygous	3A	DAS	9.0	PH
CYP17A1[l]	c.62G>A	p.(Arg21Lys)	AR/heterozygous	3A	NS-DSD	6.0	M, PH
CYP11A1[i]	c.235G>A	p.(Val79Leu)	AR/heterozygous	3B	NS-DSD	9.0	PH
CYB11B1	c.542G>C	p.(Arg181Pro)	AR/heterozygous	3B	NS-DSD	9.0	PH
CYP11B1	c.1182C>G	p.(Asn394Lys)	AR/heterozygous	3B	NS-DSD	8.5	M, UUDT
Hypogonadotropic Hypogonadism							
ANOS1	c.739C>T	p.(Arg247*)	XLR/hemizygous	5	LHD	7.0	M, BUDT
ANOS1[j]	c.1952G>C	p.(Arg651Pro)	XLR/hemizygous	3A	LHD	5.0	BS, PH, BUDT
PROK2	c.297dupT	p.Gly100Trpfs*22	AD/heterozygous	4	NS-DSD	3.0	BS, M, PH
CHD7[i]	c.524C>T	p.(Ser175Leu)	AD/heterozygous	3A	NS-DSD	9.0	PH
CHD7[g]	c.7945G>A	p.(Val2649Ile)	AD/heterozygous	3A	LHD	8.5	M, UUDT
CHD7[e]	c.6304G>T	p.(Val2102Phe)	AD/heterozygous	3A	NS-DSD	3.0	BS, M, PH
POR [b]	c.830+2dup	p.(?)	AR/heterozygous	3A	NS-DSD	7.0	M, DH
POR[j]	c.1664A>T	p.(Gln555Leu)	AR/heterozygous	3B	LHD	5.0	BS, PH, BUDT
POR[e]	c.683C>T	p.(Pro228Leu)	AR/heterozygous	3B	NS-DSD	3.0	BS, M, PH
FGF8 [g]	c.551G>A	p.(Arg184His)	AD/heterozygous	3A	LHD	8.5	M, UUDT
WDR11	c.1066G>A	p.(Val356Ile)	AD/heterozygous	3A	NS-DSD	6.0	M, PH
KISS1R[l]	c.1167C>A	p.(Cys389*)	AR/heterozygous	3A	NS-DSD	6.0	M, PH
SPRY4	c.722C>A	p.(Ser241Tyr)	AD/heterozygous	3A	NS-DSD	2.5	BS, M, PH, UDT
LHB	c.114_115delinsGA	p.Glu39Lys	AR/heterozygous	3B	DGD	9.0	Anorchia
PROKR2[m]	c.889G>A	p.(Val297Ile)	AD/heterozygous	3B	NS-DSD	9.0	PH
FGFR1	c.449-7C>T	p.(?)	AD/heterozygous	3B	LHD	7.0	M, BUDT
SOX10[k]	c.1284G>T	p.(Met428Ile)	AD/heterozygous	3B	NS-DSD	2.0	BS, M, PH, BUDT
SOX10[k]	c.1241A>C	p.(His414Pro)	AD/heterozygous	3B	NS-DSD	2.0	BS, M, PH, BUDT
Other							
DHCR7[m]	c.1337G>A	p.(Arg446Gln)	AR/heterozygous	5	NS-DSD	9.0	PH
DHCR7 [e]	c.964-1G>C	p.(?)	AR/heterozygous	5	NS-DSD	3.0	BS, M, PH
MAMLD1	c.176delC	p.(Thr59Met)	XLR/hemizygous	4	NS-DSD	6.0	BS, PH
MAMLD1	c.605C>T	p.(Thr202Met)	XLR/hemizygous	3A	NS-DSD	10.0	MH
MAMLD1[f]	c.2149G>A	p.(Gly717Ser)	XLR/hemizygous	3A	DAS	9.0	PH
MAMLD1	c.605C>T	p.(Thr202Met)	XLR/hemizygous	3A	NS-DSD	6.0	M, PH
RSPO1	c.4C>T	p.(Arg2Trp)	AR/heterozygous	3B	NS-DSD	10.5	BUDT
RSPO1	c.115G>A	p.(Ala39Thr)	AR/heterozygous	3B	NS-DSD	6.0	BS, PH

[a-m]: Each letter represents a patient with more than one variant identified.

* Class 3 variants of uncertain clinical significance (VUS) were divided into 3A and 3B depending on whether the phenotype was consistent or not.

Abbreviations: BS – bifid scrotum, M – micropenis, DH – distal hypospadias, MH – middle hypospadias, PH – proximal hypospadias, UUDT – unilateral undescended testis, BUDT - bilateral undescended testes.

Table 2. Validated and reported gene variants identified in XY boys by either single gene analysis, seven gene panel or fifty-six gene panel

Conclusions

The extent of undermasculinisation in boys with DSD seems to be unrelated to the presence of molecular genetic or endocrine abnormalities. The increased use of NGS, needs to be coupled with rigorous and standardised processes for interpretation of results.

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Sex differentiation, gonads and gynaecology or sex endocrinology

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