Investigation of primary adrenal insufficiency (PAI) in children with 46,XY differences in sex development (DSD)

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Background

- When a baby presents with atypical genitalia, the most important diagnosis to consider is 21-hydroxylase deficiency (21OHD, CAH, 46,XX)
- However, primary adrenal insufficiency (PAI) can also occur in 46,XY children with differences in sex development (DSD), although this is less common
- Known causes of 46,XY DSD-PAI include:
  - High blocks in steroidogenesis (*STAR*, *CYP11A1*)
  - Steroidogenic enzyme defects (*HSD3B2*, *CYP17A1*, *POR*)
  - Syndromes (Smith-Lemli-Opitz, *DHC7R*; IMAGe, *CDKN1C*; MIRAGE, *SAMD9*)
  - Defects in steroidogenic factor-1 (*NR5A1*)
- The relative prevalence of these conditions is not known, nor how best to investigate for them in 46,XY DSD

Aims

- To establish the prevalence of PAI for all children with a 46,XY karyotype
- To address the role of biochemical testing in diagnosing PAI in 46,XY children presenting with atypical genitalia when no other relevant associated features are present

Methods

- Case notes were reviewed of 316 children with 46,XY DSD presenting to a single tertiary centre multidisciplinary team over 25 years
- Children were identified who had been diagnosed with PAI and treated with steroid replacement
- Clinical, biochemical, and genetic data were obtained
- Basal cortisol, standard synacthen-stimulated peak cortisol and incremental rise, and basal ACTH were analysed for those children with PAI, and compared to a "control" group of children being investigated for 46,XY DSD with normal adrenal function in the first 6 months of life (n=38)
- Assays were performed on an Immulite chemiluminescent immunoassay analyser (cortisol: solid-phase, competitive; ACTH: solid-phase, two-site sequential)
- Key parameters of test performance were calculated

Results - I

- A total of **10 out of 316 (10/316, 3.2%)** children with 46,XY DSD were diagnosed with PAI

Results - II

- Five of these children (1.6%) were first diagnosed with PAI
- Three of them were 46,XY phenotypic girls in whom DSD was not suspected (two with congenital lipoid adrenal hyperplasia (*STAR*), one with complete 17α- hydroxylase/17,20 lyase deficiency (*CYP17A1*)) and two of them had syndromic features that led to the diagnosis (Antley-Bixler Syndrome (*POR*), IMAGe Syndrome (*CDKN1C*))

Subject	Diagnosis	SOR	Age at presentation	Clinical presentation	Genetics
1	STAR	F	Shortly after birth	Salt losing	+
2	STAR	F	8 days	Investigated as positive family history; Na 125, K 8.2	+
3	17α-OHD	F	5.4 years	Hypertension, shock-like collapse; K 2.9	+
4	POR	F	Shortly after birth	Antley-Bixler features, craniosynostosis, meningocele, spine, pharynx, cardiac anomalies, genitalia (bilateral UDT)	+
5	IMAGe	M	Shortly after birth	Fetal growth retardation, skeletal features, salt losing, genitalia (mild hypospadias, R UDT)	+

- Five children (1.6%) presented with 46,XY DSD/genital differences and were diagnosed with PAI through adrenal investigations
- Two of them had 3β-hydroxysteroid dehydrogenase deficiency type 2 (*HSD3B2*), two had partial 17a- hydroxylase/17,20 lyase deficiency (*CYP17A1*), and one had fetal growth restriction/PAI of unknown aetiology

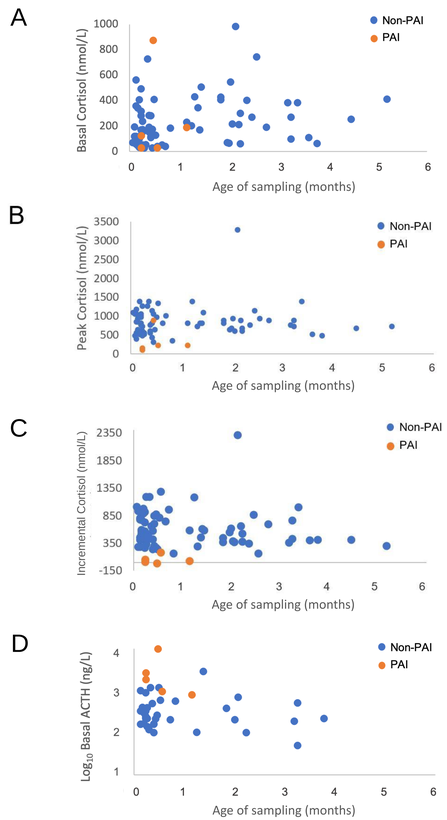
Subject	Diagnosis	SOR	Age at investigation	Basal cortisol (nmol/L)	Peak synacthen (nmol/L)	Incremental synacthen (nmol/L)	ACTH (ng/L)	Clinical presentation	USP	Genetics
6	3β-HSD2	M	15 days	874	854	-20	973	Genitalia; mild hyperpigmentation	+	+
7	3β-HSD2	M	19 days	123	123	0	266	Genitalia	+	+
8	17α-OHD	M	17 days	<28	203	175	96.1	Genitalia; mild hyperpigmentation	+	+
9	17α-OHD	M	6 days	28	74	46	183	Genitalia	+	+
10	FGR complex	M	1 month	188	206	18	79.2	Genitalia, multisystem	-	N/A

Note: Subject 6 had cortisol measured on a on Roche assay

- In some situations, hyperpigmentation, salt loss and ancestral background provided additional clues
- Urine steroid profiles and genetic testing were usually diagnostic, but take longer to obtain a result

Results - III

Scatter plots of *(A)* basal cortisol, standard synacthen-stimulated *(B)* peak cortisol, *(C)* incremental cortisol and *(D)* $\log_{10}$ basal ACTH sampled for the cohort



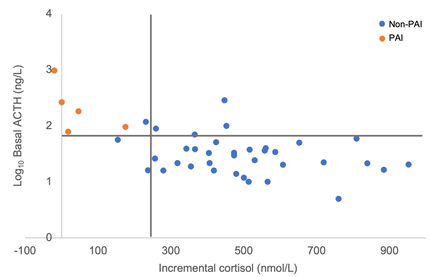
Results - IV

- In this cohort, basal ACTH (>60 ng/L) had a sensitivity of 100% and specificity of 84.2%
- Performance data for basal cortisol (<150 nmol/L) and synacthen-stimulated peak or incremental cortisol are shown in the table below

Investigation	Sen (%)	Spec (%)	Pos. LR	Neg. LR	PPV (%)	NPV (%)	Accuracy (%)
Basal cortisol (<150 nmol/L)	60.0 (14.7- 94.7)	60.5 (43.4-76.0)	1.52 (0.7-3.4)	0.66 (0.2-2.0)	16.7 (8.1-31.2)	92.0 (79.2-97.2)	60.5 (44.4-75.0)
Basal ACTH (>60 ng/L)	100 (47.8-100)	84.2 (68.8-94.0)	6.3 (3.0-13.2)	0	45.5 (28.6-63.5)	100.0	86.1 (72.1-94.7)
Peak cortisol (Synacthen) (<450 nmol/L)	80.0 (28.4-99.5)	92.1 (78.6-98.3)	10.1 (3.1-32.7)	0.22 (0.04-1.3)	57.1 (29.3-81.1)	97.2 (85.8-99.5)	90.1 (77.9-97.4)
Incremental cortisol (Synacthen) (<250 nmol/L)	100 (47.8-100)	92.1 (78.6-98.3)	12.7 (4.3-37.5)	0	62.5 (36.0-83.2)	100	93.0 (80.9-98.5)
Basal ACTH (>60 ng/L) AND Incremental cortisol (Synacthen) (<250 nmol/L)	***100 (47.8-100)***	***97.4 (86.2-99.9)***	***38 (5.5-262.9)***	***0***	***83.3 (42.0-97.2)***	***100***	***97.7 (87.7-99.9)***

Note: Sen, sensitivity; Spec, specificity; LR, likelihood ratio; P/NPV, positive or negative predictive value

- Among all permutations, ***combined basal ACTH (>60 ng/L) AND standard synacthen-stimulated incremental cortisol (<250 nmol/L)*** had the best performance in identifying PAI among 46,XY DSD children in this small cohort



Horizontal grey line: Log_{10} ACTH 1.78 (i.e. ACTH = 60ng/L); Vertical grey line: Cortisol 250 nmol/L

Conclusions

- **PAI in 46,XY DSD is an important diagnosis to consider with short- and long-term consequences, but it is uncommon (3.2% of our cohort, over 25 years)**
- **For those children presenting primarily with DSD/genital differences, basal ACTH is a useful investigation with high sensitivity, if turn-around time is quick**
- **Combined basal ACTH (>60 ng/L) AND incremental cortisol (<250 nmol/L) had the best performance in identifying PAI among 46,XY DSD children in this cohort**

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