Fig 3:
Urine steroid analysis by gas chromatography-mass spectrometry. Chromatograms for the patient and a control subject are compared. Steroid conjugates were extracted from urine, separated into two fractions by Sephadex LH-20 liquid-gel chromatography before conjugate hydrolysis and formation of MO-TMS derivatives. A,B,C: internal standards androstanediol, stigmasterol and cholesterol butyrate respectively. Metabolites of the following steroids are shown: cortisol, blue; DHA, green; pregnenolone, purple.

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A novel mutation of HSD3beta2 presenting as hypospadias with sat-wasting in a male infant

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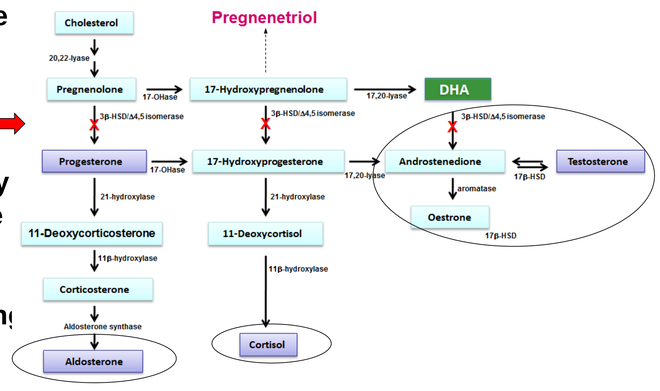
Background

Deficiency of the enzyme HSD3beta2 is a rare cause of autosomal recessive primary adrenal insufficiency associated with Disorder of Sexual Development (DSD). Cortisol, aldosterone and sex steroid deficiencies occur. Males are typically undervirilised, yet paradoxically some females may be mildly virilised through testosterone production by "backdoor" conversion pathways using iso-enzyme HSD3beta1. Salt-loss, hyponatraemia and need for mineralocorticoid replacement is variable according to specific defect. As with 21 OH-Def (CYP21) there may be mild non-classic late presenting variants.

Steroid synthesis pathways



Case Presentation

Term infant. SVD. BWt 3410g. Uneventful pregnancy. 1st child to healthy unrelated parents. Ethnicity: Black Caribbean. O/E perineal hypospadias with 1.6 cm length narrow phallus. Testes not palpable but identified as normal size at inguinal ring on USS. No Mullerian pelvis structures. No hypoglycaemia. Normal feeding. Clinically well.

Further investigations (Day 2) for "DSD":

Results:(1)

Day 2: LH <0.7 / FSH <0.7 IU/L

Day 5: Short Synacthen test: 62.5 mcg IV

- Cortisol: 0min 110 /30 min 147 / 60min 109 nmol/L (N > 550)

Genetics: Rapid PCR sex chromosomes XY (result Day 6)

Array CGH Normal Male (result Day 14)

Interpretation: Adrenal insufficiency - ? associated with hypopituitarism in view of micropenis and hypospadias. Primary adrenal insufficiency not considered. No discussion with Specialist Paeditaric Endocrine Team. Failure to assess pituitary thyroid axis

Management:
Patient started Hydrocortisone 1.25 mg oral 6-hourly
Discharged with follow-up planned :
~ 1 month neonatal clinic
+ Paeditaric Urology referral for hypospadias

Progress:

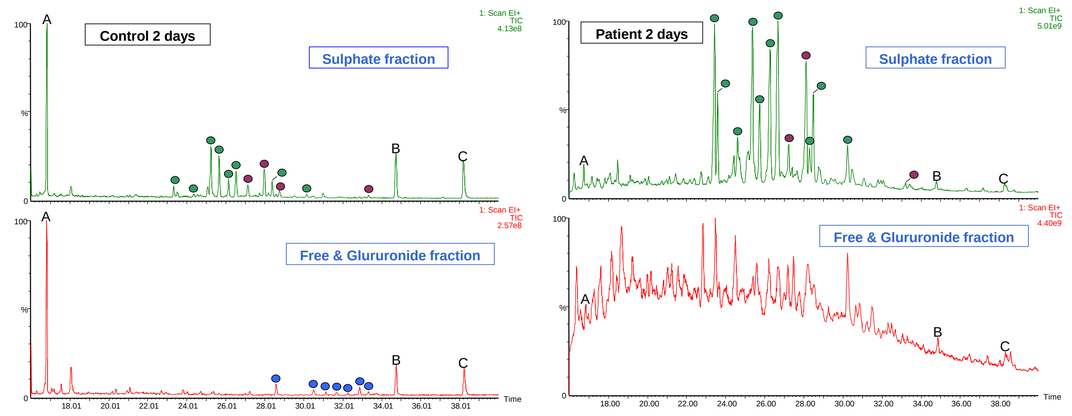
Age 26 days referred by GP to AED with h/o 1 day vomiting after feeds.
Wt 3520 g; sunken fontanelle / eyes.

Plasma Na 108, K 7.1, Urea 11.7 nmol/LCreat 51 micromol/L Urine Na <20 / K 33 mmol/L
(Plasma Renin activity 193 mU/L / Aldosterone 240 .

Management:

IV Saline 0.9% resusc. bolus then 100ml/kg /day
IV Hydrocortisone 6 hrly + Ca gluconate; Nebulised salbutamol
Convert to oral hydrocortisone 2.5 mg x 3 /day within 48 hrs.
Add Fludrocortisone + oral Nacl 30% supps. ~ 5mmol/kg/day (1 dose / feed)

Methods & Results:(2) Urine steroid profiles



Urine steroid analysis by gas chromatography-mass spectrometry. Chromatograms for the patient and a control subject are compared. Steroid conjugates were extracted from urine, separated into two fractions by Sephadex LH-20 liquid-gel chromatography before conjugate hydrolysis and formation of MO-TMS derivatives. A,B,C: internal standards androstanediol, stigmasterol and cholesterol butyrate respectively. Metabolites of the following steroids are shown: cortisol, blue; DHA, green; pregnenolone, purple.

Results:(3) Steroid hormone levels (Collected Day 2):

Testosterone 15 nmol/L ↑

Androstenedione >35 nmol/L ↑)

17 OHProg 40 nmol/L ↑) *– not available > 2 weeks later*

11DOC 49 nmol/L ↑)

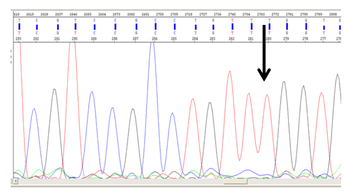
DHEAS / DHT *- requested but no results*

These results are unexpected in 3BHSD2 deficiency and could represent spurious cross-reactivity of steroids in immunoassays, or"backdoor" conversion of exaggerated DHEA levels by 3BHSD1 (Refs 1, 2)

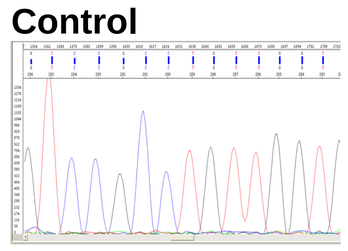
Methods &B Results:(4) DNA analysis

Patient c.65dupT

- **DNA was isolated from whole blood**
- ***HSD3B2* was amplified in 3, non-overlapping fragments followed by Sanger sequencing of individual exons and intron/exon boundaries**
- **Sequence was compared to reference sequence NG_013349.1. Nomenclature follows HGVS guidelines (www.hgvs.org)**



Control



Exon 2 of *HSD3B2* from patient (top) showing homozygosity for an insertion of one nucleotide, c.65dupT. The change leads to a frame shift mutation, p.Leu22Phefs*27

Parental genetic studies awaited

Progress at 9/2016:

Age 10 months thriving. No further illness. Developmentally normal.
Ht / Wt 50th centile.
Penile Length straight without significant chordae 3.2 cm erect
Rt testis 2ml in scrotal sac. Lt Testis still high at inguinal ring.

Treatment:

Hydrocortisone 2.5 mg oral x 3 / day
Fludrocortisone 200 mcg x 1 / day
30% NaCl 5mmol x 5 day with milk/feeds.

Conclusions & Learning points

Urine steroid profile more specific towards definitive diagnosis steroid synthesis disorder than plasma steroid hormone levels
TMSsteroid analysis better than RIA steroids
Hypospadias NOT a recognised common feature of Hypopituitarism
Disuss DSD case with Endorcine Specalist Team
Life threatening congenital disorders

References :

1) A case of 3beta-hydroxysteroid dehydrogenase type II (HSD3B2) deficiency picked up by neonatal screening for 21-hydroxylase deficiency: difficulties and delay in etologic diagnosis. Horm.Res. 2007 68: 204-8.
2) A novel homozygous Q334X mutation in the HSDB32 gene causing classic sBeta-hydroxysterod dehydrogenase deficiency: an unexpected diagnosis after after a positive newborn screen for 21-hydroxylase deficiency. Horm Res Paediatr. 2012. 77:334-8

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