CYP11A1 (side-chain cleavage enzyme) defect in three brothers causing glucocorticoid and mineralocorticoid deficiency and development of testicular adrenal rest testicular tumour

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Introduction - Aim

- **The CYP11A1 gene encodes the cholesterol side-chain cleavage enzyme, P450scc which plays a key role in the initial steps of steroidogenesis.**
- **CYP11A1 mutations result in a rare form of congenital adrenal hyperplasia with a wide clinical spectrum ranging from severe early onset primary adrenal insufficiency (PAI) in the neonatal period, with 46,XY DSD; to late-onset PAI with normal genitalia.**
- **Aim: To describe long term outcome in three family members sharing a newly described compound heterozygous CYP11A1 mutation pattern.**

Methods

- **Initial genetic studies were performed following consent from the parents and children in 2002.**
- **The following markers were used to determine which X-chromosome had been inherited by each member of the second generation: DXS989-[AHC]-DXS992-3'DYS-STR50-STR49-STR45-STR44, Four overlapping primer sets covering the 2 exons of the DAX-1 gene were then used to sequence the middle brother's DAX-1 gene: DX1, DX2, DX3 and DX4.**
- **Subsequent genetic studies were performed in 2016 as part of an initiative to assess the prevalence of CYP11A1 deficiency in PAI using HaloPlex targeted Gene Panel analysis [1]**

Family study (see Figure 1)

- **The index case II-5 was admitted aged 3.7 years with a prolonged convulsion, initially thought to be febrile in nature (temperature 38.2°C).**

- **Past medical history**
  - **Born at term weighing 2800 g; well at delivery; normal external genitalia.**
  - **Several presumed "febrile" convulsions during infancy**
  - **Admitted with collapse at 18 months , presumed to be septicaemic in cause, with three brief episodes of arm twitching. Admitted to ICU and required ventilation for "shock lung".**

- **Family history**
  - **Non-consanguineous Scottish family**
  - **Mother (30 Yrs): History of convulsions as a child, learning disability, chronic alcoholism**
  - **Father (54 Yrs): Aortic stenosis (valve replacement x 2)**
  - **Siblings:**
    - ✓ **II-1 (10.6 Yrs): History of febrile seizures as a child but normal intelligence**
    - ✓ **II-2 (8.9Yrs): Several convulsions during infancy; developmental delay; special educational needs**
    - ✓ **II-3 (8 Yrs): Frequent convulsions during infancy; hypotonic (tested for Duchenne and fragile X); also attending special school**
    - ✓ **II-4 (6.2 Yrs): Healthy**

Examination	Biochemistry
• Deeply pigmented skin • Height: 94cm [-1.5 SDS] • Weight: 13.8 kg [-1.13 SDS] • Blood pressure: 90/70 • Normal prepubertal external genitalia • Rest of clinical examination normal	• Na+= 128 mmol/l (NR:135-145) • K+ = 5,9 mmol/l (NR:3.7-4.8) • Fasting plasma glucose= 2.8mmol/l • ACTH= 1089 mU/l (N<20) • Basal/peak cortisol after synacthen 174/178 nmol/l • Plasma renin activity= 1209 µU/ml (NR:9-50)

- **Assessment in brothers II-2 and II-3 at 8 and 9 years showed that they too were pigmented with normal electrolytes; basal/peak cortisol 339/389 and 278/289 nmol/l**
- **No history to indicate salt wasting (e.g. vomiting or salt craving) but diet high in salt.**

- **Genetics 1: Glasgow (2002)**
  - **Linkage studies showed that all three brothers had inherited the same critical regions of the maternal X chromosome suggesting an X-linked disorder, but analysis of NR0B1 (DAX-1, adrenal hypoplasia) and ABCD1 (adrenoleukodystrophy) were negative. Triple A syndrome sequencing normal**

  ➔ **Conventional glucocorticoid and mineralocorticoid replacement for unclassified congenital PAI**

- **Follow-up during childhood and adolescence: All three brothers completed puberty- delayed in II-2 and II-3 with G2/G4-5 at 13.1/16.9 and 14.2/17 years.**

- **Genetics 2: London 2016**
  Studies of CYP11A1 gene showed all three brothers to be compound heterozygotes for two genetic changes:
  - ✓ **c.790_802del, K264Lfs*5 - a known disruptive variant causing frameshift and premature stop codon**
  - ✓ **rs6161 (c.940G>A, p.Glu314Lys) – a relatively common variant, previously been predicted as benign/ affects splicing when combined with the disruptive variant**

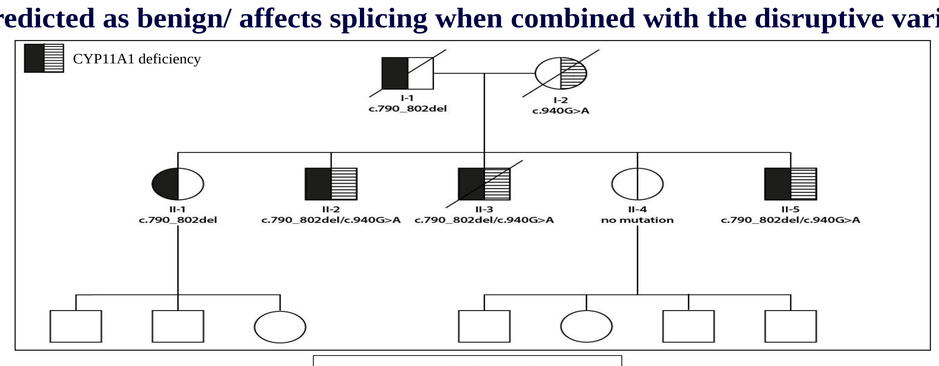

Figure 1. F Family tree

- **Adult follow-up:**
  - **In 2017 aged 37, 36 and 32 years the brothers were stable on hydrocortisone and fludrocortisone replacement, with normal pubic hair, testicular volumes (15-20 ml), and serum testosterone (27.2, 33.3 and 24.7nmol/L) but FSH values 41.2, 9.3 and 13.8 u/L.**
  - **II-3 suffered from epilepsy and died subsequently during a prolonged convulsion**
  - **II-5 had undergone L orchidectomy for suspected malignancy aged 25 years. Initial histology was reported as showing nodular Leydig cell hyperplasia, revised to testicular adrenal rest tumour (TART) in the light of CYP11A1 findings (Fig.2)**

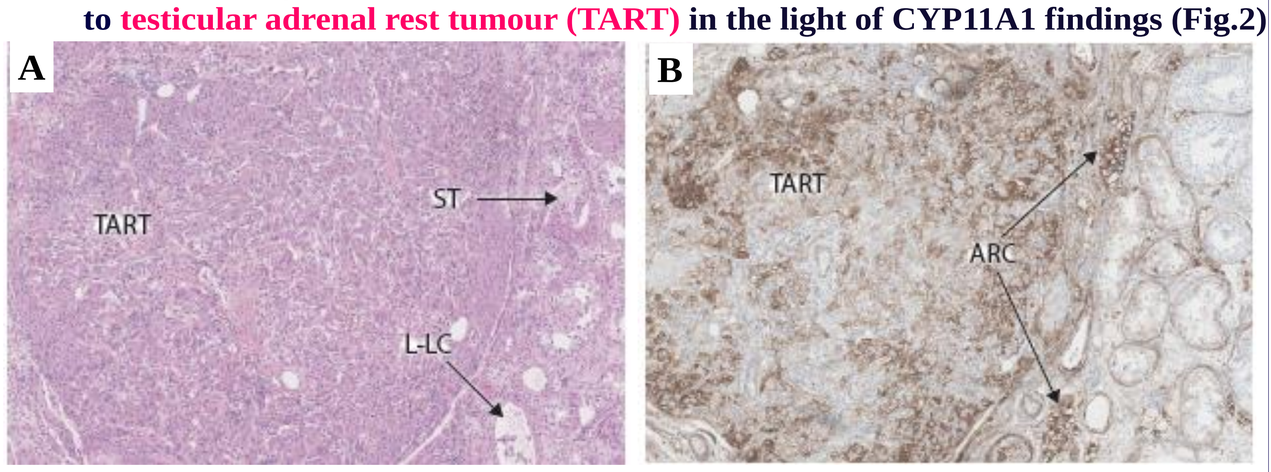

Figure 2. Testicular histology following radical orchidectomy for suspected malignancy in II-5.
A: Staining with haematoxylin and eosin shows the nodules of testicular rest cell tumour (TART), the seminiferous tubules (ST) and lipid-laden cells (L-L C)
B: Staining with CD56 shows uptake by some cells in the TART nodule, adrenal rest clumps (ARC) but not the Leydig cells or seminiferous tubules.

- **II-5 – TART and fertility issues**
  - **II-5 and his partner had been trying for a baby for 4 years and have been enrolled for treatment with in vitro fertilization.**
  - **Semen analysis showed oligo-zoospermia with abnormal morphology of the sperms and an increased risk of foetal abnormality.**

Summary and conclusions

- **Partial CYP11A1 defect is emerging as a surprisingly common cause of previously undiagnosed PAI.**
- **This kinship demonstrates the importance of precise diagnosis, which could have identified TART as the cause of testicular enlargement thus avoiding orchidectomy in the younger brother.**

References: 1.Maharaj et al Predicted Benign and Synonymous Variants in CYP11A1 Cause Primary Adrenal Insufficiency Through Missplicing. J Endo Soc 2019;3:201-221.