A novel human CYP19A1 deletion-insertion mutation reveals that the C-terminus of the aromatase protein is crucial for its activity

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Background

The steroidogenic enzyme aromatase is encoded by the *CYP19A1* gene. Aromatase activity is required for estrogen biosynthesis from androgen precursors in the ovary and several extragonadal tissues. The role of aromatase and thus estrogens for human biology is best illustrated by disease states, both deficiency and excess which might be caused by genetic disorders.

Aim

A novel deletion-insertion mutation spanning from intron 10 to the 3' UTR of the CYP19A1 gene was found in a 46,XX girl presenting with ambiguous genitalia at birth. The mother virilized during pregnancy and parents were first cousins. We investigated this novel mutation genetically and performed functional and structural studies to characterize the role of the C-terminus of the aromatase protein

Case presentation

An eight-day-old newborn was referred to our clinic for assessment of ambiguous genitalia. During the pregnancy, the mother, developed progressive signs of virilisation from 12 WA. Biochemical testing confirmed hyperandrogenism with high testosterone levels (Table1). After delivery, the virilization signs slowly decreased together with her serum testosterone levels.
Genital examination of the newborn showed a 1.5 cm clitero-phallus, with complete posterior labial fusion and a single meatus opening at the base of the genital tubercle. The genital folds were scrotalised, no gonads were palpable (Fig2).

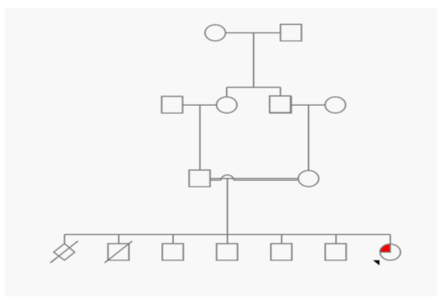

Figure 1 : *Family tree*

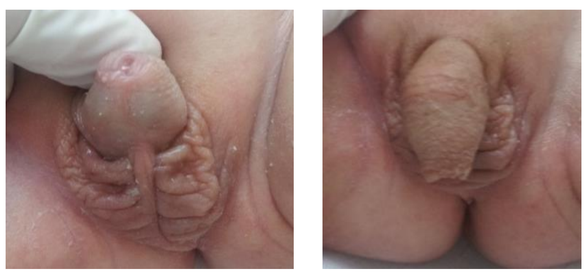

Figure 2 *: Genitalia at first examination:*

Pelvic ultrasounds showed a uterus, the gonads were not visualized. Karyotype was 46,XX. 17αOHP and rennin levels were normal, ruling out classic 21-hydroxylase deficiency. Testosterone levels were slightly elevated at birth but decreased to normal ranges by one month. Oestradiol levels were low (Table 2).

	Table1: Biochemical measurements from the mother during the pregnancy and after delivery				Table2: Biochemical measurements from the patient at different age time points		
	18 WG	4th day	10th day	1 month	1 day	9 days	4 months
Testosterone (nmol/l)	**34.3**	**47.1**	**18.1**	0.99	**3.41**	**2.03**	0.38
17 OHP (nmol/l)	0.18		8.37	0.11	55.9	11.92	1.04
DHEAS (µg/dl)	64.7		197.71	177		49.35	
Δ4Androstenedione (ng/ml)		1.39	18.76	0.91		1.39	
Cortisol (nmol/l)	204.3		447.9		504.3	305.42	
Renin						70.96	
Oestradiol (pg/ml)				**<5**		**<5**	
B-hCG (UI/L)		30.73		<1.20			
A-FP (ng/ml)		51.63		5.20			

17OHP: 17 α-Hydroxyprogesterone, DHEAS: Dehydroepiandrosterone Sulfate, βHCG: beta Human chorionic gonadotrophin, αFP: Alpha-foeto-protein

Genetic analysis

Direct sequencing of the *CYP19A1* gene revealed a deletion of 2081 nt starting in intron 10 to the 3' UTR corresponding to c.1263+354_*922del (Fig3).
Minigene experiments confirmed that this deletion prevented splicing leading to a shorter protein of 426 aa (p.P423_H503delinsRALP). Aromatase activity of WT and mutant CYP19A1 was assessed in transiently transfected HEK293 cells using radiolabeled androstenedione as substrate and the tritiated water release assay to measure conversion to estrone. Compared to WT, the mutant aromatase enzyme showed complete loss of activity. Structure analysis suggested that the C-terminal membrane anchor and heme binding cysteine residue were deleted in the mutated protein. The mutated protein was predicted not to bind heme and would therefore have no enzymatic activity (Fig4).

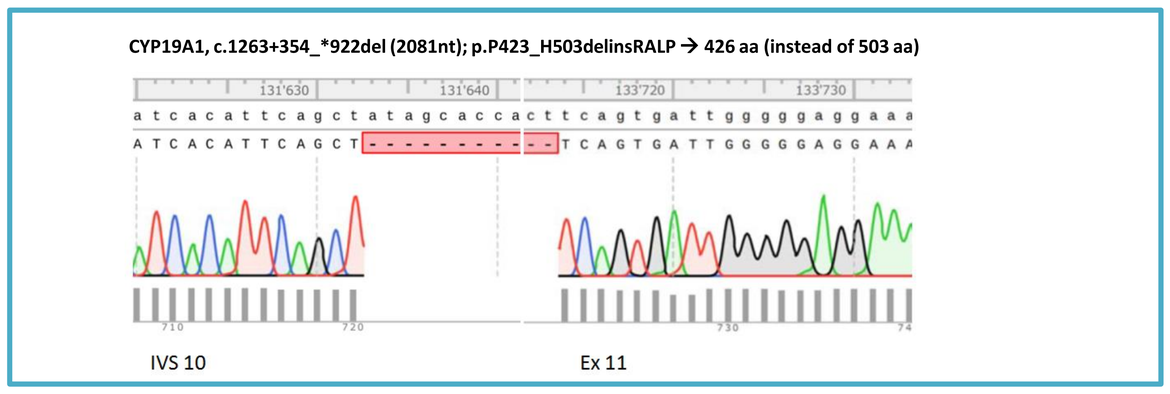

Figure 3**:** *2081-nt deletion, including IVS10 (911nt) to ex11 (1171nt)*

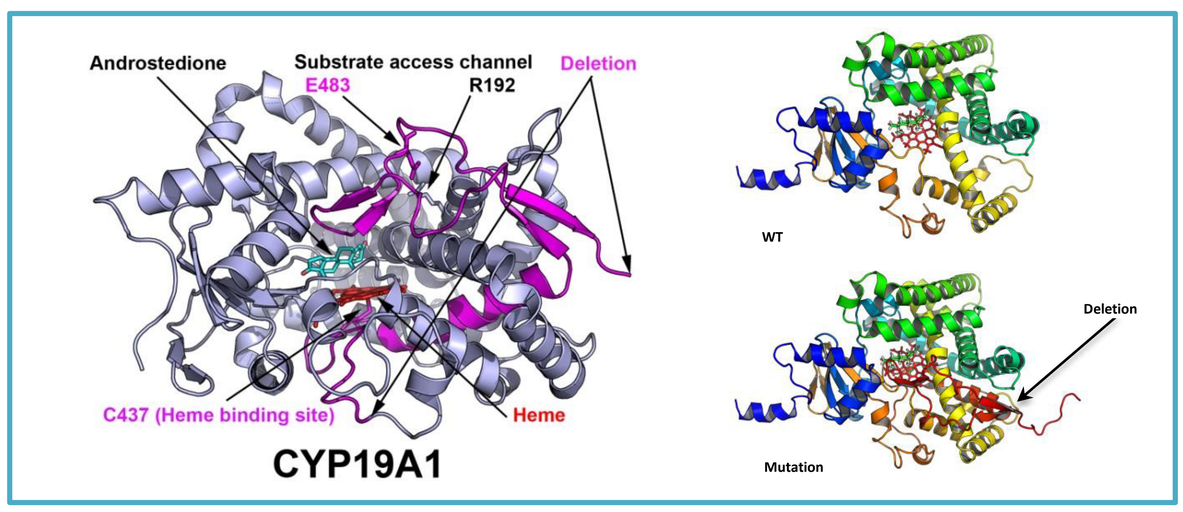

Figure 4*: X-Ray Crystal structure of human aromatase showing missing amino acids in truncated protein. Aromatase is shown as a ribbons model in light blue, missing amino acids are shown in magenta. Heme (red) and androstenedione (cyan) are shown as stick models. In the truncated protein structrally important residues involved in heme binding (cysteine 437) and substrate access (glutamic acid 483) are missing. Cysteine 437 anchors the active site heme and is critical for P450 activity. The glutamate 483 – arginine 192 interaction forms the substrate access channel in aromatase and loss of glutamate 483 will result in an open binding site without control over substrate entry or exit. The resultant protein will have total loss of activity and is expected to be denatured and cleaved by proteases in the cell.*

Conclusion

The c.1263+354_*922del *CYP19A1* mutation codes for a C-terminally truncated aromatase protein which causes a severe phenotype of aromatase deficiency in humans. In line with the phenotype, this aromatase mutation has no activity *in vitro* indicating that the C-terminus of the aromatase protein is crucial for its activity.

DOI: 10.3252/pso.eu.54espe.2015
Poster presented at: 54th Annual Meeting 9-11 October ESPE 2015 BARCELONA European Society for Paediatric Endocrinology