Cytochrome P450 Oxidoreductase Deficiency: Novel Cause of Ambiguity with Primary Amenorrhea

Sir,

P450 oxidoreductase deficiency (PORD) is an uncommon variant of congenital adrenal hyperplasia. It is an autosomal recessive condition caused by mutation in the POR (Cyp 450 oxidoreductase) gene which encodes an electron donor for all microsomal Cyp 450 enzymes and several non-Cyp 450 microsomal enzymes.[1] It has an extremely variable phenotype ranging from adrenal insufficiency, skeletal malformations similar to Antley–Bixler syndrome to genital ambiguity present in both sexes. Since it affects the activity of a combination of steroidogenesis enzymes (21-hydroxylase, 17α-hydroxylase/17,20-lyase, and aromatase), it shows a complex pattern of biochemistry and a broad spectrum of clinical severity.

Patient Case Presentation

We present a 17-year-old female born of third-degree consanguineous marriage, who came with concerns of poor breast development and primary amenorrhea. She had development of pubic hair at 13 years of age and presently she was tallest among her peers. Parents had noticed clitoromegaly at birth, but there was no progressive increase in size after birth or at puberty, and no consultation was sought for the same. There was no history of any maternal drug intake or maternal antenatal virilization. There was no history of steroid insufficiency or any significant family history.

On examination, she had a height of 164.3 cm with mid-parental height of 153.5 cm. She had a eunuchoid habitus (US: LS ratio: 0.82) and no evidence of hyperpigmentation. Her supine blood pressure was 100/70 mmHg measured in the right upper limb. The breasts were Tanner stage 2 with poor contour whereas pubic hair development was Tanner stage 4. Her genital examination showed a virilization of Prader stage 3 with posterior fourchette fusion and clitoromegaly (clitoral width of 2.5 cm) with single perineal opening. There were no palpable gonads. Her karyotype was 46 XX, and her hormone profile is shown in Table 1.

Ultrasonography of the abdomen as well as a computed tomography scan of the pelvis was done which showed bilateral multicystic ovaries as depicted in Figure 1.

Urinary Steroid Profiling

Urinary steroid profiling was done using gas chromatography mass spectrometry (GCMS), and the result is depicted in Figure 2.

Diagnostic ratios showed relative elevation of progesterone metabolites: pregnanediol (PD), pregnanetriol (PT), as well as the pregnenolone metabolites pregnenidiol (5PT) and the 17-OH-pregnanolone.

Mineralocorticoid metabolites were elevated over cortisol metabolites indicating decreased 17α-hydroxylase activity. This indicates combined impairment of CYP21A2 and CYP17A1 activities according to the diagnostic precursor/substrate ratios, highly indicative of P450 oxidoreductase (POR) deficiency.

Genetic Analysis

Initially, CYP19A1 (aromatase) gene was sequenced but it failed to show any mutation. In view of characteristic urinary steroid profile, sequencing analysis of the POR gene was done which confirmed compound heterozygosity with both novel mutations).

1. g. 26,140 G > A, c. 430 G > A, p.G144S (Exon 4)
2. g. 31,082 G > A, c. 1,265 G > A, p.W422X (Exon 11).

Discussion

PORD is an autosomal recessive disease due to mutation in the POR gene which encodes an electron donor protein required for multiple steroidogenic as well as other enzymes of drug metabolism and musculoskeletal development. The POR gene is located on Chromosome 7q11.23.[1] As multiple enzymes are involved in various combinations, there is a high degree of phenotypic and biochemical variability. It

Table 1: Investigations

Parameters (serum)	Value	Range
TSH	2.6 (mIU/ml)	0.4-4 (μIU/ml)
FSH	33.99 (IU/ml)	2.5–10 (mIU/ml)
LH	55.73 (IU/ml)	2.5–10 (mIU/ml)
17-hydroxyprogesterone progesterone (ng/ml)	3.42	<2
Testosterone (ng/ml)	22.26	0.2–0.8
DHEAS (mcg/dl)	117	
Androstenedione (ng/ml)	1.94	<0.3
Progesterone (ng/ml)	2.7	
Estradiol (pg/ml)	10	
8 am cortisol (μg/dl)	14.5	5–25
ACTH stimulated cortisol (μg/dl)	18.08	>18

DHEAS: Dehydroepiandrostenedione sulfate, TSH: Thyroid-stimulating hormone, FSH: Follicle-stimulating hormone, LH: Luteinizing hormone, ACTH: Adrenocorticotropic hormone

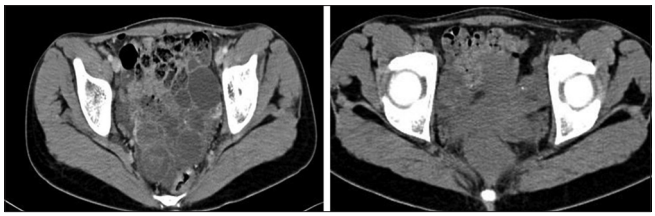

Figure 1: Magnetic resonance imaging showing the multicystic ovaries. Right hand side panel showing the effect of estrogen therapy

is a variant of congenital adrenal hyperplasia with genital ambiguity of variable degree found in both sexes. It is also the only congenital adrenal hyperplasia with associated skeletal malformations similar to Antley–Bixler syndrome which is caused due to mutations in the fibroblast growth factor receptor 2 gene.[2] As in our case, not every case of PORD is associated with skeletal malformations, thus resulting in missed diagnosis. The characteristic malformations of mid-facial hypoplasia, radio-humeral synostosis, and phalangeal abnormalities seen in the Antley–Bixler syndrome are due to lack of electron donor for the enzyme lanosterol 14α-demethylase which is required for cholesterol biosynthesis.

In 1985, Peterson *et al*. had first reported a 46 XY undervirilized infant with low sex steroids and elevated 17-hydroxyprogesterone and 17-hydroxypregnenolone levels suggestive of a combined deficiency of 17α-hydroxylase and 21-hydroxylase.[3] Flück *et al*. found POR mutations in three children with Antley–Bixler syndrome as well as in one woman with primary amenorrhea and polycystic ovaries without skeletal malformations.[4] To date, around 20 mutations in fifty patients have been described. A genotype phenotype correlation was studied in thirty patients by Krone *et al*. which showed a poor correlation.[5] The mutation p.A287P is more common in Caucasians whereas the mutation p.R457H is seen in the Japanese population. Till date, no patient with null mutations on both alleles has been described as such phenotype is incompatible with life.[5] In our patient, there was a compound heterozygosity with novel mutations on both alleles. The limitation in our case is lack of functional studies for this novel mutation.

Characteristic biochemistry which suggests a diagnosis of PORD is a raised or even normal 17OHP but never rose to the extent observed in 21-hydroxylase deficiency. In general, a cortisol deficiency especially a low adrenocorticotropic hormone stimulated cortisol is observed; however, characteristic salt wasting is not seen. A characteristic feature of PORD is a nonprogressive clitoromegaly with pubertal hypogonadism suggestive of intranatal virilization followed by deficient production of sex steroids due to 17–20 lyase and aromatase deficiency. Serum steroids analysis may be misleading; however, the analysis of urinary steroid metabolites by GCMS shows diagnostic ratios characteristic of combined defect especially the ratio of pregnenolone metabolite (pregnanediol) to cortisol metabolites and the ratio of progesterone metabolites (PD) to cortisol metabolites. The cortisol

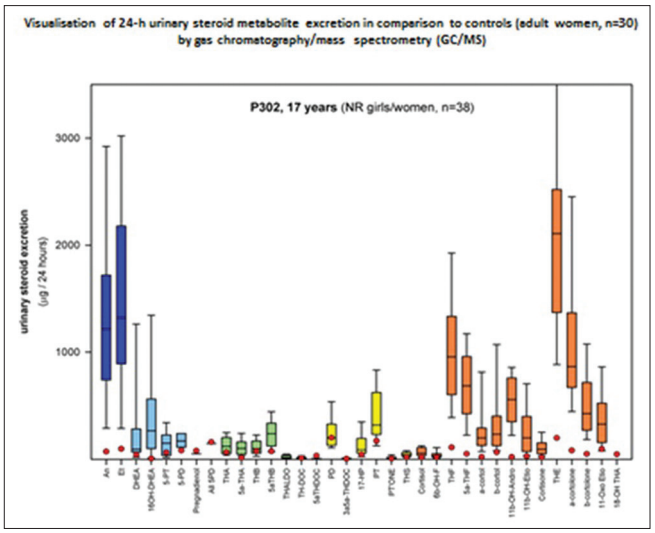

Figure 2: Urinary steroid profiling

metabolites taken are tetrahydrocortisone + tetrahydrocortisol + 5α-tetrahydrocortisol.[5] These ratios are diagnostic right from antenatal period with characteristic maternal urinary profile with low estriol metabolites to all age-groups.[6] Thus, urinary steroid profiling can aid as a screening as well as diagnostic tool for suspected PORD.

The unique features of our case are no maternal antenatal virilization, lack of skeletal malformations, and glucocorticoid deficiency. Hence, a high index of suspicion along with urinary steroid profiling is required for diagnosis in such cases.

Our patient was started on estrogen supplements, following which her ovaries regressed in size as shown in Figure 1 right hand panel. She was then subsequently referred for feminizing genitoplasty.

This case depicts the importance of having a high degree of suspicion in cases of primary amenorrhea with genital ambiguity and multicystic ovaries for PORD. It also brings to light the importance of urinary steroid profiling as a diagnostic modality for steroidogenic defects.

Conclusion

PORD can manifest clinical features similar to those in 21-OHD and in aromatase deficiency. As in our patient, POR mutations may underlie in patients with clinical diagnosis of aromatase deficiency, in whom aromatase gene mutations have not been identified. PORD should be considered as one of the differential diagnoses in cases of primary amenorrhea with genital ambiguity and multicystic ovaries.

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Conflicts of interest
There are no conflicts of interest.

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