

Genetic and Clinical Features of P450 Oxidoreductase Deficiency

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Key Words

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Abstract

P450 oxidoreductase (POR) deficiency is an autosomal recessive disorder of steroidogenesis with multiple clinical manifestations. POR is the electron donor for all microsomal P450 enzymes, including the three steroidogenic enzymes P450c17 (17 α -hydroxylase/17,20-lyase), P450c21 (21-hydroxylase), and P450aro (aromatase). Since the first description of POR mutations in 2004, about 50 patients have been reported. Serum steroid profiles indicate partial deficiencies in 21-hydroxylase, 17 α -hydroxylase and 17,20-lyase. The 17-OH progesterone levels are elevated, as in 21-hydroxylase deficiency, while androgen levels are low; cortisol may be normal but is poorly responsive to adrenocorticotrophic hormone. Most patients also have associated skeletal malformations (craniosynostosis, radio-ulnar synostosis, midface hypoplasia, bowed femora) termed Antley-Bixler syndrome. Antley-Bixler syndrome with normal steroidogenesis is caused by autosomal dominant gain-of-function mutations in fibroblast growth factor receptor 2. Males with POR deficiency are often undervirilized, while females can be virilized. The prognosis for patients with POR deficiency appears to depend on the severity of the bony malformations and

their timely treatment. The potential impact of POR mutations on drug metabolism by other hepatic P450 enzymes requires further investigation. Given the varied physical and biochemical phenotype of POR deficiency and the risk of adrenal insufficiency, clinicians should be alert to this potential diagnosis.

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Introduction

Most forms of congenital adrenal hyperplasia (CAH) are caused by mutations in genes encoding steroidogenic enzymes; mutant genes result in diminished or absent enzymatic activity, so that the clinical signs and symptoms are caused by accumulation of some steroidal precursors and/or decreased production of the principal steroidal end products. Two forms of CAH are not caused by mutations in steroidogenic enzymes: congenital lipoid adrenal hyperplasia, caused by mutations in the steroidogenic acute regulatory protein [1], and the recently described P450 oxidoreductase (POR) deficiency [2–5]. POR deficiency is the most complex of the various forms of CAH, because it affects the activity of several steroidogenic enzymes, yields a complex and variable pattern of abnormal steroid hormones, has a broad spectrum of clinical severity, and affects a number of ‘non-endocrine’ systems, including skeletal development and drug me-

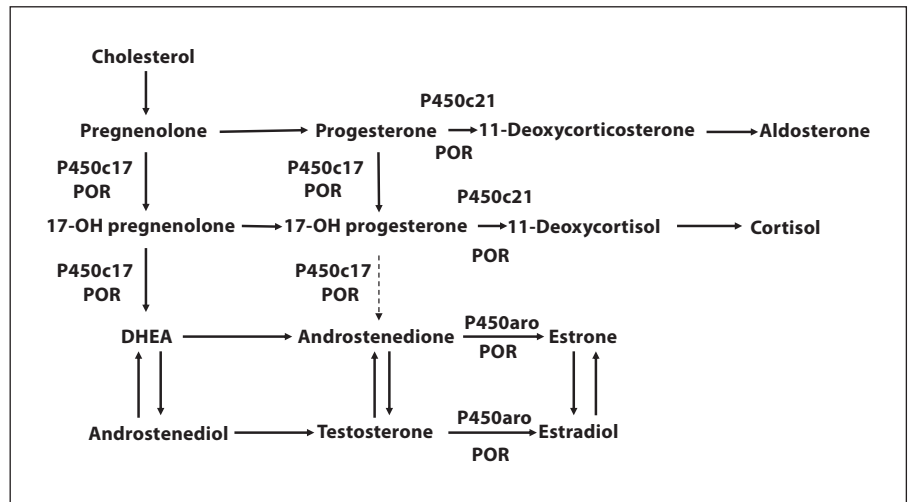


Fig. 1. Simplified steroid biosynthetic pathway indicating the steps where POR acts as a cofactor. POR supports the activities of P450c17, P450c21, and P450aro. Because the 17,20-lyase activity of human P450c17 does not effectively convert 17-OH progesterone (17-OHP) to androstenedione, 17-OHP accumulates when the 21-hydroxylase activity is impaired. The 17,20-lyase activity of P450c17 is more sensitive to perturbations in electron transfer

than is its 17 α -hydroxylase activity, so that the synthesis of DHEA, androstenedione, testosterone, and estradiol is more severely affected. Thus, the typical patient with POR deficiency will have a mildly elevated 17-OHP and low 19-carbon steroids. The combined partial impairment of 17 α -hydroxylase activity and 21-hydroxylase activity may also compromise cortisol synthesis.

tabolism. However, POR deficiency appears to be fairly common, with about 50 cases being described in the 3 years following the first report, hence endocrinologists, geneticists, and others need to become familiar with this newly characterized disorder.

P450 Enzymes and POR

Cytochrome P450 enzymes, named for their characteristic spectral shift at 450 nm, perform a wide variety of oxidative reactions. The human genome encodes 57 distinct P450 enzymes; 7 of these are type I, found in mitochondria, and 50 are type II, found in the endoplasmic reticulum. Type I P450 enzymes receive electrons from NADPH via an electron transfer chain consisting of two proteins, ferredoxin and ferredoxin reductase, whereas type II P450 enzymes receive electrons from NADPH via a single redox partner, POR [6]. Three of the 50 type II P450 enzymes are involved in steroidogenesis: P450c21, the adrenal 21-hydroxylase; P450c17, which catalyzes both 17,20-lyase and 17 α -hydroxylase activities, and P450aro, which aromatizes androgens to estrogens (fig. 1).

POR is an 82-kDa flavoprotein associated with the endoplasmic reticulum. Human POR contains 680 amino acids, whereas POR from rodents and most other species contains 677 amino acids. The human gene was identified on chromosome 7q11.2 as part of the Human Genome Project. The gene consists of 15 protein-coding exons spanning 32 kb (named exons 1–15), but it also has a non-coding exon (termed exon 1U) located 38 kb upstream [7].

Unlike ferredoxin reductase, which has only one flavin adenine dinucleotide (FAD) molecule to accept electrons from NADPH, POR has a molecule of FAD and one of flavin mononucleotide (FMN) on two distinct domains (fig. 2). This enables POR to donate electrons directly to the P450 enzyme without an intermediate such as ferredoxin. A flexible hinge region separates the two domains: once FAD receives an electron from NADPH, the hinge flexes and allows the FAD domain to align with the FMN domain and pass along its electron. Further flexion then permits the FMN domain to associate with the redox-partner-binding site of the P450 enzyme. Cytochrome *b*₅ has an allosteric effect that promotes the interaction of POR with P450c17 and some of the hepatic P450 enzymes [8–10] and can sometimes function as an alternative donor of the second electron in the POR cata-

lytic cycle [11]. The relative abundance of POR in relation to the P450 can influence the enzyme activity: addition of POR increases the 17,20-lyase reaction over the 17 α -hydroxylase activity of P450c17 [12, 13].

POR Deficiency

In 1985, Peterson et al. [14] reported a 46,XY undervirilized infant with low levels of 19-carbon steroids but elevated 17-OHP and 17-OH pregnenolone levels, suggesting combined deficiencies of 17 α -hydroxylase, 17,20-lyase, and 21-hydroxylase. Other similar cases were subsequently reported [15–20]; many of these patients had skeletal malformations consistent with Antley-Bixler syndrome (ABS). POR deficiency was suggested early on as the most logical explanation for this steroid profile [21], but following reports that POR ablation in the mouse confers embryonic lethality, the hypothesis was thought unlikely [22, 23]. The lethality appears to be due to extrahepatic POR deficiency, since a liver-specific POR knockout mouse has a severely impaired drug metabolism but normal development and reproductive capacity [24, 25]. Despite the mouse data, Flück et al. [2] found POR mutations in 3 children with ABS and in 1 woman with primary amenorrhoea and polycystic ovaries without skeletal malformations. Other investigators [3, 4, 7, 26–29] have subsequently found POR mutations in patients with the ABS phenotype plus abnormal genitalia and/or disordered steroidogenesis as well as in some patients without bony anomalies. Approximately 20 mutations in 50 patients have been described to date (table 1).

Antley-Bixler Syndrome

ABS is a skeletal malformation syndrome first described in 1975 [30] that includes craniosynostosis, midface hypoplasia, choanal atresia or stenosis, radiohumeral and/or radio-ulnar synostosis, femoral bowing and fractures, and joint contractures [30–33]. Diagnostic criteria for ABS are not definitively established, but craniosynostosis and elbow synostosis are minimum requirements [34–36]. In a review of 22 cases of ABS, Bottero et al. [33] found that >90% of the cases had craniosynostosis, midface hypoplasia, radiohumeral or radio-ulnar synostosis, joint contractures, femoral bowing, dysplastic ears, and a 'pear-shaped' nose.

The genetics of ABS was initially controversial: reports of affected siblings [37, 38] and parental consan-

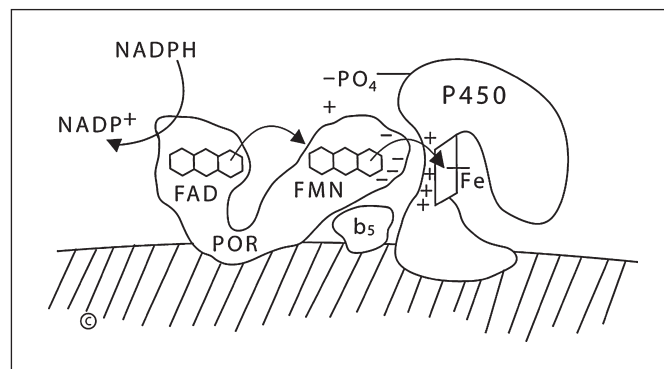


Fig. 2. Diagram demonstrating the role of P450 oxidoreductase (POR) in electron transfer by microsomal (type II) P450 enzymes. NADPH interacts with POR, bound to the endoplasmic reticulum, and gives up a pair of electrons (e^-) which are received by the flavin adenine dinucleotide (FAD) moiety. Electron receipt elicits a conformational change in the flexible hinge region of POR, so that the electrons pass from the FAD domain to the flavin mononucleotide (FMN) domain. Following another conformational change that returns the protein to its original orientation, the FMN domain of POR interacts with the redox partner binding site of the P450 enzyme. The interaction of POR and the P450 is co-ordinated by negatively charged acidic residues on the surface of the FMN domain of POR and by positively charged basic residues in the redox partner binding site of the P450. In the case of human P450c17, this interaction is facilitated by the allosteric action of cytochrome b_5 .

guinity [39] suggested autosomal recessive inheritance, but autosomal dominant fibroblast growth factor receptor 2 (FGFR2) mutations have been found in a number of cases [19, 34, 36, 40, 41]. Fibroblast growth factors (FGFs) are mitogens involved in bone growth and development [42]. FGFs can bind four different tyrosine kinase cell surface receptors (FGFRs). Dominant gain-of-function mutations in FGFRs (primarily FGFR2) cause a number of phenotypically diverse craniosynostosis syndromes, including Pfeiffer, Apert, Jackson-Weiss, and Crouzon syndromes [43–47]. The same mutation can cause two different clinical syndromes, supporting the concept that these syndromes represent phenotypic variations of the same genetic disorder [45–47].

Unlike the other craniosynostosis syndromes, ABS patients have a high incidence of genital anomalies that ranges from 31 to 64%, including hypoplastic labia majora, clitoromegaly, male genital hypoplasia, and cryptorchidism [19, 32, 33].

It was observed that while cases of ABS with FGFR2 mutations had normal genitalia [34, 36, 40, 41], reported

Table 1. Reported patients with P450 oxidoreductase (POR) deficiency

Patients	Chromosomal sex 46,XX/46,XY	POR mutations	Phenotype			References
			ABS features	Abnormal genitalia	Abnormal steroids	
4	2/2	7/8 ^a	3	3	4	2
3	2/1	6/6	1	2	3	3
2	1/1	4/4	2	1	2	4
19 (32) ^b	6/10 ^c	34/38 ^a	19 (32) ^b	12 ^c	10 ^c	26
10	6/4	19/20 ^a	9	9	10	27
3	2/1	6/6	0	2	3	28
7	2/5	14/14	5	2	7	29
1	1/0	1/2 ^a	1	1	1	7
49	22/24	91/98	40/49	32/49	40/49	

ABS = Antley-Bixler syndrome.

^a POR mutations not identified on all alleles.

^b 19 out of 32 patients with the ABS phenotype had POR mutations.

^c Karyotype, genital phenotype or steroid profile not known for all patients.

cases of ABS with apparent autosomal recessive inheritance had genital abnormalities [19, 38, 39, 43]. FGFR2 mutations were found in 7 of 16 ABS patients, but FGFR2 mutations and genital abnormalities segregated completely [19]. Since genital abnormalities are not a feature of any of the other craniosynostosis syndromes caused by FGFR2 mutations, Reardon et al. [19] proposed that ABS with genital and/or steroid abnormalities was likely due to 'digenic inheritance', involving both a factor related to FGFR2 and some other factor involved in genital development.

A phenotype similar to ABS with genital anomalies has been described in children exposed to fluconazole in utero [48–50]. Fluconazole is an inhibitor of lanosterol 14 α -demethylase (CYP51), a type II P450 enzyme that converts lanosterol to ergosterol. Speculating that a defect in cholesterol biosynthesis might cause ABS [49], Kelley et al. [20] showed that lymphoblast cells from a patient with ABS and genital abnormalities had higher levels of lanosterol than normal cells or cells from a patient with ABS and an FGFR2 mutation. Despite this apparent defect in CYP51 function, sequencing of the CYP51 gene revealed no mutation. The patient of Kelley and colleagues with ABS and genital anomalies was one of those described in the original report of POR mutations; he is homozygous for the A287P mutation which explains the decreased CYP51 activity [2].

POR Deficiency – Genetics and Biochemistry

About 26 different POR mutations have been identified to date, including 14 missense mutations, eight frameshift mutations, one deletion, and three splicing errors [2–4, 26, 27, 29]. Mutations have been found in all four POR domains. Most of the missense mutations are in the central electron transfer domain [2, 3, 26]. The most common mutation in people of European descent is A287P, while R457H is often found in Japan [4, 27, 29]. Interestingly, 12% of the reported patients have only one identified mutation [2, 7, 26, 27]. These patients are clinically indistinguishable from those with two mutations, pointing toward possible mutations in as yet unidentified regulatory regions [7]. Patients with the same mutations, even siblings, can be phenotypically different [3, 27, 28, 51]. However, patients with POR mutations always have hormonal profiles compatible with partial deficiencies of 21-hydroxylase and 17 α -hydroxylase/17,20-lyase.

Analysis of the enzymatic capacity of different POR mutants allows for correlations between genotype and phenotype. The classic in vitro assay of POR function is reduction of cytochrome *c*, using a soluble form of POR, which is not a physiological assay for membrane-bound POR and does not always correlate well with the phenotype for a given mutation [2, 3, 26]. A more physiological assay, using a system of genetically modified yeast and

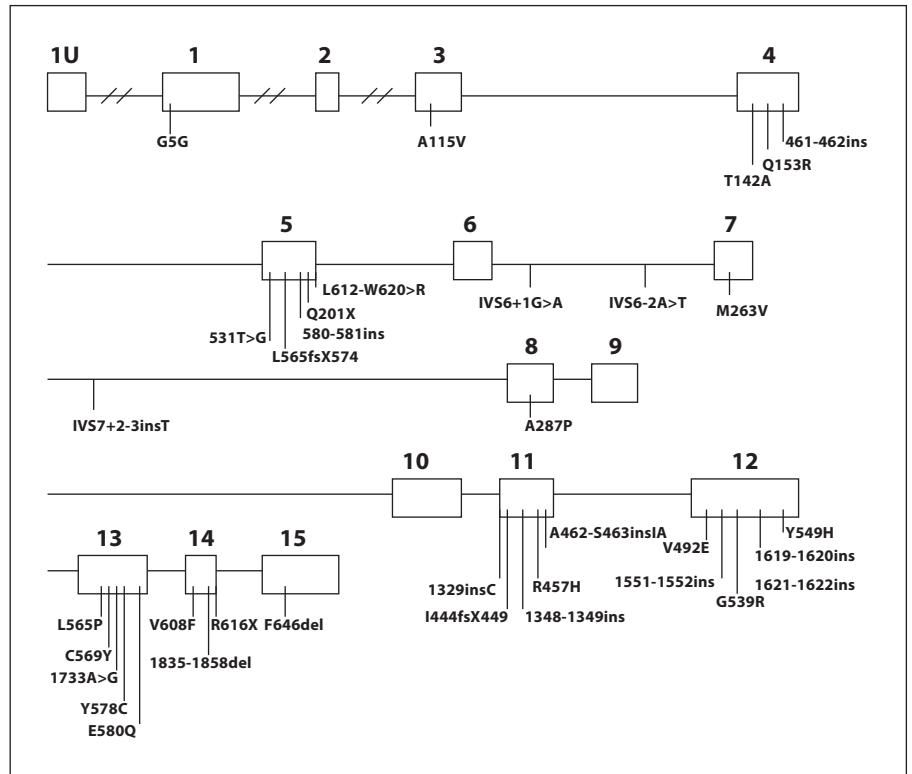


Fig. 3. Schematic of the POR gene structure, showing all exons, introns, and known sequence variations. The exons are the numbered boxes. Exon 1U is the untranslated exon approximately 38 kb upstream from the rest of the gene. The distances between exons 1U, 1, 2, and 3 are not to scale. All sequence variations are named in accordance with the recommendations of Antonarakis [61].

bacteria expressing human P450c17 and POR mutants, measures the ability of POR to support catalysis by P450c17 and consistently correlates with the phenotype [2, 26].

POR Deficiency – Endocrinology

Unlike other genetic defects of steroidogenesis, POR deficiency affects multiple enzymes that generally retain partial activity, making the steroid profiles in patients with POR deficiency variable. The activities of adrenal 21-hydroxylase (P450c21) and 17 α -hydroxylase/17,20-lyase (P450c17) are impaired. The impairment of P450c21 results in 17-hydroxyprogesterone (17-OHP) that is hyperresponsive to adrenocorticotrophic hormone (ACTH) stimulation and elevated 21-deoxycortisol. DHEA (dehydroepiandrosterone), DHEAS (DHEA sulfate), and androstenedione are low to normal because of impaired 17,20-lyase activity. This hormonal pattern, with elevated 17-OHP levels, indicates that the 21-hydroxylation by P450c21 is inhibited to a greater degree than the 17 α -hydroxylation by P450c17, but the effects of POR mutations on P450c21 have not yet been studied in vitro. The 11-de-

oxycortisol level is low. Basal, unstimulated values for cortisol are usually normal or nearly normal, but do not respond normally to stimulation with ACTH, indicating chronically compensated adrenal insufficiency. Correspondingly, the ACTH values may be high. Table 2 summarizes the plasma steroid profiles of reported patients with POR deficiency. Consistent with the inadequate cortisol response to ACTH stimulation, 5 out of the 49 reported cases had clinical signs of adrenal insufficiency [2, 4, 27], and an additional patient died of sepsis while on glucocorticoid and mineralocorticoid replacement therapy [26]. Thus assessment of adrenal function with ACTH stimulation testing and, if indicated, physiological glucocorticoid replacement are appropriate in POR deficiency.

POR Deficiency – Phenotype

The bony malformations of POR deficiency are indistinguishable from those of ABS caused by FGFR2 mutations and include midface hypoplasia, craniosynostosis, pear-shaped nose, choanal stenosis or atresia, low-set ears, and radiohumeral or radio-ulnar synostosis [26].

Table 2. Plasma steroid hormone profiles of reported patients with P450 oxidoreductase (POR) deficiency

Study and patient number	Steroid hormone levels								
	cortisol basal	cortisol stimulated	17-OHP basal	17-OHP stimulated	DOC	DHEA	DHEAS	A	T
Flück et al. (2004) [2]									
1	↓	↓	↑						↔
2	↔		↑	↑		↓		↓	
3									
4	↔	↓	↑	↑	↑			↓	↔
Arlt et al. (2004) [3]									
1									↔
2			↑						
3			↑				↔	↔	↔
Huang et al. (2005) [26]									
1	↓	↔					↔	↔	↔
2	↔		↑	↑		↓		↓	
3			↑						
4	↔		↔			↔	↔	↔	
16	↔	↓	↑						
31	↔		↑				↔	↔	↔
32	↔	↓	↑	↑			↓	↓	↓
Fukami et al. (2005) [27]									
1	↔	↓	↑	↑	↑	↓		↓	↓
2	↔		↑		↑	↔		↔	↔
3	↔	↓	↑	↑	↑	↓		↔	↑
4	↔	↓	↑	↑					↔
5	↔	↓	↑	↑	↑	↔		↓	↓
6	↔	↓	↑	↑	↑	↓		↔	↔
7	↔	↓	↑	↑	↔	↔		↓	↔
8	↔	↓	↑	↑					↔
9	↔	↓	↑	↑	↔	↔		↑	↔
10	↑	↔	↑		↑	↔		↑	↑
Fukami et al. (2006) [28]									
1	↔	↓	↑	↑	↑				↔
2	↔	↓	↑	↑	↔	↔		↑	↔
3	↔	↓	↑	↑	↔	↔		↔	↔
Scott et al. (2007) [7]									
1			↑	↑			↔	↑	↔

This table includes only reported patients for whom there are some published plasma hormone data. Numbers in bold denote females; ↔ means the value was in the normal range; ↓ means the value was below the normal range; ↑ means the value was above the normal range. Blank cells indicate that this value was not reported.

Stimulated = Value after stimulation with ACTH; 17-OHP = 17-OH progesterone; DOC = deoxycorticosterone; DHEA = dehydroepiandrosterone; DHEAS = dehydroepiandrosterone sulfate; A = androstenedione; T = testosterone.

Femoral bowing, femoral fractures, and arachnodactyly are also frequently observed. Although the pathogenesis of these bone malformations is unknown, there are several elements pointing to connections between cholesterol

biosynthesis and skeletal development: First, the known human disorders of cholesterol biosynthesis are all associated with skeletal abnormalities. The best-known of these is Smith-Lemli-Opitz syndrome, caused by muta-

tions in the gene encoding 7-dehydrocholesterol reductase [52]. Second, cholesterol is required for normal activity and signal transduction of the hedgehog proteins which are crucial for the regulation of growth and morphogenesis of embryonic structures. Third, a bony phenotype similar to ABS is seen in infants with in utero exposure to fluconazole [48–50]. Lanosterol 14 α -demethylase, a cytochrome P450 enzyme involved in cholesterol biosynthesis and which requires POR as an electron donor, is inhibited by fluconazole and has decreased activity in POR deficiency [2, 20]. These data suggest that abnormalities in cholesterol biosynthesis, caused by POR deficiency, could be responsible for the skeletal abnormalities seen in many patients with this disease.

Unlike the common single enzyme defects in adrenal steroidogenesis, which cause either virilization in girls or undervirilization in boys, POR deficiency can cause abnormal genital development in both sexes. Boys are often undervirilized, because decreased 17,20-lyase activity prevents the formation of 19-carbon androgen precursors. The 17,20-lyase activity of P450c17 is more sensitive than the 17 α -hydroxylase activity to a deficiency in POR [12, 53, 54]. In contrast, girls with POR deficiency are frequently virilized, but with no postnatal progression, unlike girls with untreated 21-hydroxylase deficiency [55]. There are two hypotheses as to the origin of this virilization, although neither has been definitively proven [3, 51, 56]. POR deficiency impairs the activity of P450aro (aromatase) which converts fetal 19-carbon androgen precursors to estrogens. Placental aromatase deficiency leads to maternal virilization and low maternal estriol and estrone [57] levels, both of which have been described in POR deficiency [2–4, 27–29, 51]. However, the main fetoplacental substrates for P450aro, 16 α OH-DHEA and androstenedione, are low in POR deficiency, and the usual urinary metabolites seen in placental aromatase deficiency are not always found in POR deficiency [3]. An alternate hypothesis for the source of androgen excess in girls with POR deficiency is the ‘backdoor pathway’ to androgen production [29, 51]. In this pathway, 21-carbon steroid precursors are 5 α -reduced and ultimately converted to dihydrotestosterone, bypassing the conventional precursors androstenedione and testosterone [56]. This is the main mechanism of androgen production in some animal species [58]; since human steroidogenic tissues contain the necessary enzymes [56], it has been proposed that the elevated levels of 17-OHP in POR deficiency provide substrate for this pathway [3, 29, 51, 56]. Evidence supporting this theory includes: a urinary metabolite of this pathway has been detected in the mother of a POR-

deficient fetus [51], urinary analysis of 22 patients with POR deficiency showed decreased products of the conventional androgen biosynthetic pathways (etiocholanolone and 11-OH androsterone) but increased androsterone which can be derived from the backdoor pathway [29], and P450c17 has greater affinity for the 5 α -reduced form of 21-carbon steroids than for 17-OHP and 17-OH pregnenolone [59]. The exact role of this pathway in human androgen production, especially in the setting of disordered steroidogenesis, remains unclear.

POR Deficiency – Diagnosis and Treatment

Because of the variability in phenotype and steroid hormone profile in POR deficiency, the diagnosis is not as straightforward as in other causes of CAH. DNA sequencing has assumed an important role in the diagnosis of this disorder. Insufficient cortisol production, especially during periods of stress, is always possible, and all patients with POR deficiency should undergo an ACTH stimulation test and receive hydrocortisone replacement if indicated. Aldosterone deficiency with salt wasting has not yet been described in this disorder but is theoretically possible, because 21-hydroxylase activity requires POR.

In pregnancy, estriol is the product of aromatization of fetal androgen precursors (DHEAS), and it is measured as part of screening for trisomy 21 and open neural tube defects. In the absence of fetal anomalies on ultrasound, low estriol is caused by adrenal steroid biosynthetic defects that cause impaired DHEAS production (steroid sulfatase deficiency, 17 α -hydroxylase deficiency, steroidogenic acute regulatory protein deficiency), adrenal hypoplasia (hypopituitarism, isolated ACTH deficiency), Smith-Lemli-Opitz syndrome, and placental aromatase deficiency. POR deficiency, because of its effect on the 17,20-lyase activity of P450c17, is known to cause low maternal estriol levels in pregnancy [51] and must also be considered in this differential diagnosis when evaluating low estriol levels in a pregnant woman.

The bony anomalies of POR deficiency require orthopaedic management. Earlier reports on ABS described a high mortality rate (50–85%), generally attributed to upper airway obstruction [33, 60]. Bottero et al. [33] observed that most of the patients who died in infancy had choanal obstruction (75%) and that early surgical correction of airway problems led to improved outcomes. Of the 50 reported patients with POR deficiency, only 3 died in

infancy [2–4, 7, 26–29]. The reason for the lower death rate in patients with confirmed POR deficiency is unknown: it may reflect a less severe disease than in patients with FGFR2 mutations, better care in more recently diagnosed patients, better care by endocrinologists, or ascertainment bias, as it is difficult to get DNA for research from deceased patients. Nevertheless, the morbidity and mortality associated with bony abnormalities require prompt intervention.

Bony abnormalities may be absent or subtle in POR deficiency, presenting a diagnostic challenge for the clinician. The reported phenotypic spectrum of patients with POR mutations and no bony problems includes completely asymptomatic patients detected by neonatal screening for 21-hydroxylase deficiency, asymptomatic patients whose mothers were virilized during pregnancy, a virilized female infant, and an adult woman with primary amenorrhoea and cystic ovaries [2, 3, 28, 29]. In all of these cases, steroid hormone analysis led to the suspicion of POR deficiency which was then confirmed by mutation analysis. Given the broad range of clinical manifestations already documented in POR deficiency, it is reasonable to suspect that there are a large number of undiagnosed patients with mild or normal phenotypes. POR deficiency must be considered in the evaluation of any child with sexual ambiguity or a history of maternal virilization during pregnancy. Mutation analysis is indicated in patients with compatible steroid hormone profiles.

Another potentially important clinical consideration is the effect of POR mutations on non-steroidogenic P450 enzymes. Most drugs are metabolized by a small number

of hepatic P450 enzymes that require the activity of POR. Sequence variations in hepatic P450 enzymes are known to cause variability in drug metabolism between individuals, but the impact of variations in POR on these hepatic enzymes has not been investigated. The liver-specific POR knockout mouse has a severely impaired hepatic drug metabolism [24]. The available clinical reports of POR deficiency do not permit a clinical assessment of drug metabolism in POR-deficient patients.

Conclusions

Unlike the other causes of CAH, POR deficiency has consequences beyond abnormal steroidogenesis: skeletal development is often abnormal, enzymes involved in cholesterol biosynthesis are affected, and there is potential for altered drug metabolism. Most reported mortality is related to the skeletal malformations, thus prompt and expert management of patients with upper airway obstruction is essential. Patients are also at risk of adrenal insufficiency and should undergo ACTH stimulation testing, especially before surgery to correct bony abnormalities. Clinicians should also remain alert to any clinical evidence of abnormal drug metabolism in patients with POR mutations.

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