CASE REPORT

Disorder of sex development as a diagnostic clue in the first Spanish known newborn with P450 oxidoreductase deficiency

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SUMMARY

We report the first known case of p450 oxidoreductase deficiency (PORD) in a Spanish boy who presented ambiguous genitalia at birth as a unique feature. He had palpable gonads in the inguinal canal and a normal 46, XY karyotype. Blood tests showed increased lanosterol and androgen precursors (17-OH-pregnenolone and 17-OH-progesterone) and low adrenal androgens (dehydroepiandrosterone and its sulfate). Blood pressure and serum electrolytes were normal. As he had low-testosterone response to human chorionic gonadotropin stimulation but responded to exogenous testosterone with phallic growth, male sex was assigned. Testosterone/dihydrotestosterone ratio and inhibin B were normal. Adrenal insufficiency was detected by corticotropin test. Hydrocortisone replacement treatment was administered. Congenital adrenal hyperplasia was ruled out and molecular analysis of POR gene showed the missense mutation p.Gly539Arg in compound heterozygosity located at splice acceptor site of intron 2 and the coding variant p.Gly80Arg. Surgery for cryptorchidism and hypospadias was performed.

BACKGROUND

Although often difficult to achieve, early diagnosis of a disorder of sex development (DSD) is of utmost importance to establish a proper sex assignment, administer hormone supplementation therapy and perform surgical genitalia reparation when appropriate. Adrenal and gonadal steroid secretion should be measured to rule out congenital adrenal hyperplasia and other less common enzymatic defects of steroidogenesis.¹ Increased steroidogenic precursors may indicate a p450 oxidoreductase deficiency (PORD) origin.² POR-dependent activities involved in the biosynthesis of cholesterol are CYP51A1 (lanosterol-14 α -demethylase) and SQLE (squalene monooxygenase), while CYP17A1 (17 α -hydroxylase/1720-lyase), CYP21A2 (21 α -hydroxylase) and CYP19A1 (aromatase) are involved in steroidogenesis^{3–4}.

We present a PORD case with DSD as a unique manifestation at birth.

CASE PRESENTATION

We present a full-term Spanish baby with an uneventful pregnancy despite the detection of ambiguous genitalia at the 20th week. He was the first child of a healthy, Caucasian, non-consanguineous, Spanish couple with no family history of ambiguous genitalia, neonatal death or skeletal abnormalities. No maternal virilisation or drugs

ingestion during pregnancy was detected. Amniocentesis showed a normal male karyotype 46,XY. Delivery was normal, birth weight was 2.450 g (–1SD), height 49.5 cm (0.6SD) and head circumference 32.5 cm (–1.2SD). Physical examination confirmed abnormal external genitalia: scrotal hypospadias, micropenis, labioscrotal folds and a single urethral meatus at median raphe. Both gonads were palpable at inguinal canal (figure 1). No other dysmorphic features were apparent.

INVESTIGATIONS

Blood pressure was normal. Abdominal ultrasound showed normal adrenal glands and absence of internal female genitalia. Blood test performed 6 h after birth showed normal electrolytes with increased androgenic precursors. Baseline corticotrophin adrenocortico tropic hormone (ACTH) was high and cortisol was normal (table 1).

Testosterone response to human chorionic gonadotropin (HCG) stimulation test (500 IU/48 h, 9 days) made on day 20 after birth was low. Testosterone/dihydrotestosterone ratio was normal, thus ruling out 5- α -reductase deficiency. The baby also responded to exogenous testosterone (25 mg/week 3 doses), with corpus cavernosum enlargement, precluding androgen resistance. Inhibin B was normal (158 ng/L, reference range: 110–575). All these results were decisive for male sex assignment.

At 3.5 months of age, androgen precursors remained elevated (17-hydroxyprogesterone (17-OHP) 249.9 nmol/L) with low adrenal androgens, dehydroepiandrosterone sulfate 0.135 pmol/L and androstenedione 0.839 nmol/L. Gonadotropin

Figure 1 Appearance of the genitalia at birth.

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Table 1 The patient's hormone laboratory results

Hormone data (units) (RR)	Patient age			
	6 h of life	20 days (HCG test)	1 month (after testosterone)	3.5 months
17-OHP (nmol/L) *(RR per age)	1133.2 (150.6±51.5)	173 (19.97±4.67)	94.8 (15.20±4.26)	249.9 (9.22±1.71)
17-OHPreg (nmol/L) (0.42–27.06)	98.6		32.3	
DHEA-S (pmol/L) *(RR per age)	4.61 (6.63±2.76)		0.54 (1.79±1.02)	0.13 (0.67±0.3)
Androstendione (nmol/L) *(RR per age)	0.003 (7.8±4.2)	0.594 (2.28±0.76)	0.909 (2.06±1.24)	0.839 (1.27±0.63)
Testosterone (nmol/L) *(RR per age)	13.3 (8.3±3.6)	7 (5.56±1.17)	7.7 (5.75±1.87)	1.9 (3.91±1.49)
DHT (nmol/L) (<17)	1.3	0.5	0.5	
FSH (IU/L) †(RR per age)	0.31 (1.22–5.19)		2.23 (0.19–2.97)	2.03 (0.19–2.97)
LH (IU/L) †(RR per age)	<0.1 (4.85–10.02)		3.64 (0.04–3.01)	29.25 (0.04–3.01)
ACTH (pmol/L) †(RR per age)	70.2 (2.1±34.8)		31.9 (3.5±21.3)	117.26 (1.8±26.7)
Cortisol (nmol/L) *(496±215.2)	295.2	336.6	380.7	303.5
Na/K (mmol/L) (135–145/3–5)	138/5.3		140/5.4	140/5.8
Cholesterol (mmol/L) (3.89–5.18)			2.1	2.8

*Mean±1 SD.

†Mean±2 SD) from normal data for healthy infants.⁵

ACTH, adrenocorticotrophic hormone; DHEA, dehydroepiandrosterone sulphate; DHT, dihydrotestosterone; FSH, follicle stimulating hormone; HCG, human chorionic gonadotropin; LH, luteinising hormone; RR, reference ranges.

levels were normal and ACTH elevated with low-to-normal cortisol (table 1). Molecular study of CYP21A2 was negative. We observed a low level of total cholesterol with an increase of lanosterol, but with normal levels of 7-dehydrocholesterol, hence Lemli-Opitz syndrome was ruled out. Serum cortisol after low-dose corticotropin test was 289.1 nmol/L (normal response >500 nmol/L). Potassium level and plasma renin activity were high. Mutational analysis was performed using PCR. This study was followed by direct sequencing of the POR gene, which revealed a missense mutation p.Gly539Arg (c.1615G>A) in compound heterozygosity located at intron 2 splice acceptor site and the coding variant p.Gly80Arg. The diagnosis of PORD was confirmed. The genetic study of progenitors showed that the father carries the variant at the acceptor site of intron 2 and the mother the missense mutation p.Gly593Arg.

DIFFERENTIAL DIAGNOSIS

PORD should be considered in cases of ambiguous genitalia with XY karyotype, among other steroidogenesis disorders, gonadal dysgenesis and androgen action disorders. Testosterone response to HCG may be helpful to establish a working diagnosis.

TREATMENT

The patient initiated replacement therapy with hydrocortisone (15 mg/m²/day) and fludrocortisone (0.05 mg/day).

Multiple corrective surgeries of ambiguous genitalia were performed as follows: bilateral orchiopexy, two-stage penoscrotal urethroplasty and correction of urethral structure and post-operative fistulae.

OUTCOME AND FOLLOW-UP

At the age of 4 months, cranial dysmorphism was detected resembling a craniosynostosis, later confirmed with the premature fusion of the metopic suture (trigonocephaly), which did not require surgical treatment. Other mild characteristic facial dysmorphic features became apparent: large domed forehead, flat nose and midface hypoplasia with proptosis and dysplastic ears.

When the patient was 3 years old, he was admitted to the hospital for an acute adrenal crisis coinciding with a urinary

tract infection which was solved without complications. He is now 7 years old and receives replacement treatment with hydrocortisone 15 mg/m²/day and fludrocortisone 0.05 mg/day.

DISCUSSION

Cytochrome PORD is a rare autosomal recessive disorder caused by mutations in the gene encoding an electron donor for all microsomal P450 enzymes and several non-P450 enzymes.

Clinical evaluation should be conducted promptly after the detection of genital ambiguity in a newborn, including family and prenatal history, and a thorough physical examination. First-line testing includes karyotype, SRY (sex-determining region Y) detection, imaging (abdominopelvic ultrasound) and hormone assessment (17-OHP, testosterone, gonadotropins, antimüllerian hormone). Adrenal and gonadal steroid secretion should be measured to rule out congenital adrenal hyperplasia and other less common enzymatic defects of steroidogenesis.⁶ Findings indicating impaired syntheses of cholesterol and adrenal androgens may suggest multiple enzymatic deficiencies (21 α -hydroxylase, 17 α -hydroxylase/17 β -lyase and aromatase), which may be explained by an impaired electron transfer from P450 oxidoreductase required for the normal activities of all these enzymes. PORD should be considered in these cases, although other dysmorphic features may not be relevant or inexistent in the newborn. PORD causes genital ambiguity in both sexes. Male undervirilisation is explained by the decrease of 1720-lyase activity of P450c17 leading to a reduced production of androgens. Affected girls present virilised external genitalia suggesting intrauterine androgen excess that does not progress postnatally, with low-circulating androgens.⁷

Mutations in POR gene are diverse, including missense, non-sense, frameshift and splice site mutations.⁸ Mutations with partial activity may lead to subtle clinical manifestations,⁹ suggesting that PORD may be more common than it is reported. Thus, the incidence of POR deficiency is actually unknown. The most common mutation in Caucasian patients is A287P, while R457H is most commonly found in the Japanese population.^{8 10 11} Patients with R457H mutation have more severe skeletal features and are often born to mothers who virilised during pregnancy, whereas patients with A287P mutation are

less severely affected and their mothers do not virilise during pregnancy.¹²

Our patient presented two different mutations (compound heterozygosis), one transmitted from each parent. Huang *et al* (2004) tried to determine the activity of every enzyme in every mutation in vitro. As they observed, the mutation found in our patient (G539R) in vitro lost >90% activity in the 1720-lyase assay but retained 46% activity in the hydroxylase assay, indicating that the mutant may still affect nicotinamide adenine dinucleotide phosphate binding. Furthermore, identification of the mutation in our patient allowed us to perform prenatal diagnosis in his sister. In 2011, the second child of this couple was born. She is a healthy girl, who carries the missense mutation p.Gly593Arg.

As in the other clinical features of PORD, the degree of impaired steroidogenesis also varies between different POR mutations. Cortisol deficiency can range from clinically insignificant to life-threatening. Adrenal insufficiency is a potential risk in the majority of patients with POR, but there is no correlation between POR genotype and the presence and severity of adrenal insufficiency. Patients with PORD often have normal baseline cortisol secretion but this cortisol production fails to increase in response to ACTH. Some patients had an adrenal crisis,¹³ and even in several cases sudden death occurred.¹⁴ Replacement therapy should be individualised on the basis of the cortisol response to ACTH stimulation. Mineralocorticoid deficiency does not seem to be a part of the biochemical presentation in PORD as described in literature. However, as our patient presented high serum potassium, replacement therapy was initiated, and renin levels remained stable.

The pathogenesis of the skeletal malformations observed in some patients with PORD is unclear. It appears to be related to the role of cholesterol biosynthesis in bone formation, as seen in other disorders of cholesterol biosynthesis such as Smith-Lemli-Opitz syndrome. In PORD, skeletal manifestations may result from the impaired activity of both lanosterol 14 α -demethylase (CYP51) squalene epoxidase (a non-P450 enzyme), and retinoic acid metabolism catalysed by CYP26 isozymes that also depend on electron transfer from POR.¹⁵ Similarly, this has been observed as a teratogenic effect in fetuses exposed to fluconazole, an antifungal agent that acts by inhibiting CYP51 activity.¹⁶

POR is also required by all drug-metabolising P450 hepatic enzymes. Hepatic P450 cytochromes are much more ancient and robust than microsomal enzymes involved in steroidogenesis. Thus, hepatic enzymes are not as susceptible to impairment due to a single mutation. To date, our patient has shown no adverse/idiosyncratic effects to the exposure of the life-long medication he has received (acetaminophen, anaesthetics, antibiotics and anti-inflammatory).

Contributors RC was involved in conception and design, SA was involved in the acquisition of data, DS-G in drafting the manuscript, BE in molecular diagnosis. Furthermore all authors made the critical revision of the manuscript.

Competing interests None.

Patient consent Obtained.

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Learning points

- Cytochrome P450 oxidoreductase deficiency (PORD) is a rare autosomal recessive disorder.
- PORD has a broad range of clinical manifestations due to an impaired steroidogenesis: ambiguous genitalia, adrenal insufficiency and skeletal malformations.
- Disorder of sex development can be the unique initial manifestation of cytochrome PORD.
- Adrenal insufficiency is a potential risk in the majority of these patients.

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