Urine Steroid Hormone Profile Analysis in Cytochrome P450 Oxidoreductase Deficiency: Implication for the Backdoor Pathway to Dihydrotestosterone

Keiko Homma, Tomonobu Hasegawa, Toshiro Nagai, Masanori Adachi, Reiko Horikawa,
Ikuma Fujiwara, Toshihiro Tajima, Ryoujun Takeda, Maki Fukami, and Tsutomu Ogata

Departments of Laboratory Medicine (K.H.) and Pediatrics (T.H.), Keio University School of Medicine, Tokyo 160-8582,
Japan; Department of Pediatrics, Dokkyo University School of Medicine Koshigaya Hospital (T.N.), Koshigaya 343-8555,
Japan; Division of Endocrinology and Metabolism, Kanagawa Children's Medical Center (M.A.), Yokohama 232-8555,
Japan; Division of Endocrinology and Metabolism, National Center for Child Health and Development (R.H.), Tokyo
157-8535, Japan; Department of Pediatrics, Tohoku University School of Medicine (I.F.), Sendai 980-0878, Japan;
Department of Pediatrics, Hokkaido University School of Medicine (T.T.) Sapporo 060-8638, Japan; Department of
Pediatrics, Shirakawa Kousei General Hospital (R.T.), Shirakawa 961-0907, Japan; and Department of Endocrinology
and Metabolism, National Research Institute for Child Health and Development (M.F., T.O.), Tokyo 157-8535, Japan

Context: Although the "backdoor" pathway to dihydrotestosterone has been postulated in the fetal-to-early-infantile period of patients with cytochrome P450 oxidoreductase deficiency (PORD), clinical data in support of this pathway remain limited.

Objective: The objective of this study was to obtain clinical evidence for the presence of the backdoor pathway in PORD.

Setting: This was a collaboration study between laboratories and hospitals.

Subjects: Twenty-two Japanese patients with molecularly confirmed PORD and 1763 control subjects participated in this study.

Intervention: Urine steroid profile analysis was performed by gas chromatography/mass spectrometry. In five patients and 776 control subjects, urine samples were obtained before 12 months of age.

Main Outcome Measure: The main outcome measure was identification of a urine steroid(s) indicating the backdoor pathway.

Results: In the PORD patients, pregnanediol, pregnanetriolone, and pregnanetriol were obviously elevated, and the urine steroid ratios reflecting CYP17A1 and CYP21A2 activities were decreased throughout the examined ages. Furthermore, etiocholanolone and 11-hydroxyandrosterone, which should originate almost exclusively from androstenedione in the conventional "frontdoor" pathway, were grossly normal or somewhat decreased since early infancy, whereas androsterone, which can be derived not only from androstenedione and dihydrotestosterone in the conventional frontdoor pathway but also from 5α-pregnane-3α,17α-diol-20-one in the backdoor pathway, was increased during early infancy and remained grossly normal thereafter. Thus, the androsterone to etiocholanolone ratio was increased during early infancy and remained grossly normal thereafter. 5α-Pregnane-3α,17α-diol-20-one was elevated throughout the examined ages.

Conclusions: The increased androsterone excretion during early infancy, as compared with the etiocholanolone and 11-hydroxyandrosterone excretions in the same period, suggests the presence of the backdoor pathway in PORD. (*J Clin Endocrinol Metab* 91: 2643–2649, 2006)

C YTOCHROME P450 OXIDOREDUCTASE (POR) deficiency (PORD) is an autosomal recessive disorder caused by mutations in the gene encoding a flavoprotein that serves as an electron donor to all microsomal P450 enzymes such as CYP51A1 (lanosterol 14α-demethylase) involved in cholesterol biosynthesis and CYP17A1 (17α-hydroxylase and 17,20 lyase), CYP21A2 (21-hydroxylase), and CYP19A1 (aromatase) involved in steroidogenesis (1–3). Clinical phenotypes of this condition are highly variable, and include abnormal skeletal development referred to as Antley-Bixler syndrome and insufficient glucocorticoid production with increased 17α-hydroxyprogesterone (17-OHP) in patients of both sexes, undermasculinization during the fetal and pubertal periods in male patients, and virilization during the fetal life and poor pubertal development without worsening of virilization in female patients, together with maternal virilization during pregnancy (1–7). Because the complete absence of POR activity is assumed to be lethal (1), some residual POR activity should be present in the patients. Indeed, all the reported patients have at least one missense mutation that is likely to preserve some residual activity (2–7).

Therefore, PORD is a unique disorder that can lead to impaired fetal sex development in both sexes. In particular,

whereas masculinization can be preserved normally in a relatively large fraction of male patients, virilization is exhibited by nearly all female patients (2–7) except for one adult woman who presented with primary amenorrhea (2). The defective sex development is primarily consistent with impaired gonadal CYP17A1 activity resulting in compromised testosterone (T) production in genetic males and defective placental CYP19A1 activity leading to accumulation of placental T as well as androstenedione (Δ^4A) that is transferred into the fetal and maternal circulations in both genetic male and female patients (2–4). In this situation, genetic male patients have nearly normal to underdeveloped external genitalia because of the combined effects of reduced gonadal androgens and accumulated placental androgens, whereas genetic female patients have virilized genitalia because of the effects of placenta-derived androgens. However, the virilization in genetic female patients appears to be inexplicable by defective placental CYP19A1 function alone: 1) in CYP19A1 deficiency, only female infants with nearly complete loss of enzyme activity (<1%) have virilization (8); and 2) in PORD, the supply of dehydroepiandrosterone (DHEA), the precursor of Δ^4A and T, from the fetus to the placenta is reduced (4, 9).

Thus, the backdoor pathway to dihydrotestosterone (DHT) has been postulated in PORD (3, 10). The backdoor pathway, which has been demonstrated in the tammar wallaby pouch young testis (11) and in the immature mouse testis (12), is driven by the accumulation of 17-OHP, leading to the sequential conversion of 17-OHP into DHT via a T-independent route. Although the backdoor pathway is mediated by CYP17A1 as well as 5α-reductase (presumably type 1) and other enzymes, the substrate for CYP17A1 in the backdoor pathway, 5α-pregnane-3α,17α-diol-20-one, is known to have a much higher affinity for CYP17A1 than 17-OHP (13). Thus, the backdoor pathway would function better than the conventional frontdoor pathway in PORD. In this study, we report urine steroid profile data obtained in PORD patients that suggest the presence of the backdoor pathway in this condition.

Patients and Methods

Patients

Twenty-two Japanese patients with PORD (11 genetic male patients with 46,XY and 11 genetic female patients with 46,XX) were studied (Table 1). Fifteen of the 22 patients have been reported previously (2, 4, 6, 7). In all the patients, molecular analysis indicated the diagnosis of PORD, and the patients were divided into two groups in terms of the mutation type: 1) those with two missense mutations, mostly homozygotes for R457H with some residual activity (2, 3) (cases 1–3 and cases 12–17) (group 1); and 2) those with one missense mutation, mostly R457H, and one non-missense mutation that probably has no or very little residual activity (cases 4–11 and cases 18–22) (group 2). Skeletal phenotype was much milder in group 1 than in group 2, masculinization in genetic male patients was somewhat better preserved in group 1 than in group 2, and virilization in genetic female patients and maternal virilization during pregnancy appeared to be similar between groups 1 and 2; case 21 only exhibited normal female genitalia. This study was approved by the institutional review board committee of each investigator, and the mutation analysis was performed at the National Research Institute for Child Health and Development after obtaining informed consent from each patient or the parents.

Urine steroid hormone profile analysis

Urine steroid hormone profile was determined by gas chromatography/mass spectrometry (14), using 24-h urine samples or random spot urine samples. The age at the time of urine sampling in each case is shown in Table 1. Analyzed urine steroids included pregnanediol (PD), pregnenetriol (PT5), pregnanetriolone (Ptl), pregnanetriol (PT), tetrahydrocorticosterone (THB), tetrahydro-11-dehydrocorticosterone (THA), tetrahydrocortisol (THF), tetrahydrocortisone (THE), DHEA metabolites (Ms) (the sum of DHEA, androstenediol, 16α-hydroxy-DHEA, 16β-hydroxy-DHEA, 16-oxoandrostenediol, and androstenetriol), 11-hydroxyandrosterone (11-OHAn), etiocholanolone, androsterone, 5α-pregnane-3α,17α-diol-20-one, and 5β-pregnane-3α,17α-diol-20-one (Fig. 1) (the formal steroid names are described in Ref. 14). This analysis was performed after obtaining written permission from each patient or the parents.

For comparison, cross-sectional data obtained from 854 males and 909 females were used (part of the control data has been reported previously) (4, 7, 14, 15). Only a single urine sample was obtained from each subject. The subject age at the time of urine sampling and the urine collection methods are summarized in Table 2. The control urine samples were collected from healthy neonates, children with microhematuria or mild upper respiratory infection at the recovered phase, and healthy volunteers of various ages. The urine sampling was approved by the institutional review board committee at Keio University Hospital; each subject or the parents agreed to the urine sampling on the condition that the sample is only used to make the control data for the diagnosis of adrenal disorders after discarding personal information except for age and sex.

Results

PD, PT5, Ptl, PT, THE, DHEA-Ms, 11-OHAn, etiocholanolone, androsterone, 5α-pregnane-3α,17α-diol-20-one, and 5β-pregnane-3α,17α-diol-20-one were measurable since birth, as was aldosterone-M. However, THB, THA, and THF, as well as the Ms of pregnenolone, deoxycorticosterone, and 11-deoxycortisol, could not be measured precisely during the first 6 months of age because of interference from unknown steroids that are probably derived from the fetal adrenocortex. Overall, the results were similar between genetic male and female patients and between groups 1 and 2 patients, including the particular case 21 with apparently normal female genitalia.

Representative results for the assessment of the conventional frontdoor pathway are shown in Fig. 2A (the data are expressed using a logarithm scale). Throughout the examined ages, PD, Ptl, and PT were obviously elevated, and PT5 tended to be increased in the PORD patients, whereas THF and THE, as well as aldosterone-M, remained grossly normal, and THB and THA tended to be elevated (data not shown). Furthermore, the (THF + THE) to (THB + THA) ratio reflecting CYP17A1 (17α-hydroxylase) activity was reduced, whereas the PT to PD ratio remained within the normal range (data not shown). Similarly, the THE to PT ratio reflecting CYP21A2 activity, the DHEA-Ms to PT5 ratio reflecting CYP17A1 (17,20 lyase) activity for the Δ^5-steroid, and the 11-OHAn to PT ratio reflecting CYP17A1 (17,20 lyase) activity for the Δ^4-steroid were all decreased in the PORD patients.

Representative data for the evaluation of the alternative backdoor pathway are shown in Fig. 2B (the data are expressed using a logarithm scale, except for the androsterone to etiocholanolone ratio, which is expressed using a linear scale). Throughout the examined ages, DHEA-Ms tended to be low, 11-OHAn was normal or low, and etiocholanolone

**TABLE 1.** Summary of patients examined in the present study

Case	POR mutation	Skeletal anomaly	External genitalia	Other urogenital feature	Maternal virilization	Age at sampling of 24-h urine	Age at sampling of Random spot urine	Ref.
Genetic male patients: 46,XY								
Group 1: two missense mutations								
1	R457H/R457H	Absent	Normal	IA	No		3 months, 12 months	7
2	R457H/R457H	Absent	Normal		Yes		22 yr	Unpublished
3	R457H/R457H	Absent	Normal		Yes		23 yr	Unpublished
Group 2: one missense and one other types of mutations								
4	R457H/Q201X	Overt	Normal		No		15 yr	Unpublished
5	R457H/Q201X	Overt	Normal		No		16 yr	Unpublished
6	R457H/A462_S463insIA	Overt	MP		Yes	3 months	10 d, 1 month, 2 months, 3 months (2×), 4 months (2×)	Unpublished
7	R457H/I444fsX449	Overt	Normal		No		14 yr (2×)	6
8	R457H/L612_W620delinsR	Overt	MP, CO (R)		No		23 yr, 28 yr, 29 yr	4
9	R457H/L612_W620delinsR	Overt	MP, HS, CO (L)	VUR (R)	No	18, 19, and 27 yr	23 yr, 25 yr, 26 yr (2×)	4
10	R457H/G5G[a]	Overt	CO (B)		No		17 yr	4
11	Y578C/I444fsX449	Overt	Normal		Yes		17 yr	4
Genetic female patients: 46,XX								
Group 1: two missense mutations								
12	R457H/R457H	Borderline	CM, LF (complete)	PCO (B)	Yes		1 month, 4 months	7
13	R457H/R457H	Borderline	CM, LF (complete)	PCO (B)	Yes		3 months, 4 months (2×)	7
14	R457H/R457H	Mild	CM, LF (complete)		Yes	2 yr	3 yr	4
15	R457H/R457H	Mild	CM, LF (complete)		No	8, 9, 10, and 11 yr	13 yr	4
16	R457H/R457H	Borderline	CM	VUR (B), PCO (L)	Yes		14 yr, 17yr	4
Group 2: one missense and one other types of mutations								
17	R457H/E580Q	Borderline	CM, LF (incomplete)		No		4 months, 8 months	Unpublished
18	R457H/IVS6+1G>A	Overt	CM, LF (complete)		Yes		5 yr	2
19	R457H/L565fsX574	Overt	CM, LF (complete)		Yes		10 yr	6
20	R457H/I444fsX449	Overt	CM, LF (complete)	PCO (L)	Yes	13 yr		4
21	R457H/Q201X	Overt	Normal		No		12 yr	Unpublished
22	R457H/?[b]	Overt	LF (partial)	VUR (R)	No		3 yr	4

MP, Micropenis; CO, cryptorchidism; HS, hypospadias; CM, clitoromegaly; LF, labial fusion; IA, imperforate anus; VUR, vesicoureteral reflux; PCO, polycystic ovary; R, right; L, left; B, bilateral. Cases 2, 3, and 16; cases 4, 5, and 21; and cases 8 and 9 are siblings.
[a] This silent substitution may affect pre-mRNA splicing.
[b] No mutation has been identified in one allele.

FIG. 1. Simplified schematic representation indicating the steroid metabolism pathway. CYP51A1 (lanosterol 14α-demethylase), CYP17A1 (17α-hydroxylase and 17,20 lyase), CYP21A2 (21-hydroxylase), and CYP19A1 (aromatase) are POR-dependent enzymes. The conventional frontdoor pathway is shown by the *solid lines* with *arrows*, and the alternative backdoor pathway is indicated by the *thick dotted lines* with *arrows*. In the backdoor pathway, the conversion of 17-OH progesterone into 5α-pregnane-3α,17α-diol-20-one is mediated by 5α-R (5α-reductase) and 3α-HSD (R) (reductive 3α-hydroxysteroid dehydrogenase), that of 5α-pregnane-3α,17α-diol-20-one into androsterone by CYP17A1 (17,20 lyase), and that of androsterone into DHT by 17β-HSD (R) (reductive 17β-hydroxysteroid dehydrogenase) and 3α-HSD (O) (oxidative 3α-hydroxysteroid dehydrogenase). 17-OH progesterone is also converted into 5β-pregnane-3α,17α-diol-20-one by 5β-reductase and 3α-HSD (R). Of the urine steroids analyzed in this study, those measurable since birth are surrounded by *solid squares*, and those measurable after 6 months of age are surrounded by *broken squares*. Note that androsterone can be derived from both the frontdoor and the backdoor pathways.
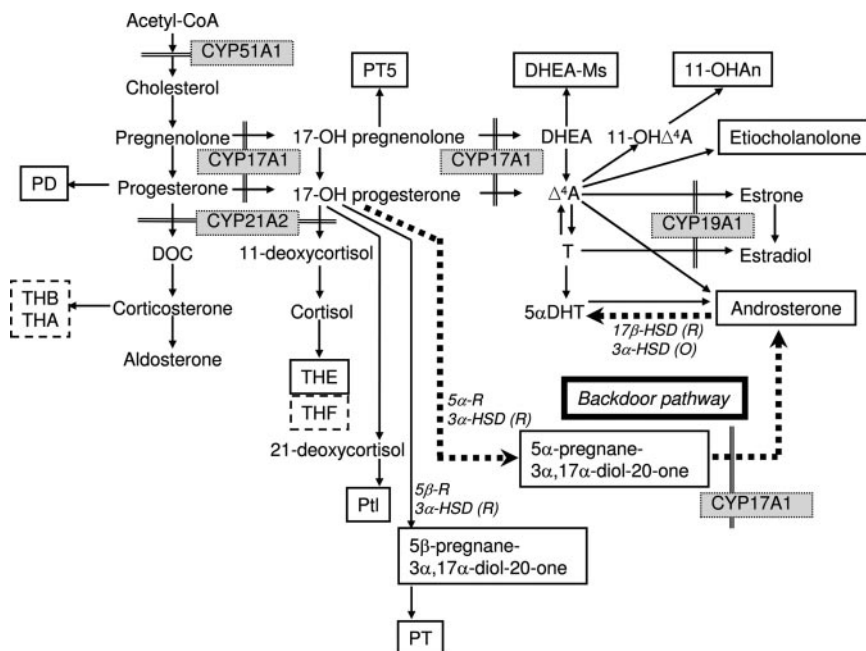
remained grossly normal in the PORD patients. By contrast, androsterone was increased during early infancy and remained grossly normal thereafter. Thus, despite the increased PT excretion, the androsterone to PT ratio remained normal during early infancy, although it decreased thereafter. Furthermore, the androsterone to etiocholanolone ratio tended to be increased during early infancy and remained grossly normal thereafter. 5α-Pregnane-3α,17α-diol-20-one was elevated throughout the examined ages, as was 5β-pregnane-3α,17α-diol-20-one.

Discussion

The urine steroid profile analysis revealed the combined CYP17A1 and CYP21A2 deficiency characteristic of PORD, in the conventional frontdoor pathway. Although several data may be confounding, they would not be inconsistent with PORD. For example, the normal THF and THE excre-

tions would be explained as the consequence of steroidogenic adjustment to normalize the cortisol production under residual CYP17A1 and CYP21A2 activities and mild adrenocorticotropic hormone excess (2–7). Furthermore, because CYP21A2 is the sole POR-dependent enzyme involved in the mineralocorticoid biosynthesis, this would explain why THB and THA excretions tended to be increased in the presence of normal THF and THE excretions. In addition, because 17-OHP is markedly elevated, this would account for the normal PT to PD ratio and the normal to low-normal 11-OHAn and etiocholanolone excretions in the presence of residual CYP17A1 activity. Therefore, the results provide further support for the urine steroid profile analysis as a highly useful method for the diagnosis of PORD (4, 6, 7, 9, 16, 17).

Notably, urine androsterone excretion was elevated during early infancy in the PORD patients, despite the impaired CYP17A1 activity in the frontdoor pathway. By contrast, DHEA-Ms and 11-OHAn derived from the frontdoor pathway were at a low or low-normal range throughout the examined ages. Furthermore, etiocholanolone remained grossly normal throughout the examined ages including early infancy, and the androsterone to etiocholanolone ratio was elevated during early infancy. In this regard, of androsterone and etiocholanolone, which are considered to be net androgen Ms derived from both androgen precursors and active androgens, androsterone can be derived not only from the conventional frontdoor pathway via Δ⁴A as well as DHT but also from the backdoor pathway via 5α-pregnane-3α,17α-diol-20-one, whereas etiocholanolone as well as DHEA-Ms and 11-OHAn should originate almost exclusively from the frontdoor pathway (9–13) (Fig. 1). Consistent with this, etiocholanolone remained grossly normal since birth, despite the elevation of 5β-pregnane-3α,17α-diol-20-one in our study. This suggests that etiocholanolone production in the backdoor route, *i.e.* the conversion of 5β-

TABLE 2. The number of urine samples obtained from control subjects

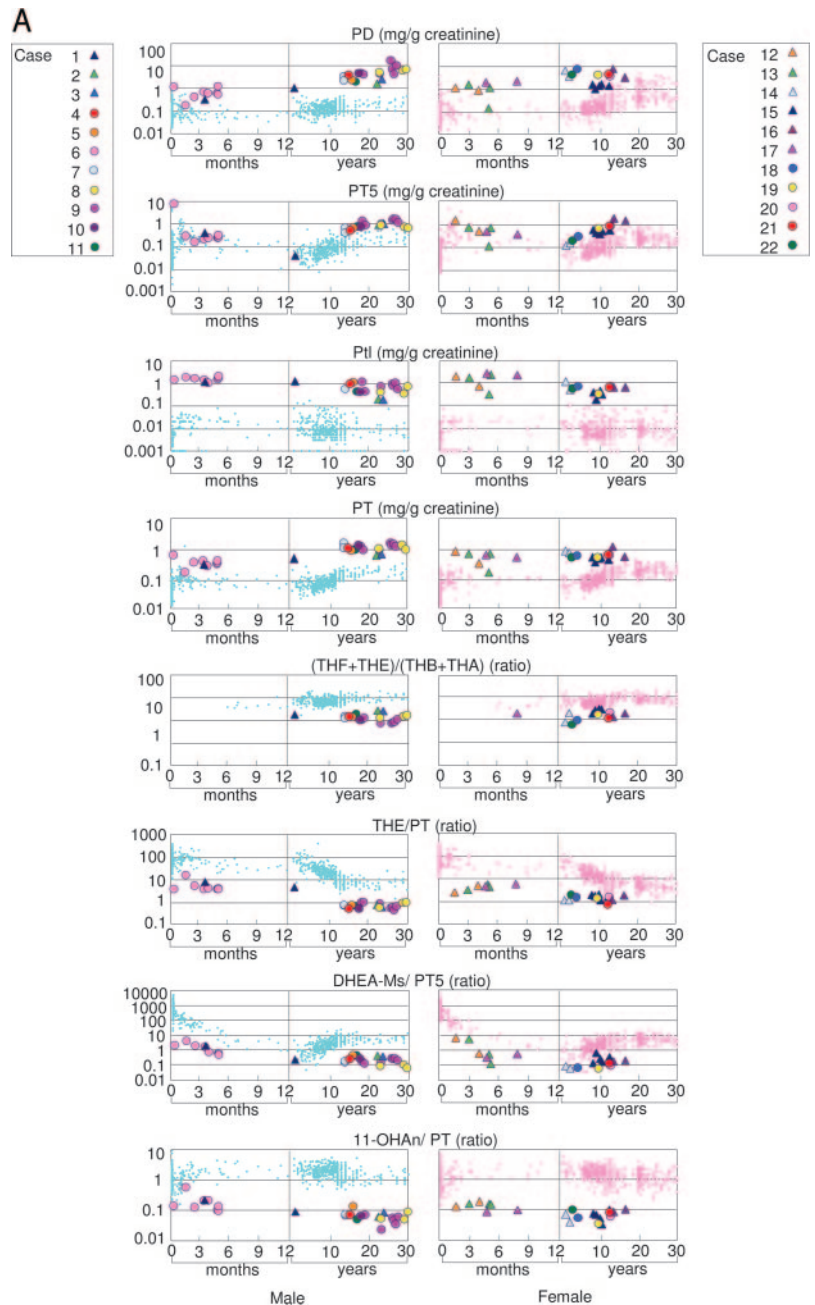| Age at sampling	24-h urine	First morning urine	Random spot urine	Total
Male				
0 ~ <1 month	0	0	366	366
1 month ~ <12 months	0	0	59	59
1 yr ~ <6 yr	9	0	39	48
6 yr ~ <12 yr	34	207	10	251
12 yr ~ <20 yr	40	59	0	99
20 yr ~ <30 yr	19	12	0	31
Total	102	278	474	854
Female				
0 ~ <1 month	0	0	311	311
1 month ~ <12 months	0	0	40	40
1 yr ~ <6 yr	7	0	9	16
6 yr ~ <12 yr	38	224	3	265
12 yr ~ <20 yr	45	84	0	129
20 yr ~ <30 yr	125	23	0	148
Total	215	331	363	909

FIG. 2. Representative results of urine steroid profile analysis. A, The results for the assessment of the conventional frontdoor pathway. B, The results for the assessment of the alternative backdoor pathway. Cases 1–11 are genetic male patients, and cases 12–22 are genetic female patients. The *triangles* indicate group 1 patients with two missense mutations, and the *circles* represent group 2 patients with one missense and one non-missense mutation. Of the 22 cases, cases 1, 6, 12, 13, and 17 have been studied before 12 months of age, and the remaining 17 cases have been studied after 1 yr of age when the backdoor pathway is unlikely to exist. The *light blue circles* and the *pink circles* show the data obtained from 854 control males and 909 control females, respectively. Urine samples from the control subjects have been obtained before 12 months of age in 425 males and 351 females, and after 1 yr of age in the remaining 429 males and 558 females. Note that all the data are expressed using a logarithm scale except for the androsterone to etiocholanolone ratio that is expressed using a linear scale. (*Figure continues on next page.*)

pregnane-3α,17α-diol-20-one into etiocholanolone by CYP17A1, remains scanty, if any.

Thus, the transient increase in androsterone secretion during the first several months of life implies the operation of the backdoor pathway during this time period (10). Consistent with our results, Shackleton *et al.* (9) have identified the backdoor pathway-derived steroids, including androsterone, in the urine of the mother of a fetus with PORD. In this context, because androsterone is a potent androgen and an efficient DHT precursor, even a small excess would have clinical effects. Thus, even if the backdoor pathway is a quantitatively minor source of 19-carbon steroids relative to the frontdoor pathway, it would be relevant to the relatively well-preserved masculinization in genetic male patients and

to the virilization in genetic female patients and in mothers of affected fetuses.

The backdoor pathway would primarily exist in the steroidogenic tissue(s) that transiently expresses several key enzymes including CYP17A1 and 5α-reductase to convert the accumulated 17-OHP into DHT (10) (Fig. 1). Thus, fetal to infantile gonads and/or adrenals would be the candidate tissue in the human (10). For the gonads, however, although steroidogenic cells are well developed in the fetal testis, they are barely present in the fetal to prepubertal ovary (18), yet the urine steroid profiles were similar between the male and female patients in this study. This may argue against the gonads being the major site for the backdoor pathway. For the adrenals, the fetal zone may be involved in the transient
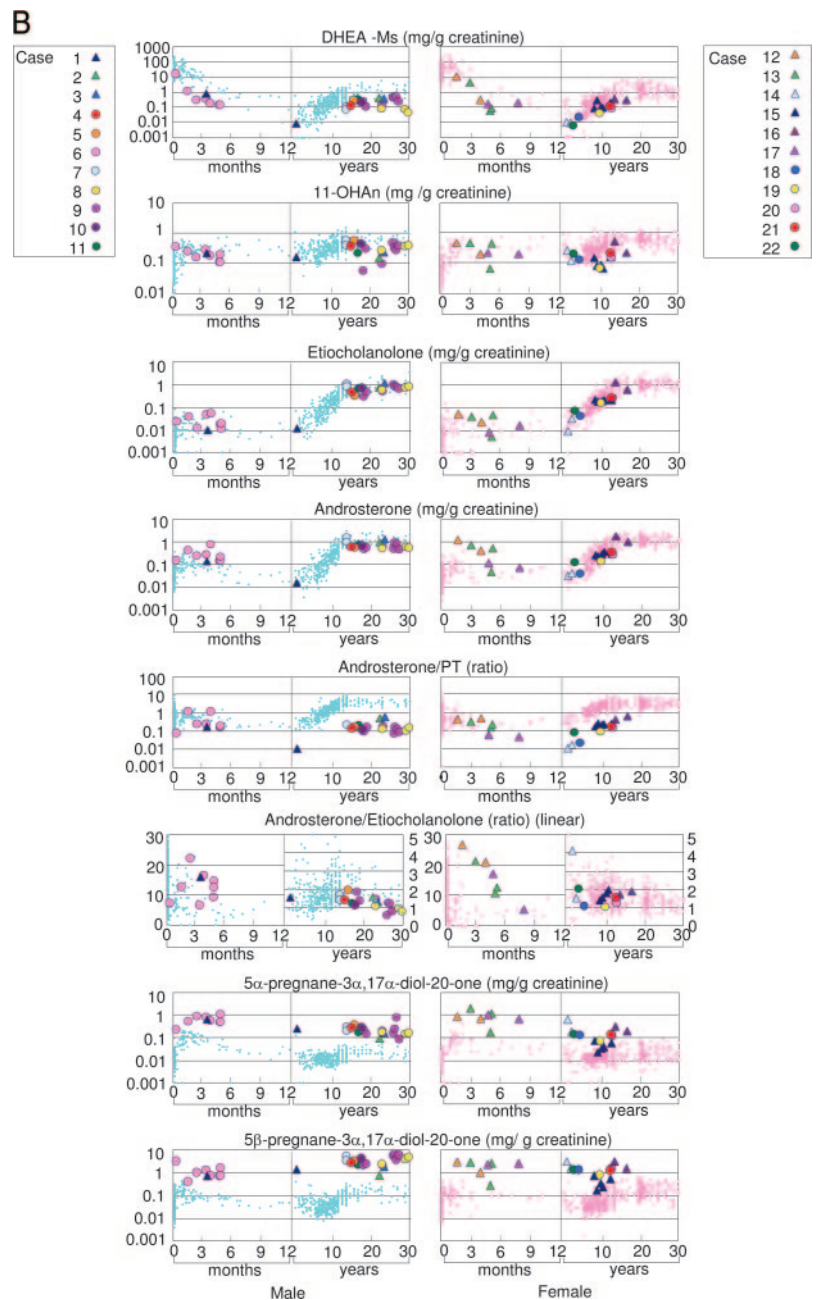
FIG. 2. *Continued*

activity of the backdoor pathway, because it disappears shortly after birth (19). In this context, because 3β-hydroxysteroid dehydrogenase activity is reduced in the fetal zone (19), it is unlikely that the fetal zone alone can produce a sufficient amount of 17-OHP to drive the backdoor pathway. However, because the fetal zone has abundant CYP17A1 activity (19), this may facilitate the conversion of the permanent zone derived 17-OHP into DHT via the backdoor pathway. In addition, other tissues/organs such as the fetal liver may also be relevant to the backdoor pathway. Therefore, this matter awaits further studies.

The urine steroid profile appeared grossly similar between groups 1 and 2. Thus, urine steroid profile analysis, although highly useful for the diagnosis of PORD, is unlikely to serve

to differentiate between the two groups of PORD. This would be consistent with the extent of defective genital development of the patients and maternal virilization during pregnancy being similar between the two groups, although masculinization in genetic male patients tended to be better preserved in group 1 than in group 2 (Table 1). Indeed, the urine steroid profile and the total amount of androgens derived from the conventional frontdoor pathway, the backdoor pathway, and the placenta could be similar between the two groups, primarily because of the complexity of steroidogenesis in PORD (Fig. 1). For example, the amount of 17-OHP is determined by the balance between synthesis mediated by POR-dependent CYP17A1 and degradation mediated by POR-dependent CYP17A1 and CYP21A2, and such

enzymatic reactions would also depend on the amount of substrates and products as well as the residual enzyme activity. In this regard, because cholesterol production relevant to the development of skeletal lesion should be carried out in a simple manner (Fig. 1), this would explain why skeletal development was clearly different between groups 1 and 2. However, other genetic and/or environmental factors would also be relevant to the genital development, because case 21 with normal female genitalia had the similar steroid profile pattern.

Two points should be made with regard to the present study. First, urine steroid profile analysis was performed for random spot urine samples in most of the patients and for different types of urine samples in the control subjects, although random spot urine samples were primarily examined before 12 months of age in both the patients and the control subjects. This may have reduced the accuracy of the data, especially the excretion dosage data even after the correction with creatinine, because of the circadian rhythms of steroidogenesis. Second, urine steroid profile analysis in the early infantile period was carried out only in five cases, with repeated examinations in case 6, and most patients received the examinations at later age. Thus, further studies in the fetal-to-early-infantile period are necessary to provide more evidence for the presence of the backdoor pathway.

In summary, the results suggest that the backdoor pathway to DHT exists in the fetal-to-early-infantile period of PORD patients. Further studies will clarify the relevance of backdoor pathway to abnormal genital development in PORD as well as to virilization in 21-hydroxylase deficiency and other pathological conditions such as polycystic ovary syndrome.

Acknowledgments

References

1. **Miller WL** 2004 P450 oxidoreductase deficiency: a new disorder of steroidogenesis with multiple clinical manifestations. Trends Endocrinol Metab 15: 311–315
2. **Flück CE, Tajima T, Pandey AV, Arlt W, Okuhara K, Verge CF, Jabs EW, Mendonca BB, Fujieda K, Miller WL** 2004 Mutant P450 oxidoreductase causes disordered steroidogenesis with and without Antley-Bixler syndrome. Nat Genet 36:228–230
3. **Arlt W, Walker EA, Draper N, Ivison HE, Ride JP, Hammer F, Chalder SM, Borucka-Mankiewicz M, Hauffa BP, Malunowicz EM, Stewart PM, Shackleton CH** 2004 Congenital adrenal hyperplasia caused by mutant P450 oxidoreductase and human androgen synthesis: analytical study. Lancet 363: 2128–2135
4. **Fukami M, Horikawa R, Nagai T, Tanaka T, Naiki Y, Sato N, Okuyama T, Nakai H, Soneda S, Tachibana K, Matsuo N, Sato S, Homma K, Nishimura G, Hasegawa T, Ogata T** 2005 Cytochrome P450 oxidoreductase gene mutations and Antley-Bixler syndrome with abnormal genitalia and/or impaired steroidogenesis: molecular and clinical studies in 10 patients. J Clin Endocrinol Metab 90:414–426
5. **Huang N, Pandey AV, Agrawal V, Reardon W, Lapunzina PD, Mowat D, Jabs EW, Van Vliet G, Sack J, Fluck CE, Miller WL** 2005 Diversity and function of mutations in P450 oxidoreductase in patients with Antley-Bixler syndrome and disordered steroidogenesis. Am J Hum Genet 76:729–749
6. **Adachi M, Tachibana K, Asakura Y, Yamamoto T, Hanaki K, Oka A** 2004 Compound heterozygous mutations of cytochrome P450 oxidoreductase gene (POR) in two patients with Antley-Bixler syndrome. Am J Med Genet A 128:333–339
7. **Fukami M, Hasegawa T, Horikawa R, Ohashi T, Nishimura G, Homma K, Ogata T** 2006 Cytochrome P450 oxidoreductase deficiency in three patients initially regarded as having 21-hydroxylase deficiency and/or aromatase deficiency. Pediatr Res 59:276–280
8. **Grumbach MM, Auchus RJ** 1999 Estrogen: consequences and implications of human mutations in synthesis and action. J Clin Endocrinol Metab 84:4677–4694
9. **Shackleton C, Marcos J, Arlt W, Hauffa BP** 2004 Prenatal diagnosis of P450 oxidoreductase deficiency (ORD): a disorder causing low pregnancy estriol, maternal and fetal virilization, and the Antley-Bixler syndrome phenotype. Am J Med Genet A 129:105–112
10. **Auchus RJ** 2004 The backdoor pathway to dihydrotestosterone. Trends Endocrinol Metab 15:32–438
11. **Wilson JD, Auchus RJ, Leihy MW, Guryev OL, Estabrook RW, Osborn SM, Shaw G, Renfree MB** 2003 5α-Androstane-3α,17β-diol is formed in tammar wallaby pouch young testes by a pathway involving 5α-pregnane-3α,17α-diol-20-one as a key intermediate. Endocrinology 144:575–580
12. **Mahendroo M, Wilson JD, Richardson JA, Auchus RJ** 2004 Steroid 5α-reductase 1 promotes 5α-androstane-3α,17β-diol synthesis in immature mouse testes by two pathways. Mol Cell Endocrinol 222:113–120
13. **Gupta MK, Guryev OL, Auchus RJ** 2003 5α-Reduced C21 steroids are substrates for human cytochrome P450c17. Arch Biochem Biophys 418:151–160
14. **Homma K, Hasegawa T, Masumoto M, Takeshita E, Watanabe K, Chiba H, Kurosawa T, Takahashi T, Matsuo N** 2003 Reference values for urinary steroids in Japanese newborn infants: gas chromatography/mass spectrometry in selected ion monitoring. Endocr J 50:783–792
15. **Homma K, Hasegawa T, Takeshita E, Watanabe K, Anzo M, Toyoura T, Jinno K, Ohashi T, Hamajima T, Takahashi Y, Takahashi T, Matsuo N** 2004 Elevated urine pregnanetriolone definitively establishes the diagnosis of classical 21-hydroxylase deficiency in term and preterm neonates. J Clin Endocrinol Metab 89:6087–6091
16. **Cragun DL, Trumpy SK, Shackleton CH, Kelley RI, Leslie ND, Mulrooney NP, Hopkin RJ** 2004 Undetectable maternal serum uE3 and postnatal abnormal sterol and steroid metabolism in Antley-Bixler syndrome. Am J Med Genet A 129:1–7
17. **Shackleton C, Marcos J, Malunowicz EM, Szarras-Czapnik M, Jira P, Taylor NF, Murphy N, Crushell E, Gottschalk M, Hauffa B, Cragun DL, Hopkin RJ, Adachi M, Arlt W** 2004 Biochemical diagnosis of Antley-Bixler syndrome by steroid analysis. Am J Med Genet A 128:223–231
18. **Grumbach MM, Hughes IA, Conte FA** 2002 Disorders of sex differentiation. In: Larsen PR, Kronenberg HM, Melmed S, Polonsky KS, eds. Williams textbook of endocrinology. 10th ed. Philadelphia: Saunders; 842–1002
19. **Fusher DA** 2002 Endocrinology of fetal development. In: Larsen PR, Kronenberg HM, Melmed S, Polonsky KS, eds. Williams textbook of endocrinology. 10th ed. Philadelphia: Saunders; 811–841