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# Cytochrome P450 Oxidoreductase Gene Mutations and Antley-Bixler Syndrome with Abnormal Genitalia and/or Impaired Steroidogenesis: Molecular and Clinical Studies in 10 Patients

Maki Fukami, Reiko Horikawa, Toshiro Nagai, Toshiaki Tanaka, Yasuhiro Naiki, Naoko Sato, Torayuki Okuyama, Hideo Nakai, Shun Soneda, Katsuhiko Tachibana, Nobutake Matsuo, Seiji Sato, Keiko Homma, Gen Nishimura, Tomonobu Hasegawa, and Tsutomu Ogata

*Department of Endocrinology and Metabolism (M.F., N.S., T.Og.), National Research Institute for Child Health and Development, Tokyo 154-8567; National Center for Child Health and Development (R.H., T.T., Y.N., T.Ok., N.M.), Tokyo 154-8535; Departments of Pediatrics (T.N.) and Urology (H.N.), Dokkyo University School of Medicine Koshigaya Hospital, Koshigaya 343-8555; Department of Pediatrics (S.So.), Saint Marianna University School of Medicine, Kawasaki 216-8511; Division of Endocrinology and Metabolism (K.T.), Kanagawa Children's Medical Center, Yokohama 232-8555; Departments of Pediatrics (N.M., S.Sa., T.H.) and Laboratory Medicine (K.H.), Keio University School of Medicine, Tokyo 160-8582; and Division of Radiology (G.N.), Tokyo Metropolitan Kiyose Children's Hospital, Kiyose 204-8567, Japan*

**We report on molecular and clinical findings in 10 Japanese patients (four males and six females) from eight families (two pairs of siblings and six isolated cases) with Antley-Bixler syndrome accompanied by abnormal genitalia and/or impaired steroidogenesis. Direct sequencing was performed for all the 15 exons of cytochrome P450 oxidoreductase gene (*POR*), showing two missense mutations (R457H and Y578C), a 24-bp deletion mutation resulting in loss of nine amino acids and creation of one amino acid (L612_W620delinsR), a single bp insertion mutation leading to frameshift (I444fsX449), and a silent mutation (G5G). R457H has previously been shown to be a pathologic mutation, and computerized modeling analyses indicated that the 15A>G for G5G could disturb an exonic splicing enhancer motif, and the remaining three mutations should affect protein conformations. Six patients were compound heterozygotes, and three patients were R457H homozygotes; no mutation was identified on one allele of the remaining one patient. Clinical findings included various degrees of skeletal features, such as brachycephaly, radiohumeral synostosis, and digital joint contractures in patients of both sexes, normal-to-poor masculinization during fetal and pubertal periods in male patients, virilization during fetal life and poor pubertal development without worsening of virilization in female patients, and relatively large height gain and delayed bone age from the pubertal period in patients of both sexes, together with maternal virilization during pregnancy. Blood cholesterol was grossly normal, and endocrine studies revealed defective CYP17A1 and CYP21A2 activities. The results suggest that Antley-Bixler syndrome with abnormal genitalia and/or impaired steroidogenesis is caused by *POR* mutations, and that clinical features are variable and primarily explained by impaired activities of POR-dependent CYP51A1, CYP17A1, CYP21A2, and CYP19A1. (*J Clin Endocrinol Metab* 90: 414–426, 2005)**

ANTLEY-BIXLER SYNDROME (ABS) is a rare congenital malformation disorder characterized by craniofacial dysmorphism and skeletal features, such as brachycephaly, radiohumeral synostosis, and multiple joint contractures (1). ABS is a heterogeneous disorder, and it occurs with and without abnormal genitalia in both sexes (1). ABS without abnormal genitalia appears to be an autosomal dominant disorder, and *de novo* heterozygous mutations of the gene for fibroblast growth factor receptor 2 (*FGFR2*) have been identified in more than 10 patients (2–4). Thus, this condition may be regarded as a variant of Pfeiffer syndrome (5). ABS with abnormal genitalia is likely to be an autosomal recessive disorder, and it is usually associated with impaired steroidogenesis (3). Although this implies the relevance of steroidogenic impairment to the development of abnormal genitalia, it remains to be determined why abnormal genitalia take place in both sexes.

Flück *et al.* (6) have recently identified mutations of cytochrome P450 oxidoreductase gene (*POR*) in three unrelated ABS patients (two males and one female) with ambiguous genitalia and impaired steroidogenesis. The *POR* gene consists of 15 exons and shows a ubiquitous expression pattern (7, 8). POR is a flavoprotein that accepts a pair of electrons from nicotinamide adenine dinucleotide phosphate, reduced (NADPH) with flavin-adenine dinucleotide (FAD) and flavin mono-

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Abbreviations: $\Delta^4$A, Androstenedione; ABS, Antley-Bixler syndrome; BA, bone age; BL, birth length; BW, birth weight; DHEA, dehydroepiandrosterone; DHEA-S, DHEA sulfate; DHT, dihydrotestosterone; $E_2$, estradiol; ESE, exonic splicing enhancer; FAD, flavin-adenine dinucleotide; FMN, flavin mononucleotide; hCG, human chorionic gonadotropin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NADPH, nicotinamide adenine dinucleotide phosphate, reduced; *POR*, cytochrome P450 oxidoreductase gene; SC35, splicing component 35 kDa; SDS, SD score; SF2/ASF, splicing factor-2/alternative splicing factor; SLO, Smith-Lemli-Opitz (syndrome); SNP, single-nucleotide polymorphism; SQLE, squalene epoxidase; SRp, serine/arginine-rich protein(s); T, testosterone; TE, testosterone enanthate.

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nucleotide (FMN) being the ports of entry and exit, respectively, and transfers electrons to all microsomal P450 enzymes, including CYP51A1, CYP17A1, CYP21A2, and CYP19A1 (9–11), as well as to several non-P450 enzymes, such as squalene epoxidase (SQLE) (12). Indeed, defective CYP51A1 and CYP17A1 activities have been indicated in *POR* mutation-positive patients (6, 13).

Subsequent studies have detected *POR* mutations in an additional five patients (two males and three females) from four families (14, 15). Although impaired steroidogenesis was invariably manifested by the five patients, typical ABS with abnormal genitalia was exhibited by one female only. ABS-compatible skeletal features were absent or equivocal in one of the two males and in the remaining two females, and abnormal genitalia were absent in the two males. These findings, in conjunction with lack of skeletal and external genital abnormalities in a *POR* mutation-positive female with steroidogenic impairment described by Flück *et al.* (6), suggest that *POR* mutations lead to a relatively wide phenotypic spectrum, including ABS with abnormal genitalia.

However, molecular and clinical studies have been performed on only a few subjects with ABS accompanied by abnormal genitalia and/or impaired steroidogenesis, so that several matters remain to be clarified, including the frequency and the type of *POR* mutations and the cause and the character of clinical features. To examine these matters, we studied 10 patients with ABS and abnormal genitalia and/or impaired steroidogenesis.

## Patients and Methods

### *Patients*

Ten Japanese patients (four males and six females), 2–29 yr old, were investigated (Table 1). Cases 1 and 2 were siblings, as were cases 3 and 4. The remaining six cases were isolated. Male patients were ascertained in their late teens because of hypomasculinization (cases 1 and 2), familial study of a severely affected younger sister (case 4), and skeletal anomalies (case 5). Female patients were identified at birth due to virilization of external genitalia (cases 3 and 6–10). Mental development was apparently normal, and karyotype and routine laboratory tests were normal in all the 10 cases. Cases 3, 7, and 9 had adrenal crisis at the time of infections, and case 3 died of adrenal failure. There was no particular episode, such as intoxication to drugs, including barbitals used for anesthesia in eight cases who received surgical treatment. According to the hospital records, maternal virilization, such as hirsuitism and voice deepening, was observed during pregnancy of cases 3, 4, 6, 9, and 10, and placental dysfunction was present during pregnancy of case 7. In case 1, maternal blood estriol was recorded as being low in the third trimester. The mother of case 7 experienced one miscarriage. There was no intrafamilial consanguinity.

### *Molecular and modeling analyses of POR*

This study was approved by the Institutional Review Board Committee at the National Center for Child Health and Development. After obtaining written informed consent, leukocyte genomic DNA was amplified by PCR for all the 15 exons and their flanking introns of *POR*, with primers designed by Web Primer (http://genome-www2.stanford.edu/cgi-bin/SGD/web-primer) using genomic and cDNA sequences (Ensembl Genome Browser, http://www.ensembl.org/, accession numbers AC005067, AC006330, and NM_000941.1). The primer sequences are shown in Table 2, together with the annealing temperatures and the PCR product sizes. Subsequently, the PCR products were subjected to direct sequencing from both directions on a CEQ 8000 autosequencer (Beckman Coulter, Fullerton, CA). To confirm a heterozygous mutation, the corresponding PCR products were subcloned with a TOPO TA Cloning Kit (Invitrogen, Carlsbad, CA), and normal and mutant alleles were sequenced separately. When possible, the corresponding PCR products were digested with appropriate restriction enzymes. Moreover, single-nucleotide polymorphisms (SNPs) were genotyped to evaluate the presence or absence of mutation-specific haplotypes. For controls, DNA samples of 100 normal Japanese individuals were used with permission.

Furthermore, exonic splicing enhancer (ESE) motif and three-dimensional protein conformation were analyzed for *POR* mutations. ESEs are distinctive exonic sequences of approximately 6–8 nucleotides that play a critical role in the accuracy or efficiency of pre-mRNA splicing (16). The ESE motif was examined by ESEfinder (http://exon.cshl.edu/ESE/, release number 2.0) using four motif-scoring matrices to predict functional ESEs recognized by four serine/arginine-rich proteins (SRp): splicing factor-2/alternative splicing factor (SF2/ASF), splicing component 35 kDa (SC35), SRp 40 kDa (SRp40), and SRp 55 kDa (SRp55), with the threshold value being 1.956 for SF2/ASF, 2.383 for SC35, 2.67 for SRp40, and 2.676 for SRp55 (17). The protein conformation was analyzed by EsyPred3D (http://www.fundp.ac.be/urbm/bioinfo/esypred/) (18).

### *Clinical assessment*

Clinical assessment was primarily performed for skeletal abnormality, sex development, and growth pattern. For skeletal assessment, physical examination and bone survey were performed. Conduction deafness was evaluated by auditory brain stem response in patients with hearing difficulty. Tracheal anomaly was investigated by fluoroscopy and/or endoscopy in patients with severe stridor. For sex development evaluation, external genitalia were observed clinically. Penile length, clitoral size, testis volume, and Tanner pubertal stage were assessed by the Japanese reference data (19–21). Internal genital structure was examined by ultrasounds and/or magnetic resonance imaging. For growth evaluation, birth length (BL) and birth weight (BW) were assessed by the gestational age-matched Japanese standards, and present actual height and weight were evaluated by the longitudinal Japanese standards (22). Bone age (BA) was determined by the Tanner-Whitehouse 2 method standardized for the Japanese (23). In addition, cardiac abnormality was evaluated by auscultation, electrocardiography, and/or echocardiography; and renal malformation was examined by ultrasounds and/or magnetic resonance imaging. Voiding cystourethrography and iv pyelography were also performed in patients with episodes of urinary tract infections.

### *Cholesterol-related studies*

Blood total cholesterol and high-density lipoprotein (HDL) cholesterol were obtained. When possible, the activity of low-density lipoprotein (LDL) receptor was examined for peripheral lymphocytes by a flow cytometric assay (24).

### *Endocrine studies*

Basal blood ACTH, renin activity, and various adrenal steroids were measured, as were basal blood LH, FSH, testosterone (T), and estradiol ($E_2$). When possible, an ACTH stimulation test [250 $\mu g/m^2$ (maximum, 250 $\mu g$) bolus iv; blood sampling at 0 and 60 min], a GnRH test [100 $\mu g/m^2$ (maximum, 100 $\mu g$) bolus iv; blood sampling at 0, 30, 60, 90, and 120 min], and a human chorionic gonadotropin (hCG) test [3000 IU/$m^2$/dose (maximum, 5000 IU) im for three consecutive days; blood sampling on d 1 and 4] were performed. Furthermore, urine steroid hormone profile was determined for random urine samples by gas chromatograph-mass spectrometry. The results were compared with the age- and sex-matched reference data (25–28).

## Results

### *Molecular and modeling analyses of POR*

*POR* mutations are summarized in Table 3, and representative results are shown in Fig. 1. Fifteen of the 16 alleles from eight families were found to have mutations: 10 alleles bore a missense mutation (R457H) at exon 11; two alleles bore a single bp insertion mutation leading

**TABLE 1.** Summary of clinical findings

| | Case 1 | Case 2 | Case 3 | Case 4 |
|---|---|---|---|---|
| Present age (yr) | 26.0 | 29.0 | 10.9 | 17.9 |
| Familial/isolated | Familial-A | Familial-A | Familial-B | Familial-B |
| Karyotype | 46,XY | 46,XY | 46,XX | 46,XY |
| Adrenal crisis | – | – | + (10 yr)[a,b] | – |
| Maternal virilization during pregnancy | – | – | + | + |
| Skeletal lesion | | | | |
| Brachycephaly/craniosynostosis | + (Overt, 1 yr)[c] | + (Overt) | + (Overt) | + (Overt) |
| Midface hypoplasia | + (Overt) | + (Overt) | + (Overt) | + (Overt) |
| Conduction deafness | +[d] | +[d] | – | – |
| Radiohumeral synostosis | – | + (10 yr)[c] | + | + |
| Restricted elbow extension | + | + | + | + |
| Femoral bowing | – | – | – | – |
| Carpal/tarsal synostosis | + | + | – | ++ |
| Multiple digital joint contractures | + (Overt) | + (Overt) | – | – |
| Arachnodactyly | + (Overt) | + (Overt) | + (Overt) | + (Overt) |
| Syndactyly | – | – | – | + (3–4th toes, B) |
| Scoliosis | + (Overt, 13 yr)[c] | – | – | – |
| Male sex development | | | | |
| Micropenis (penile length SDS) | + (−5.7)[e] | + (−5.0) | | – |
| Cryptochidism | + (L-inguinal) | + (R-inguinal) | | – |
| Hypospadias | + (Scrotal, 19 yr)[c] | – | | – |
| Testis size (ml) | 12 (R)[f] | 25 (L)[f] | | 25 (R)[f], 20 (L)[f] |
| Pubic hair (Tanner stage) | 2[g] | 3[g] | | 5[g] |
| Female sex development | | | | |
| Clitoromegaly (clitoral length SDS) | | | + (10 yr)[c] | |
| Labial fusion | | | + (Complete) (10 yr)[c] | |
| Breast (Tanner stage) | | | 1[i] | |
| Pubic hair (Tanner stage) | | | 1[i] | |
| Ovarian cyst | | | + (L) | |
| Growth pattern | | | | |
| Birth length (SDS) | +0.2 | N.E. | +0.6 | N.E. |
| Birth weight (SDS) | −1.8 | N.E. | −0.2 | −1.3 |
| Gestational age (wk) | 38 | N.E. | 39 | 40 |
| Present actual height (SDS) | +1.2 | +1.5 | +1.2 | ±0 |
| Present actual weight (SDS) | −1.5 | −0.2 | +0.9 | −0.9 |
| Bone age (chronological age at exam.) (yr) | 13.5 (18.0) | N.E. | 7.9 (8.5) | 16.5 (17.9) |
| Other findings | | | | |
| Renal lesion | + (R-VUR) | – | – | – |

N.E., Not examined; R, right; L, left; B, bilateral; PIP, proximal interphalangeal joint; and VUR, vesicoureteral reflux.
[a] Age at the occurrence of adrenal failure.
[b] Deceased at the time of adrenal failure.
[c] Surgical intervention for the corresponding lesion at the age shown in *parentheses*.
[d] With hearing aids.
[e] TE im (250 mg/dose, every 3 wk) from 18.5 yr of age.
[f] Japanese reference data: 13–20 ml at 15 yr of age, depending on the pubertal stage; no adult data available.
[g] Japanese reference data (mean ± SD, yr): P2, 12.5 ± 0.9; P3, 14.0 ± 0.3; P4, 14.9 ± 0.3; and P5, not available.
[h] TE im (25–50 mg/dose, 10 times; total 400 mg) during 2–11 months of age.
[i] Japanese reference data (mean ± SD, yr): B2, 10.0 ± 1.4; B3, 11.6 ± 1.5; B4, 13.3 ± 1.5; and B5, 14.2 ± 1.2.
[j] On hormone replacement therapy from1 3 yr of age.
[k] On hormone replacement therapy from 15 yr of age.
[l] Japanese reference data (mean ± SD, yr): P2, 11.7 ± 1.6; P3, not available; P4, 13.9 ± 1.0; P5, not available.
[m] Left ovariectomy due to a large ovarian cyst (no cyst in the right ovary).

to frameshift and resultant premature termination (I444fsX449) at exon 11; and the remaining three alleles bore private mutations, *i.e.* another missense mutation (Y578C) at exon 13, a 24-bp deletion mutation leading to loss of nine amino acids and creation of one amino acid (L612_W620delinsR) at exon 14, and a silent mutation (G5G) at exon 1. These mutations were confirmed by sequencing of the subcloned alleles. In addition, creation of a *Bsa*XI site by R457H and that of an *Rsa*I site by Y578C were confirmed by the enzyme digestion analysis. Cases 1–5 and 9 were compound heterozygotes, and cases 6, 8, and 10 were homozygotes for R457H. In case 7, no mutation was identified on one allele. The five mutations were absent in the 100 control subjects. The ESEfinder analysis showed that the silent 15A>G mutation for G5G specifically disrupted a high-score SRp40 motif. The EsyPred3D analysis indicated that R457H caused no apparent conformational alteration, whereas Y578C affected three β-strands at the H322–V325, R457–S460, and F515–R517 regions, I444fsX449 resulted in a drastic conformational alteration, and L612_W620delinsR disrupted an α-helix in the corresponding region.

Furthermore, a novel SNP (IVS4 + 33G>A) and nine recurrent SNPs reported in the National Center for Biotechnology Information Database (http://www.ncbi.nlm.nih.gov/) were identified in a region encompassing exons 4–12

**TABLE 1.** *Continued*

| Case 5 | Case 6 | Case 7 | Case 8 | Case 9 | Case 10 |
|---|---|---|---|---|---|
| 17.5 | 2.0 | 6.7 | 9.5 | 13.2 | 15.0 |
| Isolated | Isolated | Isolated | Isolated | Isolated | Isolated |
| 46,XY | 46,XX | 46,XX | 46,XX | 46,XX | 46,XX |
| – | – | + (6 yr)[a] | – | + (5 yr)[a] | – |
| – | + | – | – | + | + |
| + (Overt) | + (Mild) | + (Overt) | + (Mild) | + (Overt) | Borderline |
| + (Overt) | + (Mild) | + (Overt) | + (Mild) | + (Overt) | – |
| – | – | +[d] | – | + (13 yr)[c,d] | – |
| – | – | + | – | + | – |
| + | + | + | + | + | – |
| – | – | + | – | – | – |
| – | + | – | + | – | |
| + (Overt) | – (Mild) | + (Overt) | + (Mild) | + (Overt) | PIP only |
| + (Mild) | – | + (Overt) | – | + (Overt) | Borderline |
| – | + (3–4th toes, B) | – | – | – | – |
| – | – | + (Overt) | – | + (Overt) | – |
| – | | | | | |
| + (B-inguinal, 6 yr)[e] | | | | | |
| – | | | | | |
| 12 (B)[f] | | | | | |
| 3[g] | | | | | |
| – | + (+5.2) (2 yr)[c,h] | – | + (9 yr)[e] | + (1 month)[e] | + (1 yr)[e] |
| – | + (Complete) (2 yr)[e] | + (Partial) | + (Complete) (9 yr)[e] | + (Complete) (1 mo)[e] | – |
| – | 1[i] | 1[i] | Budding[j] | 2[i,j] | 2[i,k] |
| – | 1[j] | 1[l] | 1[k] | 1[l] | 2[l] |
| – | – | – | – | + (L) (7 yr)[m] | + (L) |
| N.E. | N.E. | −1.5 | +0.5 | −0.4 | +0.9 |
| N.E. | −0.6 | −2.8 | −0.2 | −1.0 | +1.2 |
| N.E. | 39 | 39 | 40 | 39 | 37 |
| −1.2 | +2.1[h] | −0.4 | −0.3 | +0.8 | +2.6 |
| −2.6 | +1.1 | −1.6 | −0.3 | −0.3 | +1.5 |
| 14.0 (17.5) | 2.5 (1.3)[b] | 6.0 (6.7) | 9.5 (9.5) | 10.0 (13.2) | 13.0 (15.0) |
| – | – | + (R-VUR) | – | – | + (B-VUR) |

(Table 3). The 10 SNPs and the four mutations on exons 11–14 resided within a genomic region of roughly 6 kb, whereas G5G on exon 1 was present in a genomic region approximately 26 kb apart from exon 4. Cases 6, 8, and 10 homozygotes for R457H were also homozygous for the SNP pattern constituting the A–G–G–C–C–G–T–G–T–C haplotype (Table 3), and the genotyping results in patients and parents heterozygous for R457H were consistent with the association of R457H with this haplotype. Moreover, the results were compatible with I444fsX449 and Y578C residing on an identical haplotype, and L612_W620delinsR and the apparently normal allele of case 7 residing on another identical haplotype. The haplotype for R457H was apparently absent from the normal alleles of heterozygous parents, whereas that for I444fsX449 and Y578C was apparently present in the mother of cases 3 and 4, as was that for L612_W620delinsR in the mother of case 5.

*Clinical assessment*

The results are summarized in Table 1. Skeletal features were primarily identified in the craniofacial, elbow, and digital regions (Fig. 2A). The phenotypic severity was grossly similar among the three major affected regions of each case, but it was variable among the 10 cases with borderline manifestation in case 10, mild manifestation in cases 6 and 8, and overt manifestation in the remaining cases. Surgical intervention was carried out for craniosynostosis and scoliosis in case 1, radiohumeral synostosis in case 2, and conduction deafness in case 9. In addition, cases 7 and 9 had tracheomalacia, case 4 had bilateral micropia, and case 8 had choanal stenosis.

Formation of external genitalia and secondary sexual characteristics were variably impaired in most cases (Fig. 2B). Male cases 1, 2, and 5 exhibited undermasculinization at birth and had defective pubertal development, whereas male case 4 had apparently normal sex development. Testosterone enanthate (TE) therapy was performed for micropenis in case 1, and surgical repair was performed for hypospadias in case 1 and cryptorchidism in case 5; testicular biopsy in case 1, at the time of operation, showed hypospermatogenesis. Female cases 3 and 6–10 had variably virilized external genitalia at birth, and genitoplasty was carried out in cases 3, 6, and 8–10. Case 6 was initially reared as a male and was repeatedly treated with TE (total dosage, 400 mg). Cases 3, 9, and 10 had ovarian cysts, and case 9 underwent left ovariectomy because of a large cyst. Cases 9 and 10 had poor pubertal development with no worsening of virilization and received hormone replacement therapy from 13 and 15 yr of age, respectively.

**TABLE 2.** The primer sequences, the annealing temperatures, and the product sizes for POR

| Exon | Forward primer | Reverse primer | AT (C) | Product size (bp) | Remark |
|---|---|---|---|---|---|
| 1 | CTGACATCTGCTGTTTCTGTCTCCTAA | ACTGCTTGGAGTGGTGACAGAAGA | 60 | 281 | |
| 2 | CACGCTCATTGCACACTTTTGT | TTCCTGAGAAGAGTAAAGACATGCAG | 62 | 113 | |
| 3 | ACCAACCCTGTGTCTGCCTT | TCCTACCCACTGCCTCCATCT | 62 | 202 | |
| 4 | TCTGGTGCGGGTTGAACCTT | TGGAGGGCTGCCTTTCAGATA | 62 | 310 | 5% DMSO |
| 5 + 6 | TCAGAGCGGCCCCTGTGT | ACCTGCGGGCACTTGCTCA | 62 | 605 | |
| 7 | TGTAGTCCAACCCCTCCCTCT | ATACACGGCAGACTGAGCCT | 62 | 248 | |
| 8 + 9 | TGGAGACGGAGACTCAGATCAAA | GCAAGGGCCTCCCAGGCA | 65 | 519 | |
| 10 + 11 | AGCATAGGCCTTGTTTCCAGCA | TGTGGCTGGCAGGGCAGT | 62 | 600 | 5% DMSO |
| 12 + 13 | TGAGGTTTGGGTGCCAGGT | GCCAAGGGTGGTGCTGTGA | 65 | 705 | 5% DMSO |
| 14 + 15 | CCCTCACAGCACCACCCTTG | AAGGCCAAGCCAAACACA | 62 | 445 | |

AT, Annealing temperature; DMSO, dimethyl sulfoxide.

No structural abnormality was detected for internal genitalia of the 10 cases.

Birth size was within the normal range, except for low BW in case 7, complicated by placental dysfunction, and the SD score (SDS) for BL was larger than that for BW in five of six cases in whom both BL and BW were available. Present actual body size was within the normal range, except for tall stature in case 10 and low weight in case 5, and height SDS was larger than weight SDS in all the 10 cases. Longitudinal height data obtained in case 10 showed that her height SDS remained around +1.0 in childhood and increased to +2.6 during her early to middle teens. In addition, height SDS in case 1 increased from −0.2 to +1.2 between 18 and 26 yr of age. BA was age-appropriate in cases examined in childhood age and delayed in cases examined in pubertal age by at least 2 yr, except for fairly preserved BA in a pubertal case 4 with normal masculinization and advanced BA in an infantile case 6 treated with a large amount of TE. Other features included vesicoureteral reflux in three cases. Cardiac abnormality was absent in the 10 cases.

*Cholesterol-related studies*

Blood total cholesterol was low in case 1; low-normal in cases 2, 4, 5, 7, and 9; and normal in cases 6, 8, and 10. HDL cholesterol was low-normal in cases 1, 6, 7, and 10 (Table 4). Blood cholesterol was also measured in the parents of case 10, showing maternal hypercholesterolemia [total cholesterol, 350 mg/dl (9.1 mmol/liter); HDL cholesterol, 46 mg/dl (1.2 mmol/liter)] and paternal normocholesterolemia [total cholesterol, 193 mg/dl (5.0 mmol/liter); HDL cholesterol, 70 mg/dl (1.8 mmol/liter)]. The activity of LDL receptor was 110% for the mother of case 10 (normal adult range, 75–130%) and 68% for case 10 (normal range for her age is unknown but lower than that for adults).

*Endocrine studies*

Blood hormone data were consistent with defective adrenal and gonadal steroidogenesis (Table 4). ACTH was normal or elevated at baseline, and cortisol was normal at baseline but did not increase to the normal range after ACTH

**TABLE 3.** Summary of molecular analysis

| | Mutation | | SNP[d] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Nucleotide change[a] | Amino acid change | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Cases 1 and 2 | 1370G>A/1835 1858del[b] | R457H /L612_W620delinsR | A /A | G /G | G /A | C /G | C /C | G /G | T /C | G /T | T /C | C /T |
| Father | 1370G>A /(−) | R457H /(−) | A /A | G /G | G /A | C /G | C /C | G /G | T /T | G /G | T /C | C /T |
| Mother | 1835 1858del[b] /(−) | L612_W620delinsR /(−) | A /A | G /G | A /A | G /G | C /C | G /G | C /C | T /T | C /C | T /T |
| Cases 3 and 4 | 1329_1330insC /1733A>G | I444fsX449 /Y578C | G /G | G /G | A /A | G /G | T /T | A /A | C /C | T /T | C /C | C /C |
| Mother | 1329_1330insC /(−) | I444fsX449 /(−) | G /G | G /G | A /A | G /G | T /T | A /A | C /C | T /T | C /C | C /C |
| Case 5 | (15A>G)[c] /1370G>A | (G5G)[c] /R457H | G /A | A /G | A /G | G /C | T /C | A /G | C /T | T /G | C /T | C /C |
| Father | (15A>G)[c] /(−) | (G5G)[c] /(−) | G /A | A /G | A /A | G /C | T /C | A /G | C /C | T /T | C /C | C /T |
| Mother | 1370G>A /(−) | R457H /(−) | A /A | G /G | G /A | C /G | C /C | G /G | T /C | G /T | T /C | C /T |
| Case 6 | 1370G>A /1370G>A | R457H /R457H | A /A | G /G | G /G | C /C | C /C | G /G | T /T | G /G | T /T | C /C |
| Case 7 | 1370G>A/ (−) | R457H /(−) | A /A | G /G | G /A | C /G | C /C | G /G | T /C | G /T | T /C | C /T |
| Case 8 | 1370G>A /1370G>A | R457H /R457H | A /A | G /G | G /G | C /C | C /C | G /G | T /T | G /G | T /T | C /C |
| Father | 1370G>A /(−) | R457H /(−) | A /G | G /G | G /A | C /G | C /C | G /G | T /C | G /T | T /C | C /T |
| Mother | 1370G>A /(−) | R457H /(−) | A /G | G /G | G /A | C /G | C /T | G /A | T /C | G /T | T /C | C /T |
| Case 9 | 1329_1330insC /1370G>A | I444fsX449 /R457H | G /A | G /G | A /G | G /C | T /C | A /G | C /T | T /G | C /T | C /C |
| Case 10 | 1370G>A /1370G>A | R457H /R457H | A /A | G /G | G /G | C /C | C /C | G /G | T /T | G /G | T /T | C /C |
| Father | 1370G>A /(−) | R457H /(−) | A /A | G /G | G /A | C /G | C /C | G /G | T /C | G /T | T /C | C /T |
| Mother | 1370G>A /(−) | R457H /(−) | A /G | G /G | G /A | C /G | C /C | G /G | T /C | G /T | T /C | C /C |

The (−) symbol indicates the absence of a recognizable mutation. The order of mutations and SNPs is: 5′-G5G-SNP1-SNP2-SNP3-SNP4-SNP5-SNP6-I444fsX449-R457H-SNP7-SNP8-SNP9-SNP10-Y578C-L612_W620delinsR-3′.

[a] The A of the ATG encoding the initiator methionine residue of the predicted translation product is denoted position +1.

[b] 1835_1858delTAAAGCAAGACCGAGAGCACCTGT.

[c] This silent mutation is absent in 100 Japanese control subjects.

[d] SNP 1, 387A>G (P129P) at exon 4; SNP 2, IVS4+33G>A at intron 4; SNP 3, IVS5-72G>A at intron 5; SNP 4, IVS9-13C>G at intron 9; SNP 5, IVS10-88C>T at intron 10; SNP 6, IVS10-80G>A at intron 10; SNP 7, IVS11-34T>C at intron 11; SNP 8, IVS11-33G>T at intron 11; SNP 9, 1455T>C (A485A) at exon 12; and SNP 10, 1508C>T (A503V) at exon 12.

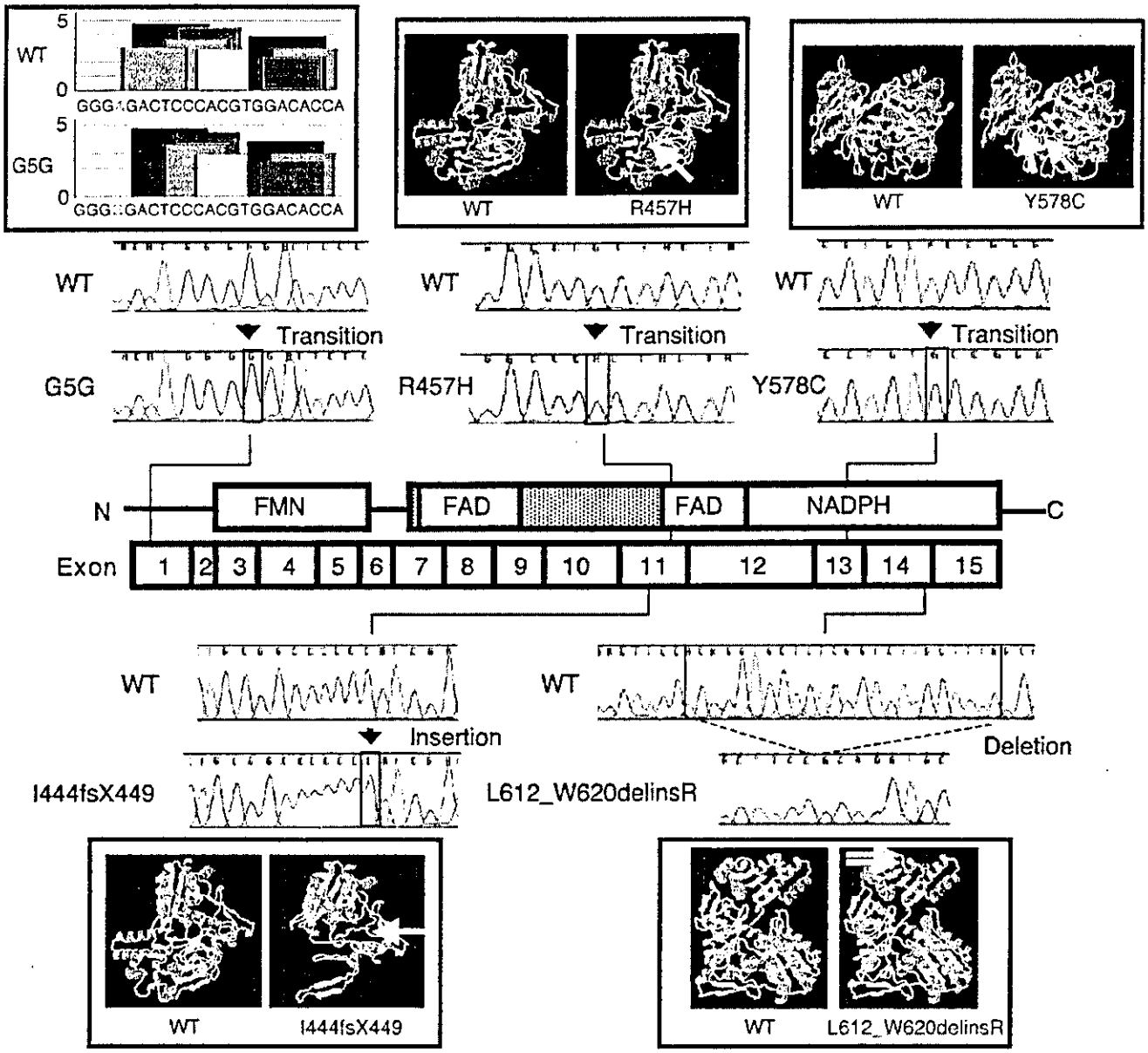


FIG. 1. *POR* mutations identified in the present study. Electropherograms indicate the DNA sequences of subcloned normal and mutant alleles. Forward sequences are depicted for G5G, R457H, Y578C, and I444fsX449, and reverse sequence is shown for L612_W620delinsR. The bar chart above the G5G electropherograms represents the results of ESE analysis, in which high scores above the threshold for SF2/ASF are shown *in red*, those for SC35 *in blue*, those for SRp40 *in green*, and those for SRp55 *in yellow*. *Each bar* is placed on the nucleotide motif for each SRp (6–8 nucleotides), and the *height of each bar* indicates the calculated score. It is predicted that the 15A>G for G5G (written *in red letters*) disrupts a high score SRp40 motif (marked with an *orange asterisk*). The ribbon diagrams above or below the electropherograms of the remaining four mutations show the results of protein modeling analysis, in which α-helices are shown *in blue*, β-strands *in yellow*, and random coils *in white*. The *white arrows* indicate the 457th residue for R457H, the 578th residue for Y578C, the 444th residue for I444fsX44, and the 612th residue for L612_W620delinsR. It is predicted that: 1) R457H results in no apparent conformational alteration; 2) Y578C affects three β-strands at the H322–V325, R457–S460, and F515–R517 regions (*orange arrow*); 3) I444fsX449 causes a drastic conformational alteration; and 4) L612_W620delinsR disrupts an α-helix in the corresponding region (*orange arrow*). In the middle of this figure, the *upper diagram* represents the cofactor binding domains (FMN, FAD, NADPH) and the connecting domain (*stippled area*), and the *lower diagram* indicates the corresponding exons of *POR*.

stimulation in most cases. Renin activity was normal at baseline, as was aldosterone. Basal and ACTH-stimulated pregnenolone, progesterone, 17-OH pregnenolone, and 17-OH progesterone were obviously elevated, as were deoxycorticosterone and corticosterone, whereas basal and ACTH-stimulated dehydroepiandrosterone (DHEA) and androstenedione ($\Delta^4$A) were normal or decreased. 17-OH progesterone was markedly elevated in all cases. Similarly,

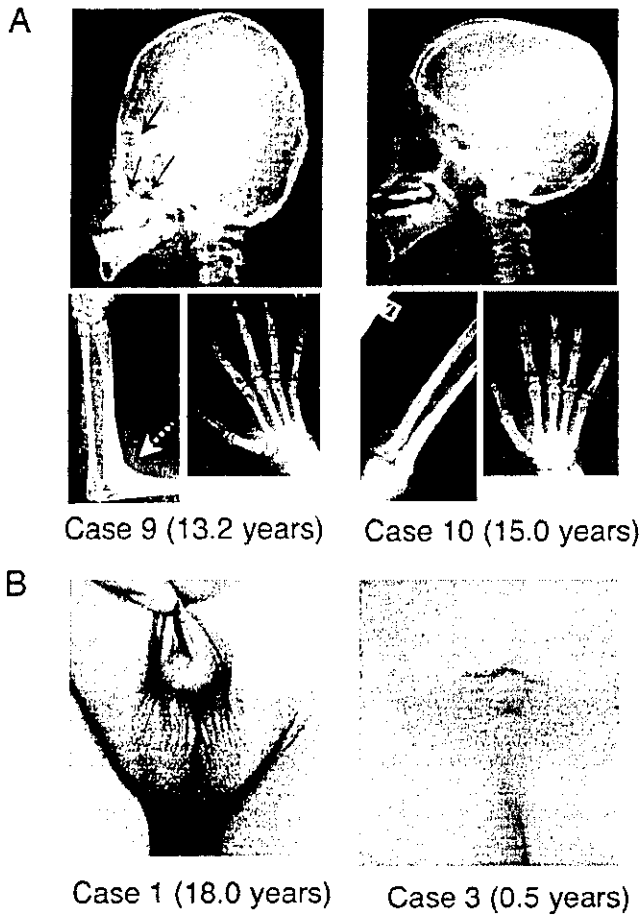


FIG. 2. Representative clinical findings. A, Skeletal features. Case 9, Craniosynostosis presents with overt brachycephaly, hypoplastic facial bones, short anterior cranial fossa (a *thick block arrow*), and deep bilateral middle cranial fossas (*thin block arrows*). Radiohumeral synostosis (a *dotted white arrow*) and arachnodactyly are also definitively manifested. Case 10, Borderline brachycephaly is seen, together with relative hypoplasia of the facial bones. Radiohumeral synostosis is absent, whereas slight arachnodactyly is suspected. B, External genitalia. Case 1, This patient with a 46,XY karyotype has micropenis, left cryptorchidism, right small intrascrotal testis, hypospadias, and sparse pubic hair. Case 3, This patient with a 46,XX karyotype shows clitoromegaly and complete labial fusion.

basal and GnRH-stimulated LH and FSH were obviously elevated in most cases, and basal T was normal or decreased in male cases and variable but grossly normal in female cases, with repeatedly measured basal T in female cases 3, 6, and 8–10 being 0.2–0.6 ng/ml (0.6–2.0 nmol/liter) in the neonatal to infantile age (reference data, not available), <0.1 ng/ml (<0.3 nmol/liter) in childhood age [reference data, <0.1 ng/ml (<0.3 nmol/liter)], and <0.1–0.5 ng/ml (<0.3–1.8 nmol/liter) in pubertal age [reference data, 0.1–0.4 ng/ml (0.3–1.3 nmol/liter)]. hCG tests yielded poor T response in male case 5 and no T response in female cases 3, 6, and 8. Basal $E_2$ was low in most cases.

Urine hormone data were also compatible with impaired steroidogenesis (Fig. 3). The steroid metabolite ratios indicating 17,20 lyase activity were at a borderline level or below the normal range in all cases examined, as were those reflecting 21-hydroxylase activity. The steroid metabolite ratios representing 17$\alpha$-hydroxylase, 11$\beta$-hydroxylase, and 3$\beta$-hydroxysteroid dehydrogenase activities grossly remained within the normal range.

## Discussion

### *POR mutations*

*POR* mutations were identified in 15 of the 16 alleles from eight families examined, and nine cases were found to be compound heterozygotes or homozygotes for five types of mutations (Table 3). Although no mutation was detected on one allele of case 7, it might be possible that a mutation resides in unexamined regions, such as the promoter and the intron sequences. Flück *et al.* (6) have also described a patient with no demonstrable mutation on one allele. Of the five mutations identified, R457H has previously been shown to be a hypomorphic mutation by the functional studies (R457H has been described as R454H in Arlt and colleagues) (6, 14). Because the R457 residue plays an essential role in the FAD binding and stabilization (10, 14), R457H may specifically disturb the interaction with FAD in the absence of a gross conformational alteration. L612_W620delinsR and I444fsX449 would drastically disrupt the POR function because of the gross conformational alterations. For Y578C, conservation of the Y578 residue among different species may argue for the functional importance of this amino acid, and the protein modeling analysis suggests the conformational change of the mutant POR protein with the cystein residue. For G5G, although this does not cause an amino acid substitution and has been described as a polymorphism in the National Center for Biotechnology Information Database (no allele frequency reported), G5G was absent in 100 control Japanese and could affect pre-mRNA splicing by disrupting the ESE motif. Indeed, it has been shown that silent, as well as nonsense and missense, mutations can affect the splicing mechanism (16). Taken together, the results would provide compelling evidence for ABS with abnormal genitalia and/or impaired steroidogenesis being an autosomal recessive disease caused by loss-of-function mutations of *POR*.

Two matters are noteworthy for the mutations. First, R457H was the most frequent mutation that was identified in 10 of the 16 alleles examined. The high frequency of R457H would be due to a founder effect in the Japanese, because R457H was associated with a specific haplotype. Indeed, R457H has also been identified in a heterozygous condition in all the three Japanese patients reported by Flück *et al.* (6) and Adachi *et al.* (15). The possibility of a hot spot mutation appears unlikely, because R457H has been detected in a heterozygous state in only one of the six non-Japanese patients from five families (6, 14), and no demonstrable fresh mutation was identified in this study. Second, the 10 cases had missense mutations in one or both alleles. In this context, the previous studies have indicated that all the nine patients have at least one missense mutations with residual POR activities (6, 14), explaining why *POR* mutation-positive patients are compatible with life, whereas *Por* knockout mice are embryonically lethal (29). Thus, although a functional study

has not been performed for Y578C, the 10 cases examined in this study must have at least one allele retaining a partial POR activity.

*Skeletal features*

Skeletal abnormality would be related to decreased activity of POR-dependent CYP51A1 (lanosterol 14α-demethylase) (11) (Fig. 4). Reduced CYP51A1 activity has been demonstrated in a *POR* mutation-positive patient (6, 13), and human fetuses exposed to a high dose of fluconazole, an antifungal agent with an inhibitory effect on CYP51A1, are known to have ABS-compatible skeletal features (30). Although the underlying mechanism remains to be determined, impaired cholesterol biosynthesis appears to play an important role in the development of skeletal abnormalities: 1) other disorders with defective cholesterol biosynthesis, such as Smith-Lemli-Opitz (SLO) syndrome, X-linked dominant chondrodysplasia punctata (CDPX2), and congenital hemidysplasia with ichthyosiform erythema and limb defects (CHILD) syndrome, also have skeletal abnormalities, although an aberrant sterol profile specific to each disorder should contribute to the development of pathognomonic skeletal features in each disorder (31); and 2) impaired cholesterol biosynthesis is known to affect Hedgehog signaling involved in skeletal development (32, 33). Thus, decreased activity of SQLE may also be relevant to the development of skeletal abnormalities in ABS, because SQLE is also a POR-dependent enzyme involved in the cholesterol biosynthesis (12).

Blood cholesterol was grossly normal in the nine cases examined. In this context, blood cholesterol value is determined by the amount of oral intake and the balance of synthesis and degradation primarily in the liver (34). In addition, an alternative pathway has been postulated for cholesterol synthesis on the basis of a substantial amount of cholesterol production in SLO syndrome patients with null mutations of *DHCR7* for 7-dehydrocholesterol reductase (13), and POR-dependent CYP7A1 (cholesterol 7α hydroxylase) is required for cholesterol degradation (34). Moreover, it has been reported that patients with CDPX2 and CHILD syndrome have minimal or no sterol abnormalities in blood but show diagnostic abnormalities in cultured lymphoblasts (35, 36). These findings would explain why blood cholesterol was grossly normal in *POR* mutations, and suggest that the impaired cholesterologenesis and resultant Hedgehog signaling defects could be present within the target skeletal cells despite the grossly normal blood cholesterol values.

Skeletal abnormality was mild in cases 6 and 8 and at a borderline level in case 10. Because cases 6, 8, and 10 were homozygous for R457H with a residual activity (6, 14), this would suggest that the severity of skeletal features is primarily determined by the degree of the residual POR activity that permits cholesterol-dependent Hedgehog signaling in the developing skeletal tissues. Consistent with this, Flück *et al.* (6) have reported that severe *POR* mutations cause skeletal abnormality and mild *POR* mutations permit apparently normal skeletal development. For the borderline skeletal features in case 10, maternal hypercholesterolemia might have played a certain role. Because fetal blood cholesterol value correlates with maternal blood cholesterol value (37), a relatively large amount of cholesterol might have been transported into the developing skeletal tissues via LDL receptor with a low normal activity, as well as via other pathways (34), mitigating the skeletal features of case 10. In support of this, maternal high cholesterol intake has mitigated skeletal features in the model mice for SLO syndrome (38). However, this notion is based on the results of a single case and remains speculative at present.

*Steroidogenic impairment and adrenogenital features*

Steroidogenesis is primarily inhibited by decreased activity of POR-dependent CYP17A1 (17α-hydroxylase and 17,20 lyase) and CYP21A2 (21-hydroxylase) (9) (Fig. 4); 17,20 lyase and 21-hydroxylase deficiencies are indicated by blood and urine hormone data, and 17α-hydroxylase deficiency is suggested by the elevated blood pregnenolone and progesterone, as well as increased blood deoxycorticosterone and corticosterone in the presence of 21-hydroxylase deficiency. Because 17,20 lyase reaction is much more severely impeded by low POR concentrations than 17α-hydroxylase reaction (39), this would explain why 17,20 lyase deficiency is more evident than 17α-hydroxylase deficiency in *POR* mutations. In addition, decreased availability of cholesterol as the precursor of sex steroids would also be relevant to defective steroidogenesis. In addition, because CYP19A1 (aromatase) activity should also be impaired in *POR* mutations (9), this would affect pubertal estrogen production.

Furthermore, two unique hormonal environments would emerge during pregnancy (Fig. 4). First, placental CYP19A1 activity would be compromised. In this context, the placenta would synthesize a certain amount of T and $\Delta^4$A from maternally derived DHEA, although DHEA of fetal origin would be reduced because of an impaired CYP17A1 activity. It is inferred, therefore, that most, if not all, of the T and $\Delta^4$A remain unaromatized and are transferred into the maternal and fetal circulations, as has been indicated in CYP19A1 deficiency (40). In addition, the placental estriol production would also be impaired, because of a compromised placental CYP19A1 activity affecting aromatization and a defective fetal CYP17A1 activity affecting the fetal 16-OH DHEA-S (DHEA sulfate) production. Second, fetal gonads may provide an alternative pathway for dihydrotestosterone (DHT) synthesis (14). It is known in the tammar wallaby that the fetal gonads can convert 17-OH progesterone to 5α-pregnane-3α,17α-diol-20-one, which has a much higher affinity for CYP17A1 than other substrates, including 17-OH progesterone (41), although such a conversion has not been demonstrated in the human. Because 17-OH progesterone is markedly increased in patients with *POR* mutations, this pathway for DHT synthesis, if it exits in the human, would be more active in patients with *POR* mutations than in normal individuals.

Adrenal crisis occurred in cases 3, 7, and 9, with sudden death in case 3. In this context, although baseline cortisol and

**TABLE 4.** Summary of blood cholesterol and hormone data

| Case no. (age at exam in yr) | 1 (18.0) | | 2 (23.5) | 3 (0.5) | | 4 (17.9) | | 5 (17.5) | | 6 (2.0) | | 7 (6.6) | | 8 (8.9) | | 9 (0.6/13.2[a]) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | B | S | B | B | S | B | S | B | S | B | S | B | S | B | S | B | S |
| Cholesterol | | | | | | | | | | | | | | | | | |
| Total (mg/dl) | 99 | | 138 | | | 121 | | 117 | | 130 | | 133 | | 170 | | 124 | |
| HDL (mg/dl) | 44 | | | | | | | | | 38 | | 34 | | | | | |
| Adrenal function | | | | | | | | | | | | | | | | | |
| ACTH (pg/ml) | **114** | | **101** | 43 | | 92 | | 50 | | 25 | | **366** | | **52** | | **85** | |
| Cortisol (μg/dl) | 10.8 | 10.1 | 16.2 | 10.7 | 12.7 | 15.3 | 17.8 | 9.7 | 12.1 | 10.4 | 14.4 | 6.3 | 5.4 | 8.4 | 10.5 | 10.4 | 13.6 |
| Renin activity (ng/ml·h) | | | | 2.8 | | 1.0 | | 1.7 | | 4.4 | | | | | | 5.9 | |
| Aldosterone (ng/dl) | 14.0 | | 13.3 | | | 9.1 | | 19.0 | | 17.0 | | 2.5 | | | | 9.4 | |
| Pregnenolone (ng/ml) | **2.6** | **7.12** | **2.8** | | | | | **2.3** | **3.9** | | | **1.3** | **1.9** | | | | |
| Progesterone (ng/ml) | **27.3** | **77.3** | | **11.0** | **73.0** | | | **16.1** | **46.3** | **5.9** | **38.0** | **16.7** | **52.1** | | | **21.0** | **82.0** |
| 17-OH pregnenolone (ng/ml) | **12.1** | **18.1** | **15.4** | 6.7 | 11.5 | **25.9** | **19.8** | **8.9** | **10.7** | **2.1** | 2.3 | **2.1** | 2.6 | **5.6** | | **23.7** | **26.7** |
| 17-OH progesterone (ng/ml) | **10.0** | **14.8** | **33.9** | **11.0** | **21.0** | **24.0** | **35.0** | **22.2** | **39.1** | **4.9** | **32.0** | **8.1** | **13.7** | **6.3** | **40.5** | **21.0** | **56.0** |
| DOC (ng/ml) | **0.6** | **1.2** | **1.7** | **1.4** | **3.6** | | | **0.7** | **1.2** | **0.8** | 0.7 | 0.3 | 0.5 | | | 0.6 | **1.3** |
| Corticosterone (ng/ml) | **62.4** | **121.0** | **50.7** | | | | | **22.2** | **69.5** | | | | | | | **25.4** | 63.8 |
| DHEA (ng/ml) | 1.4 | 1.3 | 1.4 | 0.2 | 0.4 | | | 1.4 | 2.0 | ≤0.1 | ≤0.1 | 0.9 | 0.9 | | | 2.3 | 2.0 |
| Androstenedione (ng/ml) | 0.3 | 0.4 | 1.9 | 0.5 | 0.5 | | | 0.5 | 0.7 | 0.3 | 0.3 | ≤0.1 | ≤0.1 | | | **1.8** | |
| Gonadal function | | | | | | | | | | | | | | | | | |
| LH (mIU/ml) | **14.7** | **134.5** | **21.7** | 0.3 | **16.4** | **9.3** | **35.2** | | **58.3** | 0.2 | **10.7** | <0.2 | | <0.2 | 6.3 | **7.7** | **50.4** |
| FSH (mIU/ml) | **21.3** | **46.8** | **37.1** | **9.1** | **32.6** | **7.2** | **11.2** | | **62.4** | **5.8** | **41.5** | 0.3 | | 2.4 | **3.8** | **12.9** | **20.7** |
| Testosterone (ng/ml) | 2.2 | | 3.9 | **0.5** | (0.5) | 3.9 | | 0.2 | 0.3 | <0.1 | <0.1 | <0.1 | | <0.1 | (<0.1) | 0.4 | |
| Estradiol (pg/ml) | ≤10 | | ≤10 | 13 | | | | | | ≤10 | | ≤10 | | ≤10 | | ≤10 | |

B, Baseline value; S, stimulated value; DOC, deoxycorticosterone. The values above the age- and sex-matched reference range are *boldfaced*, and those below the reference range are *underlined*; no reference data are available for the stimulated testosterone values in female cases 3 and 8. Stimulated values have been obtained by rapid ACTH tests for adrenal steroids, by GnRH tests for gonadotropins, and by hCG tests for testosterone.

[a] A rapid ACTH test was performed at 0.6 yr of age, and a GnRH test and cholesterol measurement at 13.2 yr of age after left gonadectomy.

[b] A rapid ACTH test was performed at 1.2 yr of age, and a GnRH test and cholesterol measurement at 15.0 yr of age.

aldosterone were grossly normal probably due to the presence of some residual POR function, cortisol response to ACTH stimulation was insufficient. Thus, steroid supplementation therapy is recommended at the stress time, to avoid adrenal crisis, although daily supplementation therapy appears to be unnecessary.

Male cases 1, 2, and 5 had congenital genital abnormalities and poor pubertal development, and cases 1 and 5 received therapy and/or surgical repair. The defective male sex elopment is consistent with impaired CYP17A1 activity, ause CYP17A1 activity is indispensable for T production oth fetal and pubertal periods (42). In this connection, T l $\Delta^4$A derived from the placenta, and maybe DHT derived n the fetal gonads as well, may have contributed to the e of severe congenital undermasculinization or pseudomaphroditism. In addition, the phenotype of case 4 sugts that male sex development can be normal in *POR* tations, although the reason for sufficient masculinization ains to be clarified. Such normal male development has also been described in the previously reported two males with *POR* mutations (14, 15).

Female cases had variable virilization at birth and poor pubertal development with no progress of virilization, which required genitoplasty in infancy in most cases and hormone replacement therapy from the pubertal age. Virilization at birth would primarily be due to T and $\Delta^4$A derived from the placenta, and maybe to DHT derived from the fetal gonads as well. Because all female cases had genital virilization, the effects of these androgens may be large enough to cause virilization, irrespective of the type of *POR* mutations. In particular, because the placenta is known to produce a large amount of estrogens that are converted from androgens by CYP19A1 (40), impaired placental CYP19A1 activity may result in production of a fairly large amount of T and $\Delta^4$A. In this context, mild virilization of case 7 may be due to placental dysfunction, because placental androgen production may remain small in this condition. Poor pubertal development with no as-

**TABLE 4.** *Continued*

| 10 (1.2/15.0^) | | Age- and sex-matched reference data | | | | | | Conversion factor to SI unit |
|---|---|---|---|---|---|---|---|---|
| B | S | B | S | B | S | B | S | |
| | | Cases 1, 2, 4, and 5 | | Case 6 | | Cases 7–10 | | |
| 173 | | 114–198 | | 45–182 | | 124–217 | | × 0.026 (mmol/liter) |
| 35 | | 35–65 | | 35–84 | | 34–84 | | × 0.026 (mmol/liter) |
| | | Cases 1, 2, 4, and 5 | | Cases 3 and 9 | | Cases 6–8 and 10 | | |
| **51** | | 25–100 | | 5–50 | | 5–50 | | × 0.22 (pmol/liter) |
| **22.4** | 28.0 | 5.0–16.5 | 18.0–27.0 | 4.2–23.0 | 32.0–60.0 | 3.0–19.0 | 17.0–40.0 | × 27.6 (nmol/liter) |
| | | 0.3–4.3 | | <16.6 | | <6.7 | | × 1.0 (μg/k·h) |
| 19.0 | | 3.6–32.4 | | 7.2–71.9 | | 2.5–36.0 | | × 0.028 (nmol/liter) |
| | | 0.2–0.8 | 0.9–2.2 | 0.2–0.9 | 0.5–2.8 | 0.2–0.5 | 0.3–1.4 | × 3.16 (nmol/liter) |
| | | 0.2–12.9 | 0.4–10.8 | 0.1–0.5 | 0.7–2.0 | 0.1–0.9 | 0.5–2.3 | × 3.18 (nmol/liter) |
| | | 0.3–3.0 | 2.2–8.6 | 0.6–8.3 | 9.0–31.8 | 0.1–1.6 | 0.4–7.3 | × 3.00 (nmol/liter) |
| **11.3** | | 0.5–1.9 | 1.0–2.3 | 0.1–1.0 | 0.8–2.1 | 0.1–0.9 | 0.5–3.5 | × 3.03 (nmol/liter) |
| **1.6** | | <0.1 | 0.2–0.4 | 0.1–0.6 | 0.4–1.1 | <0.6 | 0.3–1.2 | × 3.03 (nmol/liter) |
| **36.2** | | 1.6–3.9 | 17.2–51.0 | 0.8–17.5 | 22.5–65.3 | 1.1–13.8 | 21.5–75.5 | × 2.89 (nmol/liter) |
| 0.9 | | 1.0–4.0 | 1.9–5.1 | 0.3–5.9 | 1.0–11.1 | 0.1–1.5 | 0.2–3.2 | × 3.46 (nmol/liter) |
| **2.7** | 0.2 | 0.6–1.9 | 0.8–2.1 | 0.1–0.8 | 0.3–1.0 | 0.1–0.7 | 0.1–1.0 | × 3.49 (nmol/liter) |
| | | Cases 1, 2, 4, and 5 | | Cases 3 and 6–8 | | Cases 9 and 10 | | |
| **7.0** | **51.6** | 0.2–5.0 | 5.5–21.0 | <2.0 | 1.4–10.0 | 0.4–4.5 | 5.5–30.0 | × 1.0 (IU/liter) |
| **9.5** | **20.1** | 0.6–4.9 | 1.2–8.0 | 0.3–4.0 | 2.5–26.0 | 1.2–5.5 | 4.5–12.0 | × 1.0 (IU/liter) |
| **0.5** | | 2.8–7.0 | | <0.1 | | 0.1–0.4 | | × 3.46 (nmol/liter) |
| 56 | | 10–35 | | 10–20 | | 10–60 | | × 3.67 (pmol/liter) |

sociated virilization is explained by reduced activities of CYP17A1 and CYP19A1, because this should cause impaired production of both T and $E_2$ during puberty (42). In addition, ovarian cysts may be fairly common in *POR* mutations, because they were present in cases 3, 9, and 10, and have previously been described in two of three females with *POR* mutations (6, 14). Although the underlying factor(s) remains to be determined, the abnormal endocrine status, including hypergonadotropinism, may be relevant to ovarian cysts.

*Growth pattern*

Growth patterns are also primarily consistent with *POR* mutations. Relatively thin body habitus would be ascribed to chronic relative glucocorticoid deficiency, because chronic adrenal insufficiency usually results in weight loss (43). Large height gain in the middle-to-late teens in cases 1 and 10 and retarded BA in cases 1, 5, 9, and 10 would be explained by estrogen deficiency resulting from defective CYP19A1 activity, because lack of bioactive estrogens caused by mutations of *CYP19A1* for aromatase or *ESR1* for estrogen receptor α, though it does not affect childhood growth and BA maturation, is known to cause tall stature and delayed BA from the pubertal age (40). In this regard, although the precise mechanisms remain to be clarified, grossly age-appropriate BA in case 4 with sufficient masculinization, and advanced BA in case 6 treated with a large amount of TE, may be accounted for by a residual POR activity.

*Other findings*

Several features are also worth pointing out. First, maternal virilization was present during pregnancies of five cases. This phenomenon has also been documented in three of nine pregnancies of fetuses with *POR* mutations (6, 14, 15) and is primarily explained by the effects of T transferred from the placenta to the maternal circulation (Fig. 4). Indeed, maternal virilization is also found during pregnancy of fetuses with *CYP19A1* mutations (40). Second, maternal blood estriol was recorded as being low in the pregnancy of case 1. Low maternal blood estriol has also been reported in the pregnancies of sibs with ABS and steroidogenic impairment, although *POR* analysis has not been performed in the sibs (44). This phenomenon would be due to compromised placental estriol production caused by impaired fetal CYP17A1 activity and defective placental CYP19A1 activity (Fig. 4). Third, renal anomalies were identified in three cases. This may be related to aberrant sterol metabolism, because renal malformation has frequently been identified in SLO syndrome (45). Last,

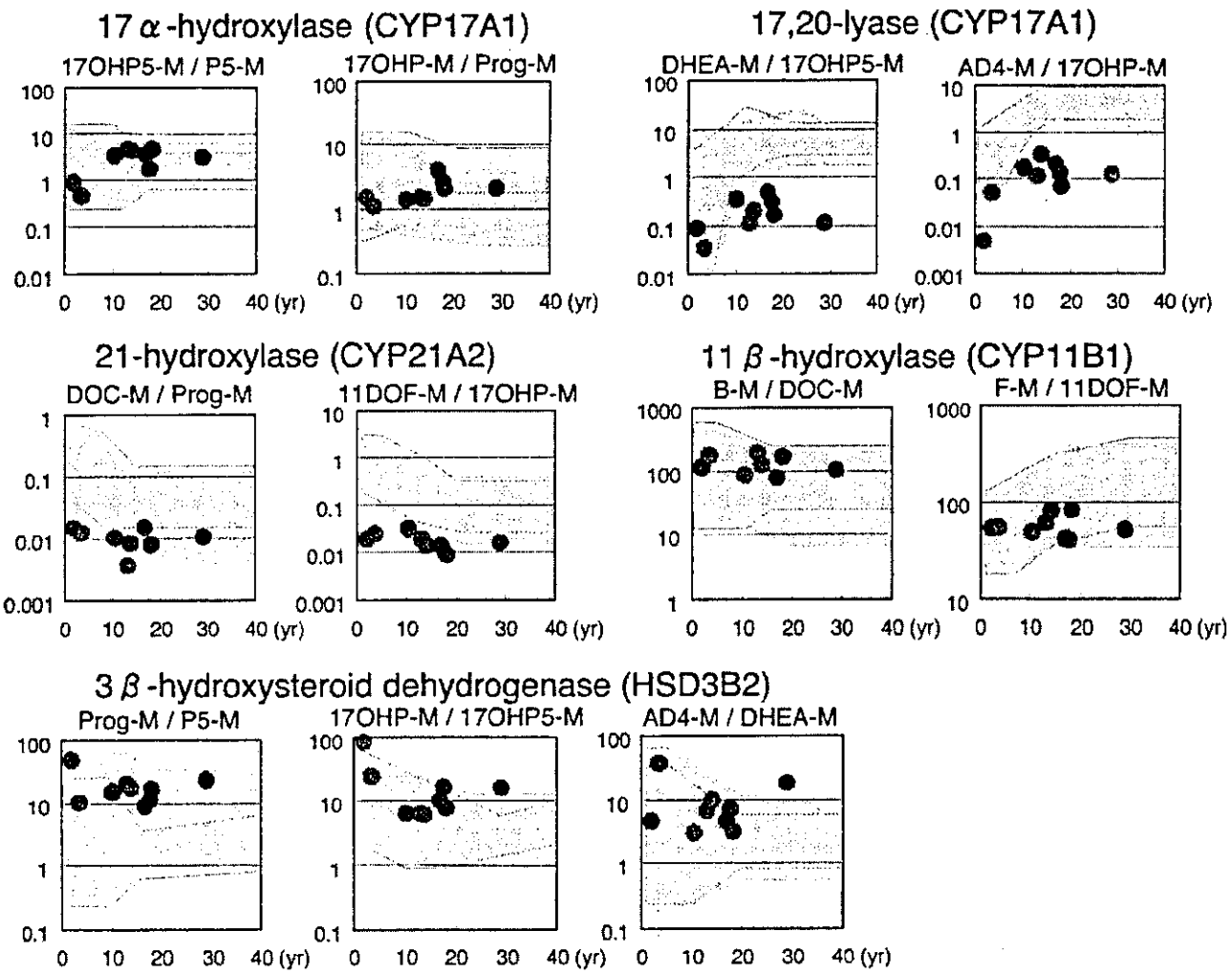


FIG. 3. Urine steroid hormone profile analysis. *Dark blue* and *red closed circles* represent male and female patient data, respectively. *Light blue* and *pink* areas indicate the normal ranges (from the minimum to the maximum) determined by the analysis of urine samples collected from 431 males and 559 females, respectively, 2–40 yr old. The age of examination is 2.0 yr for case 6 (female), 3.6 yr for case 7 (female), 10.5 yr for case 8 (female), 13.2 yr for case 9 (female), 14.0 yr for case 10 (female), 17.0 yr for case 4 (male), 17.8 yr for case 5 (male), 18.3 yr for case 1 (male), and 29.0 yr for case 2 (male). Case 3 was not studied because of sudden death, and data for CYP21A2 and CYP11B1 have not been obtained in case 5. 17OHP5-M: 17α-hydroxypregnenolone metabolites (5-pregnene-3β,17α-diol-20-one, 5-pregnene-3β,17α,20α-triol, and 5-pregnene-3β,15β,17α-triol-20-one); P5-M: pregnenolone metabolites (5-pregnene-3β,16α-diol-20-one and 5-pregnene-3β,21-diol-20-one); 17OHP-M: 17α-hydroxyprogesterone metabolites (5β-pregnane-3α,17α-diol-20-one, 5α-pregnane-3α,17α-diol-20-one, 5β-pregnane-3α,17α,20α-triol, 5α-pregnane-3α,17α,20α-triol, 5β-pregnane-3α,17α-diol-11,20-dione, 5β-pregnane-3α,17α,20α-triol-11-one, 5β-pregnane-3α,11β,17α-triol-20-one, 5β-pregnane-3α,11β,17α,20α-tetrol, and 5β-pregnane-3α,11β,15β,17α-tetrol-20-one); Prog-M: progesterone metabolite (5β-pregnane-3α,20α-diol); DHEA-M: DHEA metabolites (DHEA, 5-androstene-3β,17α-diol, 5-androstene-3β,16α-diol-17-one, 5-androstene-3β,16β-diol-17-one, 5-androstene-3β,17α-diol-16-one, and 5-androstene-3β,16α,17α-triol); AD4-M: $\Delta^4$A metabolites (androsterone and etiocholanolone); DOC-M: 11-deoxycorticosterone metabolite (tetrahydro-11-deoxycorticosterone); 11DOF-M: 11-deoxycortisol metabolite (tetrahydro-11-deoxycortisol); B-M: corticosterone metabolites (5β-tetrahydrocorticosterone, 5α-tetrahydrocorticosterone, 5β-tetrahydro-11-dehydro-corticosterone, and 5α-tetrahydro-11-dehydro-corticosterone); and F-M: cortisol metabolites (5β-tetrahydrocortisone, 5α-tetrahydrocortisone, 5β-tetrahydrocortisol, 5α-tetrahydrocortisol, cortisone, cortisol, 6β-hydroxycortisol, and 18-hydroxycortisol).

there was no history indicating abnormal responses to environmental toxins and drugs, including barbitals for anesthesia used in eight cases who underwent surgical treatment, although POR is required for microsomal P450 enzymes involved in detoxication (34). By contrast, liver-specific *Por* knockout mice show a drastically reduced pentobarbital clearance (46). Although the constructive response to barbitals between the patients and the knockout mice may be due, more or less, to the difference in species or drug dosage used, it is likely that a residual POR activity in patients is sufficient for detoxication.

## *Summary*

The present study suggests that ABS with abnormal genitalia and/or impaired steroidogenesis results from

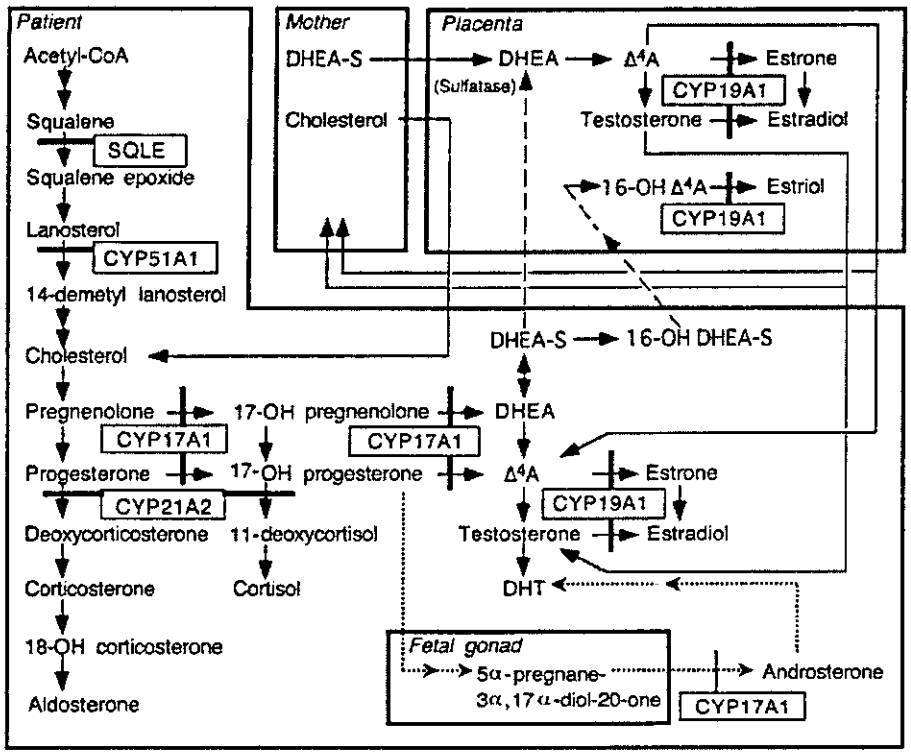


FIG. 4. Simplified schematic representation indicating impaired cholesterologenesis and steroidogenesis in *POR* mutations (DHEA, DHEA-S, $\Delta^4$A, and DHT). The activities of POR-dependent SQLE, CYP51A1 (lanosterol 14α-demethylase), CYP17A1 (17α-hydroxylase and 17,20 lyase), CYP21A2 (21-hydroxylase), and CYP19A1 (aromatase) should be impaired in patients with *POR* mutations. Only the important metabolites are shown, and the reaction steps in which some metabolites are omitted are indicated by *two tandem arrows*. Patient: Cholesterologenesis and steroidogenesis are impaired at the POR-dependent reaction steps. Fetal gonad: In the tammar wallaby, 17-OH progesterone transported into the fetal gonads can be converted into 5α-pregnane-3α,17α-diol-20-one after sequential conversion by 5α-reductase type 1 and 3α-hydroxysteroid dehydrogenase. Because 5α-pregnane-3α,17α-diol-20-one has a much higher affinity to CYP17A1 than other substrates, including 17-OH progesterone, a certain amount of androsterone may be produced and further converted into DHT after sequential conversion by 17β-hydroxysteroid dehydrogenase and 3α-hydroxysteroid dehydrogenase (this pathway is indicated by *dotted lines with arrows*, because it has not been verified in the human.) Placenta: The placenta should synthesize a certain amount of T, as well as $\Delta^4$A, using DHEA derived from maternal DHEA-S (*arrow*), although DHEA-S of fetal origin should be reduced because of decreased CYP17A1 activity (*broken line with an arrowhead*). Because the aromatization of T to $E_2$ and that of $\Delta^4$A to estrone are impaired, excessive T and $\Delta^4$A would be transferred into the maternal and fetal circulations during the pregnancy (*arrow*). Furthermore, the estriol synthesis in the placenta would be impaired because of the reduced supply of 16-OH DHEA-S from the fetus (*broken line with an arrowhead*) and compromised aromatization in the placenta. Mother: In addition to the transfer of DHEA-S from the mother to the placenta, cholesterol is transported from the mother into the fetus via placenta (*arrow*).

*POR* mutations with R457H being most frequent in the Japanese because of a founder effect, and that clinical features are variable and primarily accounted for by impaired activities of POR-dependent CYP51A1, CYP17A1, CYP21A2, and CYP19A1. Further studies including functional analysis of mutants will permit a better characterization of *POR* mutations and genotype-phenotype correlations of this condition.

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Address all correspondence and requests for reprints to: Dr. T. Ogata, Department of Endocrinology and Metabolism, National Research Institute for Child Health and Development, 3-35-31 Taishido, Setagaya, Tokyo 154-8567, Japan. E-mail: tomogata@nch.go.jp.

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# 研究構成員名簿

## 研究構成員名簿

| 氏名 | 勤務先・所属・役職 |
|---|---|
| 主任研究者 | |
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| 森　鉄也 | 慶應義塾大学医学部小児科　専任講師 |
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| 西田 光宏 | 慶應義塾大学医学部医学部小児科　医師 |
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