Clinical characteristics of cytochrome P450 oxidoreductase deficiency: a nationwide survey in Japan

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Abstract. Cytochrome P450 oxidoreductase deficiency (PORD) is a disorder of steroidogenesis that causes various symptoms such as skeletal malformations, disorders of sex development, and adrenal insufficiency. The aim of this study was to elucidate the clinical characteristics, especially age at diagnosis and treatment, of PORD from the perinatal period to adulthood in Japan. The first questionnaire was sent to 183 council members of the Japanese Society for Pediatric Endocrinology on 1 September 2018. The response rate was 65%, and a total of 39 patients with PORD were examined at 20 hospitals. The second questionnaire was sent in November 2018 to the council members examining these 39 patients with PORD. The response rate was 77%, and we received clinical information on 30 of the 39 patients. The two novel clinical findings were the age at diagnosis and the treatment of Japanese patients with PORD. In many cases, PORD can be diagnosed at <3 months of age. Hydrocortisone as the primary treatment during infancy can be used daily or in stressful situations; however, because patients with PORD generally have mild to moderate adrenal insufficiency, some might be able to avoid hydrocortisone treatment. Patients with PORD should be carefully followed up, and treatment should be optimized as for patients with other types of adrenal insufficiency. Other characteristics in the present study were similar to those described in previous reports.

Key words: Cytochrome P450 oxidoreductase deficiency, Adrenal insufficiency, Disorders of sex development, Skeletal malformations

CYTOCHROME P450 oxidoreductase deficiency (PORD) (OMIM: 613571 and 2015750) is an autosomal recessive disorder caused by loss-of-function variations in the POR gene on chromosome 7q11.23. Because the POR gene encodes an electron donor for all microsomal cytochrome P450 enzymes and several non-cytochrome P450 microsomal enzymes [1, 2], POR gene variation results in various symptoms such as skeletal malformations, disorders of sex development (DSD), and adrenal insufficiency. PORD was first reported in 2004, and more than 100 cases have since been reported worldwide [3]. Comprehensive genetic studies of Japanese [4] and Caucasian [5] patients with PORD have been published; however, the clinical characteristics, especially the age at diagnosis and treatment of PORD, have not been fully elucidated.

Materials and Methods

This study was approved by the institutional review board.
board of Kurume University School of Medicine (permission number: 18027). In this study, PORD was diagnosed when POR gene variations were identified or the patient had clinical and laboratory features conclusive for PORD. Clinical and laboratory features included three or more of the following four indexes: i) ambiguous genitalia, ii) skeletal malformations caused by impairment of CYP26 isozymes (retinoic acid metabolism) due to POR gene variations, iii) skin pigmentation, and iv) urine steroid metabolite profiling consistent with PORD. We sent the first questionnaire to 183 council members of the Japanese Society for Pediatric Endocrinology on 1 September 2018. The response rate was 65% (119/183) and spanned 20 hospitals at which 39 patients with PORD had been examined. We sent the second questionnaire to the council members examining these 39 patients with PORD in November 2018. The response rate was 77% (30/39), and data from these 30 patients with PORD were analyzed in the present study (13 males, 17 females). We collected information regarding the genotype of the POR gene as well as perinatal, childhood, pubertal, and adulthood clinical status, such as external genitalia, skeletal malformations, adrenal function, medical treatments, pubertal status, the presence of developmental delay, and maternal virilization.

**Results**

The characteristics of the patients with PORD in the neonatal period are summarized in Table 1. Thirteen males and 17 females were included in the study. The median age at diagnosis was 0.21 years (range, 0–25 years), and the median age at the time of the survey was 12.1 years (range, 0.7–46.1 years). Nine patients were diagnosed with PORD at >2 years of age (Patients 9, 10, 11, 14, 19, 20, 21, 22, and 23). Five patients (Patients 9, 11, 14, 19, and 22) were diagnosed with PORD after 2004, which was the first year of publication of PORD. Patients 20 and 21 were siblings, and they were diagnosed with PORD-like syndromes before 2004. Patient 10 may have received a late diagnosis because of the presence of ambiguous genitalia without other typical features of PORD, such as skeletal malformations or adrenal insufficiency. The patients’ clinical characteristics are summarized in Supplementary Tables 1 and 2.

**External genitalia**

The most frequent symptom at diagnosis was DSD, which was seen in 26 (86.7%) patients (male, 10/13; female, 16/17). The severity of DSD based on the external genitalia phenotype was a median of 2.5 (range, 1–3) on the Quigley scale in males and a median of 2 (range, 2–5) on the Prader scale in females (Fig. 1). The other four patients who did not show DSD were male. Four of the 17 female patients showed severe virilization with severity of 3, 4, 4, and 5 on the Prader scale. Although virilization was severe, the sex assignment was appropriate. Thirteen of 17 female patients showed moderate virilization, such as 2 or 3 on the Prader scale.

**Skeletal malformations**

Various skeletal malformations were seen in 27 patients. Major skeletal malformations were joint contracture, which was present in 19 patients, and radioulnar synostosis, which was present in 14 patients. Other skeletal malformations included midface hypoplasia, craniosynostosis, foot and hand deformities, bony union of the large joints, and femoral bending (Table 2).

**Adrenal function**

The median serum 17-hydroxyprogesterone (17-OHP) concentration at the time of diagnosis was 11.0 ng/mL (interquartile range [IQR]: 7.0–16.7 ng/mL). An adrenocorticotropic hormone (ACTH) stimulation test was performed at the time of diagnosis in 20 patients. The serum cortisol concentration was evaluated at 0, 30, and 60 minutes after stimulation. Adrenal insufficiency was diagnosed when the serum cortisol concentration was <8 μg/dL before ACTH administration and <18 μg/dL after ACTH administration. Fourteen patients (70%) were diagnosed with adrenal insufficiency based on the ACTH stimulation test result. The median ACTH and cortisol concentration at the time of diagnosis was 92.2 pg/mL (IQR: 53.2–135 pg/mL) and 8.6 μg/dL (IQR: 7.1–17.6 μg/dL), respectively. Urine steroid metabolite profiling by gas chromatography mass spectrometry [6] was performed in 20 patients. The urine steroid metabolite profile can differentiate 21-hydroxylase deficiency (21-OHD) from PORD [7, 8]. In all 20 patients, the urine steroid metabolite profile was diagnostic for PORD.

**Genetic analysis**

The POR gene was analyzed in 25 patients. Eleven

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**Table 1 Characteristics of patients with cytochrome P450 oxidoreductase deficiency in the neonatal period**

| Characteristic                  | Number (male, female) | Gestational age* (week) | Birth weight* (g) | Birth height* (cm) | Birth head circumference* (cm) | Age at diagnosis† (year) | Age at survey† (year) |
|---------------------------------|------------------------|-------------------------|------------------|-------------------|-------------------------------|-------------------------|-----------------------|
|                                 | 30 (13, 17)            | 39.5 ± 1.3              | 2,848 ± 387      | 49.4 ± 2.5        | 33.1 ± 1.5                    | 0.21 (0–25)             | 12.1 (0.7–46.1)       |
| Birth weight* (g)               | 2,848 ± 387            | 30 (13, 17)             | 49.4 ± 2.5       | 33.1 ± 1.5        | 0.21 (0–25)                   | 12.1 (0.7–46.1)         |                       |
| Birth height* (cm)              | 49.4 ± 2.5             | 2,848 ± 387             | 33.1 ± 1.5       | 0.21 (0–25)       | 12.1 (0.7–46.1)               |                         |                       |
| Birth head circumference* (cm)  | 33.1 ± 1.5             | 2,848 ± 387             | 0.21 (0–25)      | 12.1 (0.7–46.1)   |                               |                         |                       |
| Age at diagnosis† (year)        | 0.21 (0–25)            | 2,848 ± 387             | 33.1 ± 1.5       | 12.1 (0.7–46.1)   |                               |                         |                       |
| Age at survey† (year)           | 12.1 (0.7–46.1)        | 2,848 ± 387             | 0.21 (0–25)      | 12.1 (0.7–46.1)   |                               |                         |                       |

* mean ± standard deviation (SD), † median (min–max)
patients were homozygotes for the POR R457H variation, 11 were compound heterozygotes for the POR R457H variation with other variations, and 2 were heterozygotes without the POR R457H variation. One patient was not found to have POR gene variations. A chromosomal test was performed in 29 patients.

**Treatment**

A glucocorticoid (hydrocortisone) was initially administered for all 30 patients at diagnosis. Ten patients received the glucocorticoid daily, and 20 received it in stressful situations. The glucocorticoids administered to the patients at the time of the second questionnaire (i.e., during follow-up) were hydrocortisone in 26 patients and prednisolone in 2 patients. Glucocorticoids were administered only during physiologic stress in 15 patients and daily in 12 patients. Two patients did not receive glucocorticoids, and the glucocorticoid administration status was unknown in one patient.

**Puberty and adulthood**

Puberty had started in 15 patients at the time of the second questionnaire. None of the six pubertal males were administered testosterone. The luteinizing hormone, follicle-stimulating hormone, and testosterone concentrations were measured in three of the six pubertal males. The luteinizing hormone concentration was 7.0, 7.6, and 63.2 mIU/mL (reference range, 1.7–11.2 mIU/mL); the follicle-stimulating hormone concentration was 9.4, 12.3, and 99.6 mIU/mL (reference range, 2.1–18.6 mIU/mL); and the testosterone concentration was 4.69, 5.43, and 4.16 ng/mL (reference range, 1.3–8.7 ng/mL), respectively. In one male patient, only the testosterone concentration was measured and was reported as 6.28 ng/mL. Sex hormone replacement therapy was more frequent in pubertal females (7 of 9 patients, 77.8%) than in males (0 patients, 0.0%). None of the patients had children.

**Development**

Developmental delay was seen in 5 of 29 patients with PORD (1 patient was not screened for development). The degree of the developmental delay was unknown because we did not include the details in this study.

**Maternal virilization**

Maternal virilization during the perinatal period was seen in 15 patients. The major symptoms were a change of voice, acne, hirsutism, and enlargement of the nose. No maternal virilization symptoms were seen in seven patients, and the status of maternal virilization symptoms was unknown in eight patients.

**Discussion**

We surveyed Japanese patients with PORD and encountered two novel findings. First, most patients with PORD were diagnosed at the age of <3 months. This is the first study to show the mean age at diagnosis of PORD and reveal the early diagnosis. Many cases of PORD in our study were initially identified by the presence of DSD, which was also observed during the
neonatal period. Early diagnosis is important for appropriate follow-up in accordance with the natural history of PORD, such as deciding whether to prescribe daily hydrocortisone treatment or administer sex hormone replacement therapy. Second, we were able to summarize the general treatment of PORD within Japan. Because of the lack of guidelines or manuals for PORD treatment, such as the use of hydrocortisone, one of our initial aims in this survey was to reveal how hydrocortisone is administered to patients with PORD. Unfortunately, because adrenal dysfunction is not usually observed during daily life, it is often difficult to determine whether to use hydrocortisone daily, only during stressful situations, or not at all. According to a previous report, hydrocortisone should be administered if the basal serum cortisol concentration is low; it should also be administered in stressful situations if the cortisol response to an ACTH stimulation test is below normal [9]. The basal ACTH and cortisol concentrations are generally normal in patients with PORD; however, patients with PORD may need long-term glucocorticoid replacement therapy, especially during disease, inflammation, stress, or surgery [10]. When a patient is diagnosed with PORD in the neonatal or infantile period, clinicians tend to excessively administer hydrocortisone because of the risk of an insufficient cortisol status. Patients should be carefully followed up to determine whether they require hydrocortisone replacement therapy.

We also compared our results with those of previous studies. To date, more than 100 cases of PORD have been reported worldwide. The male:female ratio among patients with PORD is reportedly close to 1:1 [4], which is similar to our results. This shows that PORD is most likely inherited in an autosomal recessive manner, and the life expectancy may not be different between male and female patients.

The features of DSD in patients with PORD (75%) have been previously described [3]. The pathogenic mechanisms of DSD are associated with impaired syntheses of testosterone and dihydrotestosterone and conversion of these androgens to estrogens. The degree of external genitalia virilization is moderate in most patients of the life expectancy may not be different between male and female patients. The R457H variant of the POR gene accounts for >70% of affected alleles in the Japanese population [4]. The frequency of the POR R457H variation in East Asia is 0.0005629 as cited from the gnomAD database (https://gnomad.broadinstitute.org). The estimated frequency of PORD in Japan was 111; however, we discovered only 30 patients with PORD in the present study (30/111 = 27%). The POR R457H variation was once thought to be isolated to the Japanese population, but this variation has since been reported in Europeans and those of other ethnicities [5]. The A287P variation, which is more predominant in Caucasians, was not detected in this study. The most frequent variation in the Japanese population, the POR R457H variation, is associated with a higher prevalence of atypical genitalia in 46,XX than 46,XY patients (100% vs. 44%, respectively) [5].

Clinical differentiation between 21-OHD and PORD can be difficult because both deficiencies show similar phenotypes and a high serum 17-OHP concentration. A biochemical abnormality characteristic of PORD is a normal or slightly high 17-OHP concentration. In one study, a two-step biochemical diagnosis using urinary steroid metabolites was able to discriminate between 21-OHD and PORD with 100% sensitivity and specificity [8]. We believe that urine steroid metabolite profiling can be used to diagnose PORD.

As previously reported [4], pubertal failure is more severe in females than in males because androgen production is mediated by CYP17A1 and estrogen production is mediated by both CYP17A1 and CYP19A1. Sexual development during puberty is disturbed in patients of both sexes. This finding is consistent with insufficient sex hormone production in the gonads resulting from impaired activity of CYP19A1 and/or CYP17A1 [11].

A previous comprehensive study showed developmental delay (29%) in 17 patients with PORD [12]. In the present study, the prevalence of developmental delay was 17%, which is lower than that of the previous study but higher than that in controls. The prevalence of developmental delay is not different between controls and male patients with classic 21-OHD, which is the most common type of congenital adrenal hyperplasia and is characterized by adrenal insufficiency similar to that in patients with PORD [13]. Therefore, we believe that the developmental delay seen in our study was not associated with congenital adrenal hyperplasia. The high prevalence of developmental delay in patients with PORD may be caused by subclinical hypoglycemia due to slight adrenal insufficiency. Further investigation of the cause is needed, and a more detailed follow-up of the developmental milestones in patients with PORD is warranted.

Maternal virilization (40.8%) has been described in mothers during pregnancies of PORD-affected fetuses [3]. Hyperandrogenic manifestations in mothers have also been described during pregnancies of PORD-affected fetuses and range from acne to hirsutism until the appearance of virilization signs such as deepening of the voice [14]. A single multicenter study of 20 pregnant women showed that the presence of maternal virilization and severe birth defects detected by fetal ultrasound may
be the fundamental characteristics of fetuses with PORD [12]. However, no data in the literature show their correlation with specific \textit{POR} gene variations.

This study has several limitations. First, despite our thorough data collection, we might have missed some patients with PORD because the questionnaire was only sent to council members of the Japanese Society for Pediatric Endocrinology. Second, the collected data may be biased because this study was retrospective, the age range at the time of the survey was broad (0.7–46.1 years), and the sample size was small (30 patients). Finally, we did not collect data from the mass screening test for neonates.

In conclusion, we have described novel clinical findings, including the age at diagnosis and treatment, in Japanese patients with PORD. In many cases, PORD can be diagnosed at <3 months of age. Hydrocortisone as the primary treatment during infancy can be used daily or in stressful situations; however, because patients with PORD generally have mild to moderate adrenal insufficiency, some might be able to avoid hydrocortisone treatment. Patients with PORD should be carefully followed up, and treatment should be optimized as for patients with other types of adrenal insufficiency. Other characteristics of the patients in this study were similar to those in previous reports.

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**Disclosure**

The authors have nothing to declare.

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