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Clinical, biochemical, and genetic features of non-classical 21-hydroxylase deficiency in Japanese children

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Abstract. Non-classical 21-hydroxylase deficiency (NC21-OHD) is a mild form of 21-hydroxylase deficiency lacking apparent symptoms of androgen excess at birth. Most NC21-OHD cases are diagnosed after the onset of puberty, while a substantial number of patients are not diagnosed during childhood. Previous studies have reported ethnic differences in the prevalence of NC21-OHD. To date, the clinical features of NC21-OHD in Japanese children have not been systemically reported. Thus, we performed 3 independent analyses: retrospective analyses of newborn screening in 2 major Japanese cities (Sapporo and Niigata) and a national surveillance collecting clinical information from pediatric endocrinologists throughout the country. During the last 10 years, one case of NC21-OHD was diagnosed by newborn screening in each city, resulting in incidences of 2.0 (95% confidence interval = 0.0–5.9) and 2.1 (0.0–6.2) per 1,000,000 in Sapporo and Niigata, respectively. We collected information from 85% of the 135 Councilors of Japanese Society of Pediatric Endocrinology. Fifteen NC21-OHD patients were diagnosed during childhood, resulting in the estimated prevalence of 0.58 (0.28–1.1) per 1,000,000. Eleven patients were discovered by newborn screening, 7 patients developed hyperandrogenism symptoms (2–8 years of age, median 7), and 9 patients were treated with hydrocortisone at the time of the survey. Ten out of 13 patients showed compound heterozygosity for the P30L mutation of *CYP21A2*. Our study suggests that the prevalence/incidence of NC21-OHD is lower than that in Western countries, and that the age for initial onset of androgen excess symptoms varies during the prepubertal period.

Key words: Non-classical 21-hydroxylase deficiency, Incidence, Prevalence, *CYP21A2*, Newborn screening

CONGENITAL ADRENAL HYPERPLASIA (CAH) [OMIM code: 201910] is one of the most common autosomal recessive disorders in humans, and 21-hydroxylase deficiency (21-OHD) accounts for 90 to 95% of all CAH cases [1, 2]. Based on clinical manifestations, there are three forms of 21-OHD: the classical salt-wasting, classical simple-virilizing, and non-classical (NC21-OHD) forms.

In the severer forms (*i.e.* the above listed classical forms), prenatal androgen excess causes ambiguous genitalia in affected females, whereas, in the milder NC21-OHD form, female patients do not have genital ambiguity [1, 2]. In contrast to NC21-OHD patients with late onset of hyperandrogenism, some NC21-OHD patients, referred to as cryptic type, does not show any signs of androgen excess. From a biochemical point of view, the difference in the severity of phenotype correlates with the residual enzymatic activity of 21-hydroxylase, which is partially impaired in NC21-OHD patients [3].

The natural history of NC21-OHD during childhood has not been clarified. Owing to its mild phenotype,

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most NC21-OHD cases are diagnosed after the onset of puberty, and thus a substantial number of patients may not be diagnosed during childhood. Furthermore, the proportion of symptomatic cases of NC21-OHD remains unclear, as some effects of androgen excess, such as bone age acceleration, are not noticeable, even if carefully examined, especially in males.

To date, the clinical features of NC21-OHD in Japanese children have not been systemically reported. In Japan, newborn screening for 21-OHD has been performed since 1988 [4], and some NC21-OHD patients were discovered by newborn screening [5-8], allowing us to follow up NC21-OHD patients from childhood, when the disease is still asymptomatic.

In order to elucidate the clinical features of NC21-OHD during childhood in Japan, we performed three independent epidemiological analyses: 1) we calculated the incidence of neonatal NC21-OHD in patients with elevated levels of 17-hydroxyprogesterone (17-OHP), based on newborn screening data in two different cities, and 2) we performed a national surveillance to collect clinical information from pediatric endocrinologists throughout the country. The national surveillance would thoroughly provide the clinical features of NC21-OHD as well as its prevalence in Japan. By comparing the incidence during the neonatal period with the prevalence during childhood, we expected to estimate the prevalence of patients developing symptoms of androgen excess during childhood. Based on these independent analyses, we reported the clinical features of NC21-OHD in Japanese children.

Materials and Methods

Definition of non-classical 21-hydroxylase deficiency

In the present study, NC21-OHD was defined as the absence of symptoms of adrenal insufficiency or androgen excess during the neonatal period and the presence of biochemical abnormalities compatible with 21-OHD or of biallelic mutations in the *CYP21A2*. Symptoms of adrenal insufficiency included mucocutaneous hyperpigmentation, failure to thrive, or dehydration. Symptoms of androgen excess included virilization of the female external genitalia at birth, and growth spurt, bone age advancement, premature development of pubic hair, or menstrual irregularity. Biochemical abnormalities compatible with 21-OHD were: 1) 17-OHP basal serum level ≥ 2.0 ng/mL, 2) 17-OHP peak serum level after ACTH stimulation

≥ 10.0 ng/mL, or 3) urinary pregnanetriol/creatinine ratio ≥ 0.1 mg/g [9, 10]. The subjects in this study theoretically include the NC21-OHD cryptic type.

Newborn screening in Sapporo and Niigata

We retrospectively analyzed the clinical courses of children whose 17-OHP blood levels on filter paper were greater than the cutoff level of 21-OHD screening in Sapporo city between May 1982 and March 2010 and in Niigata prefecture between April 1989 and March 2009. Cutoff levels for the newborn screening program were determined by each regional committee. Information related to patients positive for 21-OHD in Sapporo and Niigata were sent to the Department of Pediatrics, Hokkaido University, and the Department of Pediatrics, Niigata University, respectively, and all newborn infants positive in the 21-OHD screening were examined and diagnosed at these institutions. Based on the results of the newborn screenings, we estimated the incidence (the number of newly diagnosed cases) during the neonatal period.

Nationwide survey

We asked all 135 councilors of the Japanese Society for Pediatric Endocrinology (JSPE) to provide information about patients diagnosed with NC21-OHD in the last 10 years (2001–2011). This survey potentially provides the clinical information of most children with NC21-OHD in Japan. We collected perinatal history, familial history, results from newborn screening, clinical manifestation, growth history, biochemical data, bone age, and *CYP21A2* genotype in those patients. *CYP21A2* genotypes of patients were determined by Sanger sequence. Prevalence is the number of cases who already have been diagnosed at the start of the study as well as those who were newly diagnosed during the period, and we estimated the prevalence of NC21-OHD in children under 15 years of age in Japan using the number of reported patients in this survey and the Japanese population under 15 years of age as of October 1, 2007, published by the Statistics Bureau, Ministry of Internal Affairs and Communications, Japan.

Ethical statement

Written informed consent for determining the *CYP21A2* genotype was obtained from the patients' parents.

Results

Incidence of NC21-OHD based on newborn screening in Sapporo and Niigata

A total of 498,147 and 478,337 neonates underwent newborn screening in Sapporo and Niigata, respectively. In each screening program, 26 children were diagnosed as having 21-OHD, and the incidences of 21-OHD were 5.2 (95% confidence interval = 3.2–7.2) and 5.4 (3.3–7.5) per 100,000 people in Sapporo and Niigata, respectively (Table 1). Only one patient with NC21-OHD was discovered by each screening, resulting in NC21-OHD incidences of 2.0 (0.0–5.9) and 2.1 (0.0–6.2) per 1,000,000 in Sapporo and Niigata, respectively (Table 1).

Prevalence and clinical characteristics of children with NC21-OHD as shown by the nationwide survey

Eighty-five percent of the Councilors of JSPE conducted the survey and provided us with the clinical information related to NC21-OHD patients. Fifteen patients were diagnosed with NC21-OHD during childhood in 7 institutions. All the diagnoses were confirmed by clinical, biochemical, or genetic analysis (Table 2). Age of the patients varied considerably from 1 to 30 years, and 10 patients were younger than 15 years of age at the time of the survey. We estimated the prevalence of NC21-OHD patients under 15 years of age at 0.58 (0.28–1.1) per 1,000,000.

The characteristics of the 15 children with NC21-OHD are summarized in Table 2. Eleven patients were identified by newborn screening. Seven patients devel-

Table 1 Analyses of newborn screening in Sapporo and Niigata

	Number of screened newborns	21OHD			
		Classical		Nonclassical	
		Number	Incidence (per 100,000)	Number	Incidence (per 1,000,000)
Sapporo city	498,147	25	5.2	1	2.0
Niigata city	478,337	25	5.4	1	2.1

Table 2 Summary of Japanese patients with non-21-hydroxylase deficiency

Case	Sex	Age (years)	Newborn screening for 21-OHD	Growth acceleration Y/N (years)	Bone age advancement Y/N (years)	Virilization Y/N (years)	17-OHP (ng/mL)*1		Cortisol (μg/dL)*1		CYP21A2 genotype	Age at initiation of HC replacement therapy (years)	Present dosage of replacement therapy (mg/m ² /day)
							Basal	Peak	Basal	Peak			
1	Female	1	Positive	N	NA	N	21.8	125.0	13.7	21.0	P30L/R356W	NA	NA
2	Female	4	Positive	N	N	N	110.0	NA	18.0	NA	H62L/I236N,V237E,M239K	NA	NA
3	Female	5	Positive	N	NA	N	NA	NA	NA	NA	P30L/R356W	0.1	HC 7.8
4	Female	9	Positive	N	Y (7)	Y (7)	NA	NA	NA	NA	P30L/deletion or conversion	7	HC 19.5
5	Female	10	Positive	N	NA	N	5.8	109.1	1.6	10.8	NA	NA	NA
6	Female	15	Negative	N	Y (8)	Y (8)	23.0	120.0	18.4	36.6	R435C/R435C	8	HC 7.8 + DEX 0.35
7*2	Female	18	Positive	Y (4)	Y (4)	N	18.9	29.6	NA	NA	P30L/I172N,R356W	3	HC 24.0
8	Female	22	Negative	Y (6)	N	Y (7)	56.0	190.0	18.3	30.7	IVS2-13A/C>G/deletion	8	HC 14.1
9	Female	30	NA	Y (1.5)	N	Y (4)	9.3	26.0	NA	NA	NA	4	DEX 0.57
10	Male	2	Positive	N	Y (1.5)	NA	61.7	265.0	13.1	16.3	P30L/IVS2-13A/C>G	2	HC 5.2
11*3	Male	5	Negative	N	NA	NA	NA	NA	NA	NA	P30L/R356W	NA	NA
12	Male	6	Positive	N	N	NA	7.0	95.8	NA	NA	P30L/IVS2-13A/C>G	NA	NA
13	Male	8	Positive	N	N	NA	10.9	176.0	NA	NA	P30L/G110deletion8nt	NA	NA
14*3	Male	11	Positive	N	N	NA	68.0	408.0	13.7	14.7	P30L/R356W	2	HC 3.6
15*2	Male	21	Positive	Y (7)	Y (8)	NA	110.0	156.0	12.4	13.4	P30L/I172N,R356W	8	HC 3.2 + DEX 0.32

21-OHD, 21-hydroxylase deficiency; 17-OHP, 17-hydroxyprogesterone; HC, hydrocortisone; Y, yes; N, no; NA, not applicable; DEX, dexamethasone.

*1: Data obtained from ACTH stimulation test, *2, *3: Siblings.

oped the symptoms of androgen excess, such as virilization of the female external genitalia, increased growth velocity, or bone age advancement, with the onset ranging from 2 to 8 years of age (median 7 years). All patients showed higher 17-OHP peak levels after ACTH stimulation than normal reference. These levels were moderately increased in most patients and remarkably at a similar level for classical 21-OHD in some patients. Ten out of 13 patients who were analyzed for the *CYP21A2* gene were found to be compound heterozygotes for the P30L mutation. In 9 patients, hydrocortisone replacement therapy was started from 0 to 8 years of age. Among them, 7 patients exhibited the clinical symptoms of androgen excess, and the other 2 (#3 and #14) did not have any symptoms at the initiation of the therapy. Patients #3 and #14 were started on replacement therapy based on biochemical data of reduced 21-hydroxylase activity. Indication for therapy was a low peak serum cortisol level (8.0 µg/dL) on ACTH stimulation test in patient #14, while the test was not conducted in patient #3.

Discussion

We estimated the incidence and prevalence of NC21-OHD in children based on three independent analyses (*i.e.* data from newborn screening in Sapporo and Niigata, and data from a nationwide survey). Although adult patients were not surveyed, to date our study is the largest epidemiological study of childhood NC21-OHD in Japan.

Our study suggests that the incidence and prevalence of NC21-OHD in Japanese children are much less than those in Western countries. The incidence of NC21-OHD based on the newborn screening was consistent with previous reports [5, 6]. Additionally, our national survey revealed that more than two-thirds of the patients (11 out of 15) were discovered by newborn screening, suggesting that the number of NC21-OHD patients who are newly diagnosed during childhood is limited. In western countries, NC21-OHD occurs at approximately 0.1–0.2% in the general Caucasian population but at up to 1–2% among inbred populations, such as Eastern European Jews [11]. In western countries, the V281L mutation is present in about 60% of non-classical cases [12, 13] while it is rare in Japanese patients [5, 6], as highlighted by our study. In Japanese NC21-OHD patients, P30L is the most common mutation [5, 6], and 10 out of 13 patients were compound

heterozygotes for the P30L mutation. We presume that the frequency of the P30L mutation in Japan is much less than that of the V281L mutation in Western countries, resulting in a lower incidence of NC21-OHD in Japan.

Our study revealed that the prevalence of NC21-OHD was lower (0.58/100,000) than its incidence (2.0–2.1/100,000). Although there is no statistically significant difference between the prevalence and the incidence, we cannot exclude the possibility that our survey was still insufficient to cover all medical facilities treating NC21-OHD patients. In addition, a substantial number of NC21-OHD male patients might be diagnosed with a simple virilizing form or not be diagnosed at all, resulting in a lower prevalence of NC21-OHD.

A careful monitoring of hyperandrogenism along sex and age lines is essential for managing NC21-OHD patients. Japanese NC21-OHD patients in our study exhibited virilization of external genitalia and bone age acceleration at the age of 2 to 8 years. These signs and ages at diagnosis were different from those previously reported [13, 14], *i.e.*, premature pubarche and acne around pubertal age. As widely recognized, bone age acceleration could be the sole symptom of androgen excess in male prepubertal patients. Thus, ethnic and sex differences should be considered for managing NC21-OHD children.

Our study suggests that genetic tests and physical findings are important to differentiate non-classical type from classical type. Endocrinological criteria of NC21-OHD have suggested that patients with NC21-OHD typically show peak 17-OHP values between 10 and 100 ng/mL [14–16], and that criterion has been believed to be one of the most reliable tests to distinguish NC21-OHD from classical CAH [1]. Indeed, the 17-OHP peak levels for all our patients were >10 ng/mL, but in some patients the levels were >100 ng/mL, and these were overlapping with those of classical 21-OHD. Non-classical and classical types of 21-OHD were differentiated according to the severity of the disease, and may share endocrinological characteristics. Those points suggest that comprehensive diagnostic approaches, including endocrinological tests, genetic testing, and careful observation are essential for accurate diagnosis of NC21-OHD.

Despite careful diagnostic approach, however, it is extremely difficult to distinguish NC21-OHD from the classical form in some male patients. At present, there is no decisive criterion for the diagnosis of NC21-OHD

male patients, and this might be the limitation of our study. Indeed, the number of male patients was smaller than that of female patients, and we cannot exclude the possibility that male patients with mild phenotype have a tendency to be diagnosed with simple virilizing form instead of non-classical form.

Hydrocortisone therapy may lead to suppression of the hypothalamus-pituitary-adrenal axis in patients with NC21-OHD. Indeed, after introduction of hydrocortisone therapy (24.0 mg/m²), patient #15 suffered from adrenal crisis during febrile episodes, as sufficient precaution was not taken for additional hydrocortisone in case of physical stress [7]. The therapeutic goal in childhood NC21-OHD is to use the lowest glucocorticoid dose that would adequately suppress adrenal androgens while maintaining normal growth without causing atrophy of the adrenal gland that might lead to adrenal insufficiency. Once hydrocortisone therapy is introduced, a risk of adrenal crisis in case of physical stress should always be considered.

In conclusion, to date, our study is the largest epidemiological study of childhood NC21-OHD in Japan. Consistent with previous reports, P30L is the most common mutation in Japan, and the incidence and prevalence of NC21-OHD are lower than those in Western countries. Moreover, we highlighted that the onset of androgen excess symptoms develops at various prepubertal ages. However, further studies are needed to

achieve better management of childhood NC21-OHD.

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Disclosure

None of the authors have any potential conflicts of interest associated with this research.

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