A Male Case of Nonclassical 21-hydroxylase Deficiency First Manifested in His Sixties with Adrenocortical Incidentaloma

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Abstract. Nonclassical form of 21-hydroxylase deficiency (NC 21OHD) as a frequent variant on the milder end of the disease spectrum has been widely acknowledged, but its potential contribution to adrenocortical tumorigenesis has not been fully elucidated. We report a 66-year old male case of bilateral adrenocortical incidentaloma, associated with partial 21OHD without any episodes of hypoadrenocorticism in his past history. He was demonstrated to be a compound heterozygous mutant of CYP21A2 gene (IVS2-13A/C>G/I172N). The two tumors in the left adrenal, which were interpreted as myelolipoma by imaging studies, were followed by sequential observation, whereas the contralateral large solid tumor associated with inhomogeneous radiological appearance was subsequently removed. The resected tumor was diagnosed an adrenocortical adenoma, which was devoid of P450c21 immunoreactivity. 21OHD is often associated with benign adrenocortical tumors, but bilateral adrenal tumors with heterogeneous components in both adrenals have not been reported to the best of our knowledge.

Key words: Congenital adrenal hyperplasia, Nonclassical 21-hydroxylase deficiency, Senescence, Adrenal incidentaloma, Adrenocortical adenoma

21-HYDROXYLASE deficiency (21OHD), which constitutes the majority of congenital adrenal hyperplasia (CAH), has a spectrum of phenotypes according to the clinical severity and the time of manifestation of the disease, i.e. from life-threatening, newborn-onset salt-wasting form (SW), childhood-onset simple virilizing form (SV) to later-onset nonclassical form (NC). The marked prevalence of NC in the general population has been widely acknowledged in recent years [1, 2], but reports on this type who manifested in middle aged or senior adults are still uncommon. In addition, the potential correlation of NC to the etiology of incidentally discovered adrenal mass (incidentaloma) is documented in only a handful of reports [3–5].

We herein report a case in his seventh decade of life that presented with bilateral adrenal enlargement, and was eventually detected to harbor a large adenoma in a hyperplastic adrenal cortex due to NC 21OHD, and myelolipomas in the contralateral adrenal gland.

Case report

A 66-year-old Japanese male patient was referred by his family physician to the endocrine department of Hirosaki University Hospital in June 2005 for work-up of bilaterally enlarged adrenal glands. His family history and past medical history were not contributory.
He became hypertensive in his 50s but his blood pressure was readily controllable with a single regimen of amlodipine (5 mg/day). No episodes of glucose intolerance have been reported. He experienced no spells of diaphoresis, palpitation or surge of blood pressure. He was free of symptoms such as progressive lethargy or body weight loss. His voice breaking occurred at age 13, and he did not feel any problem concerning the progression of secondary sexual characteristics compared with his peers. He fathered a son when he was 26, who thrived without notable physical problems.

On admission, he stood 155.5 cm and weighed 58.5 kg, with which a body mass index of 24.3 kg/m² is calculated. The mean blood pressure scored 128/75 mmHg (range: systolic 96–168, diastolic 52–97) in a 24-hour measurement, on the same antihypertensive as he had been taking. No Addisonian pigmentation or Cushingoid features were noted.

Thin-slice computed tomography (CT; Fig. 1) depicted the enlarged adrenals in detail. The right adrenal (solid arrow) mostly consists of a large inhomogeneous nodule, flanked by crura of cortex (arrowhead). The left adrenal contains two pieces of lipid density nodules, aligned in vertical direction (open arrows).

Fig. 1. Computed tomogram of the adrenal glands. The right adrenal (solid arrow) mostly consists of a large inhomogeneous nodule, flanked by crura of cortex (arrowhead). The left adrenal contains two pieces of lipid density nodules, aligned in vertical direction (open arrows).

No Addisonian pigmentation or Cushingoid features were noted.

Endocrinological examinations (Table 1) demonstrated a high-baseline, high-response ACTH secretion, along with partially defective cortisol secretion. A robust elevation of urinary 17-ketosteroid (17KS) by seemingly intact crura of cortex. The left adrenal contained two lipid-density nodules (35 × 33 and 14 × 14 mm). On magnetic resonance imaging (MRI; Fig. 2), the right adrenal nodule showed low signal intensity (compared with the liver) in both T1- and T2-weighted image, suggesting a tissue of cortex origin. The masses in the left adrenal exhibited high signal in both images, mimicking the signal pattern of the visceral adipose tissue.

Fig. 2. Magnetic resonance image of the adrenal glands. The right adrenal (solid arrow) appears hypointense compared with the liver, both in T1-weighted image (T1WI) and T2WI. The tumors in the left adrenal (open arrow) appear hyperintense in both images.
was noted, but 17-hydroxycorticosteroid (17OHC) and urinary free cortisol (UFC) were confined to the lower levels of the reference range. An extraordinary high level of serum 17-hydroxyprogesterone (17OHP), further exaggerated by ACTH1-24 load, was also noted, but dehydroepiandrosterone sulfate (DHEA-S) was within normal limits. Hyperrenin-hyperaldosteronemia was apparent, but renovascular stenosis and renal tumors as its differential diagnoses were excluded by imaging studies (pictures not shown).

The integration of these results, except that DHEA-S remained in the normal range, suggested partial primary adrenal insufficiency due to 21OHD. Bilateral enlargement of the adrenal glands might well be ascribed to this abnormality, and lipid-rich change of the left adrenal was interpreted as myelolipoma. However, the potential malignancy of the right adrenal mass could not be completely ruled out, due to its bulkiness and irregularity on CT scan. Therefore, right adrenalectomy was performed in October 2005.

### Pathological findings

The resected right adrenal measured 60 × 55 × 40 mm (Fig. 3), most of which appeared yellow to brown on the cut surface.

Histologically, the tumor was composed mostly of cells with dense eosinophilic cytoplasm (Fig. 4A), and was demarcated from the rest of the tissue by a well developed fibrous capsule. Infiltration of lymphocytes as well as focal lipomatous degeneration was detected sporadically. Ki67 or MIB1 labeling index of the tumor cells was as high as 5%, but the microscopic findings fulfilled only two points (‘compact cell predominance’ and ‘high nuclear grade’) out of nine in Weiss’s criteria [6]. Hence, the tumor was pathologically diagnosed adrenocortical adenoma.

The architecture of the surrounding adrenal cortex was greatly altered, with abundant formation of markedly hyperplastic nodules and intermingling of the cortex with the medulla.

Immunohistochemistry of steroidogenic enzymes was performed as described previously (Fig. 4B) [7]. It demonstrated the presence of steroid side-chain cleavage enzyme (P450scc), 3β-hydroxysteroid dehydrogenase (3β-HSD), 11β-hydroxylase (P450c11), and 17α-hydroxylase/17,20-lyase (P450c17) both in the tumor and in the surrounding cortical tissue, in the complete absence of 21-hydroxylase (P450c21).

### Gene analysis

After obtaining an informed consent from the patient, genomic analysis was conducted with DNA from the peripheral blood leukocytes, as described elsewhere [8]. The analysis disclosed two mutations of the CYP21A2 gene, IVS2-13A>C=G and I172N (Fig. 5).

As both of his parents had already deceased, we conducted allele-specific polymerase chain reaction (PCR) analysis on his genomic DNA to test whether these mutations are on the same allele or not. In brief, a forward primer that corresponds to IVS2-13A>C=G mutation on its 3’ end (CYP21S30G), and its wild type primer (CYP21S30A) were prepared (Table 2). Similarly, a

### Table 1. Laboratory data. Reference ranges are shown in brackets. Data of samples drawn at midnight are shown in parentheses. In loading tests, human CRH (100 µg) and ACTH1-24 (250 µg) were administered intravenously. FPG, fasting plasma glucose; PRA, plasma renin activity.

<table>
<thead>
<tr>
<th>Test</th>
<th>Basal blood values</th>
<th>Urine hormonal values</th>
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<tbody>
<tr>
<td>Na</td>
<td>141 mmol/l [136–149]</td>
<td></td>
</tr>
<tr>
<td>K</td>
<td>3.9 mmol/l [3.5–5.0]</td>
<td></td>
</tr>
<tr>
<td>Cl</td>
<td>103 mmol/l [98–108]</td>
<td></td>
</tr>
<tr>
<td>FPG</td>
<td>100 mg/dl [70–110]</td>
<td></td>
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<tr>
<td>HbA1c</td>
<td>5.1% [4.3–5.8]</td>
<td></td>
</tr>
<tr>
<td>ACTH</td>
<td>147 (14) pg/ml [10–60]</td>
<td></td>
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<tr>
<td>cortisol</td>
<td>15.1 (2.1) µg/dl [4–17]</td>
<td></td>
</tr>
<tr>
<td>DHEA-S</td>
<td>103 µg/dl [13–264]</td>
<td></td>
</tr>
<tr>
<td>PRA</td>
<td>4.5 ng/ml/h [0.2–2.7]</td>
<td></td>
</tr>
<tr>
<td>aldosterone</td>
<td>20.2 ng/dl [3.0–15.9]</td>
<td></td>
</tr>
<tr>
<td>free cortisol</td>
<td>16.0–29.7 µg/day [11.2–80.3]</td>
<td></td>
</tr>
<tr>
<td>aldosterone</td>
<td>5.7–8.2 µg/day [&lt;10]</td>
<td></td>
</tr>
<tr>
<td>17OHCS</td>
<td>3.5–3.6 mg/day [2.9–11.6]</td>
<td></td>
</tr>
<tr>
<td>17KS</td>
<td>21.4–23.3 mg/day [4.6–16.4]</td>
<td></td>
</tr>
<tr>
<td>metanephrine</td>
<td>0.09–0.10 mg/day [0.05–0.20]</td>
<td></td>
</tr>
<tr>
<td>normetanephrine</td>
<td>0.19–0.24 mg/day [0.10–0.28]</td>
<td></td>
</tr>
<tr>
<td>CRH test</td>
<td>0’ 30’ 60’ 120’</td>
<td></td>
</tr>
<tr>
<td>ACTH (pg/ml)</td>
<td>77 209 143 89</td>
<td>6.3 9.0 8.0 6.8</td>
</tr>
<tr>
<td>cortisol (µg/dl)</td>
<td>3.7 5.7 5.9</td>
<td></td>
</tr>
<tr>
<td>17-hydroxyprogesterone (ng/ml)</td>
<td>95.5 325 418</td>
<td></td>
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Fig. 4. Histological findings. In hematoxylin-eosin staining (A), only a few points compatible with malignancy (‘compact cell predominance’ and ‘high nuclear grade’) are noted in the tumor (×400); appearance of nontumor area (×100) is consistent with CAH. In immunohistochemistry of steroidogenic enzymes (B, ×100), complete absence of P450c21 immunoreactivity is noted.

Fig. 3. Macroscopic view of the surgical specimen. The cut surface shows yellow to brown appearance.

Fig. 5. Sequence analysis of CYP21A2 gene. Single nucleotide conversions in intron 2 and exon 4 are demonstrated.
reverse primer that corresponds to I172N on its 3' end (CYP21A40T), and its wild type primer (CYP21A40A) were prepared. PCR was performed with AmpliTaq Gold polymerase (Applied Biosystems Japan, Tokyo), four different combinations of the above primers, and PCR product of [P1, CYP21A10A] that spans exon 1 to 6 [8] as the template. The amplification setting was as follows: 95°C for 30 sec, 65°C for 30 sec, and 72°C for 1 min, for 35 cycles. As a result, the primer combinations of [CYP21S30A, CYP21A40T] and [CYP21S30G, CYP21A40A] yielded the expected PCR products, while those of [CYP21S30A, CYP21A40A] and [CYP21S30G, CYP21A40T] did not. Thus, this patient was demonstrated to be a compound heterozygous mutant of IVS2-13A/C>G/I172N.

**Postoperative course**

The patient’s postoperative course was unremarkable on small dosage (10 mg/day) of hydrocortisone replacement. It was tapered over a year, without triggering any episode of adrenocortical insufficiency. After weaning the replacement therapy, plasma levels of ACTH and 17OHP returned to the preoperative range. No emergence of Addisonian pigmentation or enlargement of the left adrenal mass was detected.

**Discussion**

We diagnosed this patient as having NC 21OHD, accompanied by an adenoma in the right adrenal gland and two myelolipomas in the left. Endocrinological findings of this patient are summarized as follows: (i) impairment of cortisol synthesis has been compensated by the rise in ACTH tonus throughout the course, barely enough to avoid the precipitation of adrenocortical insufficiency; (ii) the potential shortage of aldosterone has also been compensated by elevated drive from the renin-angiotensin system; (iii) elevation of 17KS reflects the dammed up pool of intermediary metabolites including C19 steroids; here, the unlevated level of DHEA-S may be due to low expression of DHEA-sulfotransferase, which was not measured in our present study. Alternatively, this may be a result of impaired 17,20-lyase activity, independent from 17α-hydroxylase activity, by an unknown mechanism.

The concomitance of benign adrenal tumors such as adenoma and myelolipoma is fairly common in CAH, possibly due to the chronic, strong trophic action of ACTH [3]. On the other hand, the occurrence of adrenal carcinoma in CAH is so rare [4] that some reports advocate not operating on the incidentalomas found in these patients [5]. Even in such cases, however, the importance of ruling out malignancies should not be underestimated, considering the dismal clinical outcome of patients with adrenocortical carcinoma. It is also worth taking note that flamboyant features of Cushing’s syndrome may be totally absent in cases of adrenal carcinoma occurring in CAH, masking a major hallmark frequently seen in non-CAH adrenocortical malignancy [9–11]. High signal intensity in T2-weighted MRI and rapid growth rate of the tumor are other clinical features that may be suggestive of potential adrenocortical malignancy.

The occurrence of classical CAH is approximately one in 15,000 live births as screened by neonatal hormonal examination in developed countries [2]; that of Japan is reported to be one in 15,800 [12]. This rate has ethnic differences, ranging from one in 42,000 African

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**Table 2.** Sequences of PCR primers. The first four primers are for allele-specific PCR (see text), and the last two are for generating the template for allele-specific PCR from the patient’s genomic DNA [8]. Underlined character indicates the site of point mutation.

<table>
<thead>
<tr>
<th>designation</th>
<th>sequence</th>
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<tbody>
<tr>
<td>CYP21S30A</td>
<td>5'-CTTCATCAGTTCACCACCCTCCAGCCCCCAA-3'</td>
</tr>
<tr>
<td>CYP21S30G</td>
<td>5'-CTTCATCAGTTCACCCTCCAGCCCCCAAG-3'</td>
</tr>
<tr>
<td>CYP21A40A</td>
<td>5'-TGATCTTGTCCTCCGAAGGTAGGTAACAGA-3'</td>
</tr>
<tr>
<td>CYP21A40T</td>
<td>5'-TGATCTTGTCCTCCGAAGGTAGGTAACAGT-3'</td>
</tr>
<tr>
<td>P1</td>
<td>5'-TTCCAGGCGATTCCAGGAAGGC-3'</td>
</tr>
<tr>
<td>CYP21S10A</td>
<td>5'-CCTCAGCTGACATCTCCACGA-3'</td>
</tr>
</tbody>
</table>
Americans to one in 280 Yupic Eskimos of western Alaska. In genomic studies, the rate of heterozygous carrier of 21OHD is reported to be 4.8% in New Zealand [13] to 9.5% in Austria [14]. These findings give rise to a speculation that ‘cryptic’ compound heterozygotes may be more common than previously expected, although neither of these studies succeeded in locating such a case, possibly due to their small sample size. Potential high rate of genetic 21OHD carrier reminds one of the high prevalence of adrenal incidentaloma in the general population (ca. 10% in the elderly [15]). However, whether heterozygous mutation of CYP21A2 increases the occurrence of adrenal tumors still remains to be elucidated. Patócs et al. [16] and Baumgartner-Parzer et al. [17] reported coincidentally that, in their independent series of fifty patients who harbor adrenal incidentalomas including unilateral and bilateral ones, eight (16%) were genetic carriers of various CYP21A2 mutations, and one (2%) was homozygous or compound heterozygous mutant of the gene, i.e. NC 21OHD. This rate is apparently higher than the carrier prevalence in the general population as reported above [13, 14], but a direct comparison of the occurrence of adrenal tumor in carriers vs. non-carriers of CYP21A2 mutation within a single ethnic population would be necessary to conclude this issue.

The severity of cortisol deficiency and the magnitude of 17OHP accumulation could be associated with the SV form of the disease, but the patient claimed no signs of pseudoprecocious puberty. Although his relatively low height might suggest a certain extent of androgen excess in his childhood, he seemed to go through normal puberty and his gonadal function thereafter proved sufficient to conceive offspring. The reason for the discrepancy between the hormonal profile and the phenotype is unclear. A possible explanation is that, if his present data of unelevated DHEA-S level refers to impaired 17,20-lyase activity, the rise in androstenedione level might have been negligible in his childhood.

The versatility of phenotype-genotype relationship of 21OHD has long been argued. The accumulation of in vitro expression studies has shown that IVS2-13A/C>G would result in total abrogation of the enzyme activity, and I172N in partial yet severe impairment [18]. In compound heterozygotes, the phenotype usually depends on the milder haplotype, and the present combination is expected to result in the SV form of the disease. In spite of these tentative principles, the reported numbers of SW/SV/NC cases harboring the present mutations are 2/0/2 [8], 0/6/1 [18], 1/12/0 [19], 5/14/0 [20], 0/1/0 [21], and 2/17/0 [22], further exemplifying the phenotypic variability. Tajima et al. [23] reported that one out of seven NC cases had this combination of mutations, corroborating that this is a minor yet possible genotype causing the NC form. Interestingly, five individuals of this genotype who belong to a clan showed phenotypic variability (one SW and four SV) [24]. These together imply that the phenotype is affected by factors other than genomic aberrance of CYP21A2. Hypotheses such as extraadrenal 21OHase activity, abnormalities in the putative regulatory regions remote from CYP21A2, and differences in steroid sensitivity have been suggested, but little is understood yet.

In summary, we experienced a case of bilateral adrenal incidentaloma with a background of NC 21OHD, who remained asymptomatic until his seventh decade of life. High plasma levels of ACTH and 17OHP can be a clue to NC 21OHD. Most of the adrenal incidentalomas in NC 21OHD are benign, but the indication of surgical resection should be thoroughly discussed from case to case, for there is an increased risk of overlooking carcinoma because of the absence of hypercortisolism.

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