Genetic Analysis of Two Japanese Patients with Non-classical 21-Hydroxylase Deficiency

Rui Imamine¹, Hiroshi Arima¹, Miho Kusakabe¹, Hiroshi Umeda¹, Ikuko Sato¹, Keiko Homma², Takeshi Usui³ and Yutaka Oiso¹

Abstract

We report two Japanese women with androgen excess symptoms. Analyses of 21-hydroxylase gene demonstrated that a 24-year-old Japanese woman had a homozygous mutation of IVS2-13 A/C>G, while a 25-year-old Japanese woman had a compound heterozygous mutation of I172N and E245del1nt, a novel mutation which would result in completely nonfunctional enzyme due to a frame shift. As IVS2-13 A/C>G and I172N have been classified as mutations leading to severe impairment in enzyme activity, this study not only clarified a novel mutation causing 21-hydroxylase deficiency, but also demonstrated that genotype and phenotype do not correlate well in these cases.

Key words: 21-hydroxylase deficiency, genotype and phenotype

(Inter Med 48: 705-709, 2009)
(DOI: 10.2169/internalmedicine.48.1894)

Introduction

Congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency (21OHD) is one of the most common metabolic disorders. 21OHD shows a wide range of clinical manifestations and is typically classified into three forms: classical salt-wasting form, classical simple virilizing form, and late-onset nonclassical form (1). The 21-hydroxylase gene (CYP21A2) is located on the short arm of chromosome 6 in tandem with a highly homologous CYP21 pseudogene (CYP21P) (2, 3), and most mutations in CYP21 are due to gene conversion between CYP21A2 gene and CYP21P (4). As 21OHD is an autosomal recessive disorder, clinical phenotypes depend on the activities of the less severely affected allele. Mutations in CYP21A2 could be divided into four groups based on the enzyme activity: no activity (deletion/conversion, 8bp-del, L307+T, Q318X, R356 W, E6 cluster), almost complete impairment (IVS2-13 A/C>G), severely impaired activity (I172N), and moderately impaired activity (P30L and V281L) (5, 6). There is a relatively good correlation between genotype and phenotype (7-9), and most mutations of CYP21A2 in the non-classical form are reportedly those resulting in moderately impaired enzyme activities such as P30L and V281L (7, 9).

While the overall incidence of the classical form of 21OHD is one in 15000 births, the frequency of non-classical 21OHD is estimated to be higher (1, 10). However, if the non-classical form is caused only by the known mutations defined as the less impaired enzyme activities, it might be difficult to account for the high frequency of this form. As neonatal screening does not necessarily detect the non-classical form, there might be a wider spectrum of mutations in this form. It is also possible that genotype and phenotypes might not always correlate in 21OHD, as has been suggested (11, 12).

To better understand the molecular basis of the non-classical form of 21OHD, we performed genetic analyses of two Japanese women with 21OHD who presented with late-onset symptoms of androgen excess in the present study.

¹Department of Endocrinology and Diabetes, Field of Internal Medicine, Nagoya University Graduate School of Medicine, Nagoya, ²Central Clinical Laboratories, Keio University Hospital, Tokyo and ³Clinical Research Institute, Center for Endocrine and Metabolic Disease, National Hospital Organization Kyoto Medical Center, Kyoto

Received for publication November 25, 2008; Accepted for publication January 25, 2009
Correspondence to Dr. Hiroshi Arima, arima105@med.nagoya-u.ac.jp

705
Case Report

Case 1

A 24-year-old Japanese woman visited the Department of Obstetrics and Gynecology in our hospital because of infertility in June 2007. There were no symptoms suggesting androgen excess such as ambiguous genitalia and genital virilization at birth. After she presented with accelerated growth at the age of 9-11, her height remained the same but her body weight gradually increased. She noticed hirsutism when she was 14 years old. The age at menarche was 15 years but the menstrual cycle had been irregular. There was no episode of dehydration or family history of 21OHD. She weighed 67.1 kg and her height was 157.1 cm when she visited our hospital. The blood pressure was 96/68 mmHg. The size of clitoris was 1.5×2 cm. As the basal level of plasma testosterone was elevated, she was referred to our department in October 2007.

Case 2

A 25-year-old Japanese woman was referred to our department from another hospital because of fluctuation in plasma ACTH levels in April 2006. There were no symptoms suggesting androgen excess such as ambiguous genitalia and genital virilization at birth. After she presented with accelerated growth at the age of 8-10, her height remained the same. Her body weight gradually increased when she was a junior high school student. The age at menarche was 12 years, but the menstrual cycle had been irregular. She noticed hirsutism when she was 13 years old and visited the former hospital. Administration of glucocorticoids was started based on hormonal data, which were not available when she was referred to our hospital. There was no episode of dehydration or family history of 21OHD. She weighed 70.7 kg and her height was 146.2 cm when she visited our department. The blood pressure was 127/76 mmHg. The size of clitoris was 1.0×1.5 cm.

Hormone assays

Plasma ACTH and aldosterone concentrations were measured by immunoradiometric assays. Plasma cortisol was measured by an electrochemiluminescence immunoassay. Serum dehydroepiandrosterone-sulfate (DHEA-S) was measured by a chemiluminescence enzyme immunoassay. The concentrations of plasma renin (PRC) and serum 17-OH-pregnenolone, 17-OH-progesterone, 11-deoxycortisol were determined by radioimmunoassays. Serum testosterone was determined by chemiluminescent immunoassays, while urine 17-OHCS and 17-KS were measured by colorimetric assays. ACTH stimulation test was performed using tetraacosactide acetate (Cortrosyn, 0.25 mg).

Urine pregnanetriolone measurement

Urine pregnanetriolone (Ptl) was measured using spot urine samples with gas chromatography/mass spectrometry in selected ion monitoring analysis as described previously (13, 14).

Gene analysis

After obtaining informed consent from the patients, the genomic analysis was conducted with DNA from the peripheral blood leukocytes. Mutations which have been implicated to cause 21OHD (deletion/conversion, 8 bp-del, L307+T, Q318X, R356W, E6 cluster, IVS2-13 A/C>G, I172N, P30L, V281L) were examined by either polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis, allele specific PCR or nested PCR according to the reported methods with some modifications (15, 16). The primers used for PCR-RFLP to detect IVS2-13 A/C>G were: 5’-TGGGGCATCCCCAATCCAGGTCCCT-3’ and 5’-AGACACCAGCTTGTCTGCAGGAGGC-3’. The digestion of PCR products with HhaI yields a 158 bp band in wild-type DNA and 134 and 24 bp bands in mutant allele. The primers used for PCR-RFLP to detect I172N were: 5’-TTCTCTCCTCCTACCTGCAGCATCG-3’ and 5’-GCATATTGGTCGTCCTGCCAGA-3’. The digestion of PCR products with TaqI yields a 161 bp band in wild-type DNA and 138 and 23 bp bands in mutant allele. In case 2, we also conducted direct sequencing of CYP21A2 gene followed by allele-specific PCR analysis on her genomic DNA to test whether or not two mutations recognized in PCR-RFLP were on the same allele (17).

Results

Hormone assays

Blood examination revealed that the basal levels of ACTH, 17-OH-pregnenolone, 17-OH-progesterone, 11-deoxycortisol, PRC, aldosterone, testosterone and DHEA-S were elevated in case 1 (Table 1). Sixty min after iv injection of ACTH, the values of 17-OH-pregnenolone and 17-OH-progesterone were increased up to 99.1 and 318 ng/mL, respectively. As for case 2, the hormonal data shown in Table 1 were under treatment with dexamethasone (0.75 mg/day). In both cases, the levels of urine Ptl, metabolites of 21-deoxycortisol, were elevated (Table 1).

Gene analysis

Case 1 was demonstrated to be a homozygous mutation of IVS2-13 A/C>G (Fig. 1). While gene analyses were not performed in parents of case 2, allele-specific PCR analysis on genomic DNA revealed that case 2 was a compound heterozygous mutant of I172N at exon 4 and E245del1nt at exon 7, the latter is a novel mutation (Figs. 1, 2).

Discussion

In the present study, we analyzed two Japanese women who presented with hirsutism and/or infertility in the adoles-
Table 1. Blood and Urine Examination
Case 1 had no medication while case 2 was under treatment with dexamethasone (0.75 mg/day). Values in control are shown in parentheses.

<table>
<thead>
<tr>
<th>Blood examination</th>
<th>case 1</th>
<th>case 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACTH (pg/mL)</td>
<td>283.3</td>
<td>23.9</td>
</tr>
<tr>
<td>(7.7-55.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17-OH-pregnenolone (ng/mL)</td>
<td>52.3</td>
<td>1.6</td>
</tr>
<tr>
<td>(0.1-4.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17-OH-progesterone (ng/mL)</td>
<td>131</td>
<td>13.5</td>
</tr>
<tr>
<td>(1.1-3.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11-deoxycortisol (ng/mL)</td>
<td>5.04</td>
<td>0.63</td>
</tr>
<tr>
<td>(0.11-0.60)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortisol (µg/dL)</td>
<td>16.6</td>
<td>0.6</td>
</tr>
<tr>
<td>(4.0-18.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRC (pg/mL)</td>
<td>45.39</td>
<td>44.5</td>
</tr>
<tr>
<td>(2.5-21)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aldosterone (pg/mL)</td>
<td>299</td>
<td>94.9</td>
</tr>
<tr>
<td>(29.9-159)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DHEA-S (ng/mL)</td>
<td>3410</td>
<td>67</td>
</tr>
<tr>
<td>(850-2990)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testosterone (ng/mL)</td>
<td>5.84</td>
<td>0.47</td>
</tr>
<tr>
<td>(0.13-1.08)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Urine examination    | case 1 | case 2 |%
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>17OHCS (mg/day)</td>
<td>14.1</td>
<td>1.61</td>
</tr>
<tr>
<td>(2.2-7.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17KS (mg/day)</td>
<td>72.1</td>
<td>4.69</td>
</tr>
<tr>
<td>(2.4-11.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ptl (mg/gCr)</td>
<td>2.173</td>
<td>0.261</td>
</tr>
<tr>
<td>(0.007-0.050)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 1. PCR-RFLP analysis of CYP21A2 gene. (A) The digestion of PCR products with HhaI in control yielded a 158 bp band, and in the patient, 134 and 24 (not shown) bp bands (case 1). (B) The digestion of PCR products with TaqI in control yielded a 161 bp band, and in the patient, 161, 138 and 23 (not shown) bp bands (case 2).

ence. While genital abnormalities such as ambiguous genitalia were not detected in the neonatal period in both cases, the size of clitoris was compatible to those in 21OHD patients with clitoromegaly reported previously (18) when they visited our hospital. In case 1, the basal as well as ACTH-stimulated 17-OH-progesterone levels were elevated, and serum testosterone and DHEA-S levels were high. While blood examination in the former hospital was not available in case 2, the plasma levels of 17-OH-progesterone were high even under treatment with dexamethasone. Urine Ptl, a sensitive indicator for 21OHD (14), was elevated in both cases. These two patients were born before neonatal screening for 21OHD started in Japan in 1989, and we cannot exclude the possibility that they might have had elevated 17-OH-progesterone or subtle abnormality in genitalia at birth. Nevertheless, the clinical data suggest that both cases were non-classical 21OHD. While gene analyses have been performed in patients with non-classical 21OHD (6-9, 11, 17, 19, 20), this is the first report showing genotypes of Japanese 21OHD patients with androgen excess symptoms which appeared in the adolescence.

The genetic analysis demonstrated that case 1 has a homozygous mutation of IVS2-13 A>C>G. A previous in vitro study showed that this mutation caused aberrant splicing of
Figure 2. Direct sequencing of CYP21A2 gene in case 2. Single nucleotide conversion (ATC to AAC) in exon 4 (I172N) and single nucleotide deletion (GGAGAG to GGGAG) in exon 7, a novel mutation, are demonstrated.

pre-mRNA of CYP21 (21). While this mutation is divided into a group having almost complete enzyme impairment (5, 6), it is also suggested that it might confer varying degrees of enzymatic activity (22), and a previous study reported that homozygous this mutation was found in a patient with the non-classical form as in our case (19).

In case 2, we have identified I172N in one allele and one nucleotide deletion at amino acid 245 (E245del1nt) at exon 7 of CYP21A2 in the other allele, which is a novel mutation. This nucleotide deletion would cause a frame shift and the predicted translated product is truncated at 255 amino acid, which is likely to result in completely nonfunctional enzyme as in other cases (2, 5, 23). Thus, it is likely that I172N is responsible for the phenotype in case 2, as was in the previously reported cases with the non-classical form of 21OHD in which homozygous mutation of I172N was found (20).

It is suggested that the frequency of non-classical 21OHD is higher than in the classical form (1, 10). However, the molecular basis of the non-classical form has not been fully clarified, partly because most genetic analyses have been performed in subjects screened in the neonate for 21OHD or in their families (7-9). The present cases, in which the symptoms with androgen excess were manifested in adolescence, demonstrated that the mutations which have been thought to cause the classical form could result in the non-classical form. Thus, variation in the onset could not simply be attributed to the predicted activity of the enzyme. While the mechanisms underlying the genotype-phenotype inconsistency have not been fully understood, extra-adrenal 21-hydroxylation by CYP2C19 and CYP3A4 has been reported recently (24). Furthermore, two siblings who had identical CYP21A2 genes reportedly showed different clinical forms (12). These data suggest that multiple enzymes other than CYP21A2 may exert significant 21-hydroxylation depending on the individual’s polymorphic variants of several genes. Alternatively, other than genetic factors might affect the phenotype as well. Further analyses of patients with the late onset of symptoms are required to understand how the phenotypes (classical or non-classical) are determined in 21OHD.

It is recognized that deficiency of aldosterone could be a cause of salt wasting in 21OHD. A recent study demonstrated that the aldosterone-to-renin ratio is lower even in non-classical 21OHD patients than normal subjects (25), suggesting that a greater degree of renin activity is required to maintain appropriate plasma levels of aldosterone in non-classical 21OHD. Given that the aldosterone-to-renin ratio could be a marker of 21OHD severity (25), normal (case 2) and increased (case 1) levels of plasma aldosterone as well as the fact that there was no episode of dehydration further suggest that the severity of 21OHD is mild in our cases.

In summary, mutations which have been classified as those causing severe impairment in enzyme activity are likely responsible for the late onset of 21OHD in the present cases, demonstrating that genotype and phenotype do not correlate well in our cases with the late onset symptoms. In addition, we have identified a novel mutation in CYP21A2 gene which is predicted to have no enzymatic activity. The frequency of this mutation in 21OHD remains to be clarified.

References


