Yingchun Gao¹, Jinhuan Xu¹, Chaojun Wang², Chao Gao², Jie Wu²*

¹Department of Obstetrics and Gynecology, Huai’an First People’s Hospital, Nanjing Medical University, PR China
²Department of Obstetrics and Gynecology, State Key Laboratory of Reproductive Medicine, the First Affiliated Hospital of Nanjing Medical University, Nanjing Medical University, Nanjing, PR China

Abstract

CYP21A2 mutation is the major cause of Congenital Adrenal Hyperplasia (CAH) resulted from the defect in 21-hydroxylase. In this study, we reported a patient of CAH with an unusual mutation of CYP21A2 gene. This patient was a six-year-old girl admitted to Huai’an First People’s Hospital for surgery because of malformation of external genitalia. DNA sequence analysis of CYP21A2 revealed the compound heterozygous mutations (g.6119T>A and g.6699delA) in this patient and her elder sister. Interestingly, the g.6119T>A mutation is associated with a Simple Virilizing (SV) phenotype of CAH, and the g.6699delA is a rare mutation that has not been reported so far. This case report of the patient with an unusual SV CAH identified a previously unreported mutation of the CYP21A2 gene, thus enlarging the spectrum of known mutations related with CAH. DNA sequencing of the CYP21A2 to identify rare mutations should be used for the genetic diagnosis and genetic counselling of CAH.

Keywords: Congenital adrenal hyperplasia, 21-hydroxylase deficiency, CYP21A2.

Accepted on February 6, 2017

Introduction

Congenital Adrenal Hyperplasia (CAH) is an autosomal recessive disorder mainly caused by the 21-Hydroxylase Deficiency (21-OHD) [1,2]. The impairment of 21-hydroxylase blocks the synthesis of cortisol from cholesterol and promotes the androgen biosynthesis, which leads to chronic secretion of Adrenocorticotropic Hormone (ACTH) by activating negative feedback control of hypothalamic-pituitary-adrenal axis and subsequent the adrenal hyperplasia (Figure 1) [3,4]. CAH is divided into Salt-Wasting form (SW), Simple Virilizing form (SV) and Non-Classical form (NC) based on the severity of 21-hydroxylase deficiency [2,5,6].

The 21-hydroxylase (Clinical profile and inheritance pattern of CYP21A2 gene mutations in patients with classical congenital adrenal hyperplasia from 10 families CYP21A2 active gene, (OMIM #201910), is located on chromosome 6p21.3, which is adjacent to a highly homologous pseudogene (CYP21A1P). The homology in exon and intron between CYP21A2 and CYP21A1P is up to 98% and 95% respectively, resulting in either CYP21A2 deletion or the transfer of deleterious CYP21A1P sequences to CYP21A2 after recombination or gene conservation [4,7-9].

More than 90% of patients with CAH have CYP21A2 mutations including conversions to the CYP21A1P pseudogene or large deletions [2]. High variability of CYP21A2 locus, as well as high sequence homology between CYP21A2 and CYP21A1P, is prone a incidence of this region to unequal crossing-over or gene conversion events [10]. Some pseudogene-derived mutations together with more complex gene rearrangement are common etiology of CAH [11]. More than 250 mutations have been found in CYP21A2 gene (http://www.hgmd.org). Nine common pseudogene-derived mutations (such as c.293-13A>G, c.293-13C>G, p.Pro31Leu, p.Ile173Asn, exon 6 mutation cluster) comprise 95% of the alleles [12,13].

Figure 1. Mechanism of the pathogenesis in congenital adrenal hyperplasia patients.
In this study, we present the gene mutation of a SV lineage via gene sequencing. NG_007941.2:g.6699delA is a novel mutation that has never been reported.

**Case Presentation**

The patient was a six-year-old girl admitted to the hospital for surgery because of malformation of external genitalia. On admission, the patient with 46, XX had hypertrophic clitoris (long: 4.5 cm; diameter: about 2.5 cm), ambiguous labium minor and unclear orificium vaginae. Physical examination revealed no significant abnormality. Routine and biochemical analysis of blood were both normal. Baseline serum hormonal analysis revealed that the cortisol, ACTH and T level was 251.06 nmol/L, 179 pg/ml, 4.33 nmol/L respectively. But after treatment the above three level all reduced (cortisol: 53.62 nmol/L, ACTH: 10.0 pg/ml, T: 2.31 nmol/L). B ultrasonography revealed that the thickness of the right supra-renal (adrenal) gland was about 0.6-0.7 cm and the uterus was hypoplastic. CT showed that the bilateral supra-renal (adrenal) gland was all plump, suggesting that the adrenal may be a hyperplasia. Clitoroplasty was done successfully (Figure 2).

**Table 1. Primers for amplifying 10 exons and the boundary sequences of CYP21A2.**

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>Exona</th>
<th>Products (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>5' TCGGTGGAAGGTACCTGAA 3'</td>
<td>1-5</td>
<td>1518 (P1-P2)</td>
</tr>
<tr>
<td>P2</td>
<td>5' CAGCTGCATCTCCACAGTGTGA 3'</td>
<td>2-9</td>
<td>1817 (P2-P1)</td>
</tr>
<tr>
<td>P3</td>
<td>5' CCTGCTCTGAGGACTACT 3'</td>
<td>3-10</td>
<td>2210 (P3-P4)</td>
</tr>
<tr>
<td>P4</td>
<td>5' TCTCGCACCCCAAGTATGACT 3'</td>
<td>4-10</td>
<td>2210 (P4-P3)</td>
</tr>
<tr>
<td>P5</td>
<td>5' GTTCTTCCCCAATCCAGGTC 3'</td>
<td>5-14</td>
<td>3021 (P6-P5)</td>
</tr>
<tr>
<td>P6</td>
<td>5' GGAGCCAGGATCGTTGC 3'</td>
<td>6-15</td>
<td>3021 (P6-P5)</td>
</tr>
</tbody>
</table>

**Discussion**

Congenital Adrenal Hyperplasia (CAH) owing to 21-hydroxylase deficiency is mainly caused by mutations in the CYP21A2 gene [2,14]. As there are relevance between the 21-hydroxylase deficiency caused by the mutation of CYP21A2 and severity of the clinical signs, genotyping CYP21A2 mutations has been proven to be a valuable manner for diagnosis and predicting phenotype in CAH [4,15]. The most severe SW type is caused by the mutations such as R356W and Q318X, which result in the change of protein structure and the complete loss of the enzyme activity; V281L and P30L mutations are common in the NC type and result in a reduction in 21-hydroxylase activity to 2%-50% [1,2,4,14-18].

In the present research, we identified compound heterozygous mutations (g.6119T>A and g.6699delA) in a patient with SV CAH. g.6119T>A mutation, also referred as I172N, occurring in the conserved domain of CYP21A2 and causing a loss of the hydrophobic pocket, leads to a reduction in 21-hydroxylase activity to 2%-11% in vitro studies and is associated with SV type without electrolyte disturbance [3].

The g.6699delA was also detected in this patient with SV CAH. This is a novel mutation that has never been reported before. This patient and her elder sister who also had male external genitalia, pigmentation and hypertrophy of clitoris with aging, were diagnosed with SV phenotype of CAH. g.6699delA mutation in her mother and father respectively (Figure 3A). By genotyping analysis of her parents, we could confirm that g.6699delA mutation is present in a compound
heterozygous state, in trans with the g.6119T>A mutation. Small deletion of nucleotide A at position 6698 leads to the frameshift, and, subsequently, premature stop codon at position 257. The g.6699delA may cause dramatic changes in protein structure, exerting a deleterious impact and enlarging the spectrum of known mutations related with SV CAH (Figure 3B). Functional experiments remain to be done to verify how g.6699delA functions in CAH and what kind of impact g.6699delA does on enzyme function and structure.

A recent study found that about 76% of the patients who carried 1172N mutation on one allele and a second severe mutation on the other had SV type, while 23% had the SW type [4]. Phenotype isn’t always relative accurately to the genotype suggesting diagnosis is more complex for CAH than for many monogenic disorder [19,20]. Thus combination of these two mutations screening may be an efficient mean of diagnosing SV CAH and DNA sequencing of the CYP21A2 to identify rare mutations should be used for the genetic diagnosis of CAH.

In conclusion, g.6699delA is a novel compound heterozygous mutation and enlarges the spectrum of known mutations related with CAH. Combination mutation analysis of the g.6699delA, g.6119T>A and DNA sequencing of the CYP21A2 should be considered as an important possibility for diagnosis and genetic counselling.

References


*Correspondence to

Jie Wu
State Key Laboratory of Reproductive Medicine
Department of Obstetrics and Gynecology
The First Affiliated Hospital of Nanjing Medical University
PR China