Progress in molecular-genetic studies on congenital adrenal hyperplasia due to 11β-hydroxylase deficiency

Li-Qiang Zhao, Su Han, Hao-Ming Tian
Chengdu, China

Background: 11β-hydroxylase deficiency is one of the main causes of congenital adrenal hyperplasia (CAH). It is caused by the mutation of the CYP11B1 gene that encodes the enzyme. Researches have shown that mutations of the CYP11B1 gene would result in activity decrease or inactivation of the enzyme in classical 11β-hydroxylase deficiency.

Data sources: Articles on CAH and CYP11B1 gene mutation were retrieved from PubMed and MEDLINE published after 1991.

Results: The prevalence, pathophysiology, and molecular-genetic mechanisms were summarized.

Conclusions: The disease is caused by genetic mutations of CYP11B1, and types of the mutations are varied. In classical 11β-hydroxylase deficiency, genetic mutations of CYP11B1 lead to activity decrease or loss; mutations in unclassical 11β-hydroxylase deficiency are not definite. And the relationship between genotype and phenotype is not established.


Key words: adrenal hyperplasia; 11β-hydroxylase; molecular genetics

Congenital adrenal hyperplasia and 11β-hydroxylase

Congenital adrenal hyperplasia (CAH) is a family of autosomal recessive inherited diseases, and is one of the most common inherited endocrine disorders.¹ CAH is divided into 5 types by enzyme deficiency: cholesterol desmolase deficiency; 17α hydroxylase deficiency; 3β-hydroxysteroid dehydrogenase deficiency; 21-hydroxylase deficiency; and 11β-hydroxylase deficiency. Among them, 21-hydroxylase deficiency accounts for more than 90% of CAH, while 11β-hydroxylase deficiency accounts for 5%-8% as the second commonest cause of CAH.

Clinically, CAH is characterized by ambiguous genitalia in female newborns, salt-losing in male newborns, a syndrome of hirsutism, acne and irregular periods in adolescent females. The table summarizes different presentations according to age.²

11β-hydroxylase belongs to the cytochrome P450 system (P45011β). It regulates the biosynthesis of corticosterone in zona glomerulosa and cortisol in zona fasciculata of adrenal gland, respectively. Synthesis of 11-deoxycorticosterone (DOC)-the precursor of corticosterone in zona glomerulosa is mainly regulated by adrenocorticotropic hormone (ACTH) in zona fasciculata. The deficiency of 11β-hydroxylase results in decreased synthesis of cortisol and glucocorticoids, and thus causes weakened depressive feedback of ACTH and increased synthesis of ACTH in pituitary, which leads to production of more cortisol precursor in zona fasciculata. Therefore, the substrate of 17, 20-cleavage enzyme in the noninvolved androgen synthesis pathway is increased and hyperandrogenemia appears. Besides, the increase of ACTH also leads to higher levels of DOC and 11-deoxycortisol.¹²

Clinical characteristics connected with 11β-hydroxylase deficiency

11β-hydroxylase deficiency is divided into classical and unclassical forms according to the clinical manifestations. Deficiency in 11β-hydroxylation results in virilizing CAH, usually accompanied by hypertension. Abnormal adrenal steroid secretion causes a net mineralocorticoid effect, subsequent sodium retention, and volume expansion.³

Virilization and hypertension are the main clinical characteristics of 11β-hydroxylase deficiency. Develop-

Author Affiliations: Department of Endocrinology, West China Hospital of Sichuan University, Chengdu 610000, China (Zhao LQ, Han S, Tian HM)

Corresponding Author: Su Han, 4-12, Xiaojiahe Dongsanxiang No.4, Gaoxin District, Chengdu, Sichuan 610000, China (Tel: 86-13880227998; Fax: 86-28-87062118; Email: zplhelen@126.com)

©2008, World J Pediatr. All rights reserved.
ment of the female external genitalia is affected by excess fetal adrenal androgens in fetus, causing ambiguous genitalia (female pseudohermaphroditism, or 46, XX) in all cases. However, internal genital structures are normal for females notwithstanding different degrees of virilization of their external genitalia. An 11β-OHD affected female infant is usually considered as male at birth because of the abnormal external genitalia. Persistent existing increased adrenal androgen production after birth results in premature and abnormal secondary sexual characteristics in both female and male patients. Females also manifest with increased aggressive behavior and activity in childhood. Such characteristics may include progressive penile/clitoral enlargement, emergence of axillary pubic and facial hair, acne, changing of voice, and rapid skeletal growth.

Hypertension is a common but less consistent feature compared with virilization in 11β-OHD CAH. Overproduction of DOC causes salt retention and hypertension. Elevated blood pressure is usually recognized only in late childhood or in adolescence, although it has been reported that it may appear in infants as young as 3 months. But the severity of hypertension is not necessarily correlated with the severity of other clinical features: some severely virilized females are normotensive, while mildly virilized ones might have severe hypertension. Complications of long-term uncontrolled hypertension, such as cardiomyopathy, retinal vein occlusion and blindness have been reported. Contemporary potassium depletion develops with sodium retention, but the severity of hypokalemia is variable. Renin production is suppressed by mineralocorticoid-induced sodium retention and volume expansion. Aldosterone production is low because of low serum potassium and plasma renin.

**Molecular-genetic studies of 11β-hydroxylase deficiency**

In recent years, sound and comprehensive studies have already been done in 21-hydroxylase deficiency. However, molecular-genetic studies of 11β-hydroxylase deficiency are comparatively fewer because of a lower incidence. Two genes on the long arm of chromosome 8 were confirmed to share high homology in their coding sequences, called CYP11B1 and CYP11B2, which are related to the disease. In normal adrenal tissue, CYP11B2 is only expressed at a very low level in zona glomerulosa under the stimulation of angiotensin II. Researchers have found that CYP11B2 encodes a protein with activities of 18-hydroxylase, 18-oxydase, and comparatively lower activity of 11β-hydroxylase. That's why it is called aldosterone synthase. In contrast, CYP11B1 is expressed at a high level in zona fasciculata and also appreciable in zona glomerulosa. It only encodes one protein with 1β-hydroxylase activity, and is regulated mainly by ACTH, slightly by angiotensin II. So molecular-genetic changes in CYP11B1 have been identified as the cause of 11β-hydroxylase deficiency.

**Table.** Clinical features of congenital adrenal hyperplasia

<table>
<thead>
<tr>
<th>Infancy</th>
<th>Female</th>
<th>Ambiguous genitalia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early childhood</td>
<td>Male</td>
<td>Salt loss</td>
</tr>
<tr>
<td>Late childhood</td>
<td>Male</td>
<td>Virilization, rapid growth</td>
</tr>
<tr>
<td>Adolescence/young adult</td>
<td>Female</td>
<td>Early pubic hair, rapid growth</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>Testicular masses</td>
</tr>
</tbody>
</table>

**Fig.** Summarization of mutation sites of CYP11B1. E: 1-9: exons; line between exons: introns; gray part of exon 1: 5'-noncoding region of CYP11B1; gray part of exon 9: 3'-noncoding region of CYP11B1; M: sites of base replacement and small deletions/insertions; D: sites of gross loss; R: sites of rearrangement with CYP11B2.
mutations was first reported by White et al in 1991.\(^\text{[8,9]}\) To date, 50 mutations have been reported, with 29 missense/nonsense mutations, 5\(^\text{a}\) splices, 3 small deletions, 4 gross deletions, 3 insertions, and 2 complex rearrangement mutations (including inversions).\(^\text{[4,6,10-21]}\)

These mutations usually take place in Jews, but mutation in Indians, Lebanese, Africa Negroes, and Japanese have also been reported.\(^\text{[6,10,11,13]}\) Mutation sites are summarized in Fig.

Although mutation sites spread all over the gene, it is quite notably that most reported mutations locate in exon 6, 7, and 8, which account for merely 30% of the coding sequence in CYP11B1. It is obvious that mutation distribution is not random. The following reasons are possible. First, these regions may contain sequences encoding important amino acids, whose changes could result in significant decrease or loss in enzyme activity of 11β-hydroxylase. 70% of amino acid sequences in exon 6, 7 and 8 are identical in human, ox, rat, and mouse, whereas only 44% identical in other exons, suggesting that exon 6, 7, and 8 are significantly important in maintaining the function of 11β-hydroxylase. Second, these regions are susceptible to mutations for some reasons, such as higher concentration of CpG dinucleotide. In CpG dinucleotide, methylated cytosine residual tends to mutate spontaneously while changing into thymine. Now CpG dinucleotide sequences have been established as the hot point of human mutations. And 49% of CpG dinucleotides in CYP11B1 coding sequence are located in exon 6, 7 and 8, suggesting that incidence of CpG→TpG mutation is surely higher. In comparison with the whole genome or the CYP17 gene and CYP11A gene (encoding cholesterol desmolase), the proportion of CpG dinucleotide in CYP11B1 is higher (1.5%, 2%, 3% respectively).\(^\text{[13]}\)

**Mutation mechanisms**

In reported mutations, T318M, R374Q, R384Q, R448H, and R448C are all caused by CpG→TpG transformations.\(^\text{[4,6,10-23]}\) Codon 448 is the most frequently mutated site.\(^\text{[10,12]}\) R448H is the earliest reported mutation of CYP11B1. It is a single-base replacement in exon 8, causing change of codon 448 changed from CGC into CAC. This mutation mainly takes place in Moroccan Jews, in whom the incidence of 11β-hydroxylase deficiency is relatively high, in about 1/5000 newborns. The sulfydryl of Cys450 in P450c11 is the fifth coordination site of iron atom combining site in hemachrome. This residual is completely conservative in the cytchrome P450 system, and the surrounding hemachrome combining peptides is also highly conservative. Moreover, Arg448 is conservative in the P450 enzyme system of all the eukaryotes, indicating base replacement in this site can not maintain the original function. As Arg448 locates close to Cys450, Arg448→His mutation fails in the combining of hemachrome.\(^\text{[9,12]}\) Expression experiments in vitro show that replacement of arginine by histidine in site 448 will lead to activity loss of 11β-hydroxylase. Besides, missense mutations like R448C and V441G take place in this region, and R427H is also located near the hemachrome combining site.\(^\text{[24-50]}\) Their mechanisms are quite similar to those of R448H. These mutations in the neighbourhood of hemachrome combining site indicate that this region is susceptible to mutations, and is significant in maintaining the activity of the enzyme.\(^\text{[9,10,30]}\)

Similar to R448, E371 is highly conservative in the P450 system of many species and humans, suggesting the region around E371 is functionally important. Like the mutation of R347Q (codon 372-375) in the second conservative region, E371G possibly changes the interaction with adenoxygen, and results in decreased activity of 11β-hydroxylase.\(^\text{[12,13]}\)

Moreover, V129 and A331 are conservative in the gene family (CYP11A, CYP11B1, and CYP11B2), but not in other P450 enzymes. A331, E371, and 464 where leucine insertion takes place are likely to be located in I(A331), K(E371), and L(L464) in α-helix according to the speculation of secondary and tertiary structure of 11β-hydroxylase. These sites are all located in the central core region of the conservative sequence in P450 enzyme.\(^\text{[21-25]}\) Mutations in these sites may lead to changes in the secondary and even tertiary structure of 11β-hydroxylase, and thus causing activity loss. But how V129 maintains the activity of the enzyme is not clear yet. It is presumed that V129 may be involved in substrate combination as its crystallographic data share comparability with those of bacterial enzyme P450cam, P450BM-3, and P450terp.\(^\text{[11]}\) Similar to V129, site 384 is also presumed to be involved in substrate combination. It is thought to be part of the combination region.\(^\text{[10]}\)

T319 is conservative in all the isozymes of CYP11A and CYP11B, but not in other P450 enzymes. Its location is related to transference of proton to combined oxygen molecule. The nearby T318 is completely conservative in all of the known P450 enzymes, though its function is still unknown. Mutation T318M causes activity loss of the enzyme and subsequent 11β-hydroxylase deficiency. Another mutation T318R has also been observed in this site, suggesting that codon 318 is a hot point of mutation too.\(^\text{[10]}\) However, there is an intron between T318 and T319, providing the probability that T319M mutation influences normal splicing of RNA.\(^\text{[6,12]}\) Researches have already shown
that mutations in the last nucleotide of exons would be enough to affect the correctness of RNA splicing.\[^{[29]}\]

P42 is conservative in all the isozymes of CYP11B, and is also highly conservative in other P450 enzymes that share little similarity, such as P450c17. In most of P450 enzymes, there are a number of proline residuals in this region.\[^{[27]}\] These residuals may be related to directional movement of the enzyme to the membrane, just like that in microsome P450 enzyme.\[^{[28]}\] Mutations in corresponding region of CYP21 causing slight 21-hydroxylase deficiency have confirmed the hypothesis to some extent.\[^{[28]}\]

It is common that splicing sites are involved in genetic diseases. Most mutations of splicing site are located in 3'-AG acceptor or 5'GT donor. In these illnesses, such kind of replacement affects splicing reaction of RNA, and leads to wrong transcription, and sequentially results in mistakes in expression of the right proteins. Functional studies have shown that such replacement could cause hiding of 5'splicing site or skipping of exons downstream.\[^{[2]}\] In known mutations of CYP11B1, 5 are related to splicing site mutation. In 1996, Skinner et al.\[^{[14]}\] reported a G→T mutation in the last base of exon 4 (one base upstream to the splicing site). Merke et al.\[^{[16]}\] reported a splicing mutation in the CYP11B1 gene of an African with American nationality in 1998, which was a G to A mutation in the 318 site (the CYP11B1 gene of an African with American nationality). These residuals may be located in 3'-AG acceptor or 5'GT donor. In these cases, the later one leads to skipping of exon downstream.\[^{[2]}\]

Moreover, 3 insertion mutations could cause wrong reading in DNA transcription (frame-shifting mutation) and lead to wrong production of downstream mRNA and subsequently wrong protein. For example, the insertion of two bases (GA) at site 394 changes all the DNA codes downstream to this site, making a complete change of amino acids between site 443 and 463 of hemachrome combining region and leading to corresponding function loss.\[^{[21]}\] The mechanism is similar to those of other mutations in this region, such as R448C and V441G, and the result is function loss of the final product—11β-hydroxylase. In 2001, two complex rearrangements of the CYP11B1 gene were reported independently, they were chimaera formations of CYP11B1/CYP11B2.\[^{[16,19]}\]

One of the two rearrangements was reported by Hampf et al.\[^{[16]}\] The reported chimaera consisted of 5'-regularoty sequence of CYP11B1 and 3'-structure sequence of CYP11B2, and thus expressed a protein with aldosterone synthase activity under the control of CYP11B1's promoter. As the expression of CYP11B1 is regulated by ACTH (the activation of CYP11B1's promoter is regulated by ACTH), the expression of this chimaera is also regulated by ACTH, and the aldosteronism could be controlled by glucocorticoids (glucocorticoid-remediable aldosteronism-GRA). The reported splitting sites of gene exchange mainly are located in intron 2 and 4.\[^{[25,30]}\] Such exchange would lead to a high expression of aldosterone synthase, and result in primary aldosteronism. On the other hand, this exchange may also lead to the formation of CYP11B2/CYP11B1 chimaera, which consists of the promoter of CYP11B2 and the structure sequence of CYP11B1. The expression of such chimaera is regulated by the promoter of CYP11B2 (that is by angiotensin II and K'), but not ACTH; it is expressed in zona glomerulosa, the same as CYP11B2. There is no 17α-hydroxylase in zona glomerulosa, which is necessary in the cortisol biosynthesis pathway. Furthermore, all materials are circulated centripetally from zona glomerulosa to the medulla, so 11β-deoxycortisol (substrate of 11β-hydroxylase) could hardly enter zona glomerulosa. In addition, 11β-hydroxylase expressed by CYP11B1 decreases under the regulation of aldosterone synthase promoter. The outcome is a gross reduction in cortisol and CAH is caused.\[^{[10]}\]

The CYP11B2/CYP11B1 chimaera reported by Portrat et al.\[^{[31]}\] is a combination of exon 7-9 of CYP11B1 and 5'-end of CYP11B2. It is known that amino acid residuals coded by exon 5 and 6 the CYP11B2 gene are essential to maintaining the activity of aldosterone synthase coded by the gene.\[^{[32,33]}\] Therefore, when the splitting site is located at the S'-end of exon 6, the protein coded by the chimaera will lose the function of aldosterone synthase, including activities and 18-hydroxylase, 18-oxydase, and comparatively lower activity of 11β-hydroxylase. When the patient is a homozygote, aldosterone synthase deficiency and 11β-hydroxylase deficiency are will be caused.\[^{[19]}\]

At present, genetic mutations which are related to unclassical 11β-hydroxylase deficiency are not clear. Joeherer reported 3 cases of unclassical 11β-hydroxylase deficiency, in two of which genetic mutations were shown, and the mutations were complex ones: N133H/T319M and Y423X/P42S. In vitro studies approved that N133H, T319M, and P42S greatly decreased the activity of 11β-hydroxylase.\[^{[6]}\] However, no mutation was found in the third case.\[^{[6]}\]

Moreover, there is a clear relationship between genotype and phenotype of 11β-hydroxylase deficiency. Clinical manifestations in patients with homozygous
mutations are not strictly more prominent than in those with heterozygous mutations. In the 38 patients with 11β-hydroxylase deficiency, whose mutations were all homozygous R448H, the severity of hypertension, virilism, and serum levels of 11-deoxycortisol and 11-deoxycorticosterone were significantly different.14]

Conclusion
11β-hydroxylase deficiency is an autosomal recessive inherited disease. It is the second commonest cause of congenital adrenal hyperplasia. Clinical studies of 11β-OHD CAH are far less extensive than those of 21-OHD CAH, reflecting its rarity and variable clinical manifestations. The disease is caused by genetic mutations of CYP11B1, and types of the mutations are varied. In classical 11β-hydroxylase deficiency, genetic mutations of CYP11B1 lead to activity decrease or loss; mutations in unclassified 11β-hydroxylase deficiency are not definite. And the relationship between genotype and phenotype is not established.

Funding: None.

Ethical approval: Not needed.

Competing interest: None declared.

Contributors: Han S wrote this article under the supervision of Tian HM. Zhao LQ is the guarantor.

References
22 Cingöz S, Ozkan B, Döneray H, Sakizli M. Familial pericentric inversion chromosome 3 and R448C mutation of CYP11B1 gene in Turkish kindred with 11beta-hydroxylase deficiency.
Review article

24 Rosenfield RL. Serum cortisol and 17-hydroxyprogesterone concentrations in children with classic congenital adrenal hyperplasia. J Clinic Endocrinol Metab 2002;87:2993.

Received April 2, 2007
Accepted after revision April 1, 2008