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# 17α-Hydroxylase/17,20-lyase Defects

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**Congential adrenal hyperplasia due to 17α-hydroxylase/17/20-lyase deficiency is caused by genetic defects in the gene encoding *P*450c17 (CYP17). To date, 18 different mutations in 27 individuals have been identified and all of them are located in the coding region of CYP17. Several mutations have been reconstructed in human *P*450c17 cDNA and expressed in COS cells to characterize the kinetic properties of 17α-hydroxylase and 17,20-lyase activities. The molecular bases of cases clinically reported as 17α-hydroxylase deficiency have turned out to result from complete or partial combined deficiencies of 17α-hydroxylase/17,20-lyase. The elucidation of the molecular bases generally explains the patient's clinical profiles including the sexual phenotype of the external genitalia. In one case initially reported as isolated 17,20-lyase deficiency, the molecular basis was found to be partial combined deficiency of both activities, somewhat discordant with the patient's clinical profile. However, the patient was subsequently found to have 17α-hydroxylase deficiency, suggesting involvements of age-dependent unknown factors affecting *P*450c17 activity.**

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## INTRODUCTION

A relatively rare cause of congenital adrenal hyperplasia (CAH), "17α-hydroxylase deficiency" can be characterized by a defect in either or both of the 17α-hydroxylase and 17,20-lyase activities, based on the fact that a single polypeptide, *P*450c17 catalyzes two distinct reactions: 17α-hydroxylation of pregnenolone or progesterone and the 17,20-lyase reaction of 17α-hydroxylated pregnenolone to yield the C19 steroid precursors of testosterone and estrogens [1, 2]. The CYP17 gene encoding *P*450c17 [3, 4] is a single copy gene and located on chromosome 10q24–25 [5].

Deficiency of *P*450c17 normally present in both the gonads and adrenal cortex leads to impaired production of cortisol, androgens and estrogens, with accompanying overproduction of mineralocorticoids, in particular 11-deoxycorticosterone (DOC). Consequently, affected females (46XX) have hypertension and absence of sexual development producing primary amenorrhea, while affected males (46XY) are also hypertensive and have female external genitalia. Rarely, partial deficiency of this enzyme associated with genetic ambiguity have been reported. To date, more than 120 cases of 17α-hydroxylase deficiency have been reported [6]. While most of them are clinically reported to have complete 17α-hydroxylase deficiency, about 20 cases seem to be a partial form of this trait [6]. On the other hand, a third type of deficiency, isolated 17,20-lyase deficiency, is also described in the literature. This is much less common than 17α-hydroxylase deficiency and only 14 cases have been reported [6]. Reports on genetic analysis of each form of *P*450c17 defect have been accumulated. In this article, the molecular bases of all of the reported cases of *P*450c17 deficiency are briefly reviewed.

## MOLECULAR BASIS OF 17α-HYDROXYLASE AND 17,20-LYASE DEFICIENCY

Patient CYP17 was analyzed by exonic sequencing following a conventional cloning method [3] or polymerase chain reaction (PCR) [7]. To date, 18 different mutations in 27 individuals have been identified and all of them are located in the structural gene [3, 8–22 and T. Imai, unpublished observation]. Table 1 is a brief summary of the clinical profiles and molecular defects of these patients.

### *Complete combined 17α-hydroxylase and 17,20-lyase deficiencies*

Fourteen kinds of mutations which result in virtual loss of *P*450c17 activity have been identified in patients showing typical symptoms of 17α-hydroxylase deficiency (cases 1–24 in Table 1). Briefly, 6 are mutations causing non-sense mutations by themselves [8, 14] or as a result of alterations of the reading frame [11, 12, 19].

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*Table 1. Summary of clinical profiles and molecular defects in which the structure of CYP17 gene has been analyzed*

| Case | Family | Race | Karyotype | Social sex | Mutation | Reference |
|---|---|---|---|---|---|---|
| 1 (ML) | A | Canadian | 46XY | F | 4 bp duplication (480) | 3 |
| 2 (BD) | B | Canadian | 46XY | F | Same as case 1 | 9 |
| 3 | C | Netherlander | 46XY | F | Same as case 1 | 10 |
| 4 | C | Netherlander | 46XY | F | Same as case 1 | 10 |
| 5 | D | Netherlander | 46XX | F | Same as case 1 | 10 |
| 6 | E | Netherlander | 46XX | F | Same as case 1 | 10 |
| 7 | E | Netherlander | 46XX | F | Same as case 1 | 10 |
| 8 | F | Netherlander | 46XY | F | Same as case 1 | 10 |
| 9 | G | Netherlander | 46XY | F | Same as case 1 | 10 |
| 10 | H | Netherlander | 46XY | M | Same as case 1 | 10 |
| 11 (JY) | I | Japanese | 46XX | F | Trp(17)→End | 8 |
| 12 (JK) | J | Japanese | 46XX | F | 7 bp duplication (120) | 11 |
| 13 (DG) | K | Italian | 46XY | F | Deletion and insertion (Exon 2–3) | 12 |
| 14 (DA) | K | Italian | 46XX | F | Same as case 13 | 12 |
| 15 (DM) | K | Italian | 46XX | F | Same as case 13 | 12 |
| 16 | L | Guamanian | 46XY | F | Ser(106)→Pro | 13 |
| 17 | M | Guamanian | 46XY | F | Same as case 16 | 13 |
| 18 | N | English | 46XX | F | (1) Glu(194)→End<br>(2) Arg(239)→End | 14 |
| 19 | O | Thai | 46XX | F | Deletion of<br>Asp(487)-Ser(488)-Phe(489) | 15 |
| 20 | P | Japanese | 46XX | F | His(373)→Leu | 16 |
| 21 | Q | Japanese | 46XX | F | 2-bp (GC) deletion (300, 301) | 19 |
| 22 (VB) | R | Caucasian | 46XY | F | (1) Tyr(64)→Ser<br>(2) Duplication of Ile (112) | 17 |
| 23 | S | German | 46XX | F | Arg(440)→His | 18 |
| 24 (WV) | T | Caucasian | 46XX | F | Gly(90)→Asp | |
| 25 (JG) | U | Japanese | 46XX | F | Deletion of Phe (53 or 54) | 20 |
| 26 (DL) | V | Canadian | 46XY | M | (1) Arg(239)→End<br>(2) Pro(342)→Thr | 21 |
| 27 (IS) | W | Swiss | 46XY | F | (1) Gln(461)→End<br>(2) Arg(496)→Cys | 22 |

Case 10 is a heterozygous for the mutation and thus may have a different mutation on the other CYP17 allele. All cases except 18, 22, 26 and 27 are homozygous for their respective mutations. Mutation in case 24 is based on unpublished observation by T. Imai.

Since the premature stop codon is located at the amino terminal side of the heme-binding cysteine (442) in *P*450c17, the presence of these mutations leads to absence of a functional *P*450c17 protein in the adrenal cortex and gonads. All patients are homozygous for their respective mutations except one (case 18) having two different stop codons as a compound heterozygote [14]. A 4 bp (CATC) duplication at 480 extinguishes *P*450c17 activity because it alters the reading frame, leading to a carboxy-terminal sequence that is completely different from that of normal sequence and in-frame stop codon at 506 [3, 9, 10]. The 4 bp duplication is found in members of the Mennonite religious sect residing in Canada (cases 1 and 2) and individuals in Friesland in Holland (cases 3–10), making this mutation the most widely distributed cause of *P*450c17 deficiency [3, 9, 10]. A three amino acid (Asp-Ser-Phen) deletion at 487–489 is also shown to destroy detectable *P*450c17 activity probably due to a conformational change of *P*450c17 protein [15]. These two mutations clearly tell us that the carboxy-terminal region is important for its *P*450c17 activities.

Interestingly, 4 mutations are single amino acid substitutions which completely extinguish detectable *P*450c17 activities [13, 16, 18 and T. Imai, unpublished observation]. From the molecular modeling of the human *P*450c17 sequence based on the tertiary structure of bacterial *P*450cam, it has been predicted that Ser (106) → Pro may affect substrate binding [13]. Similarly, Hls (373) → Leu has been predicted to affect the heme-binding and it has been actually demonstrated that membrane preparations from *E. coli* cells expressing the mutant form, unlike those expressing wild type, show no *P*450 nm peak upon reduction and addition of carbon monoxide [16]. The mutation of Arg (440) → His probably disturbs the heme-binding since Arg (440) may be one of crucial residues constituting the heme-binding region of *P*450c17 [18]. In case 22 in Table 1, compound heterozygous mutations were found. Upon expression of these mutant proteins in *E. coli*, Tyr (64) → Ser mutant has 15% of the wild type 17$\alpha$-hydroxylase activity, whereas the Ile (112) duplication showed no activity.

In spite of the increased levels of corticosterone and DOC, suppressed levels of aldosterone are observed in

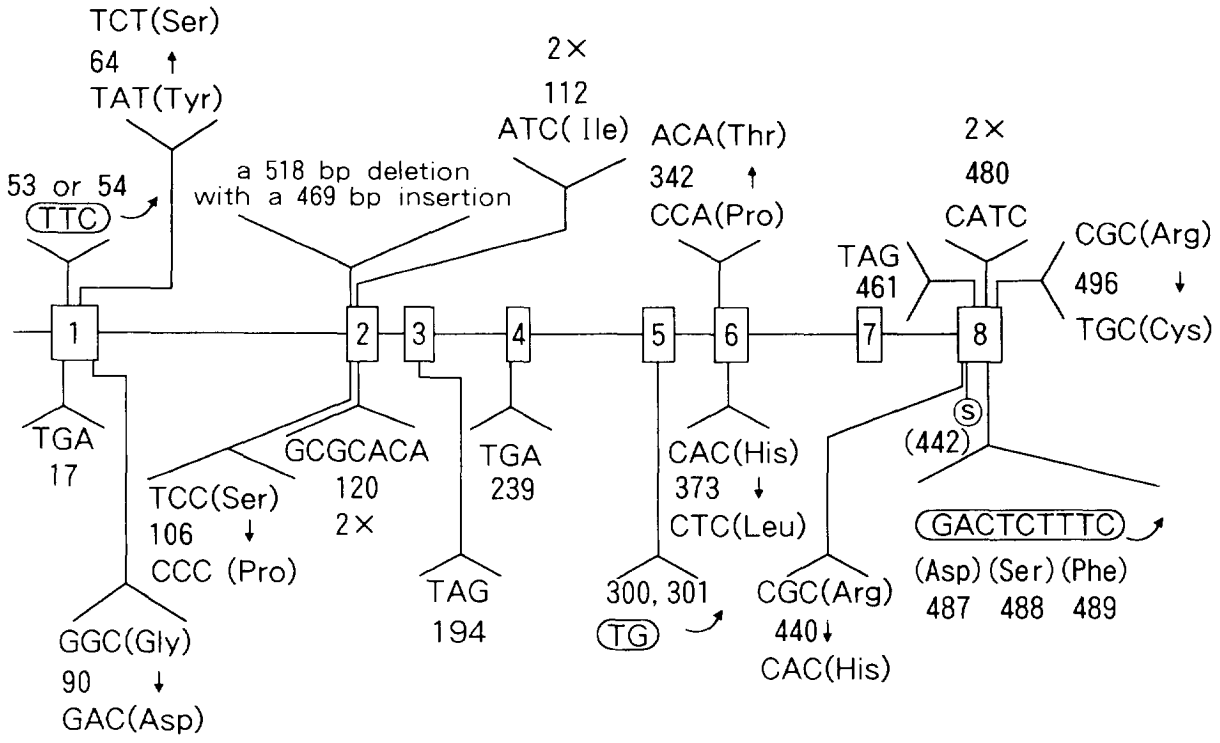


**Fig. 1. Schematic representation of the human CYP17 gene showing the position of mutations reported to date [3, 6, 8–22 and T. Imai, unpublished observations]. The numbered boxes represent the exons; the lines between them represent the introns. The circle noted in exon 8 represents the cysteine (442) required for heme binding by *P*450c17.**

most patients with 17α-hydroxylase deficiency. The suppression of aldosterone secretion from the zona glomerulosa is attributed to the suppression of the renin–angiotensin system caused by sodium retention and volume expanding actions of markedly elevated DOC and corticosterone secretion from the zona fasciculata. However, some cases with 17α-hydroxylase deficiency have elevated aldosterone levels and the mechanism remains unclear [6]. Cases 20 and 21 are such cases with exceptionally high concentrations of plasma aldosterone. However, since both patients were found to have defective *P*450c17 [16, 19] as observed in other cases with normal or suppressed levels of plasma aldosterone concentration, other mechanisms for elevated aldosterone levels should be considered.

*Partial combined 17α-hydroxylase/17,20-lyase deficiency*

Only two cases of partial combined deficiencies of both activities have been analyzed at the molecular level (cases 25 and 26). A Japanese female patient, JG has irregular menstruation, suggesting some degree of estrogen production. Sequence analysis revealed a homozygous deletion of the phenylalanine codon (TTC) at either amino acid 53 or 54 in exon 1 [20]. On the other hand, DL (case 26) is a 46XY newborn male with ambiguous external genitalia, suggesting some degree of testosterone production. The patient was found to be a compound heterozygote, carrying two different mutant alleles in the CYP17. One allele contains a stop codon (TGA) in place of arginine (CGA) at amino acid position 239 in exon 4, which makes the resultant protein truncated and nonfunctional. The second allele contains a missense mutation, namely a change from proline (CCA) to threonine (ACA) at amino acid position 342 in exon 6 [21]. Expression studies of the mutant cDNA constructs containing a change from proline to threonine at 342 or the phenylalanine deletion at either 53 or 54 in Cos 1 cells made it possible to estimate *P*450c17 activities of each individual. With respect to the requirements of 17,20-lyase activity and the sexual phenotype, DL having ambiguous genitalia has been found to have 20% of the normal level of 17,20-lyase activity. We do know that a 46XY individual (JK) (case 12), expected to have no *P*450c17 activity because of the homozygous presence of a premature stop codon has female external genitalia. In addition, the father of this patient, a heterozygous carrier for the premature stop codon and therefore expected to have 50% of normal *P*450c17 activity has apparently normal reproductive capacity. Thus, the threshold 17,20-lyase activity necessary for changing the sexual phenotype of the external genitalia from female to ambiguous is 0–20%, while that for changing from ambiguous to normal is 20–50% [21]. In genetic females, the threshold activity of 17,20-lyase for menstruation seems to be lower than expected since only 5% normal

17,20-lyase activity leads to irregular menstruation in the patient JG [20].

*17,20-lyase deficiency*

Case IS (case 27 in Table 1) was originally reported as being isolated 17,20-lyase deficiency when he was 15 years old [23]. This patient was found to be a compound heterozygote having two different mutations in the CYP17 gene. One allele contains a missense mutation from arginine (CGC) to cysteine (TGC) at 496 while the second allele has a stop codon (TAG) in place of glutamine (CAG) at 461. Expression studies to determine the effect of each mutation on the enzymatic properties of *P*450c17 revealed that in this individual, both 17α-hydroxylase and 17,20-lyase activities were dramatically reduced rather than there only being a reduction in 17,20-lyase, as initially reported [23]. However, subsequent endocrinological examinations of this patient when 25 years old have shown the absence of both 17α-hydroxylase and 17,20-lyase activities [24]. This fact suggests the involvement of age-dependent unknown factors affecting *P*450c17 activity. The electron transfer system may be one of such factors since the relative activity ratio of 17,20-lyase/17α-hydroxylase is reported to increase several fold by elevating the ratio of either NADPH-cytochrome *P*450-reductase (Red) [25] or cytochrome b5 (b5) [26]. Differences in tissue b5 or Red concentration have been suggested to be functionally associated with differences in 17,20-lyase activity in steroidogenic tissues [25, 27, 28].

## CONCLUSIONS

In the 27 patients studied biochemically to date, 18 different mutations are found suggesting that most of the reported cases of 17α-hydroxylase deficiency arise from random alterations in the structure of the CYP17 gene. An exception to this is the defect being found in 10 patients that probably reflects a "Founder effect" which arose in the Friesland region of Holland. In most cases of 17α-hydroxylase deficiency, the molecular basis of the disease clearly defines the clinical symptoms. However, the rare case which showed a clinical conversion from 17,20-lyase deficiency to 17α-hydroxylase deficiency with aging suggests that molecular basis does not always explain the clinical profiles and the necessity to consider other genetic or nongenetic factors modulating *P*450c17 activity. A precise explanation of why each mutation affects the *P*450c17 activities remains unclear except in cases with premature stop codon positioned at the amino terminal side of *P*450c17 heme-binding cysteine (442). The mechanism will only become apparent when the tertiary structure of human *P*450c17 is determined. The recent development of the bacterial system for both the expression and purification of human *P*450c17 [17] enhances the possibility of obtaining this structure.

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