

## BRIEF REPORT

# A Novel Point Mutation in P450c17 (CYP17) Causing Combined 17 $\alpha$ -Hydroxylase/17,20-Lyase Deficiency

A. M. Brooke, N. F. Taylor, J. H. Shepherd, M. E. Gore, T. Ahmad, L. Lin, G. Rumsby, M. Papari-Zareei, R. J. Auchus, J. C. Achermann, and J. P. Monson

*Center for Endocrinology (A.M.B., J.P.M.), William Harvey Research Institute, St. Bartholomew's Hospital, Queen Mary, University of London, London EC1A 7BE, United Kingdom; Department of Clinical Biochemistry (N.F.T.), King's College Hospital, London SE5 9RS, United Kingdom; Department of Medical Oncology (J.H.S., M.E.G., T.A.), Royal Marsden Hospital, London SW3 6JJ, United Kingdom; Institute of Child Health and Department of Medicine (L.L., J.C.A.), and Department of Clinical Biochemistry (G.R.), University College Hospital, London NW1 2BU, United Kingdom; and Division of Endocrinology (M.P.-Z., R.J.A.), Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, Texas 75390-8857*

**Context:** Combined 17 $\alpha$ -hydroxylase/17,20-lyase deficiency is a rare cause of congenital adrenal hyperplasia and hypogonadism. Novel single amino acid changes in P450c17 provide potentially important insights into key structural domains for enzyme function.

**Objective, Design, and Setting:** We report a novel missense mutation in P450c17 in a 17-yr-old female presenting with a malignant mixed germ cell tumor with yolk sac elements who demonstrated clinical and biochemical features of combined 17 $\alpha$ -hydroxylase/17,20-lyase deficiency.

**Methods:** Quantitative urinary steroid analysis was performed by high resolution gas chromatography. All eight coding exons of CYP17 were PCR amplified and sequenced. The position of arginine at codon 96 was modeled using the CYP17 structure 2c17 ([www.rcsb.org](http://www.rcsb.org)). The CYP17 genes were subcloned into pcDNA3, expressed in HEK-293 cells, and chromatographed.

**Patient and Results:** 17 $\alpha$ -Hydroxylase deficiency was confirmed by marked reductions in urinary and serum cortisol, androgens, and estradiol. Mutational analysis revealed a novel homozygous R96Q missense mutation in P450c17, affecting an amino acid in a key substrate-binding region of the enzyme, leading to complete inactivity.

**Conclusion:** The description of a second missense mutation at codon 96 (R96W and R96Q) in the substrate-binding region of P450c17 provides strong evidence for the key role of this amino acid in 17 $\alpha$ -hydroxylase/17,20-lyase function. An association between a malignant germ cell tumor and 17 $\alpha$ -hydroxylase deficiency has not been reported previously, although the presence of gonadoblastoma in the ovary of a patient with this condition has recently been described. (*J Clin Endocrinol Metab* 91: 2428–2431, 2006)

COMBINED 17 $\alpha$ -HYDROXYLASE/17,20-LYASE deficiency is a relatively rare cause of congenital adrenal hyperplasia first described nearly 40 yr ago (1). Genotypic females (46,XX) with this condition typically present with hypertension, hypokalemia, absent secondary sexual characteristics, and primary amenorrhea, whereas genotypic males (46,XY) demonstrate impaired virilization (complete or partial pseudohermaphroditism) and absence of pubertal development.

The gene encoding the dual function P450c17 enzyme, CYP17 (10q24.3), was cloned in 1987 and encodes eight exons over 6.4 kb DNA (2–4). Mutations in this gene were first reported in 1988 (5). Since then, approximately 45 different mutations have been described in the coding regions, splice donor sites (resulting in exon skipping), and splice acceptor sites of this gene in patients with combined 17 $\alpha$ -hydroxylase/17,20-lyase

deficiency (6). Single amino acid changes in P450c17 are relatively rare, but provide potentially important insight into key structural domains for enzyme function, and a limited number of patients with isolated 17,20-lyase deficiency due to P450c17 point mutations have been reported (7–9).

In this study, we describe a novel point mutation in a key substrate-binding region of P450c17 in a prepubertal 17-yr-old girl who presented with a malignant germ cell tumor.

### Patient and Methods

#### Laboratory analysis

Quantitative urinary steroid analysis was performed by high resolution gas chromatography as previously described (10).

#### Mutational analysis

After institutional review board approval and informed consent were obtained, genomic DNA was extracted from the patient's leukocytes. All eight coding exons of CYP17 were PCR amplified using variations of the primers and conditions described previously (11). PCRs were purified using exonuclease and shrimp alkaline phosphatase and were sequenced directly, forward and reverse, using a BigDye Terminator version 1.1 cycle sequencing kit (Applied Biosystems, Warrington, UK).

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Abbreviations: DHEA, Dehydroepiandrosterone; 17OHP, 17-hydroxyprogesterone.

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Sequencing was performed on a MegaBACE1000 capillary DNA sequencer (Amersham Biosciences, Little Chalfont, UK).

### Structural modeling and functional studies

The position of arginine at codon 96 was modeled using the CYP17 structure 2c17 ([www.rcsb.org](http://www.rcsb.org)). Images were generated with MidasPlus software on a Silicon Graphics (Mountain View, CA) octane workstation. The cDNA for CYP17 mutation R96Q was generated by overlapping PCR using oligonucleotides T7+R96Q-S (5'-CTCTGGGCAGCCTCAAATGGCAAC-3') and R96Q-AS (5'-TTGAGGCTGCCAGAGAA-GTCCTTG-5')+SP6, and pLW01-c17 as a template (12, 13). HEK-293 cells were seeded in six-well plates and transfected with 1  $\mu$ g pcDNA3-c17 plasmids using FuGene 6 as previously described (12). Cells were incubated with 2 ml complete medium containing 0.03–0.1  $\mu$ M pregnenolone with 150,000 cpm [<sup>3</sup>H]pregnenolone (PerkinElmer NEN, Shelton, CT). Aliquots (1 ml) were removed after 2–4 h, extracted, and chromatographed as described previously (12, 13).

## Results

### Case report

A 17-yr-old, phenotypically female patient from the United Arab Emirates came to medical attention because of abdominal pain and was found to have a mixed germ cell tumor with yolk sac elements. Remission was achieved with six cycles of bleomycin, etoposide, cisplatin chemotherapy (serum  $\alpha$ -fetoprotein reduced from 12,714 to 5 mU/liter). It was noted that the patient was prepubertal, with normal female external genitalia, absent axillary and pubic hair, and primary amenorrhea. Her height was at the third percentile for her age. Her parents were first cousins. Examination demonstrated palmar pigmentation, buccal and streak pigmentation, and hypertension. Abdominal computed tomography revealed bilateral adrenal hyperplasia.

Her karyotype was 46,XX. Serum LH and FSH levels were 32 and 60 IU/liter, respectively, and serum estradiol was 88 pmol/liter (24 pg/ml). Serum potassium was 2.5 mmol/liter, serum bicarbonate was 24 mmol/liter, recumbent serum aldosterone was 643 pmol/liter (23.2 ng/dl), and plasma renin activity was undetectable. Serum dehydroepiandrosterone (DHEA) sulfate, 17-hydroxyprogesterone (17-OHP), and 11-deoxycortisol were undetectable; androstenedione and testosterone were decreased at 1.1 nmol/liter (0.32  $\mu$ g/liter) and 0.4 nmol/liter (0.12 ng/ml), respectively. Serum cortisol was decreased at 15 nmol/liter (0.54  $\mu$ g/dl) with a 0900 h plasma ACTH level of 21  $\mu$ g/liter. There was no response of cortisol or any androgens to standard tetracosactrin stimulation (250  $\mu$ g, iv). A clinical diagnosis of complete 17 $\alpha$ -hydroxylase deficiency was supported by measurement of urinary steroids by gas liquid chromatography (10), which showed grossly elevated corticosterone metabolites (42,925  $\mu$ g/24 h) and pregnanediol (1,360  $\mu$ g/24 h), and absent cortisol, androstenedione, and dehydroandrosterone metabolites.

The patient commenced reverse circadian prednisolone treatment (2 mg in the morning and 4 mg in the evening), and her hypokalemia and hypertension resolved. Puberty was induced initially with 5  $\mu$ g ethinyl estradiol daily, increased gradually to 20  $\mu$ g daily over 18 months, followed by the addition of levonorgestrel. She remains in remission after 18 months of follow-up.

### Mutational analysis, modeling, and functional studies

Mutational analysis revealed a novel homozygous R96Q missense mutation in P450c17. The parents were consanguineous, but were unavailable for investigation. This R96Q change converts a highly conserved, charged, side chain amino acid (arginine) to an amino acid with a polar uncharged side chain (glutamine) in flanking strand 2 of  $\beta$ -sheet 1 (Fig. 1). This mutation showed complete loss of P450c17 function (Fig. 2).

## Discussion

We describe a novel homozygous R96Q missense mutation in P450c17 affecting an amino acid in a key substrate-binding region of the enzyme, resulting in complete absence of 17 $\alpha$ -hydroxylase/17,20-lyase activity. 17 $\alpha$ -Hydroxylase deficiency is an autosomal recessive condition that accounts for approximately 1% of all cases of congenital adrenal hyperplasia (14). The estimated incidence is 1 in 50,000 newborns (15). This condition was first described by Biglieri *et al.* (1) in an adult female (genotype 46,XX) with hypertension, hypokalemia, and sexual infantilism. This patient had increased urinary excretion of corticosterone with absence of 17 $\alpha$ -hydroxylated metabolites. As in the present case, the lack of sexual development and impaired estrogenization suggested that P450c17 has 17,20-lyase activity in addition to its 17 $\alpha$ -hydroxylase activity, and most patients reported to date have combined 17 $\alpha$ -hydroxylase/17,20-lyase deficiency due to deleterious changes in this P450c17 enzyme system. The biochemical and structural mechanisms involved in P450c17 dual function (17 $\alpha$ -hydroxylase and 17,20-lyase activity) are being elucidated, and the localization of critical domains for enzyme function has been supported by the

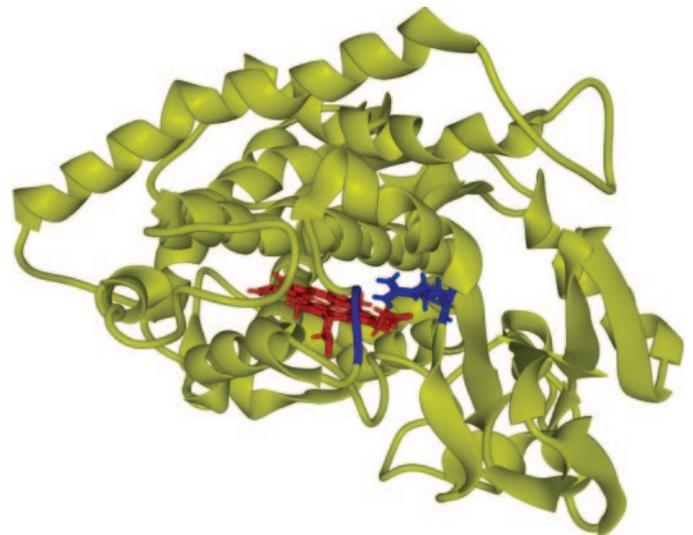


FIG. 1. Computer model of human CYP17 (RCSB pdbid 2c17). The protein backbone is shown in ribbon rendering (yellow), with heme in red. R96 (blue) protrudes into the cleft that separates the  $\beta$ -sheet domain (right) from the  $\alpha$ -helical domain (left). The guanidinium group of R96 appears to form hydrogen bonds with carbonyl groups of residues A113 and F114 (purple), but not with the side chains. Removal of this positively charged group, which occurs in mutation R96Q or R96W, is predicted to destabilize the protein by disrupting this interaction between the two domains.

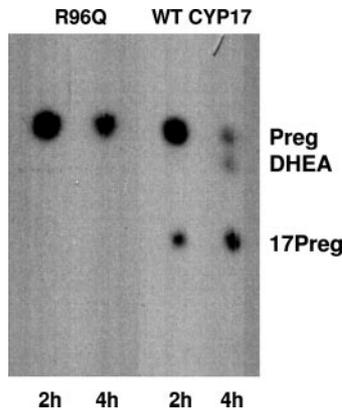


FIG. 2. Activity of the CYP17 mutation R96Q. Autoradiogram of a thin layer chromatogram used to separate [ $^3$ H]pregnenolone metabolites is shown, with the locations of steroids indicated. HEK-293 cells transfected with cDNAs for mutation R96Q or wild-type CYP17 were incubated with 0.1  $\mu$ M [ $^3$ H]pregnenolone for 2–4 h, and medium was extracted and chromatographed. Wild-type CYP17 converts pregnenolone (Preg) to 17 $\alpha$ -hydroxypregnenolone (17Preg) and then to DHEA; mutation R96Q shows no activity.

identification of a limited number of point mutations in the protein in patients with disorders of steroidogenesis.

Under normal physiological conditions, pregnenolone and progesterone undergo 17 $\alpha$ -hydroxylation to 17 $\alpha$ -hydroxypregnenolone and 17OHP, respectively. Scission of the C17,20 carbon bond in 17 $\alpha$ -hydroxypregnenolone yields DHEA; however, very little 17OHP is converted to androstenedione, because the human P450c17 enzyme catalyzes this reaction at only 3% the rate of conversion of 17 $\alpha$ -hydroxypregnenolone to DHEA (16). If neither 17 $\alpha$ -hydroxylase nor 17,20-lyase activity is present, pregnenolone is converted to mineralocorticoids. If only 17 $\alpha$ -hydroxylase activity is present, pregnenolone is converted to cortisol, and if both activities are present, pregnenolone is converted to DHEA. The P450 enzyme is bound to the smooth endoplasmic reticulum, where it accepts electrons from P450 oxidoreductase. Electron transfer for the lyase reaction is promoted by the allosteric action of cytochrome *b<sub>5</sub>*, but also requires phosphorylation of serine residues on P450c17 by a cAMP-dependent kinase. The availability of electrons seems to determine whether P450c17 performs only 17 $\alpha$ -hydroxylation or also 17,20-lyase activity. Optimal functioning of the redox partner-binding site is particularly important for the lyase reaction. Thus, point mutations (*e.g.* R347H and R358Q) that change the distribution of surface charges on the redox binding site of P450c17 impair electron transfer and can result in isolated 17,20-lyase deficiency with preserved 17 $\alpha$ -hydroxylase function (8, 9, 13).

Point mutations resulting in combined 17 $\alpha$ -hydroxylase/17,20-lyase deficiency have also provided insight into critical amino acids involved in dual-enzyme function. To date, approximately 18 different missense and in-frame point mutations have been described (7). A significant proportion of these mutations may have arisen due to a genetic founder effect, rather than through selection advantage of heterozygote carriers (17). For example, in studies of combined 17 $\alpha$ -hydroxylase/17,20-lyase deficiency in Brazil, only seven mutations from 28 unrelated alleles in 19 families were found

(11, 18). The W406R mutation was more common in southern Brazilians of Spanish origin, and the R362C mutation was more common in northern Brazilians of Portuguese origin. Interestingly, neither of these mutations has been found in Spain or Portugal, suggesting a founder arriving in Brazil many years before.

In contrast, a homozygous R96W mutation has been reported previously in two siblings of French Canadian origin with 46,XY pseudohermaphroditism and combined 17 $\alpha$ -hydroxylase/17,20-lyase deficiency (19). Although certain genetic sequences are more vulnerable to mutation, the occurrence of two different point mutations in the same amino acid suggests that this locus has a particularly important functional role and is supported by the complete loss of dual enzyme function reported in studies of P450c17 R96W activity. Furthermore, modeling of this amino acid change suggests that this amino acid lies within the flanking strand 2 of the  $\beta$ -sheet 1. Mutation of the charged side chain of arginine to glutamine would lead to complete inactivity of the enzyme.

The severity of the clinical disease tends to be milder with mutations that retain partial catalytic activity, but the age of onset of hypertension, the degree of hypokalemia, and the aldosterone production rate appear to vary, even in those with the same mutation (14). For example, up to 15% of patients with a diagnosis of 17 $\alpha$ -hydroxylase deficiency are normokalemic and normotensive at diagnosis (14) (although not all of these have had the diagnosis proven by genotyping). An elevated aldosterone level, as in our patient, is unusual, but not unique (14), and the relatively normal value of ACTH is probably due to some glucocorticoid potency of corticosterone leading to partial suppression of the hypothalamic-pituitary (HPA) axis.

This patient presented with a mixed germ cell tumor. A gonadoblastoma has previously been described in a 46,XX Brazilian patient with combined 17 $\alpha$ -hydroxylase/17,20-lyase deficiency who presented at 17 yr of age (18). There has also been another report of a 46,XX patient with 17,20-lyase deficiency presenting with giant ovarian cysts that were reduced in size by GnRH antagonist therapy (20). This association remains likely to be a chance finding, but an awareness of the possible development of gonadal tumors in patients with disordered gonadal steroidogenesis is necessary.

Our description of a second missense mutation at codon 96 (R96Q) in the substrate-binding region of P450c17 provides strong evidence for the key role of this amino acid in 17 $\alpha$ -hydroxylase/17,20-lyase function.

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Address all correspondence and requests for reprints to: Dr. J. P. Monson, Center for Endocrinology, William Harvey Research Institute, St. Bartholomew's Hospital, Queen Mary, University of London, London EC1A 7BE, United Kingdom. E-mail: j.p.monson@qmul.ac.uk.

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