

## Homozygous Mutation G539R in the Gene for P450 Oxidoreductase in a Family Previously Diagnosed as Having 17,20-Lyase Deficiency

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**Context:** Very few patients have been described with isolated 17,20-lyase deficiency who have had their mutations in P450c17 (17 $\alpha$ -hydroxylase/17,20-lyase) proven by DNA sequencing and *in vitro* characterization of the mutations. Most patients with 17,20-lyase deficiency have mutations in the domain of P450c17 that interact with the electron-donating redox partner, P450 oxidoreductase (POR).

**Objective:** Our objective was to clarify the genetic and functional basis of isolated 17,20-lyase deficiency in familial cases who were previously reported as having 17,20-lyase deficiency.

**Patients:** Four undervirilized males of an extended Bedouin family were investigated. One of these has previously been reported to carry mutations in the CYP17A1 gene encoding P450c17 causing isolated 17,20-lyase deficiency.

**Methods:** Serum hormones were evaluated before and after stimulation with ACTH. Urinary steroid metabolites were profiled by gas chromatography-mass spectrometry. Exons 1 and 8 of CYP17A1 previously reported to harbor mutations in one of these patients and all 15 coding exons of POR were sequenced.

**Results:** Gas chromatography-mass spectrometry (GC-MS) urinary steroid profiling and serum steroid measurements showed combined deficiencies of 17,20-lyase and 21-hydroxylase. Sequencing of exons 1 and 8 of CYP17A1 in two different laboratories showed no mutations. Sequencing of POR showed that all four patients were homozygous for G539R, a previously studied mutation that retains 46% of normal capacity to support the 17 $\alpha$ -hydroxylase activity but only 8% of the 17,20-lyase activity of P450c17.

**Conclusion:** POR deficiency can masquerade clinically as isolated 17,20-lyase deficiency. (*J Clin Endocrinol Metab* 93: 3584–3588, 2008)

Androgen biosynthesis requires microsomal P450c17, which converts pregnenolone to 17OH-pregnenolone (17 $\alpha$ -hydroxylase activity) and then converts 17OH-pregnenolone to dehydroepiandrosterone (DHEA) (17,20-lyase activity). Both

activities of P450c17 require electron donation from NADPH via P450 oxidoreductase (POR) (1). The 17,20-lyase activity of P450c17 is determined by three factors: a high molar ratio of POR to P450c17 (2, 3), serine phosphorylation of P450c17 (4,

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Abbreviations: DHEA, Dehydroepiandrosterone; DHEA-S, DHEA sulfate; 17OHP, 17OH-progesterone; POR, P450 oxidoreductase.

5), and the action of cytochrome b<sub>5</sub>, which promotes the association of P450c17 with POR (6–8). Mutation of the *CYP17A1* gene for P450c17 (9, 10) causes severe 17 $\alpha$ -hydroxylase deficiency, in which virtually no 17 $\alpha$ -hydroxylated steroids or sex steroids are produced.

Genetically proven isolated 17,20-lyase deficiency was first described in two patients with point mutations in the P450c17 domain that interacts with POR; these mutations retained nearly normal 17 $\alpha$ -hydroxylase activity but virtually no 17,20-lyase activity (11). Another patient with apparent isolated 17,20-lyase deficiency was reported as heterozygous for a frameshift mutation and F417C in P450c17 (12). The activity of the F417C mutant expressed in COS-1 cells and analyzed by immunoassay suggested that this mutant catalyzed 17 $\alpha$ -hydroxylase activity but not 17,20-lyase activity (12). However, computational modeling of human P450c17 (13) indicated that F417C would cause a major structural disruption of P450c17, which should eliminate both activities. Expression of P450c17 F417C in a well-established yeast system and activity assays by more sensitive conversion of radiolabeled precursors demonstrated that F417C was devoid of activity (14). Thus, combination of F417C and a frameshift should ablate all P450c17 activities, yet the patient had an elevated 17OH-progesterone (17OHP) (15). Therefore, we have reinvestigated this patient and three similarly affected family members, demonstrating that the apparent isolated 17,20-lyase deficiency was caused by a homozygous mutation in *POR*, G539R.

## Patients and Methods

### Patients

The four 46,XY patients belong to a small Bedouin clan in Southern Israel (Fig. 1A); patient 2 was the subject of the initial report (12). All the patients were followed at the pediatric endocrine clinic of Soroka Medical Center. Endocrine evaluation of patients 1–3 in early childhood revealed subnormal testosterone response to human chorionic gonadotropin and a subnormal response of cortisol to ACTH. Basal plasma DHEA and androstenedione concentrations were low and unresponsive to ACTH; basal and post-ACTH 17OHP were elevated (15). The local ethics committee at Soroka Medical Center approved the study, and the parents gave informed consent.

### Methods

Methods have been published as supplemental data on The Endocrine Society's Journals Online web site at <http://jcem.endojournals.org>.

## Results

### Clinical and hormonal findings

Patient 1 was born to healthy first-degree cousins after a normal-term pregnancy and delivery. Micropenis, inguinal testes, and an underdeveloped scrotum were noted at birth; physical examination was otherwise normal. Evaluation on d 1 showed low testosterone [52 ng/dl (1.8 nmol/liter); normal up to 345 ng/dl (12 nmol/liter)]. He is now 21 yr old, is +1.42 SD in height, and is Tanner 5 with a 7-cm penis. Patient 2, the younger brother of patient 1, was born after a normal pregnancy and uneventful delivery. He had micropenis, chordee, scrotal hypospadias, and small testes (volume 0.5

ml) in a bifid scrotum; physical examination was otherwise normal. Low concentration of testosterone [84 ng/dl (2.9 nmol/liter)] and abnormally high levels of 17OHP [11.88  $\mu$ g/liter (36 nmol/liter); normal, 0.66–2.5  $\mu$ g/liter (2–7.5 nmol/liter)] were found 48 h after birth. Cystoscopy and urethrogram showed normal internal male genital anatomy. He is now 14.6 yr old, –1.0 SD in height, and Tanner 2 with a 3-cm penis.

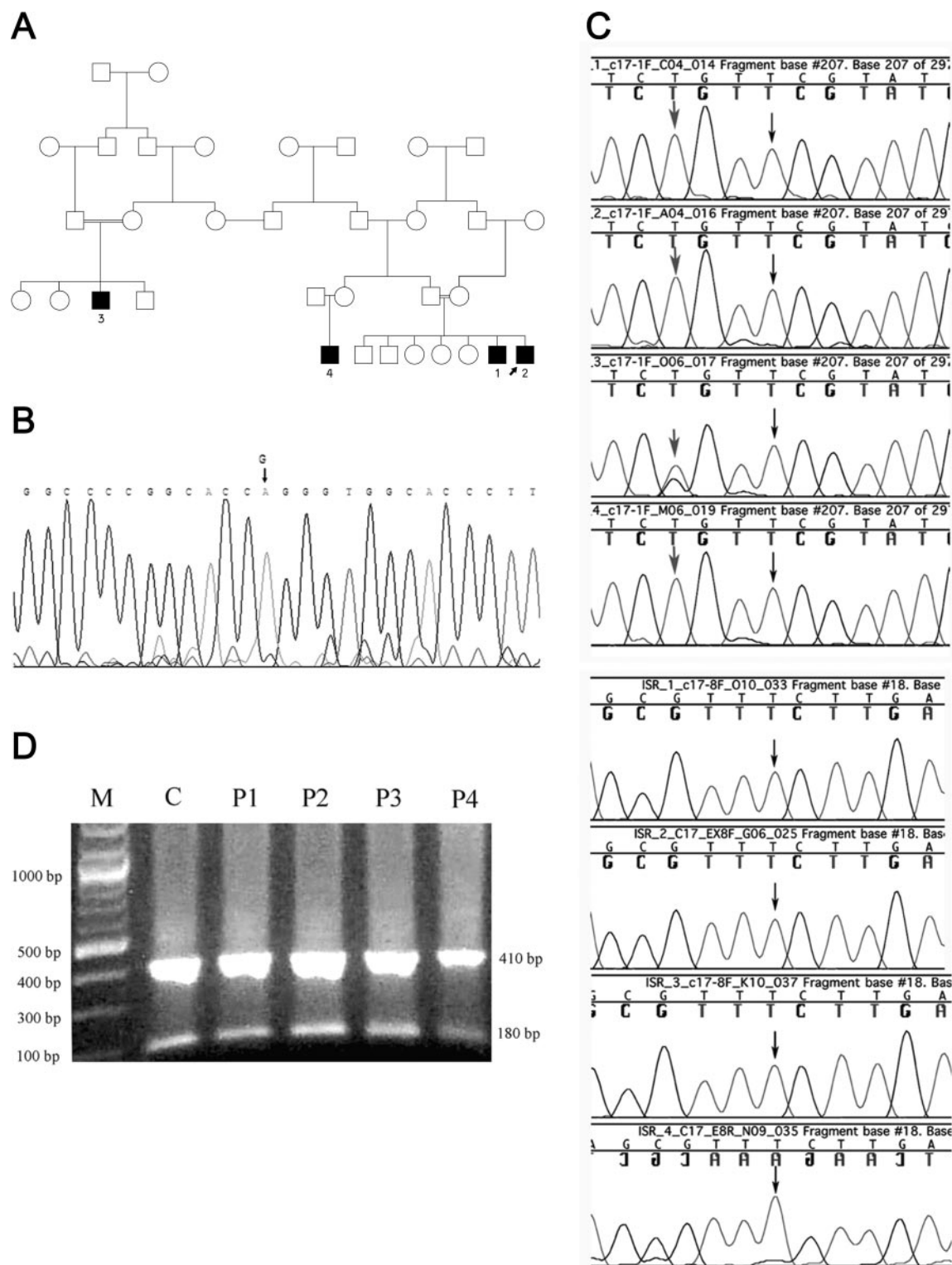
Patient 3 was evaluated at 10 months for micropenis but otherwise normal external genitalia. His parents are cousins who were followed for maternal infertility. He is now 16.5 yr old, +0.89 SD in height, and Tanner 5 with an 8-cm penis.

Patient 4 was born after normal pregnancy and uneventful delivery and evaluated on d 1 for micropenis with chordee and undescended testes; testosterone was low [20 ng/dl (0.7 nmol/liter)].

All four patients were 46,XY. None of the mothers became virilized during their pregnancies. Patient 2 underwent hypospadias repair and left orchiectomy for recurrent testicular torsion. Patient 4 underwent genitoplasty with urethral reconstruction and scrotal transposition. Linear growth of the patients was within the target range. The onset of puberty (testicular volume  $\geq 4$  cm<sup>3</sup>) was normal in patients 1 and 3, but inadequate virilization required testosterone replacement in both. Patient 2 retained one dysfunctional testis and a small penis. The patients consistently had normal blood pressure and serum electrolytes. No skeletal malformation, dysmorphism, or limited limb movements were found, and x-rays showed no radioulnar or radiohumeral synostosis. Serum cortisol responded poorly to ACTH administration. DHEA sulfate (DHEA-S) levels were low. Basal and ACTH-stimulated 17OHP levels were elevated. Testosterone increased during puberty but failed to reach normal adult male levels without supplementation (Table 1). Gonadotropins were in the normal pubertal range at onset of puberty. However, FSH levels rose steadily afterward in patients 1 and 2. Patient 1 had high FSH (36.5 mIU/liter) and LH (14 mIU/liter), and serum testosterone was low [150 ng/dl (5.25 nmol/liter)] at 21 yr of age. In patient 2, serum FSH was 19 mIU/ml, LH was 2.4 mIU/ml, and testosterone was 100 ng/dl (3.5 nmol/liter) at 14.5 yr of age.

### Gas chromatography-mass spectrometry analysis of urinary steroids

Gas chromatography-mass spectrometry profiles of urinary steroids in patients 1 and 3 were similar and typical for POR deficiency. The pattern of urinary C19 and C21 steroids revealed elevated markers of 17-hydroxylase deficiency, such as the pregnenolone metabolite 5-pregnenediol and the corticosterone metabolites *allo*-tetrahydrocorticosterone and tetrahydro-11-dehydrocorticosterone. Elevations of pregnanetriol, a metabolite of 17OHP, and pregnanetriolone, a metabolite of 21-deoxycortisol, provided signs of 21-hydroxylase deficiency. The ratios of urinary key steroid metabolites (Table 2) showed impaired activities of 1) the global 17-hydroxylase/lyase system; 2) 17-hydroxylase, particularly in the  $\Delta$ 5-pathway; 3) 17,20-lyase, particularly in the  $\Delta$ 5-pathway; and 4) 21-hydroxylase. The ratio androsterone/etiocholanolone did not indicate an active backdoor pathway to C19-steroids via 5 $\alpha$ -pregnane-3 $\alpha$ , 17 $\alpha$ -diol-20-one.



**FIG. 1.** Genetics of the affected kindred. **A**, Family tree showing the relationships among the four patients studied. The affected individuals are numbered as in the text. **B**, Sequence analysis of the *POR* gene; the mutation G→A at position 1697 in exon 12 causing the replacement of G539R is marked by an arrow on the sequence trace of patient 2. This mutation was identified in all four patients. **C**, Sequence analysis of *CYP17A1*. *Upper panel* (four tracings), Exon 1. *Black arrows* indicate that the previously reported T deletion at position 198 in *CYP17A1* exon 1 was not presented in all four patients. *Gray arrows* indicate that patients 1, 2, and 4 are homozygous for the polymorphism 195 G→T, and patient 3 is heterozygous. *Lower panel* (four tracings), Exon 8. *Black arrows* indicate that the substitution 1235 T→G, reportedly responsible for the missense mutation F147C, was not present in all four patients. **D**, Digestion of *CYP17A1* exon 8 PCR product with *Hpy188III*. M, Marker 100 bp (New England Biolabs); C, normal control; P1–P4: patients 1–4. The 591-bp PCR product of *CYP17A1* exon 8 was cut into fragments of 181 and 410 bp in all four patients and in the normal control, confirming the presence of the wild-type sequence.

**TABLE 1.** Hormone levels

	Patient 1 (16 yr)		Patient 2 (14.5 yr)		Patient 3 (15 yr)	
	Basal	pACTH	Basal	pACTH	Basal	pACTH
Cortisol ( $\mu\text{g/dl}$ )	7	7.6	8.15	10.6	7.9	8.16
17OHP ( $\mu\text{g/liter}$ )	7.73 (<2)	16.35	5.59 (<2)	19.35	13.80 (<2)	15.50
DHEA-S ( $\mu\text{g/dl}$ )	95 (80–560)		72 (80–560)		90 (80–560)	
Testosterone (ng/dl)	100 (245–1200)		110 (245–1200)		200 (245–1200)	
FSH (mIU/ml)	4.8		19		8	
LH (mIU/ml)	6.6		2.4		11	
ACTH (pmol/liter)	15 (1–10)		8.3 (1–10)			

Age-appropriate normal ranges are given in parentheses for the patients. These values were obtained when the patients were not receiving testosterone replacement therapy. The columns pACTH designate steroid hormone values in response to 0.25 mg of ACTH. Conversion factors for SI units are as follows: to convert cortisol to nmol/liter, multiply by 27.6; to convert DHEA-S to  $\mu\text{mol/liter}$ , multiply by 0.0271; to convert 17OHP to nmol/liter, multiply by 3.03; and to convert testosterone to nmol/liter, multiply by 0.0347.

### Genetic studies

Sequencing the entire coding region of *POR* in all four patients revealed a change of G→A at position 1697 (GenBank sequence NM\_000941), causing missense mutation G539R (Fig. 1B). Based on the crystallographic structure of rat *POR* and computational modeling of human *POR*, the mutated glycine is located in the NADPH binding site of *POR* and is phylogenetically highly conserved from yeast to mammals (16). Because mutations of two genes (*POR* and *CYP17A1* as reported previously in patient 2) would be most unlikely, we examined the two exons (1 and 8) of *CYP17A1* where mutations had been reported previously (12). No mutation was found in exon 1 or exon 8 in all four patients.

To provide an independent confirmation of these sequencing results, the relevant regions of *POR* and *CYP17A1* were sequenced with different primers in a second laboratory. The sequencing of *POR* exon 12 confirmed the homozygous *POR* missense mutation G539R in all four patients and confirmed that *CYP17A1* exon 1 and exon 8 did not carry mutations (Fig. 1C). The putative F417C mutation in *CYP17A1* was reported to arise from a T→G mutation, changing TCTTGA to GCTTGA (12), which would destroy the TCNNGA site recognized by restriction

endonuclease *Hpy188III*. As a third, independent test we digested PCR-amplified patient DNA with *Hpy188III*, which cut the PCR product of *CYP17A1* exon 8 from the control and all four patients into the predicted fragments of 181 and 410 bp, whereas mutation of the *Hpy188III* site would yield a product of 591 bp (Fig. 1D). Thus, sequencing in different laboratories using different primers and restriction fragment length analysis confirms that none of the patients carried the *CYP17A1* F417C mutation.

### Discussion

Patient 2 was the subject of previous clinical (15) and genetic (12) reports. Those reports diagnosed this patient as having isolated 17,20-lyase deficiency, despite an ACTH test showing hyperresponsiveness of 17OHP and subnormal responsiveness of cortisol. A genetic study reported compound heterozygosity for two *CYP17A1* mutations, a frameshift reportedly inherited from the mother and the missense mutation F417C reportedly inherited from the father (12). Immunoassays of steroids from the culture medium of cells transfected with a vector expressing the F417C

**TABLE 2.** Characteristics of the urinary steroid metabolites in patients 1 and 3: Diagnostic ratios of metabolites

	Patient 1	P95	Patient 3	P95
Age (yr)	19	15.5		
Global 17-hydroxylase/lyase activity: Bs/(An+Et)	1.8	0.3	11.0	0.3
17-hydroxylase-activity				
$\Delta 5$ -pathway: P5D/P5T-17 $\alpha$	1.5	0.4	1.4	0.3
$\Delta 4$ -pathway: PD/PT	0.0	0.1	0.2	0.1
Global: Bs/Fs	1.5	0.2	1.8	0.1
17,20-lyase-activity				
$\Delta 5$ -pathway: P5T-17 $\alpha$ /A5-3 $\beta$ ,17 $\beta$	22.6	1.6	36.2	4.8
$\Delta 4$ -pathway: PT/(An+Et)	1.7	0.2	4.4	0.2
Global: Fs/(An+Et)	1.2	2.2	6.2	2.0
21-hydroxylase-activity: 11-O-PT/a-Cl	0.8	0.0	2.1	0.0
Backdoor pathway: An/Et	0.8	1.3	1.7	1.6

Abbreviations and trivial and systematic names of steroids are as follows: An, Androsterone, 5 $\alpha$ -androstane-3 $\alpha$ -ol-17-one; Et, etiocholanolone, 5 $\beta$ -androstane-3 $\alpha$ -ol-17-one; P5D, pregnenediol, 5-pregnene-3 $\beta$ ,20 $\alpha$ -diol; P5T-17 $\alpha$ , pregnenetriol-17 $\alpha$ ,5-pregnene-3 $\beta$ ,17 $\alpha$ ,20 $\alpha$ -triol; PD, pregnanediol, 5 $\beta$ -pregnane-3 $\alpha$ ,20 $\alpha$ -diol; PT, pregnanetriol, 5 $\beta$ -pregnane-3 $\alpha$ ,17 $\alpha$ ,20 $\alpha$ -triol; A5-3 $\beta$ ,17 $\beta$ , androstenediol-17 $\beta$ , 5-androstene-3 $\beta$ ,17 $\beta$ -diol; 11-O-PT, pregnanetriolone, 5 $\beta$ -pregnane-3 $\alpha$ ,17 $\alpha$ ,20 $\alpha$ -triol-11-one; a-Cl,  $\alpha$ -cortolone, 5 $\beta$ -pregnane-3 $\alpha$ ,17 $\alpha$ ,20 $\alpha$ ,21-tetrol-11-one; THA, tetrahydro-11-dehydrocorticosterone, 5 $\beta$ -pregnane-3 $\alpha$ ,21-diol-11,20-dione; THB, tetrahydrocorticosterone, 5 $\beta$ -pregnane-3 $\alpha$ ,11 $\beta$ ,21-triol-20-one; aTHB, *allo*-tetrahydrocorticosterone, 5 $\alpha$ -pregnane-3 $\alpha$ ,11 $\beta$ ,21-triol-20-one; THF, tetrahydrocortisol, 5 $\beta$ -pregnane-3 $\alpha$ ,11 $\beta$ ,17 $\alpha$ ,21-tetrol-20-one; aTHF, *allo*-tetrahydrocortisol, 5 $\alpha$ -pregnane-3 $\alpha$ ,11 $\beta$ ,17 $\alpha$ ,21-tetrol-20-one; THE, tetrahydrocortisone, 5 $\beta$ -pregnane-3 $\alpha$ ,17 $\alpha$ ,21-triol-11,20-dione; Bs, Sum of metabolites of corticosterone (THB+aTHB+THA); Fs, sum of metabolites of cortisol (THF+aTHF+THE); P95, 95th percentile of reference values (19, 20).



mutant were interpreted as showing the mutant retained 17 $\alpha$ -hydroxylase activity but lacked 17,20-lyase activity. However, rigorous studies of the F417C mutant showed that this mutant was devoid of 17 $\alpha$ -hydroxylase and 17,20-lyase activities (14). We have now resequenced exons 1 and 8 of *CYP17A1* where the mutations were reported, and we found no mutations. This sequencing was done in two different laboratories using different primers, and the normality of the site thought to harbor the mutation at F417 was confirmed further by its sensitivity to digestion with restriction endonuclease *Hpy*188III. These results are consistent with the presence of relatively preserved 17 $\alpha$ -hydroxylase activity, as evidenced by the elevated 17OHP values. Furthermore, we have found that all four of our patients are homozygous for the *POR* missense mutation G539R. The sequencing identifying this mutation was also done in two laboratories using different primers, so one can be confident with these results.

The diagnosis of *POR* deficiency was initially missed in these patients because the disorder was not characterized until 2004 (17) and because of the borderline nature of serum hormone values and the lack of the dysmorphic features of Antley-Bixler syndrome. The *POR* mutation G539R has previously been reported in a compound heterozygous Irish patient who carried a null frameshift mutation on the other allele (16). That patient had the Antley-Bixler phenotype, whereas our homozygous G539R patients did not. It is not clear whether this phenotypic difference is due to the Irish patient having a more severe *POR* defect due to the *POR* frameshift mutation on the other allele or whether the Irish patient's phenotype was attributable to his heterozygosity for a mutation in fibroblast growth factor receptor 1 that had been reported in a single case of trigonocephaly (16).

Compared with normal *POR*, the G539R mutant supported 46% of the 17 $\alpha$ -hydroxylase activity but only 8% of the 17,20-lyase activity of human P450c17 (16). Thus, the G539R *POR* mutant would be predicted to support a level of 17 $\alpha$ -hydroxylase activity similar to that seen in heterozygotes for null mutations of *CYP17A1* but would be predicted to support minimal 17,20-lyase activity. The prediction for the net effect on P450c17 would be that *POR* mutant G539R would cause isolated 17,20-lyase deficiency, as was initially diagnosed in the index case in this clan (15). This impairment in androgen production was manifested in the undervirilization seen in our patients at birth and was further manifested at puberty. Although our three adolescent-aged patients entered puberty, its progression was slow and incomplete, associated with low testosterone values. Two patients also had high FSH values, indicating associated Sertoli cell failure. None of our patients has had adrenal insufficiency, but adrenal crisis has been reported in some cases of *POR* deficiency (18). Thus, we recommend that *POR*-deficient patients receive hydrocortisone replacement during severe physiological stress.

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