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Molecular Basis for Hypertension in the "Type II Variant" of Apparent Mineralocorticoid Excess

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Summary

The syndrome of apparent mineralocorticoid excess (AME) is a heritable form of hypertension in which cortisol acts as a potent mineralocorticoid. The type I variant results in a severe clinical and biochemical phenotype and arises because of mutations in the gene encoding the type 2 isozyme of 11β-hydroxysteroid dehydrogenase (11β-HSD2), an enzyme responsible for the peripheral inactivation of cortisol to cortisone. Only mild abnormalities of cortisol metabolism have been found in the type II variant of AME, suggesting that it may be a separate gene defect. In an extensive consanguineous Sardinian pedigree affected with "type II" AME, a novel homozygous point mutation (C945T) was found in the human 11β-HSD2 gene in four affected individuals. Thirteen family members were heterozygous for the resultant R279C amino acid substitution. The LOD score of linkage of the mutation to the disease was 3.23. Expression of the 11β-HSD2 mutant cDNA resulted in an enzyme with reduced maximum velocity, but similar substrate affinity, compared with activity of the wild-type cDNA. Affected individuals were >30 years of age and had both mineralocorticoid hypertension and evidence of impaired metabolism of cortisol to cortisone. The heterozygote state was phenotypically normal but was associated with subtle defects in cortisol metabolism. AME represents a spectrum of mineralocorticoid hypertension with severity reflecting the underlying genetic defect in the 11β-HSD2 gene; classification into distinct subtypes is inappropriate. Hypertensive populations should be screened to identify the prevalence of milder defects in 11β-HSD2 in patients currently labeled as having "essential" hypertension.

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Introduction

The syndrome of apparent mineralocorticoid excess (AME) is a heritable form of hypertension characterized by hypokalemia with suppression of plasma renin and aldosterone concentrations (Shackleton and Stewart 1990; White et al. 1997). The condition is associated with a defect in the peripheral metabolism of cortisol to hormonally inactive cortisone (Ulick et al. 1979), which is due to a defect in the activity of the type 2 isozyme of 11β-hydroxysteroid dehydrogenase (11β-HSD2). This defect in cortisol metabolism results in an increased plasma cortisol half-life; but patients are not Cushingoid, because a concomitant fall in cortisol production rate ensures normal circulating levels. The causative mineralocorticoid in AME is cortisol itself (Oberfield et al. 1983; Dimartino-Nardi et al. 1987; Stewart et al. 1988); failure of inactivation of cortisol by 11β-HSD2 results in cortisol instead of aldosterone binding to the mineralocorticoid receptor in kidney and colon.

Two types of AME have been reported. Type I AME is characterized by a marked increase in the ratio of urinary excretion of A-ring–reduced cortisol metabolites (tetrahydrocortisol [THF]+allo-THF) to cortisone metabolites (tetrahydrocortisone [THE]) (Shackleton et al. 1985; Monder et al. 1986) and arises because of mutations in the 11β-HSD2 gene (Mune et al. 1995; Wilson et al. 1995*a*, 1995*b*; Stewart et al. 1996; White et al. 1997). In contrast, although the type II variant of AME results in a similar hypertensive phenotype (Tedde et al. 1992), the urinary THF+allo-THF/THE ratio is relatively normal (Ulick et al. 1990; Mantero et al. 1994), leading some investigators to suggest that it may represent a separate gene defect (Ulick et al. 1992). We report the clinical features, biochemical studies, and molecular basis for hypertension in an extended Sardinian pedigree with AME type II.

Subjects and Methods

Clinical Summary

The extended pedigree had 37 subjects in five generations, 23 of whom were available for evaluation (fig.

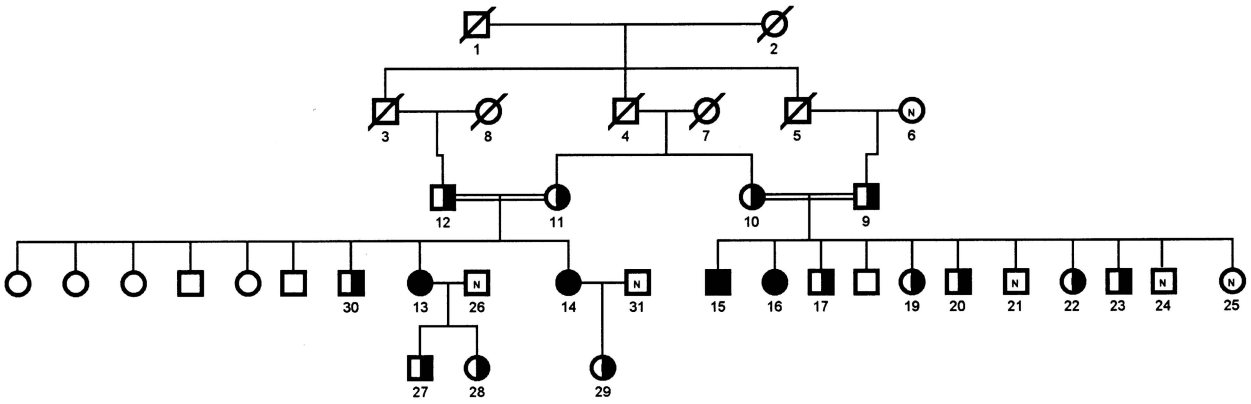

Figure 1 Family tree of a Sardinian kindred with AME. Haplotypes for the R279C mutation in the human 11β-HSD2 gene are shown as homozygous affected individuals (*blackened symbols*), heterozygous individuals (*half-blackened symbols*), or homozygous normal controls (*unblackened symbols containing* "*N*"; unblackened symbols that do not contain an "N" denote individuals who were not available for study).

1). Clinical details on patients 14–16 have been reported elsewhere (Ulick et al. 1990, 1992; Tedde et al. 1992; Mantero et al. 1994). In brief, patient 14 is a 36-year-old woman who presented, at age 27 years, with hypertension (blood pressure [BP] 160/100 mmHg) and borderline hypokalemia (3.2–3.5 mmol/liter). Her urinary THF+allo-THF/THE ratio was initially reported to be moderately elevated, at 2.56 (reference range 0.6–1.5). Patients 15 and 16 are siblings. Patient 15 presented, at age 10 years, with paralytic ileus and was found to be hypertensive (BP 240/110 mmHg) and hypokalemic (1.8–2.8 mmol/liter). A younger sister (patient 16) also was found to be severely hypertensive, at age 10 years (BP 240/110 mmHg) and to have hypokalemia (2.1 mmol/liter). Both patient 15 and patient 16 complained of fatigue, polyuria, and polydipsia and had growth retardation, nephrocalcinosis, and rickets. The THF+allo-THF/THE ratio initially was reported to be 1.41 and 1.26, respectively, in these two individuals. Patient 13 is the sister of patient 14. She is a 32-year-old woman who presented, at age 27 years, with hypertension during the third trimester of her second pregnancy. BP prior to this and, specifically, during her first pregnancy, 2 years earlier, had been reported to be normal, although she had suffered from a single episode of renal colic at age 24 years. During her pregnancy and after delivery, her BP was controlled with nifedipine in doses of 20 mg twice a day. As with patients 14–16 (Tedde et al. 1992; Mantero et al. 1996), dexamethasone in doses of 0.5 mg twice a day was shown to induce a natriuresis and to elevate plasma potassium concentrations (data not shown). When studied while she was off all therapy for 6 mo postpartum, her BP was 150/110 mmHg, her potassium was 2.9 mmol/liter, and her urinary THF+allo-THF/THE ratio was 1.44.

A more detailed and extensive biochemical and clinical characterization of the kindred recently has been completed, and the results are depicted in table 1. Patients 13–17 had therapy withdrawn for 14 d, and all subjects were evaluated on the basis of an ad lib sodium intake. Systolic and diastolic BP were recorded as the mean of three clinical BP readings. Supine plasma renin activity (PRA) was measured by radioimmunoassay (RENCTK; Sorin Biomedica). Normal reference values are 0.5–5 ng angiotensin I/ml/h. A 24-h urine collection was taken and analyzed for cortisol metabolites, by gas chromatography/mass spectrometry (GC/MS), and for urinary aldosterone (ALDOCTK; Sorin Biomedica) (reference range 5–25 μg/24 h).

Urinary Steroid Analysis

The total urinary steroid profile (conjugated and free steroids) was determined by GC/MS using a Hewlett-Packard 5970 instrument as reported elsewhere (Shackleton 1993). THE, THF, and allo-THF are included within this profile. GC/MS also was used for measurement of urinary free cortisol (UFF) and urinary free cortisone (UFE), by a different but recently published method employing stable isotope-labeled internal standards (Palermo et al. 1996).

Genotypic Studies

DNA was obtained from 23 members of the Sardinian family, from peripheral blood leukocytes (DNA extraction kit; Nucleon Biosciences). In every case, direct sequencing of the entire 11β-HSD2 gene by exon-specific PCR amplification was undertaken as reported elsewhere (Stewart et al. 1996). The PCR mixture contained 50 ng of genomic DNA, 10 mM of each dNTP, reaction

Table 1

Clinical, Biochemical, and Genetic Data for Individuals Depicted in Figure 1

Patient (Sex/ Age [years])	Genotype	THF+allo THF/THE Ratio	UFF/UFE Ratio	BP (Systolic/Diastolic)	Plasma Potassium (mmol/liter)	PRA (ng/ml/h)	Urinary Aldosterone Excretion (μg/24 h)
13 (F/32)	Homozygous R279C	1.9	7.5	150/110	2.9	1.72	.05
14 (F/36)	Homozygous R279C	4.26	1.45	160/100	2.6	.76	.05
15 (M/33)	Homozygous R279C	4.53	3	180/140	1.8	.002	.05
16(F/32)	Homozygous R279C	3.19	5.5	175/135	2.1	.046	.05
9 (M/54)	Heterozygous R279C	1.29	.37	156/108	5.3	9.09	2.37
10 (F/47)	Heterozygous R279C	.8	.7	154/96	4.8	1.12	3.52
11 (F/58)	Heterozygous R279C	1.7	.8	166/90	4.2	.54	1.22
12 (M/68)	Heterozygous R279C	1.2	.75	160/100	4.0	4.27	4.29
17 (M/29)	Heterozygous R279C	1.17	1.0	186/98	4.5	3.03	3.5
19 (F/17)	Heterozygous R279C	1.0	.5	128/80	4.5	3.28	27.1
20 (M/25)	Heterozygous R279C	.85	.63	138/88	4.6	6.88	5.94
22 (F/11)	Heterozygous R279C	.57	.56	104/64	4.7	1.11	1.4
23 (M/15)	Heterozygous R279C	.45	.76	128/80	4.3	4.61	3.3
27 (M/8)	Heterozygous R279C	.65	1.19	90/60	4.3	3.23	7.64
28 (F/4)	Heterozygous R279C	.5	1.72	80/60	4.5	3.1	11.33
29 (F/18)	Heterozygous R279C	.65	.96	120/70	4.5	4.39	2.37
30 (M/35)	Heterozygous R279C			130/70	4.2	2.64	2.31
6 (F/87)	Normal			136/80	3.8	1.58	2.69
21 (M/14)	Normal	.4	.28	110/70	4.4	2.52	1.79
24 (M/20)	Normal	.75	.65	152/90	4.5	3.91	7.96
25 (F/22)	Normal	.82	.48	110/74	4.4	2.55	1.64
26 (M/30)	Normal			120/70	4.1	.83	
31 (M/38)	Normal			115/70	3.9	4.15	4.02

buffer (10 mM Tris-HCl, 1.5 mM $MgCl_2$, and 50 mM KCl; pH 8.3), 1 unit of *Taq* DNA polymerase (Boehringer Mannheim), and 20 pmol of each primer, in a final reaction volume of 50 μl. Specifically, for the described mutation in exon 5, the primers were 5′-CAG TCC TAA TTG GCT TTG GC and 3′-GTG ATG GCA TCT ACA ACT GG; the PCR program was 35 cycles of 94°C for 20 s, 56°C for 30 s, and 72°C for 1 min. Purified PCR products were sequenced by the direct chain-termination sequencing method with fluorescent-tagged dideoxynucleotides analyzed on an automatic DNA sequencer (Applied Biosystems).

To further validate the sequencing results, 1 μg of DNA obtained from PCR amplification of exon 5 was digested with 5 units of *Hha*I (Promega) in a 20-μl volume, by use of Promega Restriction Enzyme Buffer C (10 mM Tris-HCl, 10 mM $MgCl_2$, and 50 mM NaCl) for 1 h at 37°C. The digested products were electrophoresed in 3% agarose gels.

Expression Studies

Site-directed mutagenesis.—Full-length 11βHSD2 cDNA was subcloned into pALTER vector (Promega) and was mutagenized as described elsewhere (Obeyesekere et al. 1995). The synthetic oligonucleotide—5′-TCA GTG GGA AAA GTG CAA GCA ATT GCT-3′ (corresponding to nucleotides 932–958) was used to construct the R279C mutant (C945T). The mutation was confirmed by DNA sequencing of the corresponding coding region, by means of the Sequenase 2 sequencing kit (United States Biochemical). Mutated cDNA was recloned into the mammalian expression plasmid pcDNA1 (Invitrogen) and was transfected into mammalian Chinese hamster–ovary polyoma (CHOP) cells as described elsewhere (Albiston et al. 1994).

Analysis of enzyme activity in homogenates.—Forty-eight hours after transfection, CHOP cells were washed and harvested into homogenizing buffer (0.25 M sucrose and 10 mM sodium phosphate; pH 7.4), were sonicated briefly, and then were stored at −70°C prior to use. 11β-HSD2 activity in homogenates was determined by measurement of the conversion of [^{3}H]-cortisol to cortisone, in the presence of cofactor. Specifically, the determination of 11β-HSD2 enzyme kinetics for cortisol was performed by incubation of 100 μg of transfected CHOP-cell homogenates at 37°C for 60 min, with substrate concentrations of 2 nM [^{3}H]-cortisol and 25–800 nM of unlabeled cortisol, in the presence of 500 μM NAD^+. Incubations were performed at 37°C in a total volume of 500 μl of assay buffer (50 mM Tris-HCl, 2 mM EDTA, 1 mM $MgCl_2$, 1 mM DTT, 20% glycerol, and 500 μM NAD; pH 7.4). The reaction was terminated

by the addition of 3 vol of ethyl acetate, steroids were extracted and were separated by thin-layer chromatography, and the fractional conversion of cortisol to cortisone was calculated. The transfection experiments for both wild-type and mutant 11β-HSD2 cDNAs were performed on three separate occasions, and each experiment was done in triplicate.

Western blot analysis.—CHOP-cell homogenate proteins (50 μg) from wild-type and R279C mutant 11β-HSD2 were separated by 5%–15% gradient SDS-PAGE under reducing conditions and were transferred to nitrocellulose filters (Scheicher and Schuell), for 2 h, on ice. After nonspecific sites were blocked on nitrocellulose, the blot was incubated overnight at 4°C with sheep anti-HSD2 polyclonal antibody (Shimojo et al. 1997) diluted to 1/1,000 with 0.5% skim milk powder in PBS pH 7.4 and 0.1% Tween 20. After incubation at room temperature for 60 min with the second anti-rabbit IgG labeled with peroxidase at a dilution of 1/1,000, the blots were washed in PBS/0.1% Tween 20–60 min before revelation by a chemiluminescence-detection kit (DuPont NEN) used according to the manufacturer's instructions. This was done for each of the transfection experiments, and the immunoblots were quantified by densitometry.

Statistical analysis.—Where appropriate, results were expressed as mean ± standard error (mean ± SE), and statistical analysis was undertaken by means of an unpaired *t*-test.

Results

The extended Sardinian pedigree is depicted in figure 1.

Genotypic Studies

Sequence analysis of the 11β-HSD2 gene revealed a point mutation, C945T in exon 5 of the 11β-HSD2 gene, resulting in an amino acid substitution of arginine by cysteine at codon 279 of the enzyme protein (R279C). No other mutation was found in the coding or promoter region of the 11β-HSD2 gene. This mutation results in the loss of an *Hha*I restriction site (GCG CGT GC) in exon 5; *Hha*I restriction polymorphism was used to confirm the genotype of the C945T mutation in the pedigree. Patients 13–16 were homozygous for the R279C mutation, as detected by direct sequencing (fig. 2) and confirmed by *Hha*I restriction-site polymorphism (fig. 3). Other family members were either heterozygous for the R279C mutation (n = 13 [including patient 17, who had mild hypertension]) or homozygous normal (n = 6), in keeping with an autosomal recessive mode of inheritance (tables 1 and 2). A LOD score of linkage of the R279C mutation to the disease, applied to the kindred, gave a value of 3.23 (i.e., odds for linkage >1,000:1).

Coincidentally, several differences (nucleotide substitutions at codons 451 T→G, 453 T→G, 1162 G→C, and 1210 G→C and a G insertion at codon 790) were noted with comparison of the published data for the 11β-HSD2 genomic DNA sequence deposited in GenBank. With the exception of amino acid substitution L148V in exon 2, none of these altered the coding region. These differences have been confirmed by sequencing of additional Caucasian and Afro-Caribbean controls (n = 50 in each group) and represent errors in the sequence submitted to GenBank.

Clinical and Biochemical Data

As shown in tables 1 and 2, when compared with other family members, patients 13–16 had significant suppression of the renin-aldosterone axis, reduced plasma potassium levels, and elevated BP, in keeping with a mineralocorticoid-excess state. Urinary steroid profiles showed a significant elevation of the THF+allo-THF/THE ratio and UFF/UFE ratio in homozygous affected patients, compared with both homozygous normal controls and heterozygotes; however, in affected patients the UFF/UFE ratio was more abnormal than the THF+allo-THF/THE ratio, and it was significantly higher in heterozygotes than in normal controls ($P <$ .05). In BP, plasma potassium, PRA, and urinary aldosterone, there were no significant differences between the heterozygotes and the normal controls. Three heterozygotes (patients 9, 12, and 17) had BP values consistently >160/95 mmHg, but none had evidence of mineralocorticoid excess and all had normal PRAs and aldosterone levels, suggesting an alternative cause of their hypertension. Patient 17 is a 29-year-old man with a 2-year history of hypertension, and, although his PRA and urinary aldosterone and potassium levels were normal, his UFF/UFE ratio was 1.0. Two other heterozygotes (patients 27 and 28) had a UFF/UFE ratio >1, but without evidence of hypertension or mineralocorticoid excess.

The allo-THF/THF ratio was normal in the homozygous affected patients. In comparison, in 10 patients with the so-called type 1 AME who, during the recent past, had been studied in our laboratory (C.H.L.S.), the THF+allo-THF/THE ratio was 12–70 and the UFF/UFE ratio was 8–25. The allo-THF/THF ratio was elevated, at 3.1 ± 0.7 (mean ± SE; n = 10).

Expression Studies

Conversion of cortisol to cortisone in untransfected CHOP cells was always <2%. Lineweaver-Burke plots indicated that the R279C mutant enzyme had the same affinity for F as did the wild-type enzyme (K_m =

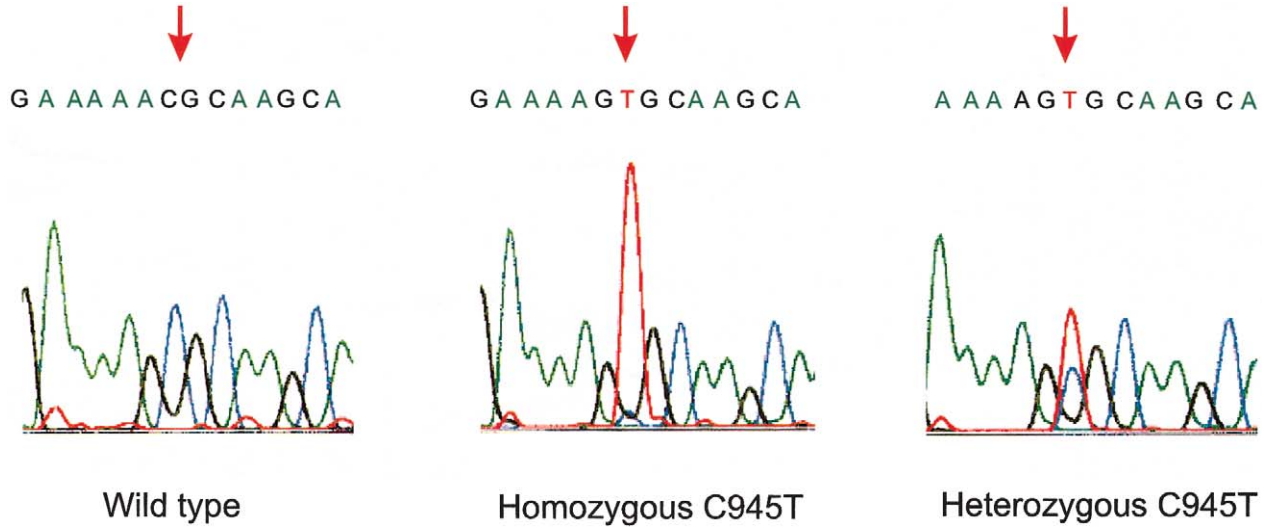

Figure 2 Automated sequence analysis of genomic DNA from part of exon 5 of the human 11β-HSD2 gene, showing homozygosity for the wild type, in a normal control (patient 26), homozygosity for the C945T mutation, in an affected individual (patient 14), and heterozygosity, in the mother (patient 11) of an affected individual.

54 nM); however, there was a difference in the maximal rate of reaction, with a decrease in maximum oxygen consumption (V_{max}), from 7.3 ± 0.9 pmol cortisone/mg/min, for the wild-type enzyme, to 4.8 ± 1.3 pmol/mg/min, for the R279C mutant enzyme (n = 3, in triplicate; $P < .05$) (fig. 4*a* and *b*). This could not be explained on the basis of transfection efficiency, in that similar levels of wild-type and R279C mutant 11β-HSD2 protein were expressed in each transfection experiment (fig. 4*c*); when quantified by densitometry, these levels were 5.3 and 5.7 arbitrary units, respectively (n = 3).

Discussion

The syndrome of AME was characterized first during the late 1970s, by Ulick and New (Ulick et al. 1979), although, in retrospect, it can be seen that the first case was reported in 1974 (Werder et al. 1974). Since then, ~50 cases have been reported worldwide (Shimojo and Stewart 1995; White et al. 1997). Patients usually present in childhood, with severe hypertension, life-threatening hypokalemia, and suppression of the renin-angiotensin-aldosterone system. AME is associated with abnormalities in the peripheral metabolism of cortisol—specifically, an increase in the excretion of the "A-ring"–reduced cortisol metabolites (i.e., THF and allo-THF) to those of cortisone (i.e., THE), suggesting defective 11β-HSD activity. Other defects in cortisol metabolism in AME also have been observed, including defective 5β-reductase activity (manifested as an increase in the allo-THF/THF ratio) (Ulick et al. 1979; Oberfield et al. 1983; Shackleton et al. 1985; Monder et al. 1986; Stewart et al. 1988). After an extensive search for the underlying mineralocorticoid, it eventually was established that cortisol itself is the offending mineralocorticoid in AME (Oberfield et al. 1983; Dimartino-Nardi et al. 1987; Stewart et al. 1988). Through these clinical studies, a vital role for 11β-HSD was uncovered. In vitro,

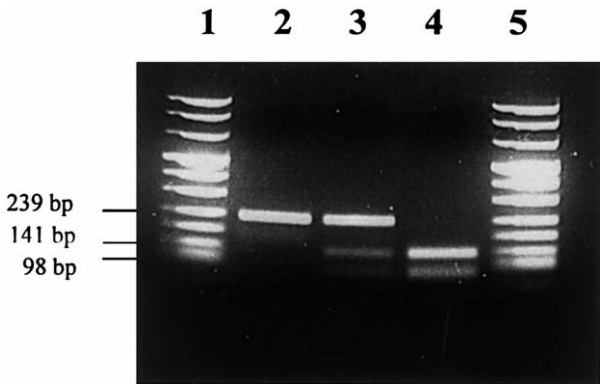

Figure 3 *Hha*I restriction-site polymorphism of exon 5 of the 11β-HSD2 gene. The expected PCR product is 239 bp, and *Hha*I digestion reveals two products of expected size, 141 bp and 98 bp. The C945T mutation results in a loss of the *Hha*I restriction site. Lanes 1 and 5, DNA molecular-weight marker VIII. Lane 2, Affected patient (homozygous for C945T), demonstrating only the undigested PCR product. Lane 3, Affected patient's mother (heterozygous), demonstrating three bands; Lane 4, Normal control (wild-type homozygous), demonstrating two bands with complete digestion.

Table 2

Systolic and Diastolic BP, Plasma Potassium, PRA, Urinary Aldosterone Excretion, and Cortisol/Cortisone Metabolites in a Sardinian Kindred with AME Type II

	Mean ± SE in		
	Homozygotes ($n = 4$)	Heterozygotes ($n = 13$)	Normal Controls ($n = 6$)
BP (mmHg):			
Systolic	166.3 ± 7.9*	133.8 ± 8.5	123.8 ± 6.9
Diastolic	121.3 ± 9.6*	81.8 ± 4.5	75.7 ± 3.3
Plasma potassium (mmol/liter)	2.3 ± .2*	4.5 ± .1	4.2 ± .1
PRA (ng/ml/h)	.63 ± .4*	2.59 ± .53	3.64 ± .65
Urinary aldosterone (μg/24 h)	.05 ± 0*	3.6 ± 1.2	5.9 ± 1.9
THF+allo-THF/THE ratio	3.4 ± .6*	.90 ± .11	.66 ± .23[a]
allo-THF/THF ratio	1.02 ± .31	1.07 ± .14	.96 ± .17[a]
UFF/UFE ratio	4.36 ± 1.34*	.83 ± .10**	.47 ± .13[a]

[a] A urinary steroid profile was available for only three normal controls.
* $P < .05$ or $P < .01$ versus normal controls and heterozygotes.
** $P < .05$ versus normal controls.

the mineralocorticoid receptor (MR) has similar intrinsic affinity for cortisol and aldosterone (Krozowski and Funder 1983; Arriza et al. 1987). In normal physiology, renal 11β-HSD "shuttles" the much higher relative concentrations of cortisol to inactive cortisone, thereby enabling aldosterone to bind to the MR (Edwards et al. 1988; Funder et al. 1988). Failure of this "protective" mechanism in AME results in cortisol acting as a potent mineralocorticoid (Stewart et al. 1988).

Two isoforms of 11β-HSD have been cloned and characterized in human tissues (Tannin et al. 1991; Albiston et al. 1994; Stewart et al. 1994*a*). 11β-HSD1 is predominantly an NADPH-dependent oxo-reductase found principally in liver, gonads, decidua, and the CNS. No mutations in the gene encoding human 11β-HSD1 have been found in patients with AME (Nikkilä et al. 1993). In contrast, 11β-HSD2 is a high-affinity, NAD-dependent dehydrogenase expressed in high amounts in fetal tissues (Stewart et al. 1994*b*) and in the "mineralocorticoid-target" tissues—kidney and colon (Albiston et al. 1994; Whorwood et al. 1995). It is this isoform that, in an autocrine fashion, protects the MR from cortisol, and mutations in the gene encoding human 11β-HSD2 have been reported in patients with AME (Mune et al. 1995; Wilson et al. 1995*b;* Stewart et al. 1996). Expression of the 11β-HSD2 mutant cDNAs results in proteins that have either severely attenuated or absent enzyme activity, with a close correlation between genotype and biochemical phenotype (Wilson et al. 1995*a;* Ferrari et al. 1996; Mune and White 1996). The condition occurs as an autosomal recessive trait, establishing AME, alongside glucocorticoid-remediable hyperaldosteronism and Liddle syndrome, as a cause of hypertension arising from a single mutation (Gordon 1995).

In 1990, the so-called type II variant of AME was described in three members of the kindred that we studied and in an additional two individuals from mainland Italy (Ulick et al. 1990). These patients differed from earlier AME patients (type I AME), in that they were reported to have relatively normal urinary THF+allo-THF/THE ratios; otherwise, their clinical features were very similar, with evidence of cortisol-induced mineralocorticoid hypertension. The main abnormality in cortisol metabolism was reported to be a generalized defect in cortisol A-ring metabolism (decreased THF+allo-THF/F ratio) (Ulick et al. 1992; Mantero et al. 1994), suggesting a defect other than 11β-HSD deficiency. We recently have suggested that this probably was not the case (Mantero et al. 1996). First, patients with type I AME have the same decrease in this "cortisol A-ring quotient" as is seen in those with AME type II (Shackleton et al. 1985; Monder et al. 1986). Second, patients were shown to have (*a*) a prolonged plasma half-life for [11α-^{3}H]-cortisol (Mantero et al. 1994) and (*b*) an increase in the UFF/UFE ratio (Mantero et al. 1996), both of which are probably more sensitive markers for the activity of 11β-HSD2 than is the THF+allo-THF/THE ratio. Finally, as this study has illustrated, the THF+allo-THF/THE ratio, although not grossly elevated, is clearly not normal in AME type II. On this note, excessive ingestion of glycyrrhetinic acid, the active ingredient in licorice, results in an acquired form of AME, which is due to inhibition of 11β-HSD2 and which causes an increase in the urinary THF+allo-THF/THE ratio (1.5–3.0), an increase similar to that seen in so-called AME type II (Stewart et al. 1987; Farese et al. 1991). It is clear, therefore, that so-called minor disturbances in cortisol→cortisone conversion, manifested as slight increases in the THF+allo-THF/THE ratio, may result in a profound clinical phenotype.

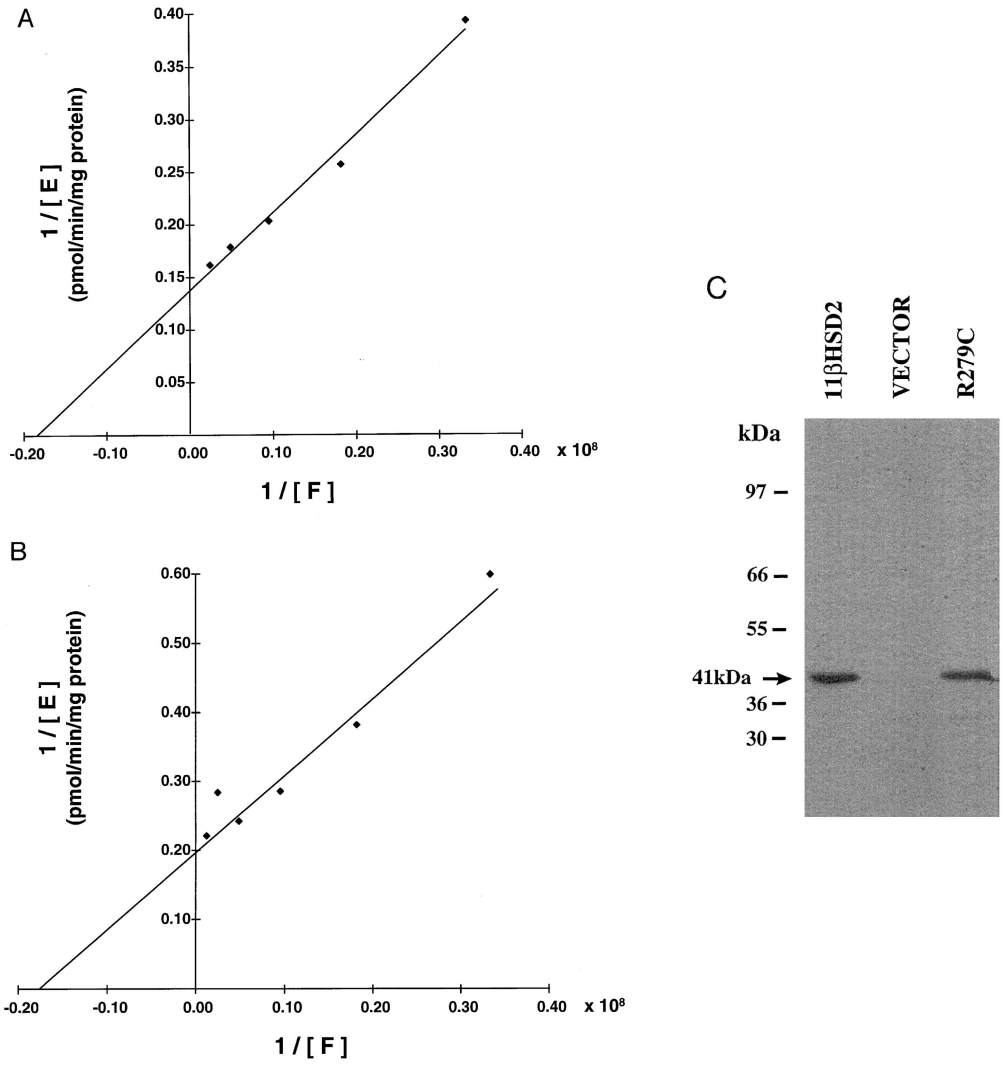

Figure 4 Kinetic analysis of 11β-HSD2 activity in CHOP-cell homogenates transfected with (*A*) wild-type 11β-HSD2 and (*B*) R279C-mutant 11β-HSD2. K_m for F was 54 nM in both instances, but V_{max} for wild-type 11β-HSD2 was 7.27 pmol cortisone/mg/min, compared with 4.84 pmol/mg/min for R279C-mutant 11β-HSD2. *C*, Western blot analysis indicating that similar amounts of 11β-HSD2 protein (wild type or R279C mutant) were expressed in CHOP cells. E = cortisone; and F = cortisol.

This study has found a novel mutation in the human 11β-HSD2 gene in a kindred with AME "type II," highlighting the artificial difference between AME types I and II. The majority of mutations causing AME are indeed Arg→Cys, reflecting the observation that the most common types of point mutations in higher eukaryotes are CpG→TpG (White et al. 1997). Compared with other reported cases of AME, the less severe biochemical and clinical features of the homozygous affected patients described in this kindred suggest that this mutation would result in only mild attenuation of enzyme activity, rather than in absent or severely reduced activity, and this was confirmed in our expression studies. The R279C mutant cDNA encoded an enzyme protein with a normal K_m for cortisol (54 nM) but with a V_{max} that was reduced by 33% of wild-type activity. In the absence of tissue from affected individuals, our studies employed rigorous kinetic analyses of mutant 11β-HSD2 activity in cell homogenates rather than in intact cells. Nevertheless precedents for mutant enzymes with alterations in V_{max}, rather than with reduced affinity for substrate, have been reported, notably in some patients with congenital adrenal hyperplasia due to homozygous mutations in type II 3β-HSD (Simard et al. 1995). It is possible that the R279C mutation results in an alteration in affinity for NAD or that the amino acid substitution causes a struc-

tural change in the protein, decreasing its activity, but further studies are required to explore this. Rather than divide AME into type I and type II variants, however, we suggest that this syndrome should be seen as a continuum, explained on the basis of mutations in the human 11β-HSD2 gene that have varying degrees of severity. On this note, a preliminary report of a "mild," isolated case of AME has been made and has been explained on the basis of a mutant 11β-HSD2 protein that has only a slightly reduced affinity (i.e., high K_m) for cortisol (Wilson et al. 1997).

The only persistent difference between our described kindred and other AME cases was the normal allo-THF/THF ratio. Coexisting defective 5β-reductase activity never has been fully explained in AME (Monder et al. 1986). It may relate to secondary changes in activity that are consequent on the profound changes in 11β-HSD2 activity. Explaining this in terms of an effect of cortisol itself, however, would seem to be unconvincing, because a decrease in the allo-THF/THF ratio (indicative of defective 5α-reductase activity) is seen in states of circulating cortisol excess (Phillipou 1982). Furthermore, licorice ingestion, which results in only mild changes in the THF+allo-THF/THE ratio, does increase the allo-THF/THF ratio (Stewart et al. 1987).

Finally, the identification of milder cases of AME highlights the need to address the prevalence of AME and the role of 11β-HSD2 in patients labeled as having "essential" hypertension. In Sardinia specifically, earlier data did report an increase in the THF+allo-THF/THE ratio in untreated hypertensives (Soro et al. 1995); the UFF/UFE ratio was not measured. Our preliminary studies in normotensive and hypertensive individuals in Sardinia have identified a heterozygote state for the C945T mutation in ~1:40 individuals (A. Li, M. Pirastu, M. Palermo, R. Tedde, and P. M. Stewart, unpublished observations), suggesting that other homozygous affected individuals will exist. The relevance of the heterozygote state remains uncertain. Although the heterozygote state has been reported to be normal in the majority of cases of AME (Mune et al. 1995; Stewart et al. 1996), two hypertensive parents of AME-affected individuals have been reported (Stewart et al. 1988; Li et al. 1997). These are of interest because, theoretically, they would possess an enzyme that has 50% of wild-type activity; and, indeed, in both cases, the THF+allo-THF/THE ratio was moderately elevated and similar to values seen our "homozygous" Sardinian kindred. In this Sardinian kindred, the heterozygote appeared to be normal. One such individual (patient 17) was hypertensive, but, in the absence of evidence of mineralocorticoid excess, it would be difficult to explain his hypertension as being due to even a partial defect in 11β-HSD2 activity. Of interest, however, was the high UFF/UFE ratio in the heterozygote state, notable in three individuals. Follow-up of these patients into late adulthood is required for definition of both the relevance of this abnormality and any subsequent development of hypertension.

AME is established as a cause of monogenic hypertension—that is, hypertension resulting from a single-gene defect. The syndrome reflects a continuum of clinical and biochemical abnormalities of varying severity, depending on the genotype; classification into distinct subtypes is inappropriate. The identification of milder forms of AME emphasizes the need to define its prevalence in hypertensive populations.

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