Apparent Mineralocorticoid Excess: Report of Six New Cases and Extensive Personal Experience

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In mineralocorticoid target tissues such as the cortical collecting duct in the kidney, the enzyme 11β-hydroxysteroid dehydrogenase type 2 (11βHSD2) is responsible for the peripheral inactivation of cortisol to cortisone, thereby protecting the mineralocorticoid receptor from inappropriate activation by cortisol. Mutations in the HSD11B2 gene cause the syndrome of apparent mineralocorticoid excess, an autosomal recessive form of inherited hypertension in which cortisol acts as a potent mineralocorticoid. Herein are described six new families with mutations in the HSD11B2 gene causing hypokalemic hypertension, with low plasma aldosterone and low renin levels in affected individuals, indicating mineralocorticoid hypertension. Profiling of urinary steroid metabolites showed decreased cortisol inactivation, with urinary tetrahydrocortisol and tetrahydrocortisone ratio (THF + 5αTHF)/THE ranging 2.4 to 40 and nearly absent urinary free cortisone in all but one case. Genetic analysis of the HSD11B2 gene from these patients with apparent mineralocorticoid excess revealed distinct homozygous point mutations in four families, a compound heterozygous mutation in one family, and a large 23-bp exonic insert with frameshift and disruption of the amino acid sequence in another family. Expression studies of mutants that were expressed in HEK-293 cells showed marked reduction or abolition of 11βHSD2 enzymatic activity. These cases are reviewed along with previous ones from the authors’ extensive personal experience to highlight the importance of 11βHSD2 in the understanding of a new biologic principle in hormone action, demonstrating that local metabolism of the glucocorticoid hormones into inactive derivatives by the enzyme 11βHSD2 is one of the mechanisms that intervene to allow specific aldosterone regulatory effects.


More than 30 yr ago, Werder et al. (1) described a case of a 3-yr-old girl who had short stature, polydipsia, and polyuria without obvious external abnormalities, including her genitalia, and who had features of mineralocorticoid hypertension with hypokalemia and metabolic alkalosis. She had suppressed plasma renin and aldosterone, and gas-chromatographic analysis of her urinary steroid profile excluded hypertensive forms of congenital adrenal hyperplasia but showed an abnormal steroid profile (1). At that time, the authors failed to realize the true nature of the condition that this girl had, a syndrome that later was characterized biochemically and recognized by New et al. (2) and Ulick et al. (3). An inherited deficiency in the enzyme 11β-hydroxysteroid dehydrogenase type 2 (11βHSD2) has been shown to cause a disorder in the peripheral metabolism of cortisol that presents with hypokalemic hypertension and suppressed circulating renin and aldosterone (1–3). The typical clinical signs and symptoms of the originally described apparent mineralocorticoid excess (AME) also included low birth weight and failure to thrive. This form of mineralocorticoid hypertension was inherited as an autosomal recessive trait. The pathogenesis of AME was found to be a deficiency of the 11βHSD2 enzyme, which converts cortisol into inactive cortisone, resulting in an excess cortisol binding to the mineralocorticoid receptor (MR) (2,3). Signs and symptoms of the syndrome can be reversed partially or fully by treatment with the MR antagonist spironolactone. Mutations in the HSD11B2 gene subsequently were demonstrated to cause the 11βHSD2 deficiency (4–22). Because plasma cortisol levels are in the nanomolar range, whereas aldosterone levels are in the picomolar range, patients with AME and deficient 11βHSD2 enzyme activity, cortisol saturates the MR. This excess stimulation of the MR results in hypokalemia, sodium retention, and volume expansion, with suppression of plasma renin and aldosterone secretion. The hallmark of the disease is a state of mineralocorticoid excess, in the absence of aldosterone and the presence of an abnormal urinary steroid profile with an increased ratio of cortisol to cortisone. Gas-chromato-
graphic analysis of the urinary steroid profile shows variably elevated ratios of urinary free cortisol to cortisone and their tetrahydrometabolites urinary tetrahydrocortisol and tetrahydrocortisone ratio (THF + 5αTHF)/THE (23,24). Herein, we describe the characterization of six new families with mutations in the HSD11B2 gene that cause AME in affected individuals. These cases are reviewed along with previous ones from our extensive personal experience to highlight the importance of 11β-HSD2 in the understanding of a new biologic principle in hormone action, demonstrating that target tissue specificity of mineralocorticoid action is enzyme, not receptor, mediated.

Materials and Methods

Case Histories and Clinical and Biochemical Analysis

Case Kindred 1. This boy (M.H.) was born with normal birth weight and height to unrelated parents from the center of France. At age 13, he was investigated for severe hypertension, morning headaches, limb paresthesia, cramps, palpitations, and tetanic convulsions (Table 1). His father and paternal grandfather also had hypertension, but nobody on his maternal side was known to have high BP. He was of relatively short stature (−0.5 SD), and his kidneys appeared normal on ultrasonography, whereas cardiac echocardiography showed left ventricular hypertrophy (LVH). Biochemical investigations showed low to normal serum potassium without metabolic alkalosis and low plasma renin (Table 1). Low aldosterone and normal deoxycorticosterone (DOC), corticosterone (B), 18-hydroxydeoxycorticosterone (18OHDQC), 18-hydroxy cortisol (18OHB), and 18-hydroxy cortisol ruled out other forms of mineralocorticoid excess or dexamethasone-sensitive mineralocorticoid excess. There was abnormally high urinary ratio of (THF + 5αTHF)/THE (Table 2). Treatment with dexamethasone (1.5 mg/d) and spironolactone (50 mg/d) resulted in normalization of his BP that was previously uncontrolled with a combination of angiotensin-converting enzyme inhibitor, β-blocker, and calcium channel blocker. Two years later, the treatment remains effective and well tolerated. He no longer had headaches or cramps, and LVH has resolved.

Case Kindred 2. This Moroccan boy (H.E.M.) was born with normal birth weight to consanguineous mother and father. At age 2, he presented high BP. His mother and father had normal BP. Physical examination and growth were normal, echocardiography showed LVH, and he had extensive nephrocalcinosis on ultrasonography (Table 1). Investigations revealed hypokalemia and metabolic alkalosis. Elevated BP excluded Bartter syndrome, and AME was suspected. Low renin and aldosterone and normal DOC, B, 18OHDQC, and 18OHB ruled out other forms of mineralocorticoid excess. The urinary (THF + 5αTHF)/THE was increased. BP normalized on salt restriction and hydrochlorothiazide. After a follow-up of 3 yr, the boy is doing well with normal BP except when indulging in excess salt intake.

Case Kindred 3. This Algerian boy (A.L.) was born with normal birth weight from healthy, consanguineous parents. At the age of 11 mo, he presented with severe dehydration, polyuria, polydipsia, and hypercalciuria. Renal ultrasound revealed the presence of nephrocalcinosis. He was severely hypertensive and hypokalemic and had moderate LVH (Table 1). Metabolic studies showed low plasma aldosterone and renin; normal DOC, B, 18OHDQC, and 18OHB were in favor of AME, and the increased urinary ratio of (THF + 5αTHF)/THE was confirmatory (Tables 1 and 2). Salt restriction alone was sufficient to normalize the BP. At 5 yr of age, BP still is normal when salt restriction is respected.

Case Kindred 4. This girl (K.I.) was born at 36 wk gestation in French Guyana with a low birth weight of 1400 g. Systemic arterial hypertension first was noticed at 4 mo of age. Echocardiography showed LVH, and kidneys were normal on ultrasound. There was no hypokalemia or metabolic alkalosis, but renin and aldosterone were low (Table 1). Her urinary (THF + 5αTHF)/THE revealed a typical pattern of AME with very low cortisone compared with normal cortisol (Table 2). Hypertension that had not been controlled by use of nifedipine then was corrected with hydrochlorothiazide. At the age of 5 yr, BP remains normal with dietary salt restriction and hydrochlorothiazide.

Case Kindred 5. These two brothers (J.C.G. and A.G.) of Portuguese origin were born from consanguineous parents. Their weight at birth was normal. One boy (A.G.) was found to have high BP and hypokalemia at age 2 yr. Hypokalemia was found in his brother at age 3 yr together with delayed growth (height −2 SD) but with normal BP. Hypertension appeared at 6 yr (Table 1). Hormonal studies revealed a pattern of hyporeninemia and hypoaldosteronemia with elevated ratios of cortisol to cortisone in the urine (Table 2). There was no metabolic alkalosis. One boy (A.G.) had a discrete renal cortical atrophy and LVH, whereas the other (J.C.G.) exhibited mild proteinuria and hypercalciuria. First dexamethasone or spironolactone alone succeeded in the control of their hypertension, but it later was necessary to associate calcium channel blockers, angiotensin-converting enzyme inhibitors, or dihydralazine. Salt restriction was also useful for J.C.G.

Case Kindred 6. This 37-yr-old woman (I.V.) with a history of low birth weight (2150 g and 43 cm) was born to unrelated parents in France. At age 4, she received a diagnosis of hypertension, hykopale-

Table 1. Clinical characteristics of individual index cases from six new families with AME

<table>
<thead>
<tr>
<th>Kindred</th>
<th>Initials</th>
<th>Gender</th>
<th>Country of Origin</th>
<th>Birth Weight</th>
<th>Age at Diagnosis of:</th>
<th>BP (mmHg)</th>
<th>K⁺ (mmol/L)</th>
<th>Metabolic Alkalosis</th>
<th>iR (pg/ml)</th>
<th>PAC (pmol/L)</th>
<th>Complications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M.H.</td>
<td>M</td>
<td>France</td>
<td>Normal</td>
<td>13</td>
<td>200/100</td>
<td>3.4</td>
<td>No</td>
<td>2.9</td>
<td>65⁹</td>
<td>LVH</td>
</tr>
<tr>
<td>2</td>
<td>H.E.M.</td>
<td>M</td>
<td>Morocco</td>
<td>Normal</td>
<td>2</td>
<td>180/120</td>
<td>2</td>
<td>Yes</td>
<td>&lt;2</td>
<td>6.4</td>
<td>Nephrocalcinosis</td>
</tr>
<tr>
<td>3</td>
<td>A.L.</td>
<td>M</td>
<td>Algeria</td>
<td>Normal</td>
<td>2</td>
<td>150/80</td>
<td>1.4</td>
<td>Yes</td>
<td>&lt;2</td>
<td>11</td>
<td>Nephrocalcinosis, LVH</td>
</tr>
<tr>
<td>4</td>
<td>K.I.</td>
<td>F</td>
<td>Guyana</td>
<td>Low</td>
<td>0.4</td>
<td>140/100</td>
<td>3.6</td>
<td>No</td>
<td>&lt;2</td>
<td>18</td>
<td>LVH</td>
</tr>
<tr>
<td>5</td>
<td>J.C.G.</td>
<td>M</td>
<td>Portugal</td>
<td>Normal</td>
<td>6</td>
<td>160/100</td>
<td>2.9</td>
<td>No</td>
<td>2</td>
<td>13</td>
<td>Proteinuria, LVH</td>
</tr>
<tr>
<td>6</td>
<td>I.V.</td>
<td>F</td>
<td>France</td>
<td>Low</td>
<td>4</td>
<td>140/60</td>
<td>2.1</td>
<td>Yes</td>
<td>&lt;2</td>
<td>25</td>
<td>Nephrocalcinosis, ESRD</td>
</tr>
</tbody>
</table>

⁹AME, apparent mineralocorticoid excess; iR, plasma immunoreactive renin (normal range, upright 7.5 to 40); LVH, left ventricular hypertrophy; PAC, plasma aldosterone concentration (normal range, upright 70 to 295).

For age, normal range is 100 to 800.
mia, and metabolic alkalosis, with low plasma renin and aldosterone levels and calcifications of her kidneys (Table 1). AME diagnosis was established on the very high cortisol/cortisone ratio in serum and (THF + 5αTHF)/TNE urine (Table 2). Hypertension responded first to spironolactone but subsequently required an association of dexamethasone and β blocker. The patient developed renal failure, and when hemodialysis was started, her hypertension was manageable without any antihypertensive drug and with fluid control only. Her mother had a history of three miscarriages and received a diagnosis of hypertension during the first of two successful pregnancies. Her grandmother had hypertension, and so did the father, who died of a cerebrovascular accident. Neither the three heterozygous children of the patient nor her normal homozygous sister have BP.

**Urinary Steroid Profile**

Urine samples were analyzed by gas chromatography—mass spectrometry similar to the method of Shackleton (23), with minor modifications as already described from our laboratories (24). Samples were quantified on a Hewlett-Packard gas chromatograph 6890 equipped with a mass selective detector 5973 and an autoinjector 7683 by selective ion monitoring. A steroid mixture that contained a known amount of all steroid metabolites to be measured was monitored on a regular basis and therefore likely to display an intermediate activity as heterozygous group (5,7,11,14,18) (Figure 1). The cells were harvested 72 h after transfection studies that used direct sequencing from these fragments. Amplified DNA (>20) of exon 5 were separated on a 1% agarose gel and purified with QIAEX II purification kit (Qiagen, Valencia, CA). Samples then were cloned in 2.1-TOPO vectors according to the manufacturer’s instruction (Invitrogen), with 4 μl of PCR product mixed in 1 μl of salt solution and 1 ml of the TOPO vector and incubated for 5 min at room temperature. The vector then was transformed in DH5α-competent cells (Invitrogen). A pUC19 plasmid was used as a control.

**Analysis of Genomic DNA**

Genomic DNA was extracted from peripheral blood leukocytes. The exons and exon-exon boundaries of the HSD11B2 gene were amplified by PCR. All reactions were performed with 10 pM of each primer in a final volume of 50 μl that contained 1.5 mM MgCl2 buffer, 0.2 mM of each dNTP, and 2.5 U of AmpliTag Gold polymerase (PE Biosystems, Foster City, CA). The DNA was amplified for 30 cycles with denaturing at 94°C for 20 s, annealing at 63°C for 1 min, and extension at 72°C for 2 min. PCR products were analyzed as published elsewhere (25). DNA was visualized by silver staining, and the variant products that were derived from patients were verified by sequencing.

Two mutations that were identified in exon 5 of HSD11B2 were not identifiable by direct sequencing. Amplified PCR fragments were subcloned into a TOPO TA cloning vector (Invitrogen, Carlsbad, CA) to allow separation of two alleles for direct sequencing from these fragments. Amplified DNA (>20) of exon 5 were separated on a 1% agarose gel and purified with QIAEX II purification kit (Qiagen, Valencia, CA). Samples then were cloned in 2.1-TOPO vectors according to the manufacturer’s instruction (Invitrogen), with 4 μl of PCR product mixed in 1 μl of salt solution and 1 ml of the TOPO vector and incubated for 5 min at room temperature. The vector then was transformed in DH5α-competent cells (Invitrogen). A pUC19 plasmid was used as a control.

**Assay for 11βHSD2 Activity**

For the expression of the HSD11B2 wild-type and mutant genes, a cDNA construct was subcloned in the expression plasmid pcDNA3, as described previously (18). Mutations were introduced by site-directed mutagenesis using the Quick Change mutagenesis kit (Stratagene, Cedar Creek, TX), and all constructs were verified by sequencing. HEK-293 cells, devoid of endogenous 11βHSD2 activity, were cultured and transfected as described previously (18). To test the activity of homozygous mutations, we used 1 μg of plasmid for transfection. We previously found that co-transfection of plasmids that express wild-type and mutant enzyme results in lower activity than transfection with the equivalent amounts of wild-type and vector plasmids (8), which is typical of members of the short-chain alcohol dehydrogenase superfamily, such as 11βHSD2, that are expressed as either dimers or tetramers and therefore likely to display an intermediate activity as heteromeric enzymes. For the compound heterozygous variants, 0.5 μg of each mutant plasmid was used. To analyze further the phenotype–genotype relationships, we performed transfection studies that used identical conditions and mutants that were identified previously by our group (5,7,11,14,18) (Figure 1). The cells were harvested 72 h after

### Table 2. In vivo and in vitro 11β HSD2 activities and identified HSD11B2 gene mutations in the six kindreds

<table>
<thead>
<tr>
<th>Kindred</th>
<th>Initials</th>
<th>(THF + 5αTHF)/TNE</th>
<th>Genotypic Status</th>
<th>Mutations</th>
<th>In Vitro Activity Compared with Wild Type (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M.H.</td>
<td>4</td>
<td>Compound heterozygous</td>
<td>Exon 2: c.547A&gt;T, p.Asp144Val + Ex 5: c.1215delITC, p.Phe367del</td>
<td>approx. 20</td>
</tr>
<tr>
<td>2</td>
<td>H.E.M.</td>
<td>26</td>
<td>Homozygous</td>
<td>Ex 5: c.1140-141insCCgGCgCTATTACCGgCC, p.Glu342fs</td>
<td>&lt;5</td>
</tr>
<tr>
<td>3</td>
<td>A.L.</td>
<td>13</td>
<td>Homozygous</td>
<td>Exon 3: c.753C&gt;T, p.Arg213Cys&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;5</td>
</tr>
<tr>
<td>4</td>
<td>K.L.</td>
<td>24</td>
<td>Homozygous</td>
<td>Exon 3: c.670T&gt;C, p.Phe189Ser&lt;sup&gt;a&lt;/sup&gt;</td>
<td>approx. 30</td>
</tr>
<tr>
<td>5</td>
<td>J.C.G.</td>
<td>42</td>
<td>Homozygous</td>
<td>Exon 5: c.1099C&gt;T, p.Ala328Val</td>
<td>&lt;5</td>
</tr>
</tbody>
</table>

<sup>a</sup>The position of the nucleotide (c.xxx) corresponds to its position in the mRNA (NM_000196 in NCBI). The numbers of the position of amino acids (p.yyy) corresponds to the number of the corresponding residue in the same reference. 11β HSD2, 11β-hydroxysteroid dehydrogenase type 2; THF and THE, urinary tetrahydrocortisol and tetrahydrocortisone, respectively (normal range of ratio 0.75 to 1.50); approx., approximately.

<sup>b</sup>In affected members of these families, a concomitant silent homozygous mutation in exon 2 that leaves the amino acid sequence intact also was identified (c. 584C>T, p.Arg213Cys).
transfection, and the oxidative activities of $11\beta$HSD2 constructs were measured using cell lysates that were incubated in a total volume of 20 μl that contained 400 μM of co-factor and 30 nCi of tritiated steroid, according to a previously reported protocol (18). Steroids were analyzed by thin-layer chromatography. Incubation time was identical in all samples and was selected to achieve conversion rates between 40 and 60% with the wild-type $11\beta$HSD2. Experiments were performed in triplicate. The relative $11\beta$HSD2 activity of mutant plasmids was calculated as the percentage of mutant to wild-type activity (approximately 20% of wild-type activity) of $11\beta$HSD2 activity (approximately 30% of the wild-type en-
zyme). Expression studies revealed almost completely abolished enzymatic activity of enzymes that bear the Arg213Cys substitution (Table 2). A homozygous T>C mutation at nucleotide 670 in exon 3, resulting in a Phe185Ser switch of the product, was identified in the proband of kindred 4 (Table 2). The two brothers of kindred 5 harbored a homozygous C>T mutation at nucleotide 1099 in exon 5 of the $11\beta$HSD2 gene, resulting in Ala328Val replacement (Table 2). In the family the two heterozygous parents, a normal homozygous brother as well as the heterozygous brother of the index case are normotensive. In the proband of kindred 6, a homozy-
gous C>T mutation at nucleotide 753 in exon 3 of $11\beta$HSD2 was identified, causing Arg213Cys substitution (Table 2). In affected members of kindreds 3 and 4, a concomitant silent homozygous C>A mutation at nucleotide 584 in exon 2 also was identified along with the other mutations. This mutation leaves the AA sequence intact (Thr156Thr).

**Genotypic Studies**

**HSD11B2 Were Identified in Each AME Kindred**

The proband in kindred 1 was found to have a compound heterozygous mutation (Figure 1). The mother hosted an A>T mutation at nucleotide 547 in exon 2, leading to Asp144Val substitution, and the father had a deletion of 3 bp (TTC) at nucleotide 1215 in exon 5, resulting in Phe367 deletion (Table 2). In kindred 2, the mother and the father of the index case both harbored the same heterozygous mutation in exon 5, and the patient is believed to be homozygous for this mutation. It consists of an insertion of 23 bp at position 1140 in exon 5, which at codon Glu342 induces a frameshift that results in the translation of a protein of 402 amino acids that are highly different from the sequence of $11\beta$HSD2 (Table 2). In the proband of kindred 3, sequencing of the DNA showed a homoy-
gous C>T mutation at nucleotide 753 in exon 3, causing Arg213Cys substitution (Table 2). In kindred 5, two siblings harbored a homozygous C>T mutation at nucleotide 584 in exon 2, which at codon Glu342 also was identified along with the other mutations. This mutation leaves the AA sequence intact (Thr156Thr).

**In Vitro Enzymatic Activity**

In vitro expression studies revealed almost completely abolished enzymatic activity of enzymes that bear the Arg213Cys (kindreds 3 and 6), Ala328Val (kindred 5), and Glu342fs (kindred 2) mutations, with a relative conversion of $[^3]H$cortisol to $[^3]H$cortisone <5% of wild-type (Table 2, Figure 2). Site-directed mutagenesis for the compound heterozygous mutations in kindred 1 (Asp144Val and Phe367del) showed a markedly reduced $11\beta$HSD2 activity (approximately 20% of wild-type activity) of the expressed compound heterozygous transfection product (Table 2, Figure 2). Expression of the Phe185Ser mutant showed residual activity of approximately 30% of the wild-type enzyme. Expression studies of mutations that were described previously (5,7,11,14,18) also are reported in Figure 2.

**Genotypic–Phenotype Correlation**

The relationship between in vitro enzymatic activity of expressed mutant plasmids as a measure of an intermediate phe-
notype and birth weight, serum potassium, BP, and the urinary (THF + 5αTHF)/THE as the distant phenotype is summarized in Figure 2. Residual activity of expressed mutants was associated with urinary ratios of (THF + 5αTHF)/THE <10 in af-

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**Results**

**Clinical and Biochemical Data**

Affected children displayed varying degrees of signs and features of AME (Table 1). Plasma renin activity and serum aldosterone were decreased significantly in all patients, indicating a suppression of the renin-aldosterone axis. However, hypokalemia and metabolic alkalosis were not present in all cases (Table 1). The defect in the conversion of cortisol to cortisone was reflected by the significantly increased ratios of (THF + 5αTHF)/THE (Table 2). Other features were the presence of LVH and nephrocalcinosis in some but not all patients. One patient (kindred 6) received a diagnosis of AME at 37 yr of age, although hypertension was diagnosed in early childhood. This patient later developed hypertensive ESRD.

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The syndrome of AME is a form of low-renin hypertension that is caused by congenital deficiency in the activity of the enzyme HSD11B2. The molecular basis of the syndrome of AME was elucidated for the first time a little more than a decade ago (5,7,11,14,18). AME was elucidated for the first time a little more than a decade ago (5,7,11,14,18). The syndrome of AME is a form of low-renin hypertension that is caused by congenital deficiency in the activity of the enzyme HSD11B2. The molecular basis of the syndrome of AME was elucidated for the first time a little more than a decade ago (5,7,11,14,18). The syndrome of AME is a form of low-renin hypertension that is caused by congenital deficiency in the activity of the enzyme HSD11B2. The molecular basis of the syndrome of AME was elucidated for the first time a little more than a decade ago (5,7,11,14,18). The syndrome of AME is a form of low-renin hypertension that is caused by congenital deficiency in the activity of the enzyme HSD11B2. The molecular basis of the syndrome of AME was elucidated for the first time a little more than a decade ago (5,7,11,14,18).
decade ago (4–6). Subsequently, we and others have identified mutations in the gene that encodes 11βHSD2 (7–22), revealing with the inclusion of the families described here >35 different nonsilent mutations in the HSD11B2 gene (Figure 1). Normally plasma cortisol levels are in the submicromolar range, whereas aldosterone levels are subnanomolar. In AME, a deficiency of the 11βHSD2 enzyme results in reduced oxidation of cortisol to inactive cortisone and thus to excess cortisol binding to the MR. Moreover, even in 11βHSD-protected cells, intracellular levels of glucocorticoid are approximately 10 times those of aldosterone (26). In AME, a deficiency in 11βHSD2 enzymatic activity further elevates intracellular glucocorticoid levels but crucially does not generate the levels of NADH that are needed to keep the cortisol–MR complexes inactive. The resultant MR activation produces Na⁺ retention, volume expansion, hypokalemia and suppression of plasma renin and aldosterone secretion. The hallmark of the disease is a state of excess mineralocorticoid activity in the absence of aldosterone and the abnormal urinary steroid profile with increased ratio of cortisol to cortisone metabolites or urinary free cortisol to cortisone.

**HSD11B2 Gene Mutations**

The disorder is inherited as an autosomal recessive trait; therefore, described cases are reported mostly in homozygous carriers with consanguineous parents. Most of the known mutations have been found in exon 3, 4, or 5 of the HSD11B2 gene, with the exception of the Arg74Gly and Pro75,1nt mutation in exon 1 (21) and Leu114,46nt mutation in exon 2 (18). A few mutations were found to leave the amino acid sequence unchanged but potentially causing aberrant splicing (21). For instance, sequence analysis of a de novo base transversion, 771C>G in exon 4 (Val254Val), creates a canonical donor splice site (TGC to TGG) (21).

Arginine is the residue that most frequently is mutated in HSD11B2, usually substituted by cysteine (Figure 1). This is not surprising, because the C-to-T transition can be considered a typical CpG consequence mutation (27,28). In fact, C-to-T transitions happen relatively frequently, because C is deaminated spontaneously to uracil, which in turn is removed by specific cellular enzymes. However, if C is methylated at the 5 position, then its deaminated form is no longer a U but a T; therefore, it is not so efficiently removed, although the mismatch usually is repaired efficiently. Dinucleotides of the sequence 5’-CG-3’ are small palindromes that in vertebrates are recognized by the methyltransferase that methylates C residues at the 5’ position in transcriptionally inactive DNA segments. Therefore, CG dinucleotides are preferred substrates for the C-to-T conversion and on the complementary strand G-to-A conversion (27,28).

**Genotype–Phenotype Correlations in AME**

When in vitro enzymatic activity is completely abolished as a consequence of mutations in the HSD11B2 gene, homozygous carriers of the mutation invariably show classical AME with severe hypokalemia, hypertension, and a markedly increased (THF + 5αTHF)/THE (4–13,15,17–19,21,22). In milder cases with isolated hypertension and normal or low-normal potassium, the identified mutations produce some but not complete reduction in 11βHSD2 activity (probands of kindred 1 and 4 [14]). This genotype–phenotype correlation in AME also is supported by our own experience when birth weight, BP, serum potassium, and in vivo 11βHSD2 activity as assessed by the urinary (THF + 5αTHF)/THE are correlated with the genotype and in vitro 11βHSD2 activity. The strength of the current phenotype–genotype analysis resides in the fact that all mutants were generated with the same in vitro mutagenesis process and expressed in the same cell system, and enzymatic assays were done according to the same protocol. Previous reports on the possible phenotype–genotype relationship of 11βHSD2 were based on reviews of published data with in vitro enzymatic assay from transfection experiments from different cell systems, using in part whole cells and in part homogenates (29). The findings presented in this analysis give insight into the variable phenotypic expression of HSD11B2 gene mutations in different kindreds, which can vary from classic AME syndrome to low-renin hypertension without hypokalemia. The pitfalls of this phenotypic variability can be emphasized by the description in the mid 1990s of a type II variant of AME with mild abnormalities of cortisol metabolism that was believed to be a separate gene defect (30), whereas, later, a homozygous point mutation (C945T) that results in Arg279Cys amino acid substitution was found in the human HSD11B2 gene of affected individuals (16).

There seems to be a clear correlation between in vitro 11βHSD2 activity and both in vivo 11βHSD2 activity as assessed by the urinary (THF + 5αTHF)/THE and serum potassium. The relationship between 11βHSD2 activity and hypertension or LVH is less evident. This discrepancy can be explained by the differences in the age of reported cases, drug treatment used before the appropriate diagnosis was made, and duration of hypertension.

The association of hypokalemia and the presence—but not absence—of metabolic alkalosis with nephrocalcinosis in our patients is intriguing. Nephrocalcinosis is a characteristic feature of Bartter syndrome (31) but is not found in Gitelman syndrome (32), although in both Bartter and Gitelman, hypokalemia and metabolic alkalosis are common (33). The distinguishing factor between Bartter and Gitelman is the urinary calcium excretion, which is high in the former and low in the latter (34). Inhibition of 11βHSD2 by licorice has been shown to increase urinary calcium excretion (35). Nephrocalcinosis usually reflects a serious metabolic defect. Therefore, the combination of hypokalemia, metabolic alkalosis, and hypercalciuria is likely to expose the complete lack of 11βHSD2 activity, whereas the absence of nephrocalcinosis and metabolic alkalosis could suggest some residual in vivo activity that is not revealed by the in vitro expression studies shown in this report. However, two probands (J.C.G. and A.L.) displayed hypercalciuria, whereas another one (I.V.) had repetitive and unexplained episodes of hypocalcemia as a young girl and later even under hemodialysis. Nephrocalcinosis was present in two of these probands (A.L. and I.V.), but hypercalciuria was not noticed in the third case.
of nephrocalcinosis (H.E.M.). These observations argue in favor of an in vivo interaction of 11HSD enzymatic activity and calcium regulation.

**11βHSD2 and Essential Hypertension**

Whereas mutation or inhibition of 11βHSD2 has been shown clearly to produce a congenital or acquired syndrome of mineralocorticoid excess, the question that remains is the extent to which subtle abnormalities in MR/11βHSD2 mechanisms may contribute to essential hypertension. There is the suggestion that the heterozygote state might predispose to “essential,” low-renin hypertension in adult life (8), but the limited information that was available in our kindreds did not allow us to corroborate this notion. Unfortunately, we did not have sufficient data to compare BP values within the normal range between healthy homozygous and heterozygous carriers.

We previously reported a case of AME with a homozygous gene mutation that resulted in a mild deficiency in 11βHSD2 (14), whose phenotype was hypertension and suppressed plasma renin levels but without hypokalemia and growth retardation. Similarly, the case of kindred 1 reported herein received a diagnosis of hypertension as an adolescent and had mild hyperkalemia and only mild suppression of circulating plasma aldosterone. These cases illustrate that mutations that lead to decreased 11βHSD2 activity can masquerade as essential hypertension. It is therefore possible that a subset of patients among the essential hypertensive population may have a subtle form of AME; there have been few attempts to analyze whether an association exists between HSD11B2 gene activity and essential hypertension.

Although patients with essential hypertension lack overt signs of mineralocorticoid excess, the demonstration of more subtle changes such as low renin levels in some patients, a positive correlation between BP and serum Na⁺ levels, plus a negative correlation with potassium levels in other patients, suggests that a mineralocorticoid effect may be contributing to hypertension in a subset of these individuals. Some authors reported that the half-life of cortisol is significantly prolonged (36) and the excretion of urinary cortisol metabolites is increased (37) in some patients with essential hypertension.

Preliminary data suggest that impaired 11βHSD2 activity is associated with an increased susceptibility of BP to salt load (38), an observation that is in line with the well-established concept that low-renin hypertension generally is considered a salt-sensitive form of high BP (39). It is interesting that this propensity for increased salt sensitivity in association with decreased activity of the 11βHSD2 enzyme may be genetically determined by variants in the HSD11B2 promoter or by the presence of undetected mutations in the HSD11B2 gene itself (38,40), an issue that deserves further investigation. This hypothesis is supported by the clinical response in two of our patients (kindred 2 and 3), who showed a good BP response to salt restriction.

It is interesting to note that one of our patients, whose AME was diagnosed in the fourth decade of life, went on to develop ESRD. One of the leading causes of ESRD in black individuals is low-renin hypertension, a form of salt-dependent hypertension; it therefore is conceivable that impaired 11βHSD2 activity might be more prevalent among this population. Watson et al. (41) reported a genetic association of a HSD11B2 flanking microsatellite and hypertension in black patients with ESRD.

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