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Apparent Mineralocorticoid Excess and the long term treatment of genetic hypertension

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Highlights

AME is a hypertensive disorder owing to 11ßHSD2 deficiency.

More than 40 mutations in the HSD11B2 gene causing AME have been identified.

Chronic hypertension in AME leads to the early development of end organ damage.

Spironolactone treatment of AME normalizes blood pressure and improves growth.

Renal transplantation of patients with AME and renal failure led to cure of AME.

Abstract:

Apparent Mineralocorticoid Excess (AME) is a genetic disorder causing severe hypertension, hypokalemia, and hyporeninemic hypoaldosteronism owing to deficient 11 beta-hydroxysteroid dehydrogenase type-2 (11ßHSD2) enzyme activity. The 11ßHSD2 enzyme confers mineralocorticoid receptor specificity for aldosterone by converting cortisol to its inactive metabolite, cortisone and inactivating the the cortisol-mineralocorticoid receptor complex.

The 20 year follow-up of a consanguineous Iranian family with three sibs affected with AME shows the successes and pitfalls of medical therapy with spironolactone. The three sibs, (female, male, female) were diagnosed at the ages of 14, 11, and 4 years, respectively. At diagnosis, hypertensive retinopathy and left ventricular hypertrophy were present in the eldest female and retinopathy was noted in the male sib. Spironolactone treatment resulted in decreased blood pressure and rise in serum potassium levels.

The older female, age 36, developed reduced left ventricular function with mitral and tricuspid regurgitation and renal failure after her second pregnancy. She was treated with renal transplantation resulting in cure of AME with decreased blood pressure and weaning from antihypertensives. Her younger sibs, age 34 and 26, do not have end organ damage.
Early and vigilant treatment improves morbidity in patients with AME. Mineralocorticoid receptor antagonists normalize blood pressure, correct hypokalemia and reduce hypertensive end-organ damage in patients with AME. Low dose dexamethasone can be considered, though the response may be variable. Future directions of therapy include selective mineralocorticoid antagonists.

**Key words**: low renin hypertension, 11β-hydroxysteroid dehydrogenase type 2, spironolactone
1. Introduction:

Apparent mineralocorticoid excess (AME) is a rare genetic form of hypertension with hypokalemic metabolic alkalosis and hyporeninemic hypoaldosteronism due to a deficiency of enzymatic activity of 11β-hydroxysteroid dehydrogenase type 2 (11βHSD2). [1] Prenatal and postnatal growth failure and juvenile hypertension are seen in the most severe phenotypes. As a result of chronic hypertension, end organ damage can occur with the renal, neurological, cardiovascular, and ocular systems being the most sensitive to damage.

Mineralocorticoid receptor specificity for aldosterone is conferred by the 11βHSD2 enzyme. This enzyme shows high expression in aldosterone-selective tissues such as the kidneys. [2, 3] Cortisol is secreted at concentrations approximately 1,000 times higher than aldosterone. The 11βHSD2 enzyme converts cortisol to the inactive metabolite, cortisone. (Figure 1) Since cortisone is unable to bind to the mineralocorticoid receptor, this enables aldosterone to selectively interact with the mineralocorticoid receptor. [1,2] This inactivation of cortisol by 11βHSD2 is considered to be responsible for protecting the mineralocorticoid receptors from cortisol overload. [4] By measuring the release of tritiated water after tritiated cortisol infusion in patients with AME, it was found that the metabolism of cortisol to biologically inactive cortisone was decreased and that the serum cortisol half-life was prolonged in these cases. [5-7] As in vivo [3H] aldosterone binding studies in a rat model show that renal mineralocorticoid receptor are approximately 90 percent occupied by cortisol acting as an antagonist, in the presence of functioning 11βHSD2, an alternative hypothesis to explain the protective mechanism of 11βHSD2 arose. [8] While 11βHSD2 converts cortisol to its inactive metabolite, it generates NADH which alters the redox state of the cortisol-mineralocorticoid receptor complex rendering it inactive. [8]

2. History of Apparent Mineralocorticoid Excess:

Although the clinical features of AME were first described in 1974 by Werder et al. [9], the first biochemical and hormonal description of the disorder was made in 1977 by New et al. in a 3 year old female with severe hypertension from the Zuni tribe. [10] The phenotype and genotype of 13 other
pediatric patients with AME were subsequently described by Dave-Sharma et al. [11] Birth weights were significantly lower compared to their unaffected sibs and they were also reported to be short and hypertensive for age. Variable damage of one or more organs (kidneys, retina, heart, and central nervous system) was found in all of the patients except one. [3] The urinary metabolites of cortisol demonstrated an abnormal ratio with an elevated ratio of cortisol to cortisone metabolites, i.e. a ratio of tetrahydrocortisol plus 5α-tetrahydrocortisol to tetrahydrocortisone of 6.7–33 compared to the normal ratio of 1.0. Of this cohort, three patients are known to have died in adolescence of cardiac complications. The first adult presentation of AME was later described by Stewart et al in 1988 showing cardiac, renal and retinal manifestations of chronic hypertension along with biochemical studies. [12]

Licorice abuse has been known to cause blood pressure elevation, sodium retention and potassium wasting, similar to symptoms seen in AME. Studies of the affinity of licorice derivatives, glycyrrhizic acid and glycyrrhetic acid, for mineralocorticoid receptor and glucocorticoid receptors show a low but sufficient affinity for the mineralocorticoid receptor and act as potent competitive inhibitors of 11βHSD2. [13]

3. Molecular Genetics:

The HSD1β2 gene is located on the long arm of chromosome 16 (16q22) and is approximately 6 kb in length containing five exons. To date, more than 40 causative mutations in the HSD1β2 gene have been identified in patients affected with AME. [4, 14] Patients with AME are often found to be the offspring of consanguineous families with homozygosity of mutations in the HSD1β2 gene. [11, 15] AME is more commonly the cause of hyporeninemic hypertension in certain ethnic groups, such as Native American and Omani populations. [11, 16, 17]

4. Treatments:
The goal of treatment of AME is to normalize blood pressure and to correct hypokalemia. Spironolactone, a mineralocorticoid receptor antagonist, binds competitively and protects the receptors against any excess mineralocorticoid activity. Blood pressure normalizes with spironolactone treatment at doses ranging from 2-12.5 mg/kg/day, improving growth and reversing hypertensive retinopathy and left ventricular hypertrophy. Reversal of bilateral nephrocalcinosis in our patients receiving spironolactone was also seen. [11]

Dexamethasone has been used to successful lower blood pressure in adult patients with AME at an initial dose of 1.5-2 mg/day followed by a maintenance dose of 0.5 mg/day. [12, 18] Treatment with dexamethasone alone often does not control hypertension in the long term. [18] Dexamethasone was shown to significantly enhance expression of 11βHSD2 mutant Tyr338His suggesting that it acts as a pharmacologic chaperone for 11βHSD2 with this mutation. [19] Dexamethasone has also been shown to aggravate the disease in children with severe phenotypes by worsening hypertension and potassium wasting [10] consistent with its activating mineralocorticoid activity through binding to the mineralocorticoid receptor. [20, 21] This negative effect was noted in the first patient hormonally characterized with AME with an E356-1 frameshift mutation in the HSD11b2 gene. Thus, treatment of AME with dexamethasone remains controversial and should be initiated in a monitored setting. This difference in response to dexamethasone suggests that the binding of dexamethasone and its activation may vary based on the mutation in the HSD11b2 gene.

Cure of AME with renal transplantation has been reported in two adult patients with AME who developed renal failure requiring transplantation. [1, 22] The patients received renal transplantation owing to renal failure and subsequently no longer demonstrated clinical signs of AME, with remission of low renin hypertension and hypokalemic alkalosis when medical treatment with spironolactone was discontinued.

5. Clinical update on a family with AME:
We report on the 22 year follow up of a consanguineous Iranian family with three affected sibs diagnosed and treated at different ages. The three sibs (IV1, IV2, and IV5) from a consanguineous Iranian family (figure 2) were diagnosed with AME at the ages of 14.2, 11.6, and 4.2 years, respectively. Blood pressures and hormonal studies at diagnosis are shown in Table 1. Diagnosis was confirmed by urinary studies showing an increased ratio of cortisol to cortisone metabolites and genetic sequencing revealing homozygous R337C mutations within exon 5. [23] (Figure 3) Spironolactone treatment resulted in a decrease in blood pressure and rise in serum potassium levels in all 3 affected sibs. [23] Clinical evaluation at 1.3 years after diagnosis revealed hypertensive retinopathy and left ventricular hypertrophy in the eldest female and retinopathy was noted in the male sib (IV2). [11] No end organ damage was noted in the youngest sib (IV5) at that time. [3]

5.1 Follow up of older female sib

The eldest female (IV1), currently at age 36 years, had been treated with spironolactone at a dose of 25 mg twice daily since diagnosis at 14.2 years. At the time of presentation, she had growth retardation, severe hypertension (220/140 mm Hg, urinary incontinence due to polyuria, small kidneys with creatinine clearance of 43 mL/min/1.73 m²) and severe retinopathy. [11] Despite spironolactone therapy, her final adult height of 155 cm was 7 cm below her target height of 162 cm. (Figure 4A) While spironolactone was administered, her blood pressure improved to 120/70 mm Hg, electrolytes became normal and creatinine was 1.5 - 2.4 mg/dL. No menstrual irregularities were noted. The patient had two miscarriages and during the third pregnancy was under supervision of author (M.R.A). At the time of normal vaginal delivery, the dosage of spironolactone was doubled for a presumed increase in cortisol production during stress and for prevention of hypertension crisis. Her renal function after delivery was similar to before delivery. At this time, therapy with spironolactone was continued as the fetus was female and there was no risk of undervirilization. During the fourth pregnancy, since spironolactone crosses the placenta and causes undervirilization of male fetuses owing to its anti-androgen effects, spironolactone was discontinued by local physicians as the fetus was male and serum potassium
concentrations were normal. During this time period, she was lost to follow up of the author (M.R.A). Both pregnancies are the result of a non-consanguineous marriage and the offspring were normotensive without electrolyte disturbances. After delivery at 32 years of age, the renal failure progressed, blood pressure rose and complications appeared and she underwent hemodialysis. When the patient IV1 was recalled for follow up after 2 years, her general condition was very poor. She was lethargic, her blood pressure was 150/90 mm Hg. Serum creatinine concentration was 12.3 mg/dL (normal range 0.5-1.5 mg/dl) at entry and hemodialysis was continued while awaiting renal transplantation. Prior to hemodialysis, the blood urea nitrogen concentration was 50 mg/dL (6 – 20) while serum creatinine concentration was 8.1 mg/dL and urine Ca/Cr ratio was 2.2/43.9 (0.05) because she was anuric. (Table 2) She had reduced left ventricular systolic function (ejection fraction of 25%) with moderate mitral and severe tricuspid regurgitation. Unilateral renal transplantation from an unrelated donor was performed. Cyclosporine, mycophenolate mofetid, and prednisone were prescribed to prevent transplant rejection. After renal transplantation and discontinuation of spironolactone, blood pressure decreased to 80/60 mm Hg. (Table 2) Sodium concentration was 141 mEq/L, potassium concentration rose to 3.9 mEq/L and creatinine concentration decreased to 0.94 mg/dL. Echocardiogram after transplantation showed resolution of mitral and tricuspid regurgitation. Cyclosporine, Cellcept and prednisone are required for immunosuppression post renal transplantation.

### 5.2 Follow up of male sib

The male sib, age 34 years, presented with generalized paralysis, hypertension and hypokalemia at 11.6 years old. He has been treated with spironolactone at a dose of 25 mg twice daily since diagnosis for 19 years. Under spironolactone therapy, he reached a final adult height of 171.5 cm, appropriate for his target height of 172 cm. (Figure 4B) He developed nephrocalcinosis initially at 17 years and demonstrated an urinary calcium to creatinine ratio of 0.4. Hydrochlorothiazide treatment was initiated owing to hypercalciuria and nephrocalcinosis. Currently, he has normal renal function with a blood urea nitrogen concentration 17 mg/dL (6-20) and serum creatinine concentration of 1 mg/dL (0.5-1.5 mg/dl).
Electrocardiogram does not show signs of left ventricular hypertrophy. At the age of 17, development of secondary sexual characteristics was below expected for age. He had no facial hair. No gynecomastia was noted. Due to his discontent with his appearance, testosterone enanthate 250 mg was administered intramuscularly monthly for 3 months during adolescence without an increase in male secondary sexual characteristics and therefore, discontinued. During evaluation for infertility, a sperm count revealed azoospermia. After a 6 month course of low dose dexamethasone (0.5 mg daily) in place of spironolactone therapy, his sperm count did not improve. On dexamethasone, his blood pressure rose to 160/108 mmHg. As his hypertension worsened and no sperm production was seen on dexamethasone, dexamethasone was discontinued and spironolactone was reinitiated.

5.3 Follow up of youngest female sib

The youngest female sib, age 26 years, had been treated with spironolactone, 25 mg twice daily, since diagnosis at 4.2 years. No end-organ damage was noted on follow-up studies at age 24 years. She reached a final adult height of 160 cm, appropriate for her target height of 162 cm. (Figure 4C) She had normal kidney function with blood urea nitrogen of 12.5 mg/dL (6 – 20), creatinine of 0.8 mg/dL and no hypercalciuria with a urinary calcium to creatinine ratio of 0.04. (Table 2) Electrocardiogram showed no signs of left ventricular hypertrophy.

She became pregnant as a result of a nonconsanguineous marriage at 25 years old. Spironolactone treatment was discontinued in the first trimester and blood pressure was controlled with methyldopa. A slight rise in blood urea nitrogen to 32 mg/dl and creatinine to 0.9 mg/dl was seen post-partum.

6. Conclusion and Future Directions:

Early genetic diagnosis of Apparent Mineralocorticoid Excess by genetic sequencing of the $HSD11b2$ gene confirming hormonal findings allows for early treatment of these patients. It has been previously shown that spironolactone treatment improves blood pressure and corrects electrolyte
disturbances of patients with AME. [11] In this consanguineous Iranian family with three affected sibs who began treatment at varying ages, we demonstrate the impact of spironolactone therapy on the course of disease. The eldest female diagnosed at 15 years old developed reduced left ventricular function and renal failure necessitating renal transplantation as a result of severe hypertension due to discontinuation of spironolactone due to noncompliance even after the delivery of her male fetus. In contrast, no end-organ damage was noted on follow-up studies of the youngest sib diagnosed and treated at 4 years of age. As these sibs have the same genotype and the probability of modifier genes is low, similar degrees of end organ damage would be expected if untreated. The early implementation of treatment minimized these complications to varying degrees based on age of initiation of treatment.

The eldest sib was cured with renal transplantation resulting in a decrease in blood pressure and weaning from antihypertensive medications. This case represents the third reported case of a successful renal transplantation in a patient with AME. [1, 22] Normalization of blood pressure and electrolytes after renal transplantation suggests that the kidney is the main site of 11βHSD2 enzymatic activity though 11βHSD2 present also in the colon. As chronic hypertension is associated with significant comorbidity and mortality, early renal transplantation may reduce end organ damage and prolong the lifespan of patients with AME. This benefit must be weighed against the risks associated with organ transplantation and the side effects of anti-rejection therapy.

Early initiation and vigilant treatment with mineralocorticoid receptor antagonists of patients affected with AME prevents and improves the morbidity and mortality of end-organ damage such as renal or cardiovascular damage as seen in this consanguineous Iranian family with three affected sibs. When treatment with mineralocorticoid receptor antagonists is initiated before impairment of renal function occurs and if the patient's compliance is good, this treatment can control the disease for many years.

In addition to mineralocorticoid receptor antagonizing properties, spironolactone competes with testosterone for binding to the androgen receptor, has inhibitory effects on 5α-reductase, competes for binding to SHBG, and inhibits enzymes involved in androgen biosynthesis. [24] Spironolactone, therefore exhibits anti-androgenic effects. The male sib exhibited undermasculinization as a side effect of
spironolactone treatment for AME. Although less potent, eplerenone is a selective mineralocorticoid receptor antagonist that does not possess anti-androgenic side effects. A trial of eplerenone is being considered. Risk of undervirilization of the eldest sib’s male fetus with spironolactone led to the interruption of spironolactone therapy. Currently, eplerenone is not indicated for pediatric use. Future studies should be directed at elucidating the etiology of this difference.

The role of the mineralocorticoid receptor and $\beta$HSD2 in essential hypertension is being increasingly recognized. Further, $\beta$HSD2 enzyme deficiency accelerates atherogenesis and causes proinflammatory changes in the endothelium of mouse models which was reduced by eplerenone. [25]

Treatment with mineralocorticoid receptor antagonists such as spironolactone has been shown to lower blood pressure in patients with essential hypertension, heart failure and hypertension resistant to conventional antihypertensives. [26-28] The presentation of the mild form of AME mimics essential hypertension as there are no electrolyte abnormalities. Mild mutations were identified in patients with mild clinical markers of AME (low aldosterone, hypertension, abnormal cortisol to cortisone ratio); in vitro studies demonstrated a very mild alteration in enzyme activity. [23, 29] Spironolactone is not a first line antihypertensive for the management of essential hypertension. Thus, mild AME should be differentiated from essential hypertension as hypertension owing to mild AME would benefit from spironolactone therapy. Polymorphisms of the $HSD11\beta2$ gene have been associated with hypertension. [30]
References


**Figure Legends:**

Figure 1: The 11βHSD2 enzyme functions to protect the mineralocorticoid receptor by converting cortisol to its inactive metabolite, cortisone. This inactivation occurs through the conversion of the hydroxyl group at position C\textsubscript{11} to an oxo group.

Figure 2: Pedigree of a consanguineous Iranian Family

There are 3 affected sibs with Apparent Mineralocorticoid Excess in generation IV. A consanguineous union in generation III is denoted by a double bar.

Figure 3: Mutation, R337C, in exon V of the gene encoding 11βHSD2 found in this consanguineous Iranian family affected with AME. The \textit{HSD11β2} gene is 6.2 kb long with five exons and has been mapped to chromosome 16q22.

Figure 4: Growth chart of patients, IV1, IV2, IV5

Figure 4A shows the growth chart of patient IV1; growth improved with initiation of spironolactone, but she did not achieve her target height. Figure 4B shows the growth chart of patient IV2 who reached target height while being treated with spironolactone. Figure 4C shows the growth chart of patient IV5 who reached target height while being treated with spironolactone. The length of the bar labeled Spironolactone indicates the duration of treatment.
Fig 1
Fig 3
Fig 4
Table 1: Baseline hormonal and genetic data of patients with AME.

*In vitro* expression studies using cDNAs with the mutations found in the *HSD11β2* gene of patients [4]

DOC, deoxycorticosterone; B, corticosterone; Aldo, aldosterone; PRA, plasma renin activity; THF, tetrahydrocortisol; THE, tetrahydrocortisone; F, cortisol; E, cortisone

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age at Diagnosis (yr)</th>
<th>BP DOC (ng/dl)</th>
<th>B (µg/dl)</th>
<th>Aldo (ng/dl)</th>
<th>Cortisol (µg/dl)</th>
<th>PRA (ng/ml/h)</th>
<th>THF 1+5α THF/THE</th>
<th>HSD11B2 Mutation</th>
<th>In vitro expression % F→E²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Range</td>
<td></td>
<td></td>
<td>18 ± 17</td>
<td>65 ± .74</td>
<td>7.2 ± 4.3</td>
<td>11.7 ± 5.7</td>
<td>0.5-6.5</td>
<td>1.0</td>
<td></td>
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<tr>
<td>IV1</td>
<td>14.2</td>
<td>220/160</td>
<td>17</td>
<td>0.3</td>
<td>1</td>
<td>17</td>
<td>7.9</td>
<td>8.91</td>
<td>R337C/R337C</td>
</tr>
<tr>
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<td>170/110</td>
<td>22</td>
<td>0.1</td>
<td>6</td>
<td>7.7</td>
<td>0.11</td>
<td>6.85</td>
<td>R337C/R337C</td>
</tr>
<tr>
<td>IV5</td>
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<td>160/100</td>
<td>36</td>
<td>1.1</td>
<td>5</td>
<td>14</td>
<td>0.07</td>
<td>6.7</td>
<td>R337C/R337C</td>
</tr>
</tbody>
</table>

* Serum steroids and PRA were measured while patient was on spironolactone therapy.
Table 2: Follow-up of patient with AME after 20 years of treatment with spironolactone.

Urinary Ca/Cr, urinary calcium to creatinine ratio; HTN retin, hypertensive retinopathy; LVH, left ventricular hypertrophy

<table>
<thead>
<tr>
<th>Patient</th>
<th>Final Ht (cm)</th>
<th>Target Ht (cm)</th>
<th>BP at last visit (mm Hg)</th>
<th>Blood Urea Nitrogen (mg/dL)</th>
<th>Creatinine (mg/dL)</th>
<th>Urinary Ca/Cr ratio</th>
<th>HTN retin</th>
<th>LVH</th>
<th>Fertility</th>
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<td>155</td>
<td>162</td>
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<td>50* 12.8**</td>
<td>8.1* 0.94**</td>
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<td>172</td>
<td>130/60 160/108 ***</td>
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<tr>
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<td>162</td>
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<td>0.04</td>
<td>No</td>
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<td>No</td>
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</tr>
</tbody>
</table>

* Prior to renal transplantation

** After renal transplantation and discontinuation of spironolactone

*** On dexamethasone