Refresher Course

Apparent Mineralocorticoid Excess

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INTRODUCTION

Apparent Mineralocorticoid Excess (AME) is a genetic disorder that typically causes severe hypertension in children, pre- and post-natal growth failure, hypokalemia, low to undetectable levels of renin and aldosterone; and is caused by a deficiency of 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2). This potentially fatal disease is caused by autosomal recessive mutations in the *HSD11B2* gene. The exploration and elucidation of this disease has opened up a new area in receptor biology as a result of the demonstration that the specificity of the mineralocorticoid receptor (MR) function depends on a metabolic enzyme rather than the receptor itself. In a broader context, the discovery of the basis for AME has led to the possible link of 11β-HSD to common essential hypertension as well as a clearer understanding of normal human physiology.

Historical Background

Although Werder, et al.[1] described a patient with similar clinical features in 1974; the first biochemical description of this disease was made in a 3 years old Native American child from the Zuni tribe[2]. Detailed clinical and endocrine evaluations of this child established the presence of features that could not be explained by any known syndrome.

Aldosterone regulates electrolyte excretion and intravascular volume by stimulating increased resorption of sodium from the urine. It is the most potent endogenous mineralocorticoid; yet, despite strong evidence of mineralocorticoid excess and hyperaldosteronism, aldosterone was undetectable in the prismatic case and in similar cases that were subsequently identified. Thus, it was initially thought that the condition was caused by an unknown mineralocorticoid[2,3], however, attempts to identify one were unsuccessful. With the use of tritiated cortisol, it was observed that the metabolism of cortisol to biologically inactive cortisone was decreased and serum cortisol half-life was prolonged, whereas the conversion of cortisone to cortisol was normal[4]. This implies that 11β-hydroxysteroid dehydrogenase, the enzyme that converts cortisol to cortisone, may be deficient.

It was also postulated that specificity of the MR was lost in these patients, allowing cortisol to bind to the MR and act as a ligand[4-6]. Several studies demonstrated that the MR has equal affinity for aldosterone and cortisol *in vitro*, however *in vivo* the MR clearly favors the binding of aldosterone[7-10]. Since normal circulating levels of cortisol are 100- to 1,000-fold higher than those of aldosterone, it appears that endogenous exposure of the MR to cortisol could preempt the ability of aldosterone to be its ligand if specificity is lost. In 1982, it was proposed that cortisol was the mineralocorticoid inducing hypertension. Biochemical findings were in agreement with this theory since AME patients treated with cortisol had increased hypertension and hypokalemia[5,6].

The role 11β-HSD plays in MR specificity has been confirmed from studying the effects of the drug carbenoxolone and extracts from the root of the licorice plant, *Glycyrrhiza glabra*. Licorice ingestion in large amounts can cause sodium retention, elevated blood pressure and potassium wasting, resulting in a hypertensive state resembling AME[11]. Glycyrrhetinic acid, a hydrolytic derivative of the active steroid in licorice extracts, is a known competitive inhibitor

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of 11β-HSD[12,13]. Carbenoxolone is a semi-synthetic analog of glycyrrhetinic acid and also can induce AME-like side effects, including sodium retention, hypokalemic alkalosis, low levels of plasma renin, and hypertension. There is marked increase in the ratio of urinary cortisol:cortisone metabolites in humans treated with carbenoxolone and in AME patients, supporting the hypothesis of abnormal 11β-hydroxysteroid dehydrogenation[4].

Since defective 11β-HSD appeared to be the likely candidate for the basis of AME, researchers sought to isolate the protein and cDNA of the enzyme. This search first led to the discovery of 11β-HSD type 1, or hepatic 11β-HSD[14]. The identification of the NADP-dependent 11β-HSD type 1 was made in rat liver homogenates. Subsequent studies on 11β-HSD type 1, however, found the enzyme to be expressed primarily in the liver, gonads, lung, decidua, pituitary, and cerebellum, where there is little mineralocorticoid[15]. 11β-HSD1 has also been shown to favor oxo-reductase activity, which serves to reactivate cortisone to cortisol. In addition, no mutations in the corresponding human gene for 11β-HSD1 were found in AME patients suggesting the presence of another 11β-HSD isoform[16]. Because aldosterone acts primarily through its effects on the renal distal convoluted tubules and cortical collecting ducts, it is predicted that both the isozyme and the MR should exist in these locations. A separate NAD^+-dependent isoform (type 2) that co-localized with the MR in the distal nephron was subsequently discovered by Naray-Fejes-Toth[17-21]. It possessed all the properties necessary for protecting the MR, i.e., a very high affinity for endogenous glucocorticoids, a high abundance in target cells, and unidirectional dehydrogenase activity. These data suggested that a defect in type 2 isoform was responsible for AME.

In 1995, cDNA encoding 11β-HSD type 2 was cloned, sequenced[22], and localized to chromosome 16q22 by Krozowski, et al.[23] and by Agarwal, et al[24]. The 11β-HSD2 enzyme is NAD^+-dependent and appears to have dehydrogenase activity only[22,25]. *HSD11B2*, the gene encodes this enzyme is approximately 6 kb in length and contains five exons[24]. The Michaelis-Menten constant (K_m) of 11β-HSD2 for cortisol is 1-100 nM[20,25-27], indicating that 11β-HSD2 has a significantly higher affinity for cortisol than does 11β-HSD1. Later studies found human 11β-HSD2 mRNA expressed in the kidney cortex and medulla, the sigmoid and rectal colon, and the salivary glands[15]; and in the pancreas, prostate, ovary, small intestine, placenta, spleen, and testes[22]. The presence of both 11β-HSD2 and mineralocorticoid receptors has also been detected in fetal skin, upper gastrointestinal tract, and lung[28].

In 1995, Wilson, et al. identified the first mutation in the *HSD11B2* gene in a consanguineous Iranian family with three sibs suffering from AME (a C to T transition resulting in a R337C mutation)[29]. Eighteen mutations have subsequently been identified in the *HSD11B2* gene in patients affected with AME, most of which completely abolish the activity of 11b-HSD2 (Figure 1).[30-36,37,41]

Epidemiology

AME is rare, having been identified in only approximately 60 patients worldwide in the past 20 years. To date, most patients with AME who have had molecular genetic analysis have been homozygotes for one of the different mutations. Rare autosomal recessive mutations are classically explained by consanguinity, endogamy (a high coefficient of inbreeding), or by a founder effect. In eight families with members affected by AME, seven appeared to fit one of these three explanations[30]. Four families came from ancestry where consanguinity and tribal inbreeding were the custom, and three came from a Zoroastrian population that originated in Iran and was driven out by Muslims in the seventh century. In the remaining family, African Americans from North Carolina, consanguinity was not proven.

Clinical and Biochemical Features

AME usually presents early in life with clinical features including severe hypertension, failure to thrive, and persistent polydipsia and polyuria. Biochemical profiles demonstrate metabolic alkalosis, severe hypokalemia and hypoaldosteronemia. Plasma renin activity (PRA) is suppressed, suggesting a volume-expansion hypertension, which responds to dietary sodium restriction (therefore, it is also called salt-sensitive phenotype).

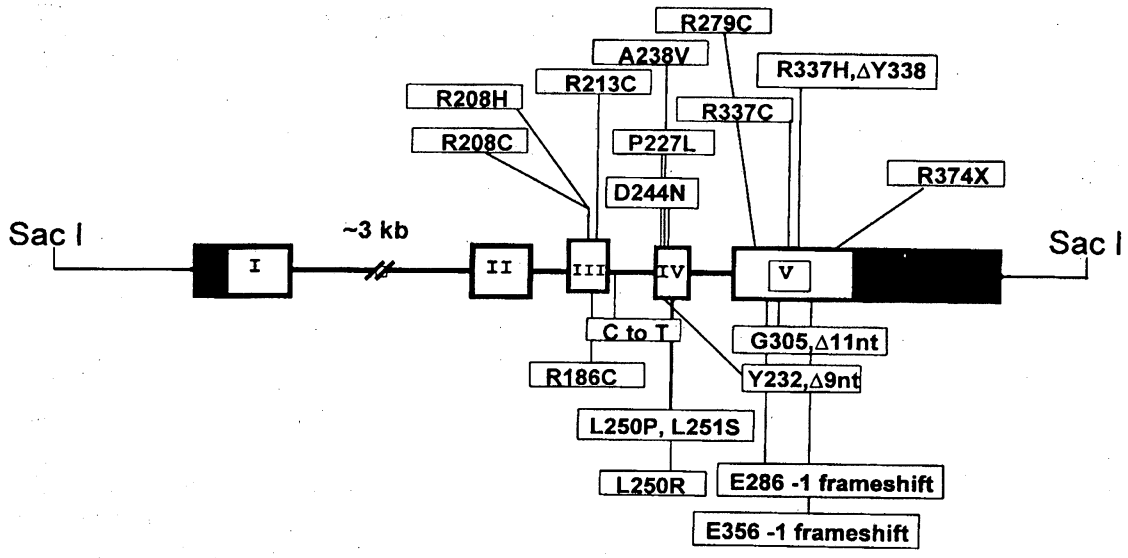

Figure 1. Mutations in the gene for 11β-hydroxysteroid dehydrogenase type 2 in patients with apparent mineralocorticoid excess (see Table for references). The *HSD11B2* gene has five exons, is 6.2 kb long, and has been mapped to chromosome 16q22. All mutations found in affected patients are homozygous except for two patients, who are compound heterozygotes (D244N/L250R and Y232,Δ9nt/G305,Δ11nt).

Biochemical diagnosis of AME can be made by measuring the ratio of urinary metabolites of cortisol to cortisone, often measured as an increase in the sum of the urinary tetrahydrocortisol and allotetrahydrocortisol, divided by the concentration of tetrahydrocortisone (THF+5αTHF/THE). In addition, Dave-Sharma, et al. reported a consistent elevation of tetrahydrocortisol/tetrahydrocortisone (THF/THE), with a predominance of THF in 14 patients affected by AME[31]. The ratio of THF+5αTHF to THE was 6.7 to 33, whereas the normal ratio is 1.0. The optimal diagnostic test is to measure the generation of tritiated water in urine samples when 11-tritiated [11-^{3}H] cortisol is injected, as described by Hellman, et al[38]. Infusion of [11-^{3}H] cortisol revealed the conversion of cortisol to cortisone to be 0-6% in typical AME patients, whereas the normal conversion is 90-95%. In addition, an assay that distinguishes 5α- and 5β-tetrahydrometabolites may be used to demonstrate that 5β-reductase activity is reduced or inhibited in AME[39,40].

AME patients reported worldwide exhibit characteristic signs of a severe 11β-HSD2 defect[1,2,4,16,29-32,37,39-53]. Birth weights are lower than in their unaffected sibs, and the patients are short, underweight, and hypertensive for age. Severe hypertension and hypokalemic alkalosis are associated with end organ damage in AME patients, particularly of the retina, kidney, cardiovascular and central nervous systems. Cardiac damage is manifested mainly in the form of concentric left ventricular hypertrophy with increased left ventricular mass. Renal manifestations are seen in the form of hypercalciuria, nephrocalcinosis, and in some cases renal insufficiency. Hypertensive retinopathy is consistently found, ranging from mild to moderate grades. CNS involvement in these patients ranges from developmental delay to various types of EEG abnormalities indicating seizure disorders or generalized cerebral dysfunction. This is a particularly important observation in light of the emerging CNS roles for mineralocorticoids[54,55].

While cortisol is synthesized in the fetal adrenal gland, circulating fetal cortisone is of maternal origin. It is likely that transplacental passage of maternal cortisol to the fetus is blocked by the abundant 11β-HSD type 2 activity in the placenta[56]. A deficiency of 11β-HSD2 in the fetal placental unit may lead to intrauterine growth retardation (IUGR) and low birth weight, as evidenced by the low birth weight of AME patients compared to the normal birth weights of unaffected siblings[31]. However, this hypothesis requires further investigation, because in a large experience of pregnant mothers treated with dexamethasone who carried fetuses affected with 21-hydroxylase deficiency congenital adrenal hyperplasia, those fetuses treated through pregnancy were of normal birth weight[57-60]. Thus dexamethasone, which passes to the fetus, does not appear to cause low birth weight.

Mild Form of AME

All of the AME patients reported until 1998 had the characteristic signs of a severe 11β-hydroxysteroid dehydrogenase 2 defect. As in many other disorders, milder forms subsequently emerged after severe classic forms were characterized, as a spectrum of the disease. A variant of AME has been reported in which the urinary THF + 5α-THF/THE ratio is relatively normal, but the phenotype is essentially the same as in patients with classic AME[61-63]. Further, another variant, whereby phenotype is normal but subtle defect in cortisol metabolism is the hallmark, has also been identified[36].

The first mild form of AME was reported by Wilson, et al. in 1998[37]. Asymptomatic hypertension was diagnosed in an American girl at the age of 12.5 years during a routine physical examination in 1995. She had normal serum electrolytes, undetectable plasma renin and serum aldosterone, normal urinalysis results, normal urine culture, normal intravenous pyelograms, a normal arteriogram of the kidney, a normal renal scan, and a normal heart size on chest X-ray. Birth weight was 7 lb 1 oz. The parents were consanguineous Mennonites of Prussian descent. The only family member with hypertension was the maternal grandmother. Although the patient lacked hypokalemia, low birth weight, and had only mild hypertension, the diagnosis of AME was established by genetic analysis. She exhibited only a moderately abnormal ratio of cortisol to cortisone metabolites (THF+5αTHF/THE was 3.0) and metabolism of cortisol to cortisone as determined by the measurement of tritiated water release after [11-^{3}H] cortisol infusion (58%) (typical patients show <6%) (Table 1). The patient clearly had AME based on biochemical evidence and later was proven to be homozygous for the P227L mutation in the *HSD11B2* gene.

Kinetic analysis showed an intermediary K_m in this patient as compared to a severe AME patient. In whole cells, the P227L construct gave a K_m for cortisol of 300 nM as compared to a K_m 62 nM for the wild-type construct; in cell homogenates, the P227L construct gave a K_m for cortisol of 350 nM and a K_m of 54 nM for the wild-type construct. This was in contrary to severe AME patient who demonstrated a K_m of 1010 nM as compared to the normal control of 110 nM[64,65] The K_m of 300 nM in this patient suggested a less severe defect than in other patients, resulting in her mild phenotype.

Thereafter, an investigation of heterozygote frequency in this inbred population where the patient came from was performed[66]. The result was astonishing, considering there were only approximately 60

patients known worldwide with apparent mineralocorticoid excess, the heterozygote frequency of 3.0% found in this population was very high.

Treatment

The treatment of AME is primarily aimed at correcting hypokalemia and hypertension. Spironolactone, a mineralocorticoid receptor antagonist, is the accepted medication of choice. In addition to spironolactone, other medications may also be used, such as the potassium-conserving diuretic amiloride, the adrenergic blocking agent atenolol, and ACE-inhibitor enalapril. Triamterene has caused a patient to go into shock and should be avoided. A reduction in dietary sodium and supplemental potassium intake have been shown to have a beneficial effect as well. Glucocorticoids of all types will aggravate the disease[2,6,28,31].

The follow-up studies of complications in 14 AME patients have been reported[31]. AME patients were treated with spironolactone and demonstrated improvements in their clinical symptoms. The dose range of spironolactone was wide (2-10 mg kg^{-1} day^{-1}) because it was started at a very low dose and gradually increased until the desired blood pressure response and serum potassium concentration were achieved. As hypercalciuria and nephrocalcinosis are consistent features of this disease, a thiazide diuretic was added to most of the patients' treatment regimens after years of treatment with spironolactone. This resulted in a reversal of bilateral nephrocalcinosis of the kidneys reported in these patients. Thiazide diuretics also aid in lowering blood pressure, and in some patients may allow for the dose of spironolactone to be reduced. This is particularly important in patients manifesting the anti-androgenic side effects (e.g., gynecomastia) of spironolactone. In one patient who was poorly compliant with the prescribed low-salt diet, the use of low-dose furosemide to control the excess sodium, in addition to spironolactone and potassium supplementation, proved to be beneficial for blood pressure control (personal communication). However, the use of a loop diuretic must be carefully exercised, since it can aggravate hypokalemia and alkalosis. Improved growth and the reversal of hypertensive retinopathy and left ventricular hypertrophy in some of the patients further demonstrate that proper treatment and meticulous compliance are able to control this disease[31].

Liddle's syndrome is a disorder similar to AME in its severe hypertensive sequelae, often resulting in kidney failure. In one such case reported, the patient underwent kidney transplantation, which resulted in complete disappearance of her hypokalemia and hypertension[67]. In a case recently reported, kidney transplantation was also curative of AME[68]. The patient had been hypertensive from the age of 19 and after AME was diagnosed at 28 years old, she was successfully treated with atenolol, enalapril, and dexamethasone. At 31, she developed end-stage renal failure and underwent kidney transplantation; her own kidneys were left intact. Serum potassium, PRA, and blood pressure were consistently normalized due to expression and activity of 11β-HSD2 by the new kidney. The urinary ratio of cortisol to cortisone was improved. Thus, kidney transplantation may provide a cure for AME.

In the review of AME patients in literatures worldwide, 5 died of AME-related illnesses (and 2 diagnosed genetically were stillborn)[37]. In some cases early and vigilant treatment of AME patients may prevent or may improve the morbidity and mortality of end organ damage such as renal or cardiovascular damage and retinopathy. The outcome of treatment studied in more patients may establish what treatment is optimal.

AME and Essential Hypertension

Essential hypertension has been estimated to occur in 15 million residents in the United States, and approximately 40% are associated with low renin.

Several studies provide evidence that reduced activity of 11β-HSD2 may be a factor in patients diagnosed with essential hypertension[69,70]. An association between a microsatellite markers near the *HSD11B2* gene and African Americans with a salt-dependent hypertensive end-stage renal disease has been demonstrated[71]. These data suggest a contribution of variants of the *HSD11B2* gene to enhance the blood pressure response to salt. In addition, Lovati, et al. have shown an association between a polymorphic microsatellite marker near

Table 1. Review of AME patients worldwide: signs and biochemical features at presentation, and subsequent biochemical and genetic evaluation.

Patient	Kindred	Ethnicity	Age (yr)	Sex	Birth weight (kg)	BP (mmHg)	BP (90th percentile for age)	Serum K^+ (mmol L^{-1})	THF + 5α THF/ THE	F secretion rate (mg d^{-1})	% Conversion F-> E	*HSD11β2* mutation	Reference
1 [a]	1	American Indian	1.0	F	1.8	180/140	105/69	2.2	32.1	0.08	0	E356-1 Frameshift	30
2	2	Zoroastrian	9.0	M	2.0	250/180	110/71	3.5	9.1 [c]	0.47	0	R337H, ΔY338	30
3	3	Italian-Moroccan	4.0	M	2.3	160/110	104/67	3.1	33.0	0.72	ND	L250R/D244N	31
4	4	African American	9.3	F	2.1	130/90	109/71	2.7	8.9	0.12	2	R186C	30
5	4	African American	4.3	F	2.6	142/98	98/70	2.8	14.9	0.15	4.2	R186C	30
6	5	East Indian	0.8	M	2.0	150/100	105/67	2.4	20.1	0.05	6	R337H, ΔY338	30
7	6	East Indian	2.5	M	2.3	150/100	91/68	1.79	12.5	0.83	2	R337H, ΔY338	30
8	7	Middle Eastern	10.9	M	2.1	170/110	115/73	1.7	27.9	0.36	ND	R208C	30
9	7	Middle Eastern	9.3	M	2.4	160/118	110/71	2.9	27.3	0.24	ND	R208C	30
10	8	American Indian	3.3	M	2.0	205/130	99/60	0.9	26.8	0.51	1.5	L250P, L251S	30
11	9	Persian	14.0	F	2.2	220/160	116/79	2.8	8.91	ND	ND	R337C	29
12	9	Persian	11.6	M	2.1	170/110	110/72	2	6.85	ND	ND	R337C	29
13	9	Persian	4.0	F	2.4	160/100	98/70	3.1	6.7	ND	ND	R337C	29
14	10	Turkish	0.1	M	2.5	155/115	99/60	3.0	13.8	ND	4.5	N286-1 Frameshift	31
15	11	Mennonite	12.6	F	3.6	160/90	110/72	5.0	3.0	0.55	58.4	P227L	37
16	12	American Indian	9	M	-	170/100	110/71	-	14.4	-	-	R208C	32
17	13	Caucasian/South American Indian	3	F	-	170/110	99/71	2.3	31.3	-	-	R213C	32, 42
18	13	Caucasian/South American Indian	3.8	F	-	200/120	108/70	2	13.4	-	-	R213C	32, 42
19 [a]	13	Caucasian/South American Indian	6	M	-		-	-		-	-	ND	42
20	14	American Indian	1	F	-	142/92	105/69	-	73.8	-	-	L250P, L251S	32
21	15	European-American Indian	1.6	M	-	140/100	105/69	3.1	19.8	-	-	L250P, L251S	32, 73
22	16	Mexican American	26	F	-	180/120	123/88	-	7.9	-	-	Intron 3 (C to T)	32

Table 1. Review of AME patients worldwide: signs and biochemical features at presentation, and subsequent biochemical and genetic evaluation. (cont.)

Patient	Kindred	Ethnicity	Age (yr)	Sex	Birth weight (kg)	BP (mmHg)	BP (90^{th} percentile for age)	Serum K^+ (mmol L^{-1})	THF + 5α THF/ THE	F secretion rate (mg d^{-1})	% Conversion F-> E	*HSD11β2* mutation	Reference
23	17	Irish American	2.3	M	-	149/83	91/68	-	134	-	-	Codon 232, Δ9 nt/ Codon 305, Δ11 nt	32
24	18	Asian-Pakistani	3.5	M	-	141/117	100/70	2.1	20	-	-	R374X	33, 49
25	18	Asian-Pakistani	1.4	M	-	144/91	105/69	3	50	-	-	R374X	33, 49
26 [a,b]	18	Asian-Pakistani	-	M	-	-	-	-	-	-	-	R374X	33, 49
27 [a,b]	18	Asian-Pakistani	-	M	-	-	-	-	-	-	-	R374X	33, 49
28	19	Japanese	2	M	2.5	160/90	91/68	2.7	43.7	-	-	R208H/R337H,Δ Y338	34
29	20	Brazilian	7	F	-	160/120	110/73	1.8	29.8	-	-	A328V	35, 44
30	21	German	3	F	-	175/110	99/71	2.8	ND	-	-	ND	1
31	22	French	4	F	2.1	140/60	98/70	2	0.61 [c]	1	-	ND	46
32 [a]	23	American Indian	2.7	F	-	180/120	101/71	2.7	9.78	-	-	ND	73
33	24	Caucasian	2	M	-	110/65	91/68	2.2	15.87	-	-	ND	47
34	25	N. European	1.6	M	2.17	150/110	105/69	2.6	45	-	-	ND	51
35 [a]	26	Caucasian	0.4	M	2.36	200/100	105/69	1.8	68.8	-	-	ND	48
36	27	Caucasian	0.8	F	-	140/100	120/80	3.2	15 [c]	8	-	R374X [d]	52
37	28	Caucasian	21	M	-	200/145	121/81	1.7	13.6	-	0	ND	43
38 [a]	18	Asian-Pakistani	3.5	M	-		-	low	ND	-	-	ND	33, 49
39	29	Turkish	1.3	M	low	120/90	105/69	low	38.06	-	-	ND	50
40	30	French	2	M	3.1	140/80	91/68	2.5	22	-	-	ND	53
41	30	French	3.3	M	3.4	160/100	99/60	2.9	42	-	-	ND	53
42 [e]	31	-	3.3	-	-	-	-	-	-	-	-	ND	45
43 [e]	32	-	3.5	-	-	-	-	-	-	-	-	ND	45
44 [e]	33	-	9	-	-	-	-	-	-	-	-	ND	45
45	34	Caucasian/ Argentinean	11	M	2.1	130/90	114/74	2.8	10.4	-	0	R213C	74
46 [f]	35	Sardinian	28	F	-	160/110	140/90	2.6 [g]	4.2	-	-	R279C	36, 68, 75

Table 1. Review of AME patients worldwide: signs and biochemical features at presentation, and subsequent biochemical and genetic evaluation. (cont.)

Patient	Kin-dred	Ethnicity	Age (yr)	Sex	Birth weight (kg)	BP (mmHg)	BP (90th percentile for age)	Serum K[a] ($mmol\ L^{-1}$)	THF + 5α THF/ THE	F secretion rate ($mg\ d^{-1}$)	% Con-version F-> E	*HSD11β2* mutation	Reference
47[f]	35	Sardinian	32	F	-	150/110	140/90	2.9[g]	1.9	-	-	R279C	36, 75
48[f]	35	Sardinian	33	M	-	180/140	140/90	1.8[g]	4.53	-	-	R279C	36, 63
49[f]	35	Sardinian	32	F	-	175/135	140/90	2.1[g]	3.19	-	-	R279C	36, 63
Normal Values					>2.5			3.2-5.2	1.0	11.5	90-95%		

[a] = died (Patient 19 died of stroke and might have been affected by AME. Patients 26 and 27 were stillborn. Patient 35 died after fever, persistently raised blood pressure and hypokalemia. Patient 38, twin of patient 24, died of a diarrheal illness; AME was suspected.), [b] = placental DNA, [c] = THF/THE, [d] = personal communication from Stewart PM, [e] = unreported potential AME patients, [f] = although symptomatic, these patients were previously categorized as "type II" AME patients owing to their moderate

THF+5αTHF/THE ratios, [g] = plasma potassium.

ND = not done, BP = blood pressure, THF = tetrahydrocortisol, THE = tetrahydrocortisone, F = cortisol, E = cortisone.

the *HSD11B2* gene and reduced 11β-HSD2 activity in salt-sensitive subjects[72]. However, a recent report comparing hypertensive patients to their sibs and controls in France where variants of the 11β-HSD2 gene and their contribution to essential hypertension susceptibility did not find a correlation[40], and it was concluded that variants did not contribute substantially to essential hypertension in Caucasians. However, low-renin hypertension was not isolated for examination, which might appear in a very mild form. It may be found that mild mutations in the *HSD11B2* gene are an unrecognized cause of low-renin hypertension.

Although severe AME is a rare disorder, milder cases may occur more frequently in the general population and present with a wider spectrum of clinical findings. Therefore, AME should be considered in suppressed-renin-hypertensive children, especially since treatment with spironolactone will cause prompt remission of symptoms.

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