A Novel Form of Human Mendelian Hypertension Featuring Nonglucocorticoid-Remediable Aldosteronism

David S. Geller, Junhui Zhang, Max V. Wisgerhof, Cedric Shackleton, Michael Kashgarian, and Richard P. Lifton

Section of Nephrology (D.S.G., J.Z., R.P.L.), and Departments of Pathology (M.K.) and Genetics (R.P.L.), Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, Connecticut 06520-8029; Division of Endocrinology (M.V.W.), Henry Ford Hospital, Detroit, Michigan 48202; and Children's Hospital Oakland Research Institute (C.S.), Oakland, California 94609

Context: Primary aldosteronism is a leading cause of secondary hypertension (HTN), but the mechanisms underlying the characteristic renin-independent secretion of aldosterone remain unknown in most patients.

Objectives: We report a new familial form of aldosteronism in a father and two daughters. All were diagnosed with severe HTN refractory to medical treatment by age 7 yr. We performed a variety of clinical, biochemical, and genetic studies to attempt to clarify the underlying molecular defect.

Results: Biochemical studies revealed hyporeninemia, hyperaldosteronism, and very high levels of 18-oxocortisol and 18-hydroxycortisol, steroids that reflect oxidation by both steroid 17-α hydroxylase and aldosterone synthase. These enzymes are normally compartmentalized in the adrenal fasciculata and glomerulosa, respectively. Administration of dexamethasone failed to suppress either aldosterone or cortisol secretion; these findings distinguish this clinical syndrome from glucocorticoid-remediable aldosteronism, another autosomal dominant form of HTN, and suggest a global defect in the regulation of adrenal steroid production. Genetic studies excluded mutation at the aldosterone synthase locus, further distinguishing this disorder from glucocorticoid-remediable aldosteronism. Because of unrelenting HTN, all three subjects underwent bilateral adrenalectomy, which in each case corrected the HTN. Adrenal glands showed dramatic enlargement, with paired adrenal weights as high as 82 g. Histology revealed massive hyperplasia and cellular hypertrophy of a single cortical compartment that had features of adrenal fasciculata or a transitional zone, with an atrophic glomerulosa.

Conclusion: These findings define a new inherited form of aldosteronism and suggest that identification of the underlying defect will provide insight into normal mechanisms regulating adrenal steroid biosynthesis. **(*J Clin Endocrinol Metab* 93: 3117–3123, 2008)**

In 1955, Dr. Jerome Conn described the now classical clinical syndrome of primary aldosteronism with hypertension (HTN), hypokalemia due to renal potassium wasting, suppressed plasma renin activity (PRA), and increased aldosterone levels (1). Since this initial description, a number of forms of primary aldosteronism have been described, including aldosterone producing adenomas, bilateral adrenal hyperplasia, unilateral adrenal hyperplasia, adrenal carcinoma, and familial forms (2).

Of the familial forms, glucocorticoid-remediable aldosteronism (GRA) is the best understood. GRA is characterized by the autosomal dominant transmission of severe early onset aldoste-

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Abbreviations: bid, Twice daily; BP, blood pressure; DHEA, dehydroepiandrosterone; FH2, familial hyperaldosteronism type 2; GRA, glucocorticoid-remediable aldosteronism; 18-OH, 18-hydroxy; 18-OH-THA, 18-hydroxytetrahydro-11-dehydrocorticosterone; HTN, hypertension; meq, milliequivalent; po, orally; PRA, plasma renin activity; qd, every day.

ronism and HTN. The disorder is caused by a gene duplication occurring from recombination between the adjacent and highly homologous aldosterone synthase and steroid 11β-hydroxylase genes on chromosome 8 (respectively encoded by *Cyp11b2* and *Cyp11b1*). This duplicated gene has chimeric structure, fusing the regulatory sequences from 11-β-hydroxylase onto coding sequences that specify aldosterone synthase enzymatic activity (3, 4). The result is that aldosterone synthase activity (which catalyzes oxidation of C-18 of the steroid nucleus), which is normally confined to the adrenal zona glomerulosa, is expressed in the adrenal fasciculata under control of ACTH. This leads to synthesis of aldosterone in adrenal zona fasciculata that is no longer regulated by the renin-angiotensin system. In addition, affected subjects also produce high levels of hybrid steroids that have both C-17-hydroxylation and C-18-oxidation owing to activity of aldosterone synthase on cortisol and its precursors. High levels of these hybrid steroids (18-hydroxycortisol and 18-oxocortisol) are uniformly found in patients with the genetic mutation of GRA and have been considered a specific marker for the disease (5, 6). In these patients, administration of exogenous glucocorticoids suppresses ACTH secretion, and causes an abrupt and dramatic decrease in aldosterone secretion (7). Adrenal pathology in these patients has featured bilateral nodular hyperplasia with atrophy of the adrenal glomerulosa.

A second form of familial aldosteronism termed familial hyperaldosteronism type 2 (FH2) has been described (8). FH2 has been defined by the recurrence of aldosteronism of any form within a kindred; unlike GRA, hyperaldosteronism in FH2 is not suppressible by dexamethasone. Patients may have bilateral adrenal hyperplasia or unilateral adenoma, sometimes within the same kindred (8, 9). Unlike patients with GRA, who are typically hypertensive at birth, affected members of FH2 kindreds do not differ clinically from patients diagnosed with primary aldosteronism, presenting in adulthood. Moreover, they do not differ with respect to age at presentation, gender, serum potassium, plasma aldosterone, or adenoma size from patients with sporadic aldosteronomas (8). To our knowledge, affected individuals have not been shown to produce hybrid steroids. Studies performed primarily in one large kindred have demonstrated linkage to chromosome 7p22, but no disease-causing mutations have been identified (10, 11).

We now describe a kindred with a familial form of early and severe primary aldosteronism; the distinctive clinical, biochemical, and genetic features indicate that this represents a new disorder.

Patients and Methods

Patients

All clinical studies were approved by the Yale Human Investigation Committee. All patients gave informed consent for their participation in the study. DNA was prepared by standard methods, and single-strand conformational polymorphism of the aldosterone synthase gene was performed using primers that flank coding exons (12). Direct sequencing of this and other candidate genes was performed by amplifying coding exons using primers lying in adjacent intronic regions followed by direct DNA sequencing.

Urine steroid measurement

Quantitative analysis of steroid excretions was achieved by GC/MS by previously published methods. One method was used for analysis for all metabolites primarily excreted as conjugates (13), whereas a specific method was used for analysis of unconjugated steroids, including cortisol, cortisone, and 18-hydroxycortisol (14).

Results

Three family members with early severe aldosteronism

The index case (HPA1–1) presented at age 5 yr with headache, polydipsia, polyuria, and HTN. Details of his remarkable presentation were reported in 1959 (15) and are summarized in Table 1. Briefly, at age 5 yr, he had extraordinary HTN (230/140 mm Hg), hypokalemia [K^+ = 2.8 milliequivalents (meq)/liter], and electrocardiographic evidence of left ventricular hypertrophy. He had marked elevation of aldosterone production: his 24-h urinary aldosterone excretion was 150 μg/d (normal 1–8 μg/d). 17-ketosteroid and 17-hydroxysteroid concentrations were unremarkable, indicating that this problem was specific for adrenal aldosterone production. Because of severe and unrelent-

TABLE 1. Clinical presentation of affected individuals in kindred HPA1

Presentation	HPA1–1	HPA1–2	HPA1–3	Normal
Age (yr)	5	7	4	
SBP (mm Hg)	230	188	148	<110
DBP (mm Hg)	140	140	114	<70
Na	136	139	138	136–144 meq/liter
Serum K^+	2.8	1.8	1.9	3.5–5 mmol/liter
Serum bicarbonate	28	NA	21	23–28 mmol/dl
PRA	NA	0.3	0.2	0.4–8.8 ng/ml/h
Serum aldosterone	NA	137.4	185.1	3–39.5 ng/dl
24-h urine aldosterone	67			1–8 μg/24 h
Cortisol		7.2	4.7	3–21 μg/dl
17-OH steroids	6.3	4.4	5.4	Male 4–14 mg/d Female 2–12 mg/d
17-ketosteroids	22.4	2	2.2	Male 8–20 mg/d Female 6–12 mg/d

DBP, Diastolic BP; 17-OH, 17-hydroxysteroid; NA, not applicable; Na, sodium; SBP, systolic BP.

ing HTN, he underwent bilateral adrenalectomy at age 9 yr, which resulted in rapid normalization of blood pressure (BP) and serum potassium levels within 1 wk. The adrenal glands were markedly enlarged, with the right adrenal weighing 8.0 g and the left 9.0 g. Pathology demonstrated that the cortex comprised the major portion of the adrenal cross-section, and represented focal nodular hyperplasia, chiefly of the zona fasciculata, which contained many vacuolated lipid-laden cells with hypertrophy of the cytoplasm.

Twenty-six years later, the two daughters of the index case presented concurrently at ages 7 (HPA1–2) and 4 yr (HPA1–3) with severe HTN. BPs were 188/140 and 148/114 mm Hg, respectively. Both were markedly hypokalemic, with serum potassium levels of 1.8 and 1.9 meq/liter. Although serum bicarbonate levels were unremarkable on this blood draw, serum bicarbonates over many months averaged 34 mmol/dl. Serum aldosterone levels were markedly elevated at 137.4 and 185.1 ng/dl (normal range 3–39.5) despite suppressed PRA levels (0.3, 0.2 ng/ml·h, respectively), whereas serum 17-ketosteroid and cortisol levels were unremarkable (Table 1). Interestingly, the girls both showed relatively normal growth and development, with height and weight between the 25th and 50th percentile. Because the features resembled those of GRA, each girl was hospitalized and administered a 3-wk trial of dexamethasone (0.25–0.5 mg every 6 h). During these 3 wk, PRA levels remained suppressed at less than 0.2 ng/ml·h for each girl. The serial progression of aldosterone levels and BP are shown in Fig. 1. Aldosterone levels increased progressively during the 3 wk, and there was no improvement in systolic or diastolic BP. Importantly, because the test was done in an inpatient setting, medical compliance was ensured.

The girls were lost to follow-up for 8 yr and presented again at ages 18 and 15 yr with severe HTN despite aggressive therapy. In the intervening time, the girls had each undergone normal pubertal development; there was no evidence of hirsutism or virilism, and each had normal menses. At this presentation, subject HPA1–2 had a BP of 173/120 mm Hg, and serum K^+ was 2.9 on a regimen of propranolol 80 mg twice daily (bid), nifedipine 90 mg every day (qd), amiloride 10 mg qd, lisinopril 40 mg qd, and K-dur 80 meq three times a day. Subject HPA1–3 had a BP of 150/90 mm Hg and a serum K^+ of 2.2 despite lisinopril 40 mg qd, nifedipine 60 mg bid, amiloride 10 bid, spironolactone 50 mg orally (po) bid, atenolol 25 mg qd, and KCl 50 meq po three times a day. An echocardiogram performed on the older daughter showed concentric left ventricular hypertrophy with preserved ejection fraction. Plasma steroids from subject HPA1–3 before steroid therapy showed suppressed ACTH, marked elevations of desoxycorticosterone, 18-hydroxy(OH) corticosterone and aldosterone, minor elevations in corticosterone levels, normal cortisol levels, and decreased dehydroepiandrosterone (DHEA) levels (Table 2). Urinary steroid excretion (Table 3) essentially paralleled the serum results, with markedly increased excretion of 18-hydroxytetrahydro-11-dehydrocorticosterone (18-OH-THA) (a tetrahydro-metabolite of 18-OH-corticosterone), tetrahydroaldosterone, normal excretions of conventional cortisol metabolites (tetrahydrocortisone, tetrahydrocortisol, and 5α-tetrahydrocortisol), normal levels of corticosterone metabolites (tetrahydrocorticosterone and tetrahydro-11-dehydrocorticosterone metabolites), and reduced androgen metabolite excretions (DHEA, androsterone, and etiocholanolone). The excretion of 18-oxo-tetrahydrocortisol was markedly elevated. In addition, the urine studies demonstrated normal excretion of free cortisone and cortisol but exceptionally elevated excretion of unconjugated 18-hydroxycortisol (6126 and 3432 μg/d) and 18-oxocortisol (250 and 200 μg/d). By comparison, Rich *et al.* (16) found mean 24-h 18-hydroxycortisol and 18-oxocortisol excretion of 636 $\pm$ 523 μg/d and 148 $\pm$ 87 mg/d, respectively, in affected members of a large GRA kindred.

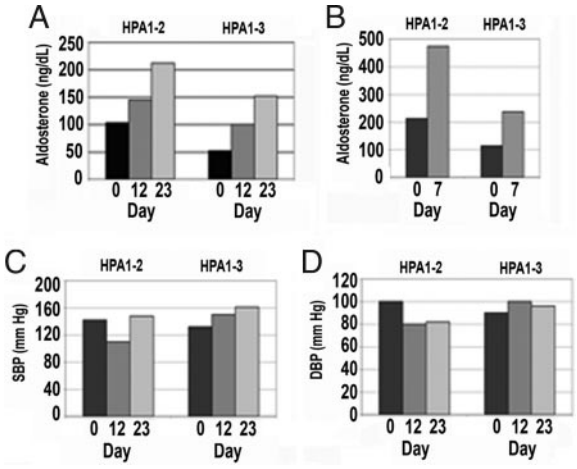

FIG. 1. Glucocorticoids fail to ameliorate aldosteronism or HTN in kindred HPA1. A, Serum aldosterone levels during the 23-d trial of dexamethasone. Serum aldosterone levels increased progressively during glucocorticoid therapy in these patients. B, A repeat 7-d trial of dexamethasone was attempted years later (see *Results*). Again, serum aldosterone levels increased steadily in the presence of administered glucocorticoid. C and D, Response of systolic and diastolic BP (SBP and DBP, respectively) to a 23-d trial of po dexamethasone in patients HPA1–2 and HPA1–3. There was no significant improvement in BP in either patient during glucocorticoid therapy.

Because these clinical features resemble GRA, a 7-d trial of dexamethasone (0.5 mg bid) was performed on each child. Again, this treatment failed to normalize BP (data not shown) and caused serum aldosterone levels to double (Fig. 1B). Remarkably, cortisol levels were not suppressed in either patient, remaining at 24.3 and 6.9 mg/ml in both subjects after 1-wk therapy. Importantly, the approximate doubling of serum aldosterone levels in these girls is consistent with the prior result from 10 yr previously (Fig. 1A), and is suggestive of a pharmacological effect of dexamethasone to increase aldosterone production. This strongly suggests the patients were compliant with the dosing and makes the nonsuppressibility of cortisol production that much more remarkable. These findings together reveal abnormalities in the biosynthesis of mineralocorticoids, glucocorticoids, and androgens in these subjects.

Because of unrelenting HTN, despite aggressive medical therapy, both subjects underwent bilateral laparoscopic adrenalectomy. BP and serum K^+ normalized in each subject within 2 wk, and they remained normotensive and normokalemic on replacement doses of prednisone and fludrocortisone. Three years later, HPA1–3 died of presumed addisonian crisis when she unaccountably stopped taking corticosteroid replacement during pregnancy. Testosterone levels in HPA1–2 have normalized, sug-

TABLE 2. Representative steroid excretions (μg/24 h) in patients HPA1–2 and HPA1–3 at ages 15 and 17 yr

Steroid	HPA1–2	HPA1–3	Normal mean (μg/24 h)[a]	Normal range (μg/24 h)
Androsterone	189	74	1257	373–3414
Etiocholanolone	396	176	1463	450–2910
DHEA	11	4.3	202	20–1139
B metabolites	566	165	446	113–607
F metabolites	4720	1653	3915	600–7313
Tetrahydroaldosterone	599	302	28	6–63
18-OH-THA	2549	1364	80	25–207
18-Oxo-THF	250	200	<10	<10
Cortisone	157	107	50	21–107
Cortisol	66	31	23	8–61
18-OH-cortisol	6126	3432	71	28–133

B metabolites represent the sum of the tetrahydro-metabolites of corticosterone and 11-dehydrocorticosterone. F metabolites are the sum of tetrahydrocortisone (THE), tetrahydrocortisol (THF), and 5α-tetrahydrocortisol (5αTHF). 18-OH-THA is the metabolite of 18-hydroxycorticosterone. 18-Oxo-THF, 18-oxo-tetrahydrocortisol.

[a] Adult females (n = 17).

gesting that the androgen deficiency encountered preoperatively in her case may have been due to stress induced by altered vascular and electrolyte homeostasis rather than a deficiency in her ability to manufacture these hormones (17).

Pathology

Inspection of the excised adrenals from these two subjects showed markedly enlarged adrenal glands with a paired adrenal weight of 81 and 39 g (normal range < 12). Light microscopical analysis of the sections revealed a thin, atrophic zona glomerulosa and diffuse hyperplasia of the zona fasciculata with many vacuolated, lipid-laden cells and hypertrophy of the cytoplasm (Fig. 2). There was no evidence of nodularity. The zona reticularis was normal in appearance but slightly decreased in size, perhaps because of the massive hypertrophy of the zona fasciculata. Electron microscopy of the hyperplastic tissue revealed a combination of platelike and tubulovesicular cristae indicative of transitional zone morphology (Fig. 3).

Genetic analysis

Figure 4 shows the extended pedigree of this kindred. The sisters of the index case are alive and well, and have no signs or symptoms suggestive of primary aldosteronism. The father of the index case died at the age of 36 yr with severe HTN and heart failure. His mother was alive and well at the age of 79 yr. The siblings and parents of the index case's father all lived beyond the age of 60 yr. The homogeneous and distinctive features of the severe aldosteronism, its pattern of transmission in the kindred, and the apparent male to male transmission together support autosomal-dominant transmission of the trait.

The production of high levels of the hybrid steroids 18-oxocortisol and 18-hydroxycortisol constitutes evidence that aldosterone synthase and steroid 17-hydroxylase activities exist together in the same cells. The findings of an atrophic zona glomerulosa and hypertrophied zona fasciculata with transitional features is consistent with this observation. The only known mechanism for such an event is the gene duplication that causes GRA. However, several features of the aldosteronism are inconsistent with this diagnosis. First, the severity of the HTN among affected family members is truly remarkable, and the uniform requirement for adrenalectomy would be remarkable for GRA. Second, the massive adrenal hyperplasia seen in subject HPA1–2 has not been described in GRA. Finally, suppressibility of aldosterone secretion with glucocorticoid administration is

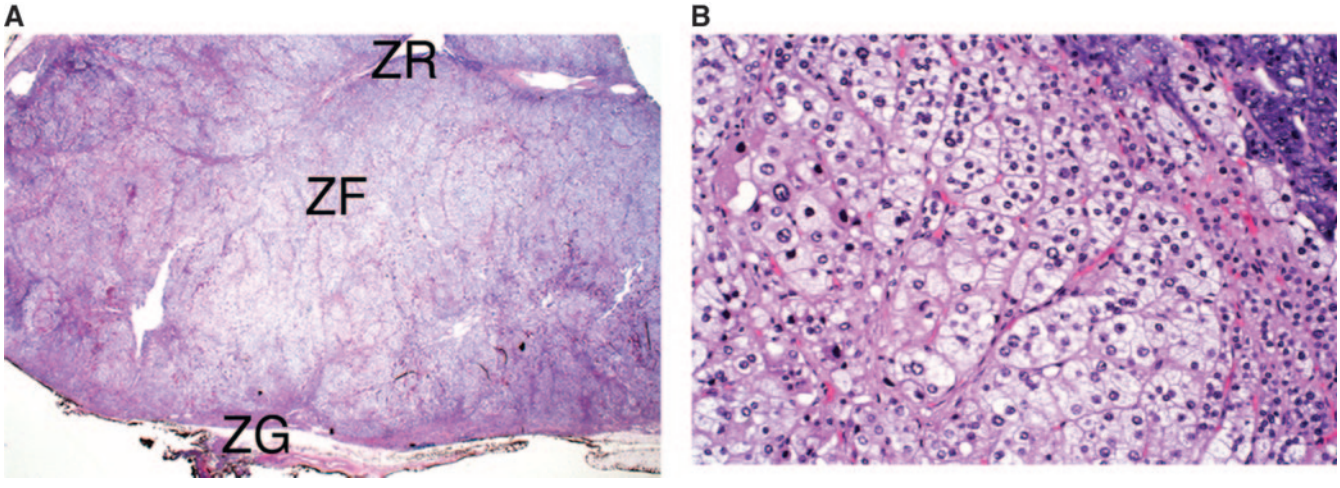

FIG. 2. Light microscopical analysis of adrenal glands from patient HPA1–2. A, Low-power magnification of the adrenal gland shows a thin, atrophic zona glomerulosa (ZG), a massive zona fasciculata (ZF), and a compressed, but present, zona reticularis (ZR). B, Higher-power magnification of the same gland shows evidence of diffuse hyperplasia without evidence of adenoma. The lipid-laden cytoplasm is indicative of high metabolic activity. Magnification (A), ×10; magnification (B), ×100.

TABLE 3. Plasma steroid concentrations in patient HPA1–3

Steroids	HPA 1–3	Normal
ACTH	<5	3–37 pg/ml
Aldosterone	74	3–16 ng/dl
Androstenedione	108	80–240 ng/dl
Corticosterone	235	66–220 ng/dl
18-OH corticosterone	239	9–68 ng/dl
Cortisol	9.7	4–11 μg/dl
DHEA	112	215–850 ng/dl
DOC	47	2–19 ng/dl
Testosterone	9.8	10–55 ng/dl

DOC, Desoxycorticosterone.

considered pathognomonic of GRA, and the inability to achieve suppression with two attempts in each of the two affected subjects effectively excludes this diagnosis.

To test whether affected individuals in this kindred harbor a novel variant of GRA, we first assessed the tightly linked *Cyp11B1* and *Cyp11B2* genes implicated in GRA. All patients with GRA have chimeric gene duplications that fuse regulatory sequences from 11-OHase to coding sequences of aldosterone synthase, resulting in ACTH-dependent expression of aldosterone synthase in adrenal fasciculata. These duplications can be conveniently screened for by Southern blotting (3). Members of this kindred did not have GRA-like duplications by this test, showing only wild-type fragments (data not shown). We considered whether novel types of mutations in these genes might be the cause of this syndrome. We performed single-stranded conformational polymorphism and direct DNA sequence analysis of these genes to determine whether intragenic mutations might be present (12). This demonstrated that the father and one daughter (HPA1–3), but not the other (HPA1–2), are heterozygous for a common synonymous single-base substitution in exon 2 of *Cyp11B2* (G1279A). Because the daughters' mother (HPA1–4), whose identity was confirmed by genotypical analysis, is not a carrier of this allele, we can conclude that the father transmitted G1279A to one of his affected daughters but not the other, thus excluding this locus and tightly linked genes as a cause of disease in this kindred (Fig. 5). *Cyp11B1* and *Cyp11B2* are extremely tightly linked on chromosome 8 (separated by only 30 kb), so this result excludes mutation in either of these genes as a cause of this disease.

The persistence of cortisol secretion despite dexamethasone therapy suggested a global dysregulation of adrenal steroid biosynthesis. Therefore, we considered a number of genes known to be involved in regulation of the development of the adrenal cortex and in regulation of adrenal steroid production as potential candidate genes. In addition to candidates such as the angiotensin II and ACTH receptors, we considered genes such as DAX-1, which has been implicated in congenital adrenal hypoplasia (18), Ad4BP, a transcription factor essential for the transcription of steroidogenic p450 genes (19), and the NGFI-B family members Nur77 and Nurr1, which have been implicated in adrenal zonation and may play a role in the regulation of aldosterone synthase (20). We screened these genes by direct sequencing of coding exons but failed to identify potential disease-causing mutations in any of these genes (data not shown).

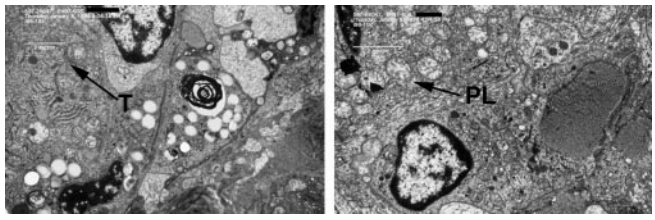

FIG. 3. Electron microscopical analysis of adrenal sections from patient HPA1–2. Electron micrographs of adrenal zona fasciculata from patient HPA1–2 demonstrate both platelike (PL) and tubulovesicular (T) cristae, consistent with transitional zone morphology. The presence of numerous lipid-laden vacuoles is suggestive of high metabolic activity. Magnification, ×4000.

Discussion

We have described a novel form of mendelian HTN caused by massive adrenal mineralocorticoid production. Although a number of characteristics of this disease are similar to the clinical picture observed in GRA, clinical, biochemical, and genetic findings make clear that this family has a distinct disorder. Like patients with GRA, these patients present with childhood HTN, elevated aldosterone levels, and high levels of the hybrid steroids 18-oxocortisol and 18-hydroxycortisol. In each case, hyperplasia observed in the adrenal zona fasciculata coupled with the atrophy of the zona glomerulosa suggests that excess hybrid steroid production is due to ectopic production of aldosterone in the zona fasciculata or in the transitional zone between these two tissues. Nonetheless, affected members of the present kindred can be distinguished from GRA on clinical, biochemical, and genetic grounds. Clinically, the degree of hyperplasia and the refractory HTN requiring adrenalectomy to control HTN in three affected members of this family distinguish this disease from reported families with GRA. Biochemically, in contrast to patients with GRA, HTN and aldosteronism in the kindred we describe here are not reversed by administration of exogenous glucocorticoids. Indeed, in two independent tests in two patients, aldosterone levels doubled in each test. In addition, these subjects also appear to have autonomous cortisol production, with suppressed ACTH and failure to suppress cortisol secretion with dexamethasone, and reduced androgen production. Finally, the absence of the gene duplication characteristic of GRA and the demonstration that the affected father transmitted different aldosterone synthase alleles to his two affected daughters definitively excludes this locus as a cause of disease in this kindred.

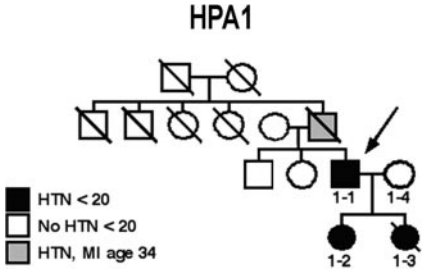

FIG. 4. Pedigree of kindred HPA1. The affected individuals are represented by *filled symbols*. The father of patient HPA1–1 died of HTN and heart failure at age 36 yr, and is the presumed source of the disease-causing mutation. His parents and siblings all lived beyond age 65 yr and are presumed unaffected. MI, Myocardial infarction.

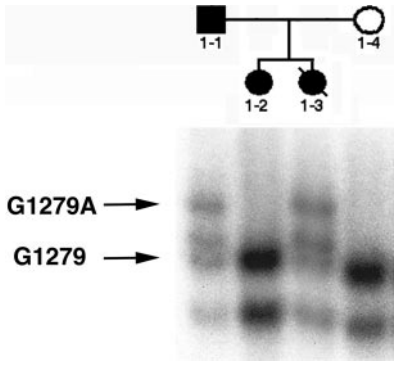

FIG. 5. Inheritance of aldosterone synthase polymorphisms in HPA1. Single stranded conformational polymorphism of *Cyp11B2* (aldosterone synthase) exon 2 demonstrated that the index case (HPA1–1) is heterozygous for the G1279A polymorphism, as is HPA1–3, but not HPA1–2. The daughters' mother, whose identity was confirmed by genotypical analysis, does not carry this allele. Thus, the affected father passed distinct *Cyp11b2* alleles to each daughter, thus excluding this locus and the nearby *Cyp11B1* locus as causes of this syndrome.

This disorder bears even less resemblance to the other form of familial hyperaldosteronism that has been described, termed FH2, an entity whose existence as an autosomal dominant trait is still uncertain. FH2 is broadly defined as the recurrence of primary aldosteronism that is not glucocorticoid remediable within an extended kindred. Patients with FH2 are generally normotensive as children, and present with HTN and either adrenal adenomas or bilateral adrenal hyperplasia as adults. Affected subjects have neither the severity of HTN nor the massive fasciculata hyperplasia found in this kindred; high levels of hybrid steroids have not been reported. It is clear that the unique clinical and biochemical features present in the cases we describe here constitute a distinct clinical entity.

From these considerations, this kindred has a new form of familial hyperaldosteronism characterized by massive overproduction of aldosterone and the hybrid steroids 18-oxocortisol and 18-hydroxycortisol, but which is not suppressible by glucocorticoids. We are aware of only two previous reports of a potentially similar syndrome. In one, an infant presented with severe HTN and laboratory indices notable for suppressed renin and hyperaldosteronemia, BP was well controlled with spironolactone monotherapy, and her mother was reported to have the teenage onset of HTN that responded only after spironolactone administration (21). In the other case, a 26-month-old girl was diagnosed with primary aldosteronism with evidence of bilateral adrenal aldosterone production, which did not respond to dexamethasone suppression, but which was successfully treated with spironolactone monotherapy; intriguingly, at the age of 4 yr, she had persistent cortisol production, with a serum level of 3.1 μg/dl, after 3-wk dexamethasone therapy (1 mg/d). Her father's history was notable for primary aldosteronism, which was successfully treated by nine-tenths adrenalectomy (21, 22). In these cases, neither hybrid steroid production nor pathology was assessed, so it cannot be determined whether these patients in fact had the same disorder. However, in each of these cases, spironolactone monotherapy was sufficient for appropriate treatment of the HTN, suggesting a much more benign disease process.

The patients we describe differ from these previously reported patients in the severity of their disease. Each affected individual in the kindred we describe had severe HTN and hypokalemia with evidence of left ventricular hypertrophy despite aggressive use of antihypertensive agents, including spironolactone. These cases are further distinguished by the presence of elevated levels of the hybrid steroids 18-oxocortisol and 18-hydroxycortisol, steroids that previously were thought to be relatively specific for the diagnosis of GRA in this context. The clinical and biochemical picture observed in our patients is consistent with ectopic expression of aldosterone synthase in the adrenal zona fasciculata, leading to conversion of synthesized cortisol to 18-hydroxycortisol, and of deoxycorticosterone to aldosterone. The atrophy of zona glomerulosa tissue in the adrenal glands of these patients coupled with the rapid clinical response to adrenalectomy makes clear that all aldosterone synthesized emanates from the hypertrophic zona fasciculata, similar to what has been previously observed in GRA. However, in contrast to GRA, the disorder described here appears to involve a global dysregulation of adrenal steroid biosynthesis. In GRA, exogenous glucocorticoid suppresses expression of not only the chimeric AldoS/11βHSD2 gene, but also the remainder of the cortisol biosynthetic machinery, resulting in an appropriate suppression of cortisol secretion by dexamethasone. In the kindred described here, the persistence of aldosterone and cortisol secretion during dexamethasone therapy implies continued expression not only of aldosterone synthase, but also of the other enzymes involved in the cortisol biosynthetic pathway, including 3βOH steroid dehydrogenase, 17α-hydroxylase, 11-βOHase, and 21α-hydroxylase. The pattern of steroid production observed here seems to parallel that observed in patients with primary aldosteronism caused by angiotensin II unresponsive adenomas, in whom 18-oxocortisol production is not suppressed by dexamethasone (14). We speculate that the individuals in the kindred we describe carry a germline defect similar to a genetic defect that led to this form of aldosterone-producing adenoma. However, the genetic basis of these adenomas is not, at present, known. Identification of the underlying gene in this kindred may provide insight into the normal mechanisms underlying regulation of adrenal steroid biosynthesis or, alternatively, might play a role in the differentiation of the zona glomerulosa and zona fasciculata.

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Address all correspondence and requests for reprints to: David S. Geller, P.O. Box 208029, Section of Nephrology, Yale University School of Medicine, New Haven, Connecticut 06520-8029. E-mail: david.geller@yale.edu.

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References

1. **Conn JW, Louis LH** 1955 Primary aldosteronism: a new clinical entity. Trans Assoc Am Physicians 68:215–231

2. **Mulatero P, Dluhy RG, Giacchetti G, Boscaro M, Veglio F, Stewart PM** 2005 Diagnosis of primary aldosteronism: from screening to subtype differentiation. Trends Endocrinol Metab 16:114–119
3. **Lifton RP, Dluhy RG, Powers M, Rich GM, Gutkin M, Fallo F, Gill Jr JR, Feld L, Ganguly A, Laidlaw JC, Murnaghan DJ, Kaufman C, Stockigt JR, Ulick S, Lalouel JM** 1992 Hereditary hypertension caused by chimaeric gene duplications and ectopic expression of aldosterone synthase. Nat Genet 2:66–74
4. **Lifton RP, Dluhy RG, Powers M, Rich GM, Cook S, Ulick S, Lalouel JM** 1992 A chimaeric 11β-hydroxylase/aldosterone synthase gene causes glucocorticoid-remediable aldosteronism and human hypertension. Nature 355:262–265
5. **Ulick S, Chan CK, Gill Jr JR, Gutkin M, Letcher L, Mantero F, New MI** 1990 Defective fasciculata zone function as the mechanism of glucocorticoid-remediable aldosteronism. J Clin Endocrinol Metab 71: 1151–1157
6. **Gomez-Sanchez CE, Montgomery M, Ganguly A, Holland OB, Gomez-Sanchez EP, Grim CE, Weinberger MH** 1984 Elevated urinary excretion of 18-oxocortisol in glucocorticoid-suppressible aldosteronism. J Clin Endocrinol Metab 59:1022–1024
7. **Litchfield WR, New MI, Coolidge C, Lifton RP, Dluhy RG** 1997 Evaluation of the dexamethasone suppression test for the diagnosis of glucocorticoid-remediable aldosteronism. J Clin Endocrinol Metab 82: 3570–3573
8. **Stowasser M, Gordon RD, Tunny TJ, Klemm SA, Finn WL, Krek AL** 1992 Familial hyperaldosteronism type II: five families with a new variety of primary aldosteronism. Clin Exp Pharmacol Physiol 19:319–322
9. **So A, Duffy DL, Gordon RD, Jeske YW, Lin-Su K, New MI, Stowasser M** 2005 Familial hyperaldosteronism type II is linked to the chromosome 7p22 region but also shows predicted heterogeneity. J Hypertens 23:1477–1484
10. **Jeske Y, So A, Kelemen L, Sukor N, Willys C, Bulmer B, Gordon RD, Duffy D, Stowasser M** 2008 Examination of chromosome 7p22 candidate genes RBaK, PMS2 and GNA12 in familial hyperaldosteronism type II. Clin Exp Pharmacol Physiol 35:380–385
11. **Stowasser M, Gordon RD** 2006 Monogenic mineralocorticoid hypertension. Best Pract Res Clin Endocrinol Metab Metabolism 20:401–420
12. **Shimkets RA, Warnock DG, Bositis CM, Nelson-Williams C, Hansson JH, Schambelan M, Gill Jr JR, Ulick S, Milora RV, Findling JW, Canessa CM, Rossier BC, Lifton RP** 1994 Liddle's syndrome: heritable human hypertension caused by mutations in the β subunit of the epithelial sodium channel. Cell 79:407–414
13. **Shackleton CH** 1993 Mass spectrometry in the diagnosis of steroid-related disorders and in hypertension research. J Steroid Biochem Mol Biol 45: 127–140
14. **Palermo M, Gomez-Sanchez C, Roitman E, Shackleton CH** 1996 Quantitation of cortisol and related 3-oxo-4-ene steroids in urine using gas chromatography/mass spectrometry with stable isotope-labeled internal standards. Steroids 61: 583–589
15. **Therien B, Mellinger RC, Caldwell JR, Howard PJ** 1959 Primary aldosteronism due to adrenal hyperplasia; occurrence in a boy aged 10 years. AMA J Dis Child 98:90–99
16. **Rich GM, Ulick S, Cook S, Wang JZ, Lifton RP, Dluhy RG** 1992 Glucocorticoid-remediable aldosteronism in a large kindred: clinical spectrum and diagnosis using a characteristic biochemical phenotype. Ann Intern Med 116: 813–820
17. **Hardy MP, Gao HB, Dong Q, Ge R, Wang Q, Chai WR, Feng X, Sottas C** 2005 Stress hormone and male reproductive function. Cell Tissue Res 322:147–153
18. **Niakan KK, McCabe ER** 2005 DAX1 origin, function, and novel role. Mol Genet Metab 86:70–83
19. **Parker KL, Rice DA, Lala DS, Ikeda Y, Luo X, Wong M, Bakke M, Zhao L, Frigeri C, Hanley NA, Stallings N, Schimmer BP** 2002 Steroidogenic factor 1: an essential mediator of endocrine development. Recent Prog Horm Res 57:19–36
20. **Bassett MH, White PC, Rainey WE** 2004 A role for the NGFI-B family in adrenal zonation and adrenocortical disease. Endocr Res 30:567–574
21. **Malagon-Rogers M** 2004 Non-glucocorticoid-remediable aldosteronism in an infant with low-renin hypertension. Pediatr Nephrol 19:235–236
22. **Greco RG, Carroll JE, Morris DJ, Grekin RJ, Melby JC** 1982 Familial hyperaldosteronism, not suppressed by dexamethasone. J Clin Endocrinol Metab 55:1013–1016