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Congenital Hyperreninemic Hypoaldosteronism Unlinked to the Aldosterone Synthase (*CYP11B2*) Gene

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Isolated hyperreninemic hypoaldosteronism presenting in infancy is usually caused by mutations in the *CYP11B2* gene encoding aldosterone synthase. We studied five patients in four unrelated kindreds with hyperreninemic hypoaldosteronism, in whom we were unable to find such mutations. All presented in infancy with failure to thrive, hyponatremia, hyperkalemia, markedly elevated plasma renin activity, and low or inappropriately normal aldosterone levels. All had normal cortisol levels and no signs or symptoms of congenital adrenal hyperplasia. All responded to fludrocortisone treatment. There were no mutations detected in exons or splice junctions of *CYP11B2*. Linkage of the disorder to *CYP11B2* was studied in two unrelated consanguineous patients and in an affected sib pair. The consanguineous patients were each heterozygous for at least one of three polymorphic microsatellite markers near *CYP11B2*, excluding linkage to *CYP11B2*. However, linkage of the disease to *CYP11B2* could not be excluded in the affected sib pair. Genes involved in the regulation of aldosterone biosynthesis, including those encoding angiotensinogen, angiotensin-converting enzyme, and the AT1 angiotensin II receptor were similarly excluded from linkage. These results demonstrate the existence of an inherited form of hyperreninemic hypoaldosteronism distinct from aldosterone synthase deficiency. The affected gene(s) remain to be determined. (*J Clin Endocrinol Metab* 86: 5379–5382, 2001)

ALDOSTERONE REGULATES ELECTROLYTE excretion and intravascular volume mainly through effects on the distal nephron, where it increases sodium resorption from, and potassium excretion into, the urine by indirectly increasing activity of the epithelial sodium channel and the sodium-potassium ATPase. Aldosterone deficiency interferes with these processes and thus results in hyponatremia, hypovolemia, and hyperkalemia (1).

In primary aldosterone deficiency (*i.e.* inability of the adrenal gland to synthesize the hormone), plasma renin activity (PRA) is elevated, and so this condition is also referred to as hyperreninemic hypoaldosteronism. Renin is a proteolytic enzyme secreted by the nephron's juxtaglomerular apparatus in response to low intravascular volume. It cleaves angiotensinogen to angiotensin I, which is converted to angiotensin II by angiotensin-converting enzyme (ACE). Angiotensin II binds to specific receptors on cells in the adrenal zona glomerulosa (the site of aldosterone synthesis), thus increasing aldosterone biosynthesis and secretion under normal circumstances (2).

Primary aldosterone deficiency can result from destruction of the entire adrenal cortex (Addison's disease) by infection, injury, or autoimmune processes; from genetic disorders affecting the entire gland (such as lipoid hyperplasia, adrenoleukodystrophy, or adrenal hypoplasia congenita); or from genetic disorders affecting specific enzymatic conversions required for aldosterone biosynthesis. Two of these, the salt-wasting forms of 21-hydroxylase and 3β-hydroxysteroid dehydrogenase deficiencies, also affect cortisol biosynthesis.

Rare patients have aldosterone deficiency with entirely normal cortisol and sex steroid synthesis. This is usually attributable to an inability to convert deoxycorticosterone to aldosterone, a three-step reaction normally mediated by the enzyme aldosterone synthase (3, 4). Two forms of aldosterone synthase (also termed: corticosterone methyloxidase) deficiency are recognized. These syndromes have identical clinical features but differ in profiles of secreted steroids. In particular, whereas excretion of 18-hydroxycorticosterone is mildly decreased in type I deficiency, urinary and serum levels of this steroid are markedly increased in patients with type II deficiency (5).

Aldosterone synthase is a mitochondrial cytochrome P450 enzyme, CYP11B2. It is encoded by the *CYP11B2* gene located on chromosome 8, band q24.3, approximately 40 kb away from the 93%-identical *CYP11B1* gene encoding the steroid 11β-hydroxylase enzyme required for cortisol biosynthesis (6, 7). Most reported patients with presumed aldosterone synthase deficiency carry mutations in *CYP11B2* (8–15).

Here, we report five patients in four unrelated kindreds with hyperreninemic hypoaldosteronism presenting in early infancy in whom we were unable to detect mutations in *CYP11B2*. Two of these patients were of consanguineous parentage, strongly suggesting that the disease was inherited in an autosomal recessive manner. In these kindreds, the presence of consanguinity permitted the use of homozygosity mapping to exclude linkage of the disorder to *CYP11B2*, demonstrating the existence of an inherited form of hyper-

Abbreviations: ACE, Angiotensin-converting enzyme; AGT, angiotensinogen; PRA, plasma renin activity.

reninemic hypoaldosteronism distinct from aldosterone synthase deficiency.

Materials and Methods

Samples for DNA extraction were obtained from patients with familial hyperreninemic hypoaldosteronism (Table 1) after obtaining appropriate informed consent.

Sequence analysis of *CYP11B2* was performed as previously described (16). Three highly polymorphic microsatellite markers near the *CYP11B2* gene were examined in two patients of consanguineous parentage (1 and 2) and in an affected sib pair (4a and 4b). These patients were also typed for microsatellite polymorphisms near genes involved in the regulation of aldosterone biosynthesis, including those encoding angiotensinogen, ACE, and the AT1 type angiotensin II receptor. The names and sequences of all oligonucleotides used in the study are listed in Table 2.

PCR was performed in 10-μl reaction volumes for the *CYP11B2* microsatellite markers and 20-μl reaction volumes for the remaining markers. Reactions contained 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 0.1% gelatin, 5 μCi [$^{33}\alpha$P]deoxy-ATP (>2500 Ci/mmol), and usually 1 μM of each primer, 125 μM deoxy-GTP, thymidine 5′-triphosphate, and deoxy-CTP, 1.25 μM unlabeled deoxy-ATP, 16 ng genomic DNA, and 0.2 U *Taq* polymerase. Cycling conditions consisted of a 30-sec denaturation at 96 C, 30 sec annealing at 55 C, and 1 min extension at 72 C for 30 cycles. A 5-min denaturation step (96 C) preceded cycling, and a 10-min primer extension step and 4-C soak followed. A modified hot start was used as previously described (16).

Samples were subjected to electrophoresis in 6% denaturing polyacrylamide gels, fixed, dried, and autoradiographed for 1–18 h.

Results and Discussion

The clinical and biochemical characteristics of five patients in four kindreds are summarized in Table 1. All developed signs of hypoaldosteronism within the first few weeks of life, including failure to thrive, hyponatremia, and hyperkalemia. All had markedly elevated PRA and low or inappropriately normal aldosterone levels. All responded to treatment with fludrocortisone by normalizing serum electrolytes and PRA.

Thus, these patients clearly had primary hypoaldosteronism with onset in early infancy. Two were of known consanguineous parentage consistent with an autosomal recessive disorder such as aldosterone synthase deficiency. All had normal 18-hydroxycorticosterone levels, suggesting that the patients did not have type II aldosterone synthase deficiency, but these results were entirely consistent with type I aldosterone synthase deficiency.

However, complete sequencing of all exons and introns 1, 4, 6, and 7, plus partial sequencing of the remaining introns (including all splice junctions) and 50 bp of the 5′ flanking region failed to reveal mutations in any of these patients. Patient 1 was heterozygous for a V386A polymorphism; but this polymorphism, by itself, has a minimal effect on enzymatic activity (8). Patient 2 was heterozygous for the K173R polymorphism, which also has no effect on enzyme activity (17). In any consanguineous kindred carrying an autosomal recessive disease, the two mutant alleles in each affected patient should be identical, and all polymorphic markers near the affected gene should be homozygous. Thus, the fact that each of these patients was heterozygous for a polymorphism within *CYP11B2* argues strongly against linkage of the disease to this gene. However, both of these polymorphisms are normally present in the closely linked *CYP11B1* gene (6) and could potentially represent recent gene conversions in

TABLE 1. Patients with hyperreninemic hypoaldosteronism

	1 (Indian)		2 (Pakistani)		3 (Caucasian)		4a (Caucasian)			4b		Normal
Consanguinity		1st cousin		1st cousin		No			No			
Age	6 mo	1 yr	3 wk	3 mo	6 wks	6 mo	3 mo	5 yr, 10 mo	6 yr, 4 mo	4–6 wk	7 mo	
Treatment	None		None		None		None	None	0.1	None		
Hydrocortisone (mg/d)				4								
Fludrocortisone (mg/d)		0.15		0.15		0.1			0.1		0.1	
NaCl (mmol/d)		20		30		23				10	10	
Weight (kg)	4.40	7.24		6.8	4.10	9.64		14.5	15.5	4.6	6.0	
Height (cm)	58	67		63	55	73		96.5	99	55	64	
BP (mm Hg)		145/75										
Serum values												
Na (mM)	128	135	111	135	108	140	120	143	139	135	132	
K (mmol/l)	5.6	5.7	8.7	5.6	7.4	4.6	6.5	4.3	4.3	6.1	4.6	
Cl (mmol/l)	97	103	80	105	80	107	93	102	107	94	96	
CO2 (mmol/l)	17	21		24	13	23	18	27	23	22	22	
DOC (pM)	333		2420						3300		4840	212–1480
Corticosterone (pM)	22,500		32,200	6,200					72,800	21,900	134,000	2300–43,300
18-OH corticosterone (pM)	610		720	180	1600				4030	2200	3750	140–6070
Aldosterone (pM)	470		200	58	1600			310	210	310	210	140–2500
Renin (ng/l/s)	125	<0.2	49.7	0.89	301	<0.2		>300	2.3	>300	21	0.6–10.3
17-OH progesterone (basal/stim) (pM)	normal		7320/13000	1760/3270	normal					normal		1210–6050♂ 390–3210♀
Cortisol (basal/stim) (nM)	330/830		830/860	300/470	630	250		360	360	440		77–630

BP, Blood pressure; DOC, deoxycorticosterone; mo, month.

TABLE 2. Sequences of oligonucleotides used to type microsatellite polymorphisms

Gene	Locus	5′ → 3′ Oligonucleotide sequence
Aldosterone synthase (CYP11B2)	D8S1704	TCTGGGTGATAGAGCAAGAC
		AGCTAAAAATTGACACTTGTTTACA
	D8S1836	CCTTCATATCCTCCATACCC
		GCTGACTCCGTCCTGTGT
	D8S1727	TCCCAACAGAGCAATGTC
		TTAAGCCACAACACAGGATG
AGT	AGT	TAGATCTCTCAGCTATTACAAGG
		GTTTCAGAGAAACTGACCTGTGG
	D1S1656	GTGTTGCTCAAGGGTCAACT
		GAGAAATAGAATCACTAGGGAACC
ACE; DCP1	D17S944	GCCCAGGAGGTTGAGACTT
		CCTTCCATAGGAACGGCT
	D17S1874	TAGGAGGCAGCAATGG
		TGTGGTATGTGTGGCA
AT1 angiotensin II receptor	AGTR1	AGGAGAAATGTTCCAAGGGACAA
		GTATTCCATGTGAAACAGCTCCA
	D3S3626	CCAGAATTATCTGTGACTCCC
		CCTATGGTGGAGGGTGAG

these patients. To definitively rule out linkage to *CYP11B2*, we examined three highly polymorphic microsatellite markers near the gene in the two consanguineous kindreds (Table 1, Fig. 1). In addition, these microsatellite markers were examined in the two patients who were siblings to see if they shared both haplotypes at this locus.

In fact, both consanguineous patients were heterozygous for at least one microsatellite near *CYP11B2*, effectively excluding *CYP11B2* from linkage to the disease in these kindreds. However, the two affected sibs carried identical alleles at each of these markers (a 1/4 chance occurrence), so we cannot formally eliminate the possibility that we missed mutations in *CYP11B2* in this kindred or in the other nonconsanguineous patient, in whom we had no way of testing for linkage.

We considered whether these patients might have had a known disorder other than aldosterone synthase deficiency. Although salt-wasting forms of congenital adrenal hyperplasia are relatively common causes of hyperreninemic hypoaldosteronism, all of our patients had normal cortisol levels, rendering this diagnosis unlikely. In particular, the normal 17-hydroxyprogesterone levels obtained in four patients rule out 21-hydroxylase deficiency, by far the most common salt-wasting form of congenital adrenal hyperplasia (18).

Normal cortisol biosynthesis was further demonstrated by cosyntropin stimulation testing and/or normal ACTH levels documented at 9 months of age or later (not shown). This suggests that none of these patients had an autosomal recessive form of adrenal hypoplasia congenita. This was important to rule out because aldosterone deficiency often presents before cortisol deficiency in this disorder (19).

Thus, it seems likely that these patients have a distinct autosomal recessive disorder that we term familial hyperreninemic hypoaldosteronism type 2, or FHHA2, to distinguish it from aldosterone synthase deficiency (FHHA1). This probably results from one or more defects in regulation of aldosterone biosynthesis. In principle, defects in genes encoding components of the renin-angiotensin system might be expected to interfere with aldosterone biosynthesis; such genes include those encoding angiotensinogen (*AGT*), angiotensin converting-enzyme (*ACE*, *DCP1*) and the AT1 type

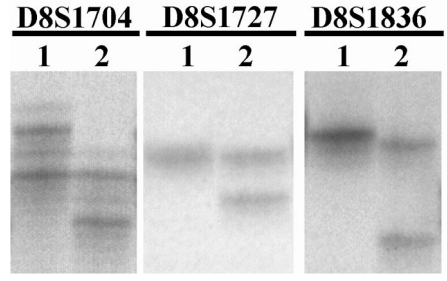

FIG. 1. Typing two consanguineous patients (1 and 2) for microsatellite polymorphisms closely linked to *CYP11B2*. Patient 1 is heterozygous for microsatellite D8S1704, whereas patient 2 is heterozygous for all three polymorphisms.

angiotensin II receptor. To evaluate this possibility, we typed the two consanguineous patients for two microsatellite polymorphisms near each of these genes. All patients were heterozygous for at least one marker at each locus, excluding these genes from linkage to the disease (not shown). In addition, we were able to exclude these genes from linkage to the disease in the affected sib pair, because the sibs carried different alleles at each of these loci.

Moreover, each of the corresponding genes has been knocked out in mice, and none of the knockouts have a phenotype of electrolyte abnormalities or hypoaldosteronism; instead, all are hypotensive (20–22). The lack of electrolyte abnormalities is consistent with the fact that aldosterone secretion is potently stimulated by hyperkalemia, which should compensate for the lack of stimulation by angiotensin II. As regards signaling steps downstream from the AT1 receptor, this receptor signals through G protein-coupled mechanisms that raise intracellular calcium levels (23, 24). These mechanisms are common to many cell types, and mutations affecting them would be expected to have much broader phenotypes than isolated aldosterone deficiency.

Possibly FHHA2 involves a *trans* acting factor affecting *CYP11B2* expression and/or development of the adrenal zona glomerulosa. At present, these factors are still being defined, and none have been identified that are uniquely expressed in the zona glomerulosa.

FHHA2 may be more common than aldosterone synthase

deficiency (FHHA1). Other than in Iranian Jewish kindreds, who all carry the same mutations (8), we failed to detect mutations in 4 of the 7 kindreds with hyperreninemic hypoaldosteronism we examined (unpublished observations). Similarly, no mutations affecting enzymatic activity could be detected in *CYP11B2* in 4 of 11 published kindreds with apparent aldosterone synthase deficiency (9–15, 25–27). Considering the likelihood of bias against publishing negative results, this proportion of kindreds without *CYP11B2* mutations is probably an underestimate. However, none of the published kindreds without detectable *CYP11B2* mutations were consanguineous, and so linkage to *CYP11B2* could not be formally excluded by the method used in the present study.

Elucidation of the cause(s) of FHHA2 awaits ascertainment of one (or more) consanguineous kindred(s) with enough affected individuals that homozygosity mapping can be used to determine the chromosomal location(s) of the affected gene(s).

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