Autosomal Dominant Pseudohypoaldosteronism Type 1: Mechanisms, Evidence for Neonatal Lethality, and Phenotypic Expression in Adults

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Autosomal dominant pseudohypoaldosteronism type 1 (adPHA1) is a rare condition that is characterized by renal resistance to aldosterone, with salt wasting, hyperkalemia, and metabolic acidosis. It is thought of as a mild disorder; affected children’s symptoms respond promptly to salt therapy, and treatment is not required after childhood. Mutations in the mineralocorticoid receptor gene (MR) cause adPHA1, but the long-term consequences of MR deficiency in humans are not known. Herein are described six novel adPHA1-causing MR mutations (four de novo) and evidence that haploinsufficiency of MR is sufficient to cause adPHA1. Furthermore, genotype–phenotype correlation is reported in a large adPHA1 kindred. A number of cases of neonatal mortality in infants who were at risk for adPHA1 were identified; coupled with the frequent identification of de novo mutations in affected individuals, this suggests that the seemingly benign adPHA1 may have been a fatal neonatal disorder in previous eras, preventing propagation of disease alleles. In contrast, it is shown that adult patients with adPHA1 are clinically indistinguishable from their wild-type relatives except for presumably lifelong elevation of renin, angiotensin II, and aldosterone levels. These data highlight the critical role of MR in the maintenance of salt homeostasis early in life and illuminate the sodium dependence of pathologic effects of renin and angiotensin II. They furthermore argue that nongenomic effects of aldosterone play no significant role in the long-term development of cardiovascular disease.

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ceptor from binding to its cognate ligand aldosterone, have been identified (6–9).

Although mutations in MR are known to cause adPHA1, many questions remain as to how the identified mutations cause the adPHA1 phenotype. The identified gene mutations all cause either a premature termination of translation of the peptide or a loss of ligand binding (5–10). Is haploinsufficiency, the loss of one of two functional MR alleles, sufficient to cause the adPHA1 phenotype, or does the mutant peptide inhibit the function of the wild-type allele as a dominant negative allele? Similarly, why does the disease process remit after the neonatal period, and what are the clinical consequences of disease, if any, in adulthood? To clarify these issues, we characterized further adPHA1 kindreds and identified six novel disease-causing mutations in MR in seven kindreds. Furthermore, we extended these and other previously described adPHA1 kindreds to gain insight into the underlying physiology of disease in patients with adPHA1.

Materials and Methods

Patients

All clinical studies were approved by the Yale Human Investigation Committee. Kindreds PHA15 and PHA16 have been described previously (5). Although not known to be related, these families live in the same village in northwestern Spain and were found to carry the identical R537X disease-causing mutation. We extended these pedigrees by recruiting relatives of known adPHA1 carriers. We prospectively extending these and other previously described adPHA1 kindreds to gain insight into the underlying physiology of disease in patients with adPHA1.

Table 1. Clinical characteristics of PHA1 index patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Symptom</th>
<th>Na (mEq/L)</th>
<th>K (mEq/L)</th>
<th>Cl (mEq/L)</th>
<th>HCO3 (mEq/L)</th>
<th>PRA (ng/ml per h)</th>
<th>Aldo (ng/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHA47-1</td>
<td>18 d</td>
<td>Failure to thrive</td>
<td>114</td>
<td>9.7</td>
<td>93</td>
<td>22</td>
<td>15176</td>
<td>1035</td>
</tr>
<tr>
<td>PHA51-1</td>
<td>1 mo</td>
<td>Failure to thrive</td>
<td>130</td>
<td>6</td>
<td>101</td>
<td>18.3</td>
<td>&gt;150</td>
<td>2048</td>
</tr>
<tr>
<td>PHA67-1</td>
<td>5 d</td>
<td>Failure to thrive</td>
<td>114</td>
<td>5.7</td>
<td>81</td>
<td>21</td>
<td>179</td>
<td>400</td>
</tr>
<tr>
<td>PHA72-1</td>
<td>4 wk</td>
<td>Failure to thrive</td>
<td>111</td>
<td>6.7</td>
<td>77</td>
<td>20</td>
<td>NA</td>
<td>&gt;300</td>
</tr>
<tr>
<td>PHA76-1</td>
<td></td>
<td>Failure to thrive</td>
<td>133</td>
<td>6.4</td>
<td>105</td>
<td>19</td>
<td>119.2</td>
<td>1382</td>
</tr>
<tr>
<td>PHA82-1</td>
<td>20 d</td>
<td>Failure to thrive</td>
<td>126</td>
<td>6</td>
<td>96</td>
<td>16</td>
<td>59.6</td>
<td>397</td>
</tr>
</tbody>
</table>

*Clinical data from PHA1 patient presentation. Normal ranges: Na+, 136 to 144 mM; K+, 3.5 to 5.0 mM; PRA, 0.5 to 3.0 ng/ml per h; aldosterone, 5 to 90 ng/dl (1 to 12 mo). Aldo, serum aldosterone; NA, not available; PHA1, pseudohypoaldosteronism type 1; PRA, plasma renin activity.
presented in the first month of life with failure to thrive, hyponecetria, and hyperkalemia despite markedly elevated renin and aldosterone levels. Each improved rapidly after the administration of sodium supplementation and resumed normal growth and development. Patient PHA67-1 was remarkable in that, in addition to the PHA1 phenotype, she presented with microcephaly and neurodevelopmental delay; her mother, who was hospitalized as an infant for severe feeding difficulties and vomiting, also has mental retardation with microcephaly.

Citing exons in MR were screened with DHPLC, and identified variants were sequenced directly. In this way, MR were identified in each PHA1 kindred (Figure 1). Patient PHA51-1 is heterozygous for a single base pair insertion in exon 6 that converts Tyr503 to an immediate stop codon (Figure 1A). Patient PHA72-1 (Figure 1B), like affected individuals from kindred PHA30 (data not shown), is heterozygous for a nonsense mutation in exon 3 that alters R590 to an immediate stop codon. The index case in kindred PHA67 and her mother are heterozygous for a nonsense G→T substitution at the final base pair in exon 5 (Figure 1C); although the expected effect of this mutation is conversion of the codon GGA coding for G589 to an immediate TGA stop codon, sequencing of MR cDNA from the index patient and her mother revealed that the actual effect of this mutation is to disrupt normal exon 5 splicing and unveil a cryptic splice site 10 bp 5′ to the normal exon 5 splice donor site, resulting in a frameshift (data not shown). Patient PHA82-1 is heterozygous for a single nonsense mutation in exon 9 that converts Q967 to an immediate stop codon (Figure 1D); this mutation is predicted to truncate helix 12, a crucial domain for steroid hormone receptor function. Affected individuals in kindred PHA76 are heterozygous for an insertion of alanine at nucleotide 2681 that results in a frameshift after H821 and a stop codon 14 amino acids later (Figure 1E). Finally, patient PHA47-1 is heterozygous for a missense mutation in exon 6 that converts serine 818 to leucine (Figure 1F). This mutation lies in the center of the hormone-binding domain of the receptor; to determine whether mutation had a functional effect on the activity of the receptor, we tested the ability of this receptor to activate transcription of a reporter gene in response to aldosterone (see Materials and Methods). In contrast to the wild-type receptor, which showed normal activation in the presence of aldosterone, we saw no activity of the S818L mutant receptor, indicating that this mutation disrupts MR activity, thereby identifying codon 818 as an essential residue for normal receptor function (Figure 1G).

The identified mutations are summarized in Table 2. Each of these mutations was not identified in 180 control chromosomes, indicating that these are not common polymorphisms. Moreover, four of the six mutations are de novo mutations, as they were absent in the parents of affected individuals. To confirm that these were in fact the biologic parents of the affected patients, we performed genotypic analysis of the parents of the index cases in kindreds PHA47, PHA51, and PHA82 and of the parents of the affected mother in kindred PHA67 using 10 or more unlinked short tandem repeat markers (see Materials and Methods). In each case, parentage was confirmed, indicating that the mutations in their children are true de novo mutations.

The identification of de novo mutations in the affected children of unaffected parents in a gene that previously was linked to the disease offers conclusive evidence that these mutations are disease causing.
Haploinsufficiency of MR Is Sufficient to Cause the adPHA1 Phenotype

PHA30 is an adPHA1 kindred that has been well described previously (13). The kindred is composed of nine affected individuals in three generations with a diagnosis of adPHA1 on the basis of findings of salt wasting and hyperkalemic metabolic acidosis despite markedly elevated serum aldosterone levels (Figure 2A). As noted above, analysis of genomic DNA in this kindred identified a heterozygous C<sup>3</sup>T substitution that converts R590 to a stop codon in all affected individuals. We gained insight into the underlying mechanisms of adPHA1 through further studies of kindred PHA30. We sequenced cDNA that was prepared from RNA that was freshly isolated from peripheral blood lymphocytes from patient PHA30-1 and compared this with genomic sequence. As expected, genomic DNA was heterozygous for the C<sup>1984T</sup> substitution. In contrast, the cDNA sequence showed evidence of only the wild-type cytosine (Figure 2B). Although it would have been interesting to reconfirm this finding via analysis of mRNA from an epithelial tissue, such as the skin or salivary glands, from this patient, we did not have access to such tissue. Nevertheless, the absence of the mutant mRNA in lymphocytes suggests that it is degraded by cellular mechanisms that prevent translation of mRNA with stop codons outside the last exon via nonsense mediated decay (15); we therefore infer that MR haploinsufficiency is sufficient to cause the adPHA1 phenotype in this kindred.

Genotype–Phenotype Correlation in adPHA1

The determination that MR haploinsufficiency causes the adPHA1 phenotype suggests that MR receptor quantity can have important effects on renal sodium handling, particularly in the neonatal period. However, there are few data on the effect of MR deficiency in adults. The ability to assign unambiguously affection status on the basis of genotype allowed us to perform genotype–phenotype correlation studies in extended adPHA1 kindreds to gain insight into this question. Kindreds PHA15 and PHA16 have been described previously (5); although not known to be related, these families live in the same village in northwestern Spain and carry the identical R537X mutation in MR. We extended the pedigrees by recruiting relatives of known adPHA1 carriers (see Materials and Methods). In total, 35 family members were studied; 14 individuals carried the mutation, and 21 individuals did not (Figure 3). In kindred PHA15, only the proband had been symptomatic with adPHA1, having presented at 8 mo of age with a clinical history of anorexia, vomiting, salt wasting, and failure to thrive. At the time, his weight and height were below the 3rd percentile, and he was started on 3 g/d Na<sup>+</sup>. He remained at the 3rd percentile for 1 yr and then exhibited catch-up growth, reaching the 25th percentile by age 3 and the 50th percentile by age 16 off

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Table 2. Mutations identified in PHA1 kindreds<sup>a</sup>

<table>
<thead>
<tr>
<th>Patient</th>
<th>Mutation</th>
<th>Mutation Effect</th>
<th>De Novo Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHA47-1</td>
<td>C2669T</td>
<td>S818L</td>
<td>Yes</td>
</tr>
<tr>
<td>PHA51-1</td>
<td>InsA1715</td>
<td>Y503X</td>
<td>Yes</td>
</tr>
<tr>
<td>PHA67-1</td>
<td>G2581A</td>
<td>Splice alteration</td>
<td>Yes&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>PHA72-1</td>
<td>C1984T</td>
<td>Arg590X</td>
<td>No</td>
</tr>
<tr>
<td>PHA30-1</td>
<td>C1984T</td>
<td>Arg590X</td>
<td>No</td>
</tr>
<tr>
<td>PHA76-1</td>
<td>InsA2681</td>
<td>fsH821</td>
<td>No</td>
</tr>
<tr>
<td>PHA82-1</td>
<td>C3115T</td>
<td>Q967X</td>
<td>Yes</td>
</tr>
</tbody>
</table>

<sup>a</sup>Each of the indicated patients was found to be heterozygous for the indicated mineralocorticoid receptor genomic sequence mutation. fs, frameshift; Ins, insertion.

<sup>b</sup>Whereas patient PHA67-1 inherited the G2581T mutation from her mother, the mother’s mutation was de novo.
sodium supplementation. The remaining genotypically affected individuals in PHA15 were asymptomatic.

The proband in kindred PHA16 presented at age 4 mo with signs of dehydration. Her younger siblings each received a diagnosis of adPHA1 as neonates because of family history and poor growth. The remaining five genotypically affected individuals in the pedigree were asymptomatic. Importantly, four at-risk children in kindred PHA16 died at an early age; one child died of burns at the age of 3 yr, and three other children (ages 1 yr, 15 d, and 8 d, respectively) died of unknown causes. No tissue was available for genotypic evaluation of these infants.

We studied various clinical indices of aldosterone activity in affected and unaffected adults in these kindreds (Figure 4). We found no significant difference in systolic or diastolic BP, serum sodium, serum potassium, fractional excretion of sodium, or transtubular potassium gradient between affected individuals and their unaffected relatives. The only biochemical marker of disease was a dramatically elevated serum aldosterone level (Figure 4). Mean serum aldosterone levels were 126 ± 31.2 ng/dl in affected individuals, compared with 8.8 ± 1.4 ng/dl in unaffected individuals (P < 0.0001). Random seated serum aldosterone levels proved to be an excellent marker for affection status: Individuals with aldosterone levels >30 ng/dl carry the R537X mutation, whereas individuals with aldosterone levels <30 ng/dl are genotypically unaffected.

Discussion

We previously showed that heterozygous loss-of-function mutations in the human mineralocorticoid receptor cause adPHA1 (5). With the inclusion of the kindreds described here, 22 different disease-causing mutations in MR have been identified in 24 adPHA1 kindreds. It has been proposed that adPHA1 is genetically heterogeneous on the basis of the failure to identify disease-causing mutations in MR after sequencing of
all exons in patients with adPHA1 (7,16). However, nonexonic mutations or large deletions would not be detected by these means, and linkage analysis, which could have excluded the gene definitively, was not performed. Therefore, disease-causing mutations in MR cannot be ruled out. Indeed, Sartorato et al. (17) ruled out an exonic mutation in an adPHA1 kindred by sequence analysis, but linkage analysis ultimately led to the identification of a large disease-causing deletion in MR. In our own experience, we have identified MR mutations in seven of nine kindreds with evidence of dominant disease transmission, and we could not exclude linkage to MR in the remaining kindreds. Consequently, we believe that mutation in MR represents the principal, if not the only, cause of adPHA1.

Haploinsufficiency for MR Is Sufficient to Cause the adPHA1 Phenotype

Many human diseases that are caused by loss-of-function mutations in nuclear receptors have been described, but the mechanism of disease differs for each receptor. For example, whereas glucocorticoid receptor (NR3C1) mutations cause familial cortisol resistance (OMIM #138040) via receptor haploinsufficiency (18,19), mutations in the thyroid hormone receptor cause generalized thyroid hormone resistance (OMIM #188570) via a dominant-negative mechanism; the mutant receptor interferes with wild-type receptor by forming a transcriptionally inactive dimer (20,21). We found wild-type but not mutant MR mRNA in peripheral blood lymphocytes of a patient with adPHA1. The absence of mutant RNA likely is due to nonsense-mediated decay, a process by which mRNA that bear premature stop codons in nonterminal exons are degraded by the cell (15). This suggests that patients who have adPHA1 and bear premature stop codons in MR, the majority of patients described, are also haploinsufficient. Although we cannot rule out other mechanisms of action by which other MR mutations cause adPHA1 in other families, it is clear that haploinsufficiency is sufficient to cause the adPHA1 phenotype. Furthermore, the finding that MR haploinsufficiency can cause a measurable salt-wasting phenotype suggests that early in life, the kidney cannot upregulate expression of the wild-type MR allele sufficiently to normalize sodium balance and that mineralocorticoid activity is limited in part by receptor levels.

Genotype–Phenotype Correlation in adPHA1

The MR has been ascribed a wide variety of physiologic roles. The study of individuals who lack one of two functional copies of MR provides a unique opportunity to study its role in human physiology. This study represents the first detailed physiologic analysis of patients with adPHA1 confirmed to have a disease-causing mutation in MR. By performing genotype–phenotype correlation studies in extended adPHA1 kindreds, we have gained insight into MR’s role(s) in human physiology. A number of conclusions regarding MR can be drawn from our findings.

adPHA1: A Fatal Neonatal Disorder?

We found striking phenotypic diversity in patients who had adPHA1 that was caused by MR mutation. adPHA1 generally is regarded to be a mild disease, and consistent with this, some of the adult patients whom we studied were entirely asymptomatic with no recollection of significant illness as infants. Conversely, the probands each were hospitalized for salt wasting, growth retardation, and failure to thrive. It is not clear why some infants come to clinical attention and others do not, but we have previously suggested that infants with intercurrent illness that impairs oral intake or produces diarrhea may be more likely to become symptomatic (5). Alternatively, it may be that most infants with adPHA1 do present, and only infants with genetic or environmental advantages survive without active intervention.

It is noteworthy that four of the patients reported here as well as one patient reported previously carry confirmed de novo mutations (5). Furthermore, two previously described patients with adPHA1 have MR mutations that are not carried by either parent, although formal genotyping to prove parental identity was not performed (7,8). Therefore, up to seven of the 22 MR mutations that have been reported may have arisen de novo. The high incidence of de novo mutations suggests impaired reproductive fitness in patients with adPHA1. Analysis of the few extended adPHA1 pedigrees available suggests no obvious impairment of fertility or failure to transmit the disease allele (Figures 1 and 3) (5,6,8–10), suggesting that fertility concerns do not account for the observed impairment in disease transmission. In contrast, we observed a number of deaths in infants who were at risk for adPHA1 (Figure 3B), a finding observed by others as well (22). Although we have no genotypic information on the deceased infants, the frequency of unexplained neonatal death in these kindreds suggests that adPHA1 is potentially lethal in neonates, and we infer that the impaired reproductive fitness most likely is due to neonatal death of these children. We believe that this inference, based on available but admittedly incomplete information, is compelling enough to recommend the prophylactic provision of salt to at-risk infants until a definitive diagnosis can be made. Furthermore, if this hypothesis is correct, we believe that in the modern medical era, more such children will survive to procreate; therefore, the incidence of the disease may well increase, forcing neonatologists to be aware of the potential perinatal dangers. The clinical severity of disease in patients with adPHA1 early in life highlights the essential role of aldosterone-sensitive sodium transport in the neonate.

adPHA1 in Adults

The findings presented also give insight into renin-angiotensin system in adult physiology. Despite an intrinsic salt-wasting predisposition, adults with adPHA1 are clinically indistinguishable from their unaffected relatives. We found no significant differences between these groups with regard to systolic BP, diastolic BP, serum sodium, serum potassium, urine sodium, and urine potassium. These patients maintain normal volume homeostasis via an approximately 14-fold elevation in serum aldosterone levels, suggesting that homeostatic mechanisms that regulate sodium balance remain intact in these individuals at the expense of a marked increase in aldosterone levels. Whether these individuals would struggle to
cope with salt deprivation or severe volume depletion is not known.

Angiotensin II has been proposed as a key molecule in the pathogenesis of hypertension, left ventricular hypertrophy, and myocardial fibrosis (23,24). Our patients had markedly elevated renin levels and presumably angiotensin II levels contributing to hyperaldosteronemia, yet we found no evidence of hypertension or congestive heart failure in affected individuals. This suggests that the effect of angiotensin II on long-term BP regulation and on myocardium is at least partially if not completely load dependent and that in the absence of aldosterone-induced volume expansion, no significant hypertension or heart failure is seen. Similarly, much has been written about nongenomic effects of aldosterone, effects of aldosterone not mediated via MR (25). The absence of significant cardiovascular findings in our patients with adPHA1, in whom serum aldosterone levels have been elevated consistently throughout their lives, suggests that nongenomic effects of aldosterone do not lead to clinically relevant cardiovascular sequelae either. We cannot exclude, however, that nongenomic effects of aldosterone affect short-term cardiovascular responses.

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