Pseudohypoparathyroidism: Inheritance of deficient receptor-cyclase coupling activity

(dominant inheritance/recessive inheritance/adenylate cyclase)

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ABSTRACT Pseudohypoparathyroidism, type I (PHP-I) is an inherited disorder of primary resistance to multiple hormones that work by stimulating adenylate cyclase. In an attempt to clarify the mode of inheritance of PHP-I, we measured the activity of the N protein, a receptor-cyclase coupling component, in erythrocyte membranes. Erythrocyte N-protein activity was reduced by ≥50% in erythrocytes of 15 PHP-I patients and was normal in 19 of their clinically normal first degree relatives. Reduced N-protein activity and the PHP-I phenotype in these families both dominant and recessive patterns of inheritance. This suggests that at least two distinct genetic loci are involved in inheritance of N-protein deficiency. In two additional families, dominant inheritance of the PHP-I phenotype was associated with normal activities of erythrocyte N protein. Thus, it appears that mutation of at least one additional genetic locus, not involving the N protein, can produce PHP-I.

Pseudohypoparathyroidism, type I (PHP-I), is an inherited disease characterized by resistance to the metabolic effects of parathyroid hormone (PTH) (1,2) and other hormones (3-6) that act by stimulating adenylate cyclase. Reported pedigrees of families that have PHP-I have suggested X-linked dominant (7), autosomal dominant (8), and recessive (9) inheritance of the disease. A reliable biochemical marker, closely related to the pathogenesis of PHP-I, should help to clarify this confusing picture.

Two recent reports (10,11) have described a biochemical defect in erythrocytes of PHP-I patients: partially deficient activity of a membrane protein, termed N, that is required for functional coupling of hormone receptors and catalytic adenylate cyclase. Generalized N-protein deficiency in endocrine target organs of PHP-I patients is an attractive hypothesis to explain the pathogenesis of the disease.

We have used erythrocyte N-protein activity as a biochemical marker to investigate the inheritance of PHP-I. The results suggest that erythrocyte N-protein deficiency and the clinical disease can be inherited by either dominant or recessive transmission. In addition, we report studies of two families in which clinically evident PHP-I is associated with quantitatively and qualitatively normal erythrocyte N-protein activities. These findings demonstrate the biochemical and genetic heterogeneity of PHP-I and suggest that mutations of at least three genetic loci can produce the disease.

METHODS

Informed Consent. Blood was drawn by venipuncture with the informed consent of both the patients and the normal volunteers. Consent was obtained by procedures approved by Committees on Human Research of the University of California, San Francisco; the Sepulveda Veterans Administration Medical Center; the University of California, Los Angeles; and the Massachusetts General Hospital.

Assays of Erythrocyte N Protein. We assayed N-protein activity in erythrocyte ghosts by two assays, exactly as described (10). In the first assay, we measured the ability of detergent-soluble extracts of erythrocyte ghosts to complement the N-protein deficiency of cyc−S49 mouse lymphoma membranes in vitro by using 100 μM isoproterenol and 100 μM guanosine-5′-O-(3-thiotriphosphate) (GTPγS) to stimulate adenylate cyclase, exactly as described (10). In the second assay, we measured the choleratoxin-catalyzed transfer of [32P]ADP-ribose from [32P]NAD to the 42,000-dalton peptide subunit of N protein in erythrocyte membranes. Results for both assays are expressed as a percentage of the activity of a standard erythrocyte membrane preparation formed by pooling membranes from erythrocytes of normal subjects (10).

In some experiments, we assessed N-protein activity in the complementation assay with other stimulators of adenylate cyclase, including 100 μM GTP (with or without isoproterenol), 10 μM prostaglandin E1 (PGE1), and 10 mM NaF. We used either of two detergents: 0.2% Lubrol 12A9, as described (10), or 0.5% sodium cholate/1.0 M NaCl. With the second detergent, reconstitution of cyc− membranes was performed by using a modification of the method described by Sternweis and Gilman (12). In this procedure, 0.5-1.0 M NaCl was present throughout the extraction and reconstitution procedure. Adenylate cyclase activity in reconstituted cyc− membranes was linear with increasing amounts of active cholate extract if it was supplemented with decreasing amounts of a cholate extract that had been heated at 90°C for 10 min to destroy N-protein activity. The total amount of active and inactive cholate extract was always 40% of the total volume.

RESULTS

We will first describe findings in PHP-I patients whose erythrocytes showed reduced N-protein activities and then in a family in whom no erythrocyte defect was found.

PHP-I with Reduced Erythrocyte N-Protein. These 10 patients (Table 1) exhibited the typical clinical and laboratory fea-

Abbreviations: PHP-I, pseudohypoparathyroidism, type I; PTH, parathyroid hormone; N protein, guanine nucleotide-binding regulatory component of adenylate cyclase; GTPγS, guanosine-5′-O-(3-thiotriphosphate); PGE1, prostaglandin E1; cAMP, cyclic AMP; cyc− and unc, N-deficient and receptor-uncoupled mutants of S49 mouse lymphoma, respectively.

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Table 1. Clinical and laboratory features of 10 PHP-I patients

<table>
<thead>
<tr>
<th>Family</th>
<th>Patient</th>
<th>Relationship</th>
<th>Age/sex</th>
<th>Skeletal abnormalities</th>
<th>Serum Ca</th>
<th>PTH response (urinary cAMP)</th>
<th>Hypothyroidism</th>
<th>Treatment</th>
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<tbody>
<tr>
<td>G</td>
<td>17</td>
<td>Propositus</td>
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<td>↓</td>
<td>N</td>
<td>D</td>
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<tr>
<td>H</td>
<td>18</td>
<td>Sister</td>
<td>35 F</td>
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<td>+</td>
<td>D</td>
</tr>
<tr>
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<td>↑</td>
<td>+</td>
<td>D</td>
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<tr>
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<td>+</td>
<td>D</td>
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<tr>
<td>I</td>
<td>21</td>
<td>Sister</td>
<td>14 F</td>
<td>S, B, C</td>
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<td>↑</td>
<td>+</td>
<td>D, T</td>
</tr>
<tr>
<td>J</td>
<td>22</td>
<td>Propositus</td>
<td>41 F</td>
<td>S, B</td>
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<td>+</td>
<td>D, T</td>
</tr>
<tr>
<td>K</td>
<td>23</td>
<td>Mother</td>
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<td>S, B, C</td>
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<td>ND</td>
<td>N</td>
</tr>
<tr>
<td>L</td>
<td>24</td>
<td>Propositus</td>
<td>32 F</td>
<td>S, B</td>
<td></td>
<td>ND</td>
<td>ND</td>
<td>N</td>
</tr>
<tr>
<td>M</td>
<td>25</td>
<td>Sister</td>
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<td>N</td>
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<td>S, B, C</td>
<td></td>
<td>↑</td>
<td>ND</td>
<td>N</td>
</tr>
</tbody>
</table>

S, Short stature; B, brachydactyly present; C, subcutaneous calcifications present; N, normal; D, vitamin D preparation; T, thyroid hormone preparation. Criteria for clinical hypothyroidism (indicated by +), decreased serum calcium, increased serum PTH, and decreased urinary cAMP response to PTH (indicated by vertical arrows) were exactly as detailed in ref. 10; ND, not done.

Skeletal and laboratory features of PHP-I—short stature and brachydactyly (all 10); hypocalcemia requiring vitamin D therapy (9 of 10); decreased urinary excretion of cyclic AMP (cAMP) in response to infusion of PTH (all of 6 tested); elevated serum PTH (5 of 8 tested). One patient (G-17, Table 1), a man whose resistance to PTH was described in the report by Albright et al. (1), subsequently underwent parathyroidectomy; his serum PTH now is low, and he continues to require treatment with vitamin D. Five patients required thyroid hormone replacement therapy for hypothyroidism.

By both the in vitro complementation and chola toxin-catalyzed radiolabeling assays, N-protein activity was substantially reduced in erythrocytes of these 10 patients (filled circles, Fig. 1), in comparison with that of erythrocytes of 16 normal subjects. For statistical comparison, these values were combined with those of five previously reported patients who have classical PHP-I (patients 1-5 in ref. 10; filled triangles in Fig. 1). In the complementation assay, erythrocytes of 15 PHP-I patients exhibited 57 ± 9% (mean ± SD) of the standard activity, as compared with 105 ± 12% in 16 normal subjects (P << 0.001). In the radiolabeling assay, erythrocytes of PHP-I patients showed 55 ± 9% of standard activity, compared with normal values of 110 ± 24% (P << 0.001).

One of these patients (J-22, see Table 1) fits the description (13) of "pseudo-PHP." The mother of a daughter who has classical PHP-I, this patient had a round face, striking brachydactyly of fingers and toes, and short stature, but no history of hypocalcemia. In spite of her apparently normal endocrine status, this patient exhibited reduced erythrocyte N-protein activity by both the complementation and radiolabeling assays (42% and 41% of standard, respectively).

We studied erythrocytes of 19 clinically normal first degree relatives of eight patients (all except patients K-23 and L-24). In every case, clinically normal relatives had normal erythrocyte N-protein activities (96 ± 11% of standard for 19 samples tested in the complementation assay; 102 ± 19% of standard for 14 samples tested in the radiolabeling assay; these means are not statistically different from those of the normal subjects tested). Thus, reduced erythrocyte N-protein activity correlated perfectly with clinical appearance of the PHP-I phenotype in these families.

Pedigrees of the I and J families (Fig. 2) suggest contrasting modes of inheritance of both the PHP phenotype and erythrocyte N-protein deficiency. In the J family, the pattern is consistent with inheritance of a dominant phenotype, first appearing in the mother (patient J-22) described above. In the I family, two siblings have PHP-I and erythrocyte N-protein deficiency, although both parents are normal both clinically and by measurements of erythrocyte N protein. This pattern is consistent with inheritance of a recessive defect, as noted in a previous report of the I family (9). In that report, incorrect paternity of the affected children was shown to be exceedingly unlikely, on the basis of testing for a variety of genetic markers, including blood groups, HLA antigens, serum proteins, and erythrocyte enzymes. Consanguinity in this family was denied, and the parents came from diverse geographic and ethnic backgrounds (9).

**PHP-I with Normal Erythrocyte N Protein.** Patients in another family who had classical PHP-I had normal erythrocyte N-protein activities, by both assays used (Table 2). The propositus showed skeletal abnormalities, including brachydactyly,

![Fig. 1. Erythrocyte N-protein activities of normal subjects (open symbols) and 15 PHP-I patients (closed symbols). (Left) N-protein activity as assessed by ability of erythrocyte membrane extracts to complement adenylate cyclase in N-protein deficient cell membranes. (Right) Incorporation of [32P]ADP-ribose from [32P]NAD into the 42,000-dalton peptide subunit of N protein; this reaction is catalyzed by chola toxin. Results are normalized to a standard membrane preparation (see Methods). Closed circles represent values for patients described in Table 1, and closed triangles represent values for patients 1-5 described in ref. 10. Bars represent the mean ± SD for each group.**

She suffered from several grand mal seizures 11 years ago, but serum calcium and skull x-rays were normal. Anticonvulsant therapy with phenytoin was started then and discontinued 5 years ago. Seizures have not recurred, and she remains in excellent health.
none of these patients showed a detectable deficiency of erythrocyte N protein.

**Qualitative Defect in N Protein?** We previously reported a kindred of PHP-I patients (family E in ref. 10) who exhibited hypocalcemia, PTH resistance, and hypothyroidism. By both the biochemical assays we used, erythrocytes of PHP-I patients in the E family, like those of the F family described above, contained N-protein activities similar to those of normal subjects. Could this be due to a qualitative defect in the N protein of these two families not detected by the complementation and ADP-ribosylation assays?

A defect in the N protein of a variant mouse lymphoma tissue culture line, termed unc, provides a precedent for such a qualitative lesion: the N protein in unc membranes contains apparently normal amounts of cholera toxin substrate peptides; both in the unc membrane and in complementing the defect of cyc–, N protein mediates normal stimulation of adenylate cyclase by all effectors except hormones (β-adrenergic amines and PGE) (14). We therefore asked whether N protein from erythrocytes of patients in the E and F families is partially “uncoupled” with respect to its ability to mediate stimulation of cAMP synthesis by hormones in the complementation assay.

The results of several experiments (Table 3) provide no support for the notion that the N protein in either the E or the F family bears a functional lesion similar to that of unc: N-protein activity solubilized by two different detergents from E and F patients’ erythrocytes exhibited quite normal capacity to mediate stimulation of adenylate cyclase by isoproterenol and PGE and by guanine nucleotides and fluoride ion as well.

We also found that N protein from erythrocytes of two “low N” patients (J-21 and I-19) was qualitatively normal by these criteria, although its activity was quantitatively reduced (Table 3, experiment 3). Erythrocyte extracts from these two patients exhibited proportionately the same reduction in ability to mediate cAMP synthesis in the complementation assay, regardless of the effector used.

Because mutations can produce peptide gene products that have altered sensitivity to inactivation by heat, we tested the thermolability of N-protein activity from erythrocytes of PHP-I patients. Heating Lubrol 12A9 extracts of normal erythrocytes at 45°C in the presence of guanylyl-5’-imidodiphosphate, a guanine nucleotide analog, caused an exponential decay in the capacity of the extracts to complement cAMP synthesis in N-protein-deficient cyc– membranes, with a t½ averaging 12 min. Under the same conditions, decay of N-protein activity from erythrocytes of 10 “low N” PHP-I patients and six “normal N” PHP-I patients (from families E and F) was similar to that of

### Table 2. Family F: Clinical characteristics and erythrocyte values

<table>
<thead>
<tr>
<th>Patient</th>
<th>Relationship</th>
<th>Age/sex</th>
<th>Skeletal abnormalities</th>
<th>Serum Ca</th>
<th>Serum PTH</th>
<th>PTH response (urinary cAMP)</th>
<th>Hypothyroidism</th>
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<th>Complementation</th>
<th>Radio-labeling</th>
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<td>D, T</td>
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<td>106</td>
<td></td>
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<td>12</td>
<td>Mother</td>
<td>45 F</td>
<td>S</td>
<td>↓↑</td>
<td>N</td>
<td>+</td>
<td>D, T</td>
<td>87</td>
<td>93</td>
<td></td>
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<tr>
<td>13</td>
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<td>S</td>
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<td>N</td>
<td>+</td>
<td>T</td>
<td>96</td>
<td>ND</td>
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<tr>
<td>14</td>
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<td>T</td>
<td>75</td>
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<tr>
<td>15</td>
<td>Son</td>
<td>2.5 M</td>
<td>N*</td>
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<td>ND</td>
<td>±</td>
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<td>134</td>
<td></td>
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<tr>
<td>16</td>
<td>Son</td>
<td>1.5 M</td>
<td>N N</td>
<td>ND</td>
<td>ND</td>
<td>±</td>
<td>93</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations and signs are as given in legend to Table 1; ±, borderline condition.

* Elevated serum phosphate.
normal controls. Thus, we found no evidence of altered thermolability of N-protein in any of the PHP-I patients.\(^b\)

**DISCUSSION**

We have found decreased N-protein activity in erythrocytes of 15 PHP-I patients and normal N-protein activity in erythrocytes of 19 phenotypically normal first degree relatives of PHP-I patients in six affected families. The close correlation between the biochemical marker and the clinical disease strongly supports the hypothesis (10) that the primary biochemical lesion in some PHP-I patients is a generalized deficiency in the activity of the N protein, an essential component of hormone-sensitive adenylate cyclase in all animal cells (15).\(^1\)

Identification of a primary biochemical defect in an inherited disease often reveals genetic and biochemical heterogeneity underlying the surface phenotype. Thus, we have studied two families with PHP-I in whom erythrocyte N-protein activities are normal [families E (10) and F (see Table 2)]. Because both families included individuals who showed clinical hypothyroidism as well as resistance to PTH, their underlying defect probably lies distal to the PTH receptor, involving regulation of cAMP accumulation in response to other hormones as well.

\(^b\)In the absence of Gpp(NH)p, N-protein activity was much more labile on heating, even at lower temperatures (e.g., 37°C). We observed no differences in the rate of N-protein inactivation, under these conditions, between normal and PHP-I extracts.

\(^1\)An alternative hypothesis is that erythrocyte N-protein deficiency results from some other feature of PHP-I. For at least two cardinal features of the syndrome, hypocalcemia and elevated serum PTH, this hypothesis is not tenable: N-protein activity was decreased in two patients who did not exhibit hypocalcemia or a history of vitamin D therapy [J-22 and a previously reported case (10)], and N-protein activity was normal in hypoparathyroid controls (10). Similarly, excess serum PTH is not necessary (patient C-17) nor sufficient [families E (10) and F] for expression of erythrocyte N-protein deficiency.

These patients could have inherited mutations that involve the catalytic unit of adenylate cyclase or that result in increased cAMP phosphodiesterase,\(^3\) or they could have N-protein lesions not detected by our assays. We ruled out one subclass of such defects, functional uncoupling of N protein from hormone receptors (see Table 3).

The contrasting “dominant” and “recessive” pedigrees of the J and I families (see Fig. 2) strongly imply biochemical heterogeneity in the mechanisms that result in reduced N-protein activity. The N protein is composed of at least two dissimilar peptide subunits, whose stoichiometry is not yet defined (17). A mutant allele producing an inactive version of one of these N peptides could explain dominant transmission of N-protein deficiency. Thus, affected heterozygotes would have ≈50% of normal N-protein activity (and presumably normal N-protein molecules) in their cells, as is the case in erythrocytes of two families that have apparently dominant inheritance of PHP-I (family J and patients A-1 and A-2 in ref. 10). Most published pedigrees show apparently dominant transmission of PHP-I (7).

In contrast, the PHP-I syndrome and erythrocyte N-protein deficiency appear to have been recessively inherited in family I (see Fig. 2). How could mutant genes produce a normal clinical phenotype and normal erythrocyte N-protein activity in the parents but combine to produce both N-protein deficiency and PHP-I in their progeny? At present, speculation must be limited by our ignorance of the number and stoichiometry of peptide gene products represented in the N protein. It seems possible that products of two mutant genes might complement each other in a negative fashion in the progeny (e.g., by producing an inactive oligomeric N protein), while the presence of either

\(^3\)We have recently isolated a mutant S49 mouse lymphoma clone that provides a precedent for this possibility: In this clone, resistance to the cAMP-elevating effects of hormones and cholera toxin is associated with a 4-fold increase in cAMP phosphodiesterase activity, while adenylate cyclase and N-protein activities are normal (16).
which may of heritage produced that is genetically dominant and that may affect a second genetic locus. This conclusion should be qualified: Because dominant disorders are not always completely expressed, it is possible that either parent in the I family could have the PHP-I genotype, although it is not expressed clinically or in measurements of erythrocyte N-protein activity. In addition, none of our results unequivocally rule out X-linked transmission of PHP-I. If the disorder were X-linked in the I family, the mother could carry the defective gene but not express it clinically or in her erythrocytes, owing to relative underrepresentation of active X chromosomes bearing the defective gene in erythrocyte precursors and hormonally responsive tissues. Finally, the I family pedigree is also consistent with dominant inheritance of a mutation in the germ cells of one of the parents, a possibility that cannot be ruled out.

In summary, the results of this study support the conclusion that deficiency of N-protein activity underlies the pathophysiology of many PHP-I patients and suggest that the PHP-I syndrome is genetically heterogeneous. It is likely that PHP-I can be produced by mutations of at least three genetic loci, two of which may affect N-protein activity. Biochemical dissection of this heterogeneity—as in the syndromes of androgen resistance (19)—may help to elucidate poorly understood or unsuspected biochemical mechanisms that are essential for normal endocrine regulation.

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