Familial pseudohyperkalemia is a "leaky red blood cell" condition in which the cells show a temperature-dependent loss of potassium (K) from red blood cells when stored at room temperature, manifesting as apparent hyperkalemia. The red blood cells show a reduced lifespan in vivo but there is no frank hemolysis. Studies of cation content and transport show a marginal increase in permeability at 37°C and a degree of cellular dehydration, qualitatively similar to the changes seen in dehydrated hereditary stomatocytosis (hereditary xerocytosis). Physiological studies have shown that the passive leak to K has an abnormal temperature dependence, such that the leak is less sensitive to temperature than that in normal cells. We performed genetic mapping on the original family and found that the condition in this kindred maps to the same locus (16q23-ter) that we have previously identified for an Irish family with dehydrated hereditary stomatocytosis, which does not show the same temperature effects.

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Materials and Methods

Clinical case. A four-generation Scottish kindred consisting of 32 subjects, of which 11 were affected, was studied. The clinical presentation has previously been described. Patients were typed according to a simple test in which aliquots of heparinized blood were stored on the
To compare temperature effects in the present family with the previously mapped DHS kindred, we examined the changes in plasma K on storage of whole heparinized blood at room temperature in FP cells, normals, and in the previously mapped DHS family (Fig 1A). In the FP cells (○), there was a marked increase in plasma K⁺ in blood stored at 20°C, reflecting previous results, while in normal subjects and the DHS family (□, △), plasma K⁺ showed no significant change with time. The OBR K⁻ influx was studied as a function of temperature in the proposita (●), three normal subjects, and an affected member of the previously mapped DHS kindred (Fig 1B). The FP cells show a slightly increased flux at 37°C, but in the interval 37°C to 20°C, the slope of the plot is significantly more shallow than that in the previously mapped DHS subject and the normal (confirming previous studies on this) family. Table 1 illustrates the minimal ion flux and content abnormality in these FP cells compared with normal and the DHS kindred. Hemoglobin levels and MCV were within the normal range. Reticulocyte counts were on average slightly high (mean, 2.5%). We have previously shown a reduced RBC lifespan, slightly abnormal MCHC and blood film, with anisopoliathocytosis, polychromaphilia, and few target cells and stomatocytes. The cells showed minimal dehydration.

The four-generation kindred was analyzed using nine different microsatellite markers of DHS locus. A summary of the results is reported in Table 2. Significant LOD scores have been obtained with most of the markers used (D16S511, D16S3037, D16S520, D16S498, and D16S3026). The highest LOD score (4.14) was obtained with marker D16S3037 at theta (θ) of recombination frequency. Additional positive LOD scores were also detected with the remaining four markers. These findings
denote typical range on all 11 affected family members; isotopic flux results denote typical value on proposita. bumetanide-sensitive NaK2Cl cotransport; and “Leak,” the ouabain MOPS with inhibitors at 0.1 mmol/L if required (see text). “Pump” denotes the ouabain-sensitive NaK pump fraction: “Cotransp,” the DHS family. We conclude that FP and DHS are almost the marker itself in the present family as compared with the can be most likely explained by the different informativity of obtained the highest score with marker D16S3037. This finding marker showing the highest LOD score was D16S520, here we DHS loci at the same chromosomal position. While in DHS the and D16S3074. These results allowed us to colocalize FP and been obtained for the following markers: D16S402, D16S511, In particular, significant LOD scores higher than three have FP with a series of microsatellite markers from the DHS locus. (16q23-qter). Clear showed that FP disease locus in this family maps at the same position as DHS on the long arm of chromosome 16 (16q23-qter).

**DISCUSSION**

Positive LOD scores have been obtained in this family with FP with a series of microsatellite markers from the DHS locus. In particular, significant microsatellite scores higher than three have been obtained for the following markers: D16S402, D16S511, and D16S3037. These results allowed us to colocalize FP and DHS loci at the same chromosomal position. While in DHS the marker showing the highest LOD score was D16S520, here we obtained the highest score with marker D16S3037. This finding can be most likely explained by the different informativity of the marker itself in the present family as compared with the DHS family.4 We conclude that FP and DHS are almost certainly caused by alterations of the same gene. The possibility of two adjacent alleles cannot formally be dismissed, but seems unlikely. In five families showing this combination, we have never observed any recombination (Grootenboer S, Delaunay J, unpublished data, January 1999).

The incidence of FP is unknown. Its occurrence must certainly be underestimated, because it is asymptomatic and its discovery is always accidental. On functional grounds, the slight hematological and ion flux abnormalities at 37°C allow it to be classified as a mild variant of the hemolytic conditions gathered under the generic label hereditary stomatocytosis syndromes. It is the temperature effects that define this syndrome, and in particular, the shallow slope profile of the OBR fluxes. The present mapping work confirms this relationship, and the comparison of temperature effects shows that different thermotrophic variants can be due to mutations of what is almost certainly the same gene.

It should be noted that the temperature profiles of the OBR K fluxes in other kindreds with FP may be different from the shallow slope variant seen here. In particular, the ouabain-plus-furosemide K efflux in the family described by Meenaghan et al6 showed a U-shaped curve, similar to that seen in normal RBCs suspended in media in which either Cl– is replaced by salicylate or thiocyanate,20 or in which Na+ is replaced by an organic cation.21 The French family described by Dagher et al8 was different again. Given these heterogeneities, it may not be appropriate to extrapolate the present mapping results to other families with the FP combination of almost-normal hematology and temperature-dependent pseudohyperkalemia.

Biochemical data and laboratory findings suggest that the function of a candidate gene should be related to monovalent cation movements across the membrane. Unfortunately, there are no known genes resembling a Na+K+ transporters or channel located within the FP/DHS locus. However, several gene fragments (expressed sequence tags) whose function remains unknown have already been mapped within this locus. Work is in progress to define the possible function of each of them and to see if one could be involved in determining FP/DHS. Recombinants with more centromeric markers (D16S503, D16S515, D16S516, and D16S3091) spanning the region of LCAT and KCC genes exclude these two genes as candidates and reduce the region for pseudohyperkalemia to 20 cM. This region has a minimum of overlapping of approximately 1.5 cM with that for xerocytosis, and most likely support the hypothesis of allelism (one gene with different mutations).

In conclusion, this work shows that a variant of that group of genetically leaky RBC conditions maps to a locus on chromosome 16 to which we have already mapped DHS. As previously suggested by hematoletic studies,17 FP and DHS may be variants of the same genetically leaky RBC disease. Much work will be necessary to indentify the assumed “FP/DHS gene,” and

<table>
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<tr>
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<th>Hb (g/dL)</th>
<th>Retic (%)</th>
<th>[Na] (mmol/L cells 2 )</th>
<th>[K] (mmol/L cells 2 )</th>
<th>Pump (mmol/L cells 2 h 1 )</th>
<th>Cotransp (mmol/L cells 2 h 1 )</th>
<th>Leak</th>
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<tbody>
<tr>
<td>FP (Edinburgh)</td>
<td>14 to 18</td>
<td>2 to 4</td>
<td>12</td>
<td>85</td>
<td>2.6</td>
<td>1.2</td>
<td>0.12</td>
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<tr>
<td>DHS (Irish family)</td>
<td>11 to 12</td>
<td>6 to 12</td>
<td>12 to 14</td>
<td>75 to 86</td>
<td>4.6 to 5.9</td>
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<tr>
<td>Normal</td>
<td>14 to 18</td>
<td>&lt;2</td>
<td>5 to 11</td>
<td>88 to 105</td>
<td>0.8 to 2.0</td>
<td>0 to 1.2</td>
<td>0.075 to 0.900</td>
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Intracellular Na and K were measured by flame photometry on fresh, washed RBCs. K influx was measured at 37°C using 86Rb as a tracer in MOPS with inhibitors at 0.1 mmol/L if required (see text). "Pump" denotes the ouabain-sensitive NaK pump fraction; "Cotransp," the bumetanide-sensitive NaK2Cl cotransport; and "Leak," the ouabain + bumetanide-resistant, residual passive leak fraction. Hematological values denote typical range on all 11 affected family members; isotopic flux results denote typical value on proposita.15

<p>| Table 1. Comparative Diagnostic Data on Present Kindred With FP and Previously Mapped DHS Family |
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to account for the simple or compound phenotypes arising from the various mutations within this gene.

ACKNOWLEDGMENT

We are grateful to the patients for their cooperation.

REFERENCES

Familial Pseudohyperkalemia Maps to the Same Locus as Dehydrated Hereditary Stomatocytosis (Hereditary Xerocytosis)