Haemoglobin Porto Alegre in a Cuban family

G. MARTÍNEZ, F. LIMA, M. WADE, M. ESTRADA, B. COLOMBO, L. HEREDERO, AND H. GRANDA

From Instituto de Hematologia e Inmunología, Apartado 8070, La Habana 8, and Departamento de Genética Médica, Instituto de Ciencias Básicas y Preclínicas Victoria de Girón, Universidad de La Habana, Cuba

SUMMARY During a screening programme for abnormal haemoglobins in Habana, one case of Hb Porto Alegre was found in 23 000 cases analysed. The ability of this variant to polymerise in vitro and the absence of clinical features in the carriers have been confirmed. These observations are now explained by the findings of high levels of glutathione in the red cells of subjects heterozygous for Hb Porto Alegre: it is suggested that the increase of glutathione is responsible for the absence of in vivo polymerisation and accounts for the lack of clinical symptoms.

Haemoglobin Porto Alegre was first described in a Brazilian family (Tondo et al., 1963). Two more cases of this abnormal haemoglobin have been found in the same country (Seid-Akhaven et al., 1973) and one in Argentina (Peñalver et al., 1971; Peñalver and de Miani, 1972).

Haemoglobin Porto Alegre presents one extra thiol group in the β-chain due to substitution of the serine in position nine for cysteine (Bonaventura and Riggs, 1967). This haemoglobin has the ability to polymerise spontaneously in the haemolysate, while in vivo it is present in the tetrameric form, which accounts for the lack of anaemia, erythrocyte morphological abnormalities, and clinical symptoms (Tondo et al., 1963). The physicochemical properties of Hb Porto Alegre have been fully described (Tondo, 1972).

During the course of a screening for abnormal haemoglobins carried out on secondary school students of Havana City, one case of Hb Porto Alegre was found in 23 000 cases analysed. We report here the study of the family carrying this abnormal haemoglobin.

Subjects and methods

Routine haematological data were obtained by standard methods (Dacie and Lewis, 1966). Red blood cells were counted in a TOA automatic cell counter (Microcell Counter, Model CC-1002). Electrophoresis was carried out in polyacrylamide (Heredero et al., 1974) and in starch gel (Smithies, 1959). Fetal haemoglobin was determined according to the method of Betke (Betke et al., 1959) and HbA2 by the method described by Bernini (Bernini, 1969). Haemoglobin purification was obtained by DEAE-Sephadex chromatography (Jonxis and Huisman, 1968). Glutathione results were noted.

Results

The index case was found because of the appearance on polyacrylamide gel electrophoresis at pH 8.6 of a fresh haemolysate, of a large amount of a fast-moving band similar to HbA3. The case was a healthy 16-year-old boy, with no previous medical history. Physical examination did not show any abnormalities and the haematological values were within the normal range. All the other members of his family were studied: the father and 4 sibs were also found to be heterozygous for the abnormal haemoglobin. The pedigree is shown in Fig. 1 and the haematological data are summarised in Table 1.

Storage of the haemolysate at −20°C for a few days produced a slow band migrating between HbS and HbC on starch gel electrophoresis at pH 8.6 (Fig. 2).
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Table 1  Haematological data of family

<table>
<thead>
<tr>
<th>Family members and age (y)</th>
<th>Hb type</th>
<th>Hb (g/dl)</th>
<th>RBC × 10^6</th>
<th>PCV</th>
<th>=CV</th>
<th>=CH</th>
<th>Ret.</th>
<th>Glutathione</th>
<th>Glutathione</th>
<th>Glutathione</th>
<th>G6PD</th>
<th>G6PD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>mm^3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>mg GSH/100 ml cells</td>
<td>reductase IU/g Hb</td>
<td>peroxidase IU/g Hb</td>
<td>IU/g Hb</td>
<td>IU/g Hb</td>
</tr>
<tr>
<td>I.1 50</td>
<td>A/PA</td>
<td>14.3</td>
<td>4.86</td>
<td>43</td>
<td>90</td>
<td>36</td>
<td>34</td>
<td>1.0</td>
<td>57</td>
<td>3.55</td>
<td>3.06</td>
<td>7.85</td>
</tr>
<tr>
<td>I.2 45</td>
<td>AA</td>
<td>13.6</td>
<td>4.73</td>
<td>38</td>
<td>81</td>
<td>27</td>
<td>34</td>
<td>1.5</td>
<td>17</td>
<td>2.01</td>
<td>3.28</td>
<td>5.80</td>
</tr>
<tr>
<td>I.3 30</td>
<td>A/PA</td>
<td>12.6</td>
<td>4.16</td>
<td>36</td>
<td>86</td>
<td>30</td>
<td>36</td>
<td>1.1</td>
<td>46</td>
<td>2.64</td>
<td>3.63</td>
<td>6.00</td>
</tr>
<tr>
<td>II.7 27</td>
<td>A/PA</td>
<td>15.6</td>
<td>4.95</td>
<td>46</td>
<td>96</td>
<td>33</td>
<td>34</td>
<td>0.9</td>
<td>51</td>
<td>2.65</td>
<td>4.06</td>
<td>6.94</td>
</tr>
</tbody>
</table>

![Family pedigree](image)

Fig. 1  Family pedigree. The arrow indicates the propositus.

![Electrophoresis](image)

Fig. 2  Electrophoresis of the carrier of Hb Porto Alegre at pH 8.6. (1) HbS heterozygote; (2) and (3) propositus.

Its amount increased with time and its appearance could be prevented by addition of dithyothreitol to a freshly prepared haemolysate. This behaviour suggested that a spontaneous polymerisation caused by the formation of S-S bridges takes place in the haemolysate. The presence of such a polymer was confirmed by gel filtration on Sephacex G-100 and by ultracentrifugation. In both cases a molecular weight consistent with the presence of a haemoglobin octamer was obtained.

The abnormal haemoglobin was purified from a 25-day old haemolysate on a Sephadex G-100 column and on a DEAE-Sephadex column using a pH gradient: under this last condition the abnormal haemoglobin eluted after HbA. The amount of the variant was found to be 37%.

It has been reported (Rucknagel et al., 1964) that the β₂ tryptic peptide is absent in the fingerprint of the Hb Porto Alegre. In our case this peptide could not be located with ninhydrin staining, but after aminoethylatation an abnormal peptide appeared in the position shown in Fig. 3. The composition of this peptide is given in Table 2. The abnormal haemoglobin was thus identified as Hb Porto Alegre (α₂β₂ 9 Ser→Cys).

Discussion

Haemoglobin Porto Alegre is a clinical and electrophoretic silent mutant. Another variant behaving in the same way has been reported, Hb Ta-Li, another β-chain variant in which the presence of a cysteine in position 83 produces polymerisation on the haemoglobin in the haemolysates (Blackwell et al., 1971). In

![Amino acid composition](image)

Table 2  Amino acid composition of abnormal β-II peptide

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>β₂ Residues expected</th>
<th>β₂ Residues observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thr</td>
<td>1</td>
<td>0.95</td>
</tr>
<tr>
<td>Ser</td>
<td>1</td>
<td>1.05</td>
</tr>
<tr>
<td>Gly</td>
<td>1</td>
<td>2.08</td>
</tr>
<tr>
<td>Ala</td>
<td>2</td>
<td>1.21</td>
</tr>
<tr>
<td>Val</td>
<td>1</td>
<td>0.97</td>
</tr>
<tr>
<td>Leu</td>
<td>1</td>
<td>0.98</td>
</tr>
<tr>
<td>Lys</td>
<td>1</td>
<td>0.94</td>
</tr>
<tr>
<td>S-Ae-Cys</td>
<td>0</td>
<td>*</td>
</tr>
</tbody>
</table>

* The peptide stained positively for tryptophane.
both cases there are no pathological consequences and no haematological abnormalities, probably because both variants are kept in the normal tetrameric form in the erythrocytes. It would be interesting to know whether cells carrying Hb Porto Alegre require a special metabolism to maintain the extra thiol groups of the variant in the reduced form: our results indicate that these cells contain higher amounts of glutathione (Table 1). In fact, glutathione levels are twice those of a normal subject, which seems to correspond to the twofold increase of \(-\text{SH}\) groups (4 in Hb Porto Alegre compared to 2 in HbA). Glutathione peroxidase and reductase, as well as G6PD and 6PGD activities were found to be normal (Table 1).

It is believed that at least one of the functions of glutathione is to protect haemoglobin as well as other thiol containing proteins from denaturation under oxidative stress (Srivastava and Beutler, 1970). Independently of the different mechanism proposed to explain this function (Blume et al., 1973), our findings suggest that higher levels of glutathione are needed in the presence of unusually high number of \(-\text{SH}\) groups. Thus it seems reasonable to propose that the increased levels of glutathione in the red cells of Hb Porto Alegre carriers is the result of the need to maintain the abnormal haemoglobin in the tetrameric functional form, though the biochemical mechanism responsible for this high concentration is not clear. Experiments are under way to clarify this point.

To explain the findings of 3 probably unrelated cases of Hb Porto Alegre, 2 in Brazil and 1 in Argentina, it has been suggested that the gene responsible for this variant could be relatively common in Portugal and in Spain (Seid-Akhaven et al., 1973). The finding of a new case in a Cuban family of Spanish ancestry, further supports this hypothesis, though the possibility of an independent mutation cannot be excluded.

References
globin Ta-Li: \(\beta83 \text{Gly} \rightarrow \text{Cys. Biochimica et Biophysica Acta}
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Requests for reprints to Dr B. Colombo, Instituto de Hematologia e Immunologia, Hospital E. Cabrera, Apartado 8070, Altahabana, La Habana 8, Cuba.