Hereditary anaemias in Portugal: epidemiology, public health significance, and control

M C Martins, G Olim, J Melo, H A Magalhães, M O Rodrigues

Abstract
A countrywide prospective study aimed at establishing the prevalence of the haemoglobinopathy genes in the Portuguese population was carried out by screening 15 208 randomly selected blood samples from young males. This male based survey provided the opportunity of assessing simultaneously the prevalence of the red cell enzyme glucose-6-phosphate dehydrogenase (G6PD) deficiency, thus giving a picture of these important hereditary anaemias in Portugal.

The results showed a low average frequency of \( \beta \) thalassaemia (0·45%) and haemoglobin \( S \) (0·32%) carriers as well as G6PD deficiency (0·51%). However, these disorders are unevenly distributed throughout the country with a higher prevalence in some areas, mainly in the south. The relationship of this pattern of haemoglobinopathies to the known haplotypes linked to \( \beta \) thalassaemia and sickle cell disease, relevant historical events, and local selective pressure was investigated. Hb D and Hb J are the commonest other structural variants. The implemented programme for control of these hereditary anaemias is described.

The first cases of Cooley's anaemia in Portugal were reported in 1938 and subsequently there have been many other reports of haemoglobin disorders among the Portuguese. More recently the common \( \beta \) thalassaemia gene mutations and the known \( \beta ^ { + } \) haplotypes have been described in the Portuguese population.

G6PD deficiency is usually also present where Hb disorders occur. 'Favism' is diagnosed occasionally throughout the country and the significance of the disorder was agreed at a recent meeting held in Albufeira in 1991.

Despite these reports, the true prevalence of haemoglobin disorders and G6PD deficiency in Portugal and their importance for public health remained uncertain. Following a national meeting, a national haemoglobinopathy group was established, and with their help a large scale random study was carried out to answer these questions.

Materials and methods
Universal conscription for military service is the rule in Portugal. The population sample studied was exclusively formed of young males attending the three military recruitment centres before any kind of selection (medical, physical, or any other kind), thus representing a random sample of the Portuguese population.

Blood samples from 15 208 young males attending the three military selection centres in Oporto (north), Coimbra (centre), and Setubal (south) were collected in tubes with EDTA as anticoagulant and analysed in the Clinical Chemistry and Haematology Laboratory of the Portuguese National Institute of Health. Red blood cell indices and haemoglobin electrophoresis were performed on all samples; 13 785 were screened for G6PD deficiency. The subjects' place of origin was noted and the results expressed in relation to administrative districts.

Red cell indices were measured using a Coulter Counter (S,90) under strict calibration and internal and external quality control (4 C Coulter Counter cell control, participation in the UK External Quality Assessment Scheme). Hb electrophoresis was carried out on cellulose acetate strips with tris-EDTA-boric acid buffer at pH = 8·9. Hb \( A_1 \) quantification was by microcolumn chromatography on samples with microcytosis and hypochromia (MCH < 28 pg and MCV < 78 fl, which we have previously found to be cut off values of MCH and MCV between Portuguese \( \beta \) thalassaemia and non-\( \beta \) thalassaemia carriers).

Whenever suggested by the electrophoretic pattern, Hb F levels were quantified by the alkaline denaturation method of Betke et al.11 The presence of Hb S was confirmed by electrophoresis on agar gel with citrate buffer, pH = 6·1, a low solubility test in a high molarity phosphate buffer with dithionite, and a positive sickling test.12 For G6PD the blood samples were screened by the NADP reduction fluorescent method.13

Results
The findings are summarised in the table. Among the 15 208 blood samples analysed for Hb disorders 0·45% showed \( \beta \) thalassaemia trait (95% confidence limits 0·45 ± 0·011) and 0·32% showed sickle cell trait (95% confidence limits 0·32 ± 0·089). The geographical distribution by administrative districts is shown in figs 1 and 2. The prevalence of both increases from the north (total prevalence less than 0·1%) towards the south (total prevalence more than 2% in three districts).

Less common structural variants of haemoglobin were also found, the most frequent being Hb D (n = 9) and Hb J (n = 13). Their
Martins, Olim, Melo, Magalhães, Rodrigues

Prevalence of both Hb disorders and G6PD deficiency by administrative district in Portugal.

<table>
<thead>
<tr>
<th>Administrative districts</th>
<th>Population (1988) (thousand)</th>
<th>No tested</th>
<th>% carriers</th>
<th>G6PD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>B¹</td>
<td>B²</td>
<td>Total</td>
</tr>
<tr>
<td>Aveiro (C)</td>
<td>667 9</td>
<td>800</td>
<td>0.37</td>
<td>0.12</td>
</tr>
<tr>
<td>Beja (S)</td>
<td>176 6</td>
<td>808</td>
<td>0.99</td>
<td>0.11</td>
</tr>
<tr>
<td>Braga (N)</td>
<td>774 9</td>
<td>396</td>
<td>0.25</td>
<td>0.00</td>
</tr>
<tr>
<td>Bragança (N)</td>
<td>184 3</td>
<td>283</td>
<td>0.90</td>
<td>0.01</td>
</tr>
<tr>
<td>Castelo Branco (C)</td>
<td>222 5</td>
<td>359</td>
<td>0.52</td>
<td>0.00</td>
</tr>
<tr>
<td>Coimbra (C)</td>
<td>446 5</td>
<td>1149</td>
<td>0.78</td>
<td>0.17</td>
</tr>
<tr>
<td>Évora (S)</td>
<td>173 6</td>
<td>669</td>
<td>1.37</td>
<td>0.71</td>
</tr>
<tr>
<td>Faro (S)</td>
<td>342 1</td>
<td>766</td>
<td>1.08</td>
<td>0.68</td>
</tr>
<tr>
<td>Guarda (C)</td>
<td>195 1</td>
<td>333</td>
<td>0.00</td>
<td>0.02</td>
</tr>
<tr>
<td>Leiria (C)</td>
<td>436 0</td>
<td>460</td>
<td>1.16</td>
<td>0.23</td>
</tr>
<tr>
<td>Lisboa (C)</td>
<td>2127 6</td>
<td>3151</td>
<td>0.32</td>
<td>0.30</td>
</tr>
<tr>
<td>Portalegre (S)</td>
<td>136 8</td>
<td>543</td>
<td>0.18</td>
<td>0.18</td>
</tr>
<tr>
<td>Porto (N)</td>
<td>1676 9</td>
<td>2029</td>
<td>0.00</td>
<td>0.05</td>
</tr>
<tr>
<td>Santarém (C)</td>
<td>460 2</td>
<td>422</td>
<td>0.94</td>
<td>0.71</td>
</tr>
<tr>
<td>Setúbal (S)</td>
<td>789 2</td>
<td>1188</td>
<td>1.16</td>
<td>0.99</td>
</tr>
<tr>
<td>Viana do Castelo (N)</td>
<td>266 5</td>
<td>641</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Vila Real (N)</td>
<td>262 2</td>
<td>433</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Viseu (N)</td>
<td>422 3</td>
<td>818</td>
<td>0.24</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>9761 2</td>
<td>15208</td>
<td>0.45</td>
<td>0.32</td>
</tr>
</tbody>
</table>

N, north, C, central, S, south.
*Poisson distribution.
95% confidence limits: B thal: 0.45 ± 0.011, Hb S: 0.32 ± 0.089, B thal + Hb S: 0.77 ± 0.014, G6PD def: 0.51 ± 0.109.

geographical distribution was more or less evenly scattered.

Out of the initial number of blood samples analysed for Hb disorders, 13 785 were screened for G6PD deficiency and 68 were found to be deficient, giving a low overall prevalence of 0.51% in males (95% confidence limits 0.51 ± 0.109). Fig 3 shows that the distribution of G6PD deficiency resembles that of ß thalassaemia and sickle cell trait, ranging from zero in several northern regions to 1.37% in the south, with 1.85% in one central province.

Discussion

Epidemiology

This study established for the first time a picture of the prevalence of hereditary anaemias in the whole of Portugal. The differences between the north and the south may be related to several factors.

Migrants from Mediterranean areas settled predominantly in the south which, unlike the north, is level and offered better conditions for settlement, agriculture, fishing, and mining (fig 4). In fact the four commonest Mediter-
Hereditary anaemias in Portugal: epidemiology, public health significance, and control

Ponds with the widespread areas of Portuguese settlement in Africa.\(^9\)\(^10\)

Malaria was formerly frequent in the low valleys of the rivers Sado, Guadiana, and Tejo (fig 4). This may have tended to maintain or increase the local frequency of \(\beta\) thalassaemia, sickle cell, and G6PD deficiency genes.

Further studies are planned to identify the mutations in the G6PD deficient samples. Information on the proportion of Mediterranean and African mutations will cast further light on the origins of these conditions in Portugal.

This study has provided the scientific basis for a National Haemoglobinopathy Control Programme which is progressively involving the primary health care services in the most affected districts and specialised clinics in the relevant hospitals.

**PUBLIC HEALTH IMPORTANCE**

The Hardy-Weinberg equation\(^11\) may be used to calculate the minimum frequency of homozygous births. However, the calculation is inaccurate since it cannot take account of irregularities of distribution. Applied to this survey and using the total figure for \(\beta\) thalassaemia + \(\beta^b\) trait per district (table) the calculation gives a minimum annual birth rate of three homozygotes in Portugal. This figure is inconsistent with the observed number of at least 300 living patients, 218 with sickle cell disease (SS or S/\(\beta\) thalassaemia).

This discrepancy is probably the result of two factors. (1) A survey of this type cannot detect 'hot spots' with a high frequency of carriers. Three sickle cell hot spots are known, Coruche and Alcacer do Sal in the low valleys of the rivers Tejo and Sado respectively, and Pias in the south-east. Others may exist. (2) The survey also omitted the specific gene contribution of immigrants from former Portuguese colonies in Africa, particularly from the Cabo Verde islands, 7% of whom carry sickle cell trait. In a recently organised national register of sickle cell patients 61.9% are African in origin.

Even without considering special populations at risk, the survey indicates the existence of between 73,795 and 76,528 carriers of \(\beta\) thalassaemia or AS in Portugal (table). These are significant figures since these genes can interact to produce severe genetic disease and also carry risks in themselves. \(\beta\) thalassaemia trait carries an important risk of misdiagnosis as iron deficiency anaemia, followed by inappropriate iron therapy, while sickle cell trait carries some anaesthetic risks and risks associated with endurance exercises (particularly in the armed forces) and must be excluded in pilots. G6PD deficiency also carries risks associated with certain drugs, particularly antimalarials.

**PROGRAMME FOR CONTROL OF HEREDITARY ANAEMIAS**

Progressive understanding of these facts is making people aware of the need for a programme for hereditary anaemias in Portugal. A
programme for the control of both the Hb disorders and G6PD deficiency, following WHO guidelines, is now being implemented in seven districts (fig 5). In each district there is a group responsible for coordination and an appropriate strategy for prevention (carrier screening, information, and genetic counselling).

This programme is based on the government health centres which serve the whole population, and the national network of district Public Health Laboratories, all of which participate in the National External Quality Assessment Scheme in Haematology.

Blood samples from health centre users in groups with a presumed high gene frequency or who, if positive, require immediate family studies and counselling (pregnant women before 3 months' gestation, young couples, immigrants of African extraction, gypsies) are analysed at the district Public Health Laboratory. The tests used at this level are cheap and simple, including red cell indices and haemoglobin S solubility test. Samples found to be positive are sent to the National Institute of Health in Lisbon for Hb A2 estimation or haemoglobin electrophoresis or both for definitive identification of Hb S.

Single carriers are counselled using an appropriate information leaflet by a trained member of the health centre team (general practitioner, nurse). A 'carrier card' is issued. This draws attention to the personal risks for the carrier, such as iatrogenic disease with iron overload in β thalassaemia trait if mistreated with iron and sickling crisis in AS in severe hypoxia or extreme physical stress.

Every carrier is requested to ask his or her family to come for study. Couples at risk identified in this way are sent to a specialist genetic counsellor available in Lisbon who also periodically visits the three district hospitals in the most affected regions. They may, when indicated, request prenatal diagnosis, performed at the National Institute of Health.

G6PD deficient subjects are given a special leaflet. They are advised not to ingest the foodstuffs and medicines listed in order to avoid a haemolytic crisis.

In every district data are collected to improve knowledge of the epidemiology of hereditary anaemias in Portugal, to find carrier 'hot spots', and to identify couples at risk.

The control programme also includes treatment of patients. Most patients attend central specialist centres in relevant hospitals in Lisbon, Coimbra, and Oporto which follow WHO guidelines, providing adequate blood, desferroxamine, and desferrioxamine infusion pumps. In Faro and Beja most patients are treated in the local hospitals and in Beja psychological and social support is also being organised. A parents' association has started at a national level.

We believe that this programme of screening and counselling, based in the primary care system, will provide a structural basis for other genetic programmes, for example, for cystic fibrosis, in the future.

The people involved at the national and district level in the Haemoglobinopathy Control Programme in Portugal are listed below.


The authors thank Dr B Modell for advice as WHO consultant and for her kind review of this paper, the Portuguese Armed Forces for allowing the blood collections, Drs M M Freitas (Instituto Nacional de Saúde, Porto), M H Bento (Lab ARS Coimbra), M C Almeida, (Lab ARS Setúbal), and I Picanço and L Batalha for technical help.

2 Ribeiro do Rosário M, Colaço FM. Nosophoria da hemoglob-


