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ORIGINAL ARTICLE

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Abstract Haemoglobinopathies are the world's most frequently found monogenic disorders. In the cases with high oxygen affinity, the decrease in the liberation of the oxygen determines a secondary erythrocytosis. In this work, we present 17 unrelated families of Caucasian race and of Spanish origin, with ten variants of haemoglobin or haemoglobinopathies with high oxygen affinity which were diagnosed in our laboratory. Of the ten haemoglobinopathies, in four (the Hb San Diego, the Hb Johnstown, the Hb Malmö and the Hb Columbia-Missouri), the change of amino acid affects zones of the contact $\alpha_1\beta_2$; in two variants (the Hb Strasbourg and the Hb Syracuse), it affects the unions with 2,3-DPG in the central cavity; in the other two (the Hb Badalona and the Hb La Coruña), the cavity of contact with the group haem is affected; in one (Hb Bethesda), it affects the zone of contact $\alpha_1\beta_{1;}$ and in one (Hb Olympia), the position 20 of the chain in the helix B in the surface of the protein is affected. In all cases, the change of amino acid, though of different form, facilitates that the quaternary structure of the haemoglobin becomes stable in its relaxed configuration so the transfer of oxygen and the P_{50} value are decreased. All cases were sent to our laboratory because of shown erythrocytosis. In the majority of them, the diagnosis was done during an analysis of routine or for being relatives of the first ones.

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Introduction

Haemoglobinopathies are the world's most frequently found monogenic disorders [1]. Single mutations, deletions or insertions in the genes that encode globin chains may lead to qualitative alteration of the genes' expression in structural haemoglobinopathies or to a reduced or nonexistent expression, as seen in thalassaemia [2].

So far, over 900 haemoglobin structural variations have been described. The structural alteration is due to a single amino acid replacement in over 95% of these variants. Depending on the replaced amino acid nature and its location, changes will be observed in the haemoglobin molecules' stability, solubility and function that finally lead to clinical haemoglobinopathies. Polyglobulia, generated to compensate for a decrease in the O_2 released into tissues, when the amino acid replacement increases Hb affinity for oxygen (O_2), is one of the clinical conditions that can be found [3]. Up to now, 89 haemoglobinopathies that show high oxygen affinity have been described. Only 18 of them affect one of the α genes and 71 in the β chain [4].

Material and methods

In our laboratory, from January 1996 to January 2006, we characterised 17 families, which are not related to each other, that show ten haemoglobin variants or haemoglobinopathies with high oxygen affinity. The haemoglobinopathies were characterised either through electrophoresis in an alkaline pH (8.6 pH) cellulose acetate or an isoelectric focussing in polyacrylamide gel (5.5–8.5 pH) or agar citrate

(pH 6.0) electrophoresis or reverse-phase high-performance liquid chromatography (HPLC) for globin chains [5] or ionic-exchange HPLC [6]. Haemoglobin stability was determined using the isopropanol test [7], while the function was established through P_{50} in the oxygen balance curve plotted by a TCS Haemox-Analyser (TCS Medical Products Co., Huntingdon Valley, PA, USA) [8]. The molecular analysis was completed by automatic sequencing of the α and/or β genes' polymerase-chain-reaction-amplified products using the ABI Prism TM dRhodamine Terminator Cycle Sequencing Ready reagents kit (PE Applied Biosystems, Foster City, CA, USA) and the sequence was studied in an ABI Prism 310 Genetic Analyser (PE Applied Biosystems) [9, 10].

Results

All the demographic data, hematrimetric results, P_{50} and chromatography findings are summarised in Table 1.

None of the patients in the study had any past or present clinical record of arterial or venous thrombosis at the time of diagnosis. All the referrals to our laboratory were due to erythrocytosis found, most often, during routine tests or through being relatives of the former patients. All patients were ethnically Caucasian and of Spanish origin.

Discussion

In haemoglobinopathies that show high oxygen affinity, the lower release of oxygen leads to tissue hypoxia that prompts erythropoietin output at renal level and induces both an increase in erythropoiesis and an offsetting secondary erythrocytosis [3].

In the haemoglobin structure, the $\alpha_1\beta_2$ bonds, the C-terminal ends of the β and α chains, the bonds to 2,3-DPG in the central cavity and the contact cavity with the haem group are significant zones not only for oxygen uptake but also for the 2,3-DPG, CO_2 and H^+ allosteric effects over that uptake. Mutations experienced by amino acids enclosed in these zones, which are critical for haemoglobin functions, can therefore determine alterations in affinity to oxygen [11]. Of the ten high-affinity haemoglobinopathies found by our laboratory, in four (Hb San Diego, Hb Johnstown, Hb Malmö and Hb Columbia-Missouri), the amino acid change affects the $\alpha_1\beta_2$ contact zones [12–15], while in two (Hb Strasbourg and Hb Syracuse) the bonds to 2,3-DPG, in the central cavity, are affected [16, 17], and in another two (Hb Badalona and Hb La Coruña) the contact cavity to the haem group [18, 19] is affected and in one (Hb Bethesda) the $\alpha_1\beta_1$ contact zone is affected [20]. But, in the Hb Olympia, the amino acid change is located in site 20 of

Table 1 Haemoglobin variants with high oxygen affinity diagnosed in the Service of Haematology of Hospital Clínico San Carlos

Fam	No of patients (age/sex)	Hb (g/dL)	Hto (%)	P_{50} (mmHg)	HPLC Hb	HPLC chains	Molecular mutation
1[a]	1 (50/V)	20	58	13.2	N	β^X; β^A; α	Hb Olympia [β20(B2) Val→Met; GTG→ATG]
2[a]	1 (25/V)	21.3	63.7	11	HbX/HbA	β^X; β^A; α	Hb Syracuse [β143(H21)His→Pro; CAC→CCC]
3[b]	3 (54/V; 26/M; 19/V)	13–17	44–52	21–22	N	β^X; β^A; α	Hb San Diego [β109(G11)Val→Met; GTG→ATG]
4[a]	1 (20/M)	18.6	60.2	20	N	β^X; β^A; α	Hb San Diego [β109(G11)Val→Met; GTG→ATG]
5[a]	1 (52/M)	16.7	48.2	14	N	β^X; β^A; α	Hb San Diego [β109(G11)Val→Met; GTG→ATG]
6[a]	1 (22/V)	18.4	55	15	N	β^X; β^A; α	Hb San Diego [β109(G11)Val→Met; GTG→ATG]
7[b]	2 (74/V; 47/M)	17–19	53–61	17–19	N	β^A; β^X; α	Hb Johnstown [β109(G11)Val→Leu; GTG→CTG]
8[a]	1 (32/M)	15.3	45.8	20	N	β^X; β^A; α	Hb Johnstown [β109(G11)Val→Leu; GTG→CTG]
9[b]	4 (41/M; 11/M; 9/M; 5/M)	14.2–17	46.6–50.2	16–19	N	β^X; β^A; α	Hb Johnstown [β109(G11)Val→Leu; GTG→CTG]
10[b]	4 (52/V; 26/M; 23/V; 21/V)	17.5–19.6	53–57.5	14–19	N	β^X; β^A; α	Hb Johnstown [β109(G11)Val→Leu; GTG→CTG]
11[a]	2 (30/V; 27/M)	18–19	60	13–15	N	β^X; α. No β^A	Hb Johnstown + β^0Tal IVS-1-nt1 (G→A)
12[b]	2 (23/V; 53/V)	17.3	49.2	12.5	HbX/HbA	β^X; β^A; α	Hb Bethesda [β145(HC2)Tyr→His; TAT→CAT]
13[c]	3 (17/M; 35/M; 666/M)	17	48–49	22	HbX/HbA	β^X; β^A; α	Hb Badalona [β31(B13)Leu→Val; CTG→GTG]
14[a]	1 (66/V)	15.9	49.5	21	N	β^X; β^A; α	Hb Strasbourg [β23(B5)Val→Asp; GTT→GAT]
15[a]	1 (25/V)	18.4	55	17	HbX/HbA	β^A; β^X; α	Hb Malmö [β97(FG4)His→Glu; CAC→CAA]
16[b]	4 (66/M; 59/V; 35/V; 29/M)	16–18	50–55	16–21	N	β^A; α^A; α^X	Hb Columbia-Missouri [α2 88(F9) Ala→Val; GCG→GTG]
17[b]	2 (22/V;50/M)	17–20	50–60	16–17	N	β^A; β^X; α	Hb La Coruña [β38 (C4)Thr[®]Ile; ACC→ATC]

[a] One generation
[b] Two generations
[c] Three generations

the chain helix B and there is no relation to any of the aforementioned zones, but it is accompanied by erythrocytosis. Replacement at this level of a polar amino acid, such as valine, by a hydrophobic one like methionine will most likely indirectly determine changes in some of those critical zones [21]. In this sense, another two haemoglobin variants affecting the site 20 of β chain Hb Trollhättan (Val→Glu) and Hb Uxbridge (Val→Gly) has been reported and in both variants there is an increase in oxygen affinity with mild erythrocytosis in the case of the Hb Trollhättan [22, 23]. In any event, an amino acid change, even through a different process, helps the haemoglobin quaternary structure to be stabilised in its relaxed configuration, thus reducing oxygen release and therefore lowering P_{50} in the oxygen dissociation curve.

Up to now, 89 haemoglobin variants that show high affinity for oxygen have been described: 18 in the α chain and 71 in the β one [4]. But two thirds of them are not accompanied by erythrocytosis, either because the affinity increase is slight or moderate and only found during in vitro studies or when molecular instability is present too, determining a concomitant haemolysis or if the mutating gene expression is low, as occurs in the α chain variations, or is reduced, as in Hb Crete [11]. Some erythrocytosis was found in all the patients reported in this paper or had been referred to our laboratory for that reason. But of the ten haemoglobinopathies, only one was found in an α chain variant. In a family affected by Hb Johnstown, an associated β^0 thalassaemia was found in two members due to IVS-1-nt1 (G→A) mutation. For this reason, all Hb was virtually Hb Johnstown in these patients. Most of the high-affinity haemoglobinopathies appear in heterozygotic individuals, while homozygotic cases are not reported, except for the Hb Tarrant found in an α chain and, for this reason, only 50% was quantified [24]. If the case found by our laboratory is added, high-affinity haemoglobinopathies associated to a β-thalassaemia are only found in five instances, all of them in a β chain and, for this reason, the Hb of these patients show high oxygen affinity. In such cases, as was to be expected, larger erythrocytosis levels are described, but significant clinical complications were not reported [11]. The notion that the homozygotic condition of variants with high oxygen affinity is incompatible to life can be questioned based on the aforementioned cases.

Erythrocytosis due to high-affinity haemoglobinopathies are usually fairly well tolerated by young patients, although thrombotic complications have been reported in elderly patients or when other vascular hazard factors are associated. Thrombotic complications have also been described for unstable variants that determine changes in the erythrocytic membrane of splenectomised patients, but none of the 34 patients in the study had either any acute clinical condition nor any thrombotic history [25, 26]. Although the high-affinity variants could in animal models determine an increase in foetal losses during pregnancy due to reduced oxygen release in the placenta, an increase in foetal losses has not been reported in families affected by high-affinity haemoglobinopathies.

Evidence of the pathological variant must be produced to diagnose secondary erythrocytosis in high-affinity haemoglobin variants. But over 30 of the aforementioned haemoglobinopathies remain undetected during conventional electrophoretic studies [11] for which reason chromatographic and molecular biology techniques have to be applied. In order to show the variant's high affinity for oxygen, however, a functional haemoglobin study is required. In all the patients studied, P_{50} was low but, in six of the ten variants found, the findings of electrophoretic and HPLC ionic-exchange studies were normal and only haemoglobin variants were disclosed by the reversed-phase HPLC.

A haemoglobin variant that shows high affinity for oxygen must be the suspected aetiology in families that report a history of erythrocytosis or young people, when there are no apparent reasons for this. Although it is rarely diagnosed, the 17 families reported that display ten different variants in the same geographic area lead us to think that the percentage of secondary erythrocytosis in haemoglobinopathies of this type could be even higher and therefore their systematic detection should be emphasised in erythrocytosis of unknown origin, more so when a family history is available.

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