

Red Cell Disorders

Genetic studies suggest a novel Portuguese origin for hemoglobin Porto Alegre

Molecular studies performed in Portuguese and Brazilian cases of hemoglobin Porto Alegre [β^9 Ser \rightarrow Cys] revealed that the mutation is in association with the Mediterranean haplotype I and framework 1 and that it is also in *cis* with an undescribed intragenic polymorphism (codon 27, GCC \rightarrow GCT). Based upon these findings, and reinforced by historical data, we suggest that hemoglobin Porto Alegre originated from a single mutational event in the Portuguese population and was then spread to South America, namely to Brazil.

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Hemoglobin (Hb) Porto Alegre [β^9 (A6) Ser \rightarrow Cys] was discovered in 1963 in a Caucasian Brazilian family¹ and was subsequently observed in members of several other families in Brazil,^{2,3} Cuba⁴ and the Canary Islands.⁵ Malcorra-Azpiazu and colleagues suggested in 1993 that the Hb Porto Alegre mutation originated in the population of the Canary Islands and was then exported to the Portuguese and Spanish colonies of South America.⁵ In this report, we describe four novel cases of Hb Porto Alegre, found in two unrelated Portuguese families, and we propose a novel mutational event for this variant.

Portuguese carriers of Hb Porto Alegre, as others, presented no clinical or hematologic features. Their red blood cell indices were obtained with an automated cell counter. Hb analysis was performed by cellulose acetate electrophoresis at pH 8.4, isoelectric focusing, and low pressure liquid chromatography (Hb-Gold, Drew Scientific Ltd., Barrow-in-Furness, Cumbria, England). Globin chains were separated by reversed phase-high performance liquid chromatography⁶ (Gold Beckman Liquid Chromatograph, Beckman Instruments Inc, Fullerton, CA, USA). All these biochemical techniques revealed the presence of one abnormal Hb variant (Figure 1). Total genomic DNA of the Hb variant carriers was isolated from peripheral blood leukocytes by a salting-out procedure.⁷ The coding regions of the *HBB* gene

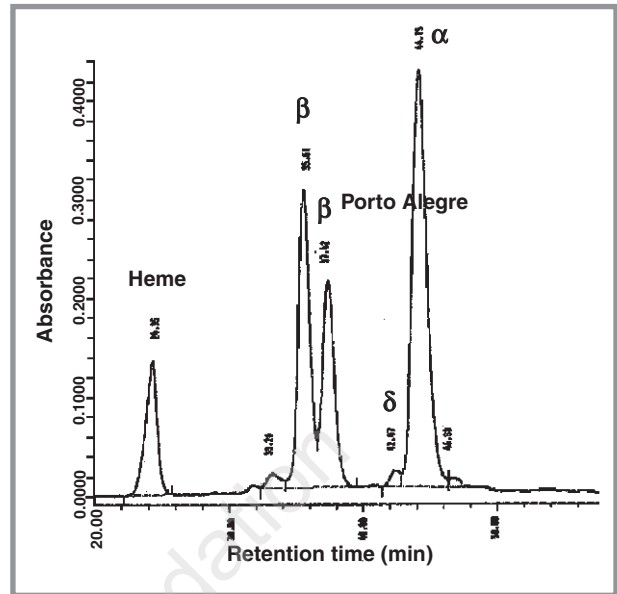


Figure 1. Reversed phase - high performance liquid chromatograph globin chain profile found in a carrier of Hb Porto Alegre. In this case, integration of the peak areas revealed 39.5% of $\beta^{\text{Hb Porto Alegre}}$ and 58.0% of β^{A} -chain.

were amplified by polymerase chain reaction (PCR). Direct sequencing of PCR products was performed using an ABI PRISM 3100 DNA Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

The *HBB* sequence analysis revealed a base substitution at codon 9 (TCT \rightarrow TGT), and consequently, the variant was identified as Hb Porto Alegre. Unexpectedly, another sequence alteration was detected in codon 27 (GCC \rightarrow GCT) of the same gene (Figure 2); this alteration does not lead to an amino acid substitution (both coding for alanine). β -globin cluster haplotype, which segregates with the variant, was assigned after examining nine restriction endonuclease sites: *HincII* (ϵ), *XmnI* (5' to $\epsilon\gamma$), *HindIII* (within $\epsilon\gamma$ and $\epsilon\gamma$), *HincII* (within and 3' to $\psi\beta$), *HinfI* (5' to β), *Avall* (within β), and *HinfI* (3' to β). The amplified products were digested with the appropriate restriction enzymes under the condi-

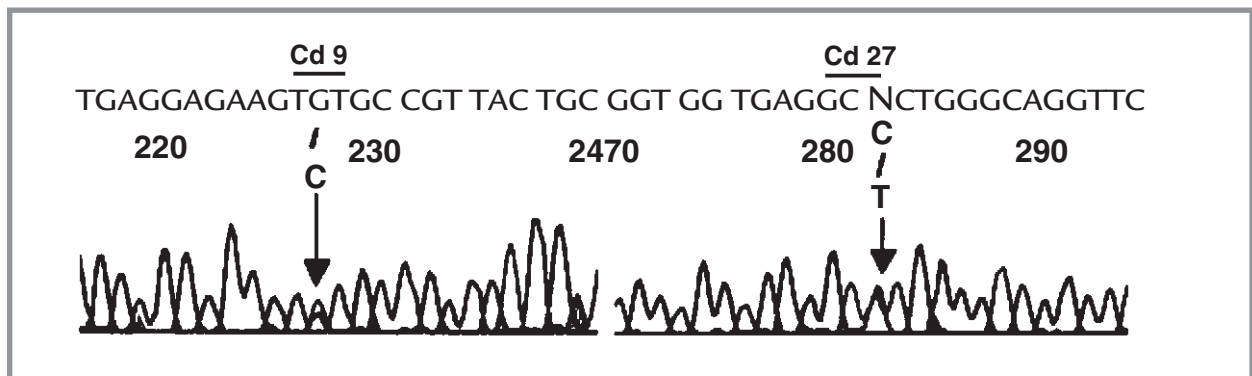


Figure 2. *HBB* sequence analysis showing the codon 9 (TCT \rightarrow TGT) mutation causing Hb Porto Alegre and the linked codon 27 polymorphism (GCC \rightarrow GCT).

tions recommended by the manufacturer. Family studies allowed the unequivocal association of Hb Porto Alegre with Mediterranean haplotype I⁸, and with framework 1⁸ and also revealed its segregation, in *cis*, with the codon 27 novel intragenic polymorphism.

Because of historical data, we hypothesized whether Hb Porto Alegre described in Brazil could have a Portuguese origin, and in order to answer this question, the same molecular approach was performed in the Brazilian family described by Gonçalves *et al.*³ As we expected, the Brazilian case presented the same, above described, genetic background.

As suggested by Malcorra-Azpiazu and colleagues in 1993,⁵ the several cases of Hb Porto Alegre described in the Canary Islands could be indicative that the mutational event giving rise to this variant had occurred there. Subsequently, the mutation could have spread to Spanish colonies in South America. This hypothesis is, in fact, compatible with historical data. The indigenous inhabitants of the Canary Islands – the Guanches – were occasionally visited by a variety of groups: Romans (40 B.C.), then the Arabs (999), and then in the 13th and 14th centuries many European conquerors, including the Portuguese. In the 15th century the Islands was contended between the Portuguese and Spaniards until 1479, when the *Treaty of Alcáçovas* declared Spanish sovereignty over the islands. These islands became an important base for voyages to the Americas.⁹ However, knowing that Hb Porto Alegre has been found in the Portuguese population, another hypothesis could be forwarded. It is possible that Portuguese carriers of Hb Porto Alegre left progeny on Canary Islands and that the mutation subsequently expanded to the South America in the 15th and following centuries. Furthermore, it is known that after the discovery of Brazil by the Portuguese in 1500, a massive transmigration of the Portuguese population to Brazil occurred until the mid 20th century.¹⁰ In conclusion, based on our molecular findings, and considering the above historical data, we propose that Hb Porto Alegre had one original mutational event in the Portuguese population and that it was spread to South America, namely to Brazil.

This hypothesis could find support from studies of the genetic background of the Spanish and Cuban Hb Porto Alegre cases and efforts were made to contact the laboratories where these cases had been characterized. We have obtained information that in Cuba another case of the variant was detected, curiously in a family of Portuguese origin with the surname Ferreira (*G. Martinez, personnel communication*). Unfortunately, none of the above mentioned cases of Hb Porto Alegre was accessible for molecular studies.

Paula Faustino,* Armandina Miranda,[†] Isabel Picanço,*
Elza M Kimura,[‡] Fernando Ferreira Costa,[§]
Maria de Fátima Sonati[¶]

*Laboratório de Biologia Molecular, Centro de Genética Humana, Instituto Nacional de Saúde Dr Ricardo Jorge (INSA), Lisboa, Portugal; †Laboratório de Hematologia, Centro de Biopatologia, INSA, Lisboa, Portugal; ‡Departamento de Patologia Clínica, Faculdade de Ciências Médicas, Universidade Estadual de Campinas (UNICAMP), Campinas, Brazil; §Centro de Hematologia e Hemoterapia, UNICAMP, Campinas, Brazil

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Correspondence: Paula Faustino, PhD, Centro de Genética Humana, Instituto Nacional de Saúde Dr Ricardo Jorge, Av. Padre Cruz 1649-016 Lisboa, Portugal. Phone: international +351.217519234. Fax: international +351.217526410. E-mail: paula.faustino@insa.min-saude.pt

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Red Cell Disorders

Simultaneous detection of α -thalassaemia and β -thalassaemia by oligonucleotide microarray

In this study, we describe a reliable microarray-based assay for the simultaneous detection of α/β -globin genotypes. The efficiency and specificity of this method were evaluated by blinded analysis of 1,880 samples. The assay provides unambiguous detection of complex combinations of heterozygous, compound heterozygous and homozygous α/β -thalassaemia genotypes.

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Thalassaemia is most common genetic disorder in Asia. The approach to dealing with the problem of thalassaemia is to prevent and control the birth of new cases. This requires an accurate identification of couples at high risk of thalassaemia. A previous study has shown that microarrays can be used to detect thalassaemia.¹ In this study, we have developed a microarray-based assay for rapid detection of the common severe thalassaemia defects found in Asia. This screening test can accurately give a specific diagnosis of the thalassaemia genotype. To identify mutations of α/β -thalassaemia, a total 38 of oligonucleotide probes are immobilized on slides and a single hybridization is performed with fluorescence-labeled multiplex polymerase chain reaction (PCR) products. Hybridization is detected by fluorescence scanning, and the α/β -globin genotypes are assigned by quantitative analysis of the hybridization results.

A pair of probes was designed for each point mutation, one complementary to the normal sequence and the other to the variant. The probes are 15~20mer oligonucleotides with the mismatch base placed in the center of the