

## HEMOGLOBIN J ROVIGO ( $\alpha^{53}$ ALA $\rightarrow$ ASP) IS NOT ASSOCIATED WITH AN $\alpha$ -GLOBIN GENE DELETION

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### ABSTRACT

A patient of Italian descent exhibited an abnormal hemoglobin with a fast electrophoretic migration, corresponding to 30% of total hemoglobin. The chromatographic analysis disclosed an  $\alpha$  globin chain mutation; the tryptic digestion of the abnormal chain followed by HPLC analysis identified Hb J Rovigo ( $\alpha$  53 Ala  $\rightarrow$  Asp). DNA digested with Bam HI restriction enzyme revealed a single 14 kb band after hybridization to an  $\alpha$  specific probe. These results demonstrate that the hemoglobin J Rovigo mutation is not associated with  $\alpha$ -thalassemia, occurring on chromosome 16, where a pair of nondeleted  $\alpha$  genes is present.

### INTRODUCTION

Hemoglobin structural mutants are very frequent, some of which reach high frequencies (Hb S, Hb C, Hb D, Hb E) because of the selective advantage afforded against malaria. Most mutants are uncommon and others are extremely rare. Hb J Rovigo is a rare  $\alpha$  chain variant which was initially described in an Italian family (Alberti *et al.*, 1974), and later was observed in Brazil in a family of Italian descent (Araújo *et al.*, 1980).

The  $\alpha$ -globin gene cluster is located on chromosome 16 and comprises one embryonic  $\zeta$  gene, one  $\psi\zeta$  and one  $\psi\alpha$  genes, one pair of functional  $\alpha_2$  and  $\alpha_1$  genes and one  $\theta$  gene of undefined activity, arranged in the following order: 5' -  $\zeta$  -  $\psi\zeta$  -  $\psi\alpha$  -  $\alpha_2$  -  $\alpha_1$  -  $\theta$ -3'.  $\alpha$  gene deletions ( $\alpha$ -thalassemias) have been observed in association with several mutants (Pich *et al.*, 1978; Lie-Injo *et al.*, 1979; Honig *et al.*, 1980; Surrey *et al.*, 1980; Moo-Penn *et al.*, 1983 and Costa *et al.*, 1991). This association benefits the

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selection of the mutant in a malarious environment, because even though the structural mutant is selectively neutral, its frequency increases passively by force of the linkage with  $\alpha$ -thalassemia. In this work we show the results of the molecular study in a patient with Hb J Rovigo which demonstrates that this abnormal hemoglobin is not associated with  $\alpha$ -thalassemia.

## MATERIALS AND METHODS

### *Patient*

C.G., a woman of Italian descent, had a hemoglobin variant of fast electrophoretic migration, corresponding to 30% of the total hemoglobin. The hematologic data were: RBC=4.6 ( $10^6/\text{mm}^3$ ), Hb=13.8 g/dl and Ht=41%. Hb A<sub>2</sub> was 5.8% by elution after electrophoresis.

### *Hemoglobin analysis*

Hemoglobin electrophoresis was performed on cellulose acetate with Tris-EDTA-boric acid buffer at pH 8.9. Globin was precipitated from the hemolysate by acetone/HCl and the abnormal globin chain was isolated by chromatography on CM-cellulose (Clegg *et al.*, 1966), and freeze-dried. The isolated fraction was submitted to tryptic digestion, and the resulting peptides were separated by HPLC (Gardiner *et al.*, 1982). The peak corresponding to the abnormal peptide was collected, evaporated and submitted to aminoacid analysis after hydrolysis with 6 N HCl.

### *DNA analysis*

DNA was extracted from peripheral blood leukocytes, digested with the restriction endonuclease BamHI, according to the manufacturer's directions (Pharmacia, Sweden), electrophoresed on 0.8% agarose and transferred to nylon filters (Southern *et al.*, 1975). A genomic DNA fragment was used as probe, radiolabelled with  $^{32}\text{P}$ .dCTP by "Random Primer", hybridized to an alpha-specific probe, washed at high stringency and autoradiographed.

## RESULTS AND DISCUSSION

Electrophoresis revealed a fast-moving abnormal hemoglobin which corresponded to 30% of total hemoglobin (Figure 1). The chromatographic separation of globin chains on CM-cellulose showed an abnormal  $\alpha$  chain (Figure 2). HPLC separation

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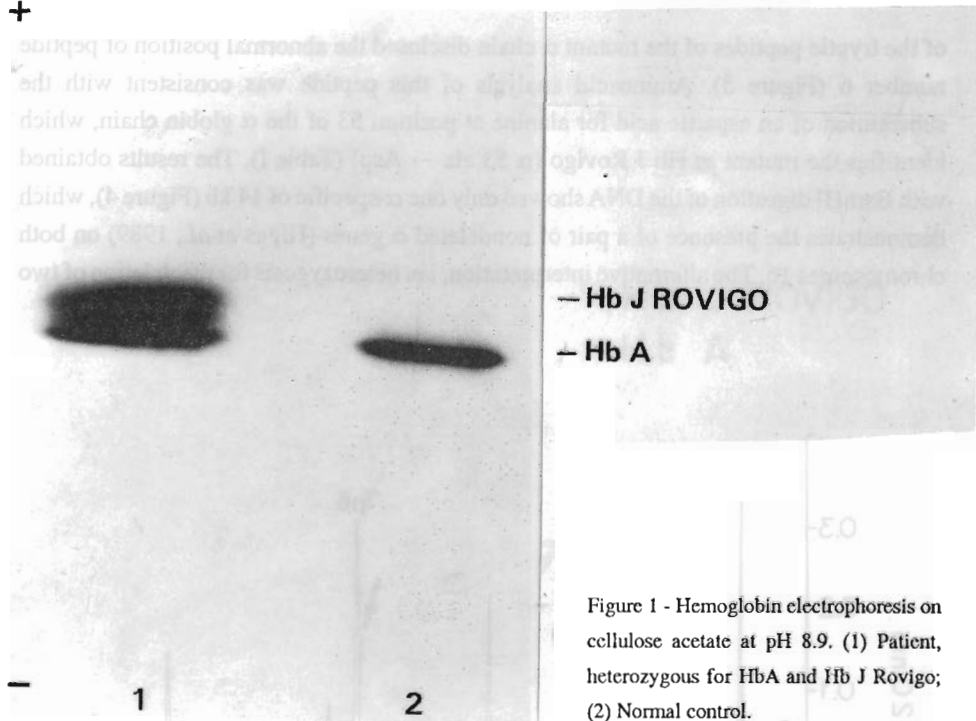


Figure 1 - Hemoglobin electrophoresis on cellulose acetate at pH 8.9. (1) Patient, heterozygous for HbA and Hb J Rovigo; (2) Normal control.

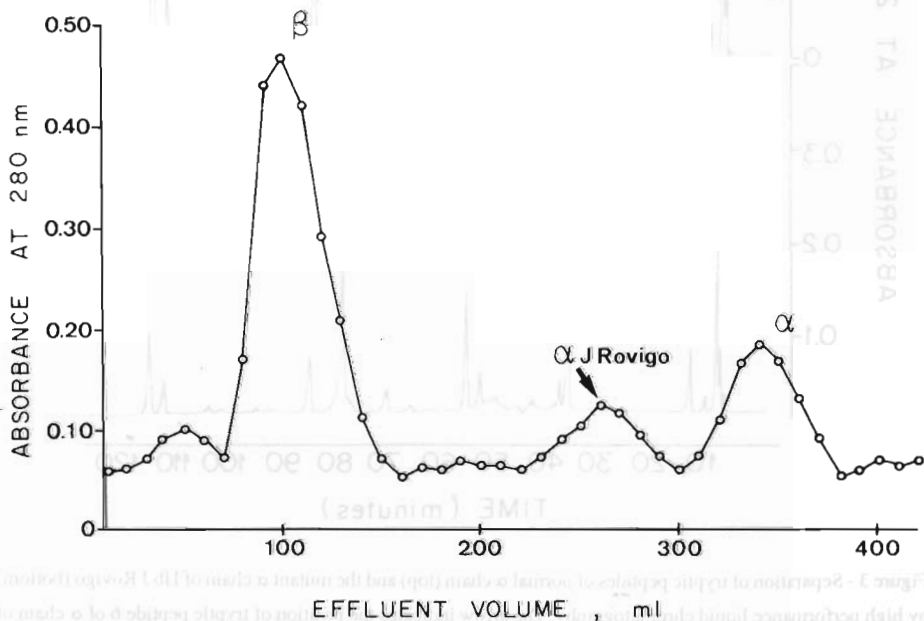


Figure 2 - Globin chain separation by chromatography on CM-cellulose with urea-phosphate buffers.

of the tryptic peptides of the mutant  $\alpha$  chain disclosed the abnormal position of peptide number 6 (Figure 3). Aminoacid analysis of this peptide was consistent with the substitution of an aspartic acid for alanine at position 53 of the  $\alpha$  globin chain, which identifies the mutant as Hb J Rovigo ( $\alpha$  53 ala  $\rightarrow$  Asp) (Table I). The results obtained with BamHI digestion of the DNA showed only one  $\alpha$ -specific of 14 kb (Figure 4), which demonstrates the presence of a pair of nondeleted  $\alpha$  genes (Higgs *et al.*, 1989) on both chromosomes 16. The alternative interpretation, i.e. heterozygosis for the deletion of two

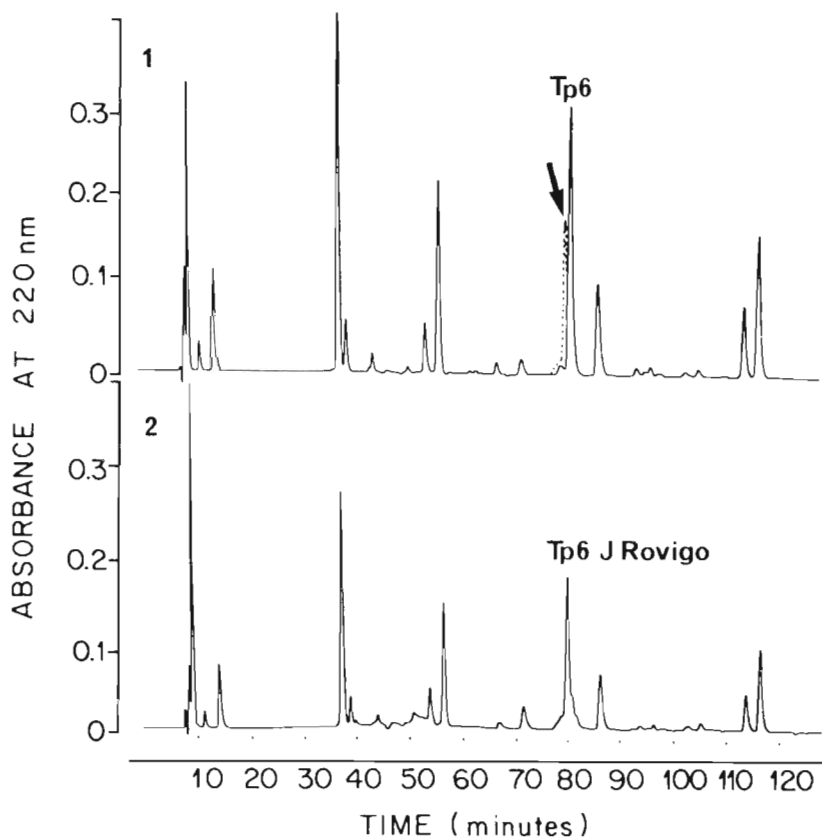


Figure 3 - Separation of tryptic peptides of normal  $\alpha$  chain (top) and the mutant  $\alpha$  chain of Hb J Rovigo (bottom) by high performance liquid chromatography. The arrow indicates the position of tryptic peptide 6 of  $\alpha$  chain of Hb J Rovigo.

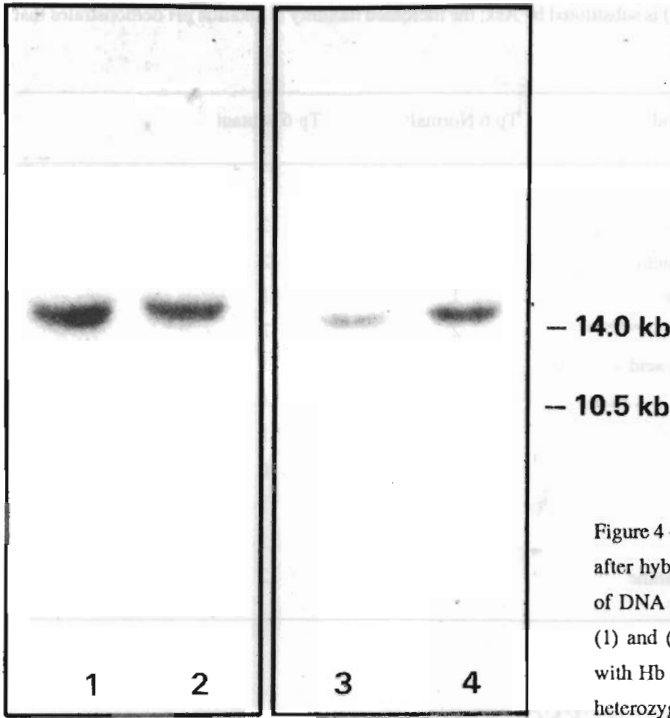


Figure 4 - Autoradiography of nylon filter after hybridization with  $\alpha$ -specific probe of DNA samples digested with Bam HI. (1) and (4): normal controls; (2) patient with Hb J Rovigo; (3)  $\alpha$  3.7-thalassemia heterozygote.

$\alpha$ -genes on one chromosome ( $\alpha^0$ -thalassemia) is not compatible with the hematologic data. Hb J Rovigo is an extremely rare mutant and only four cases have been described as yet, two of which were associated with  $\alpha^+$ -thalassemia (Alberti *et al.*, 1974; Moon-Penn *et al.*, 1978; Araújo *et al.*, 1980 and Hombrado *et al.*, 1987). In spite of the high frequency of  $\alpha$ -thalassemia in Italy, reaching about 15% in some regions (Williams *et al.*, 1990), our result does not demonstrate association of Hb J Rovigo with the deletion type thalassemia. This may explain the low frequency of the mutant, when compared with other  $\alpha$  mutants, such as Hb G Philadelphia (Surrey *et al.*, 1980), Hb Hasharon (Pich *et al.*, 1978) and Hb Stanleyville II (Costa *et al.*, 1991), for which an association of the mutation with  $\alpha$ -thalassemia has been demonstrated. The selective advantage provided by  $\alpha$ -thalassemia may have contributed to rising the frequencies of these mutants as opposed to Hb J Rovigo.

**Table I - Aminoacid composition of peptide Tp 6 from a normal  $\alpha$  chain and from an  $\alpha$  chain of Hb J Rovigo. The only Alanine (at position 53) is substituted by Asx; the increased mobility at alkaline pH demonstrates that the new residue is Aspartic acid.**

Aminoacid	Tp 6 Normal	Tp 6 Mutant
Lysine	1	1
Histidine	2	2
Aspartic acid	1	2
Threonine	1	1
Serine	2	2
Glutamic acid	1	1
Proline	1	1
Glycine	1	1
Alanine	1	0
Valine	1	1
Tyrosine	1	1
Phenylalanine	2	2

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## RESUMO

Uma paciente de descendência Italiana exibia uma hemoglobina anormal com migração eletroforética rápida correspondendo a 30% do total de hemoglobina. A análise cromatográfica mostrou tratar-se de alteração das cadeias  $\alpha$ ; pela digestão triptica seguida de análise por HPLC foi identificada como Hb J Rovigo ( $\alpha^{53}$  Ala  $\rightarrow$  Asp). A análise do DNA da paciente após a digestão com enzima BamHI produziu um único fragmento de 14 kb. Esses resultados demonstram que a mutação da Hb J Rovigo não está associada à  $\alpha$ -talassemia, ou seja, ocorre em um cromossomo 16 onde está presente um par de genes  $\alpha$ .

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