



Is the α -^{cal} mutation prevalent in central Spain?

The two structural genes for the α -globin chain are located on the short arm of chromosome 16. Normal individuals have four α genes ($\alpha\alpha/\alpha\alpha$) whereas α -thalassemias are usually produced by deletion of one, two, three or four α genes or by point mutations.¹ Deletion of both α genes within the same chromosome is commonly observed in individuals from the Mediterranean basin² and South East. In Spain, α^0 mutations are common to other populations (--SEA, --MED),³ and other mutations are characteristic of the Hispanic ethnic group in which at least four new mutations have been described.⁴⁻⁶

Sir,

Recently, we had the opportunity to study twenty-two patients with the --CAL mutation belonging to five unrelated families from the Madrid region, all within a 50 km radius of the city center. This mutation was described previously by Fortina et al.⁷ in a Calabrian family.

Hematologic data were obtained using a Coulter STKS. Globin chain synthesis was determined by the method described by Clegg.⁸

DNA was isolated from the white cells obtained from peripheral blood extracted with phenol-chloroform, digested with several different restriction enzymes and hybridized with the following probes: a 1.8 kb SAC I ζ fragment from the recombinant pRB ζ , a 1.5 kb Pst I α fragment from the recombinant pRB α , a 4.0 kb Hinf I 3'HVR and a 0.6 kb ECOR I/BAM HI from the plasmid Jw5.⁹

Hematologic data and globin chain synthesis for the patients with α^0 -thalassemia and the two with HbH disease are similar to those recorded in subjects with other mutations with HbH disease or with α -thalassemia-1.

Abnormal bands obtained with 3'HVR probe are shown in Figure 1. The deletion removes genes $\alpha 1$, $\alpha 2$, $\psi\alpha 1$, $\psi\alpha 2$, $\psi\zeta 1$ and $\zeta 2$. The Pvu II 3'HVR allele associated with this deletion is approximately 3.8 kb long.

In Spain, approximately 100 cases of heterozygotic α^0 -thalassemia have been described. These patients are from both central Spain and coastal regions refuting the hypothesis that α thalassemia is restricted to the Mediterranean coast.

In this work, we found a surprisingly high frequency of the --CAL mutation present in four generations in five unrelated families. This mutation was described in the mother and daughter of a Calabrian family in 1991.⁷

It is interesting to examine the relationship between the Spanish and Italian populations. The Spanish presence in Italy dates back to the end of the XIII century when James II of Aragon (1291-1327) was king of Sicily. From 1495 this presence was intensified when the province of Naples became linked to the Crown of Aragon (1503). During these years large numbers of Spanish troops travelled to Naples. This situation was repeated during the war (1713-1715) that ended in the Utrecht treaty and later with Elizabeth of Farnesio and her son Charles,

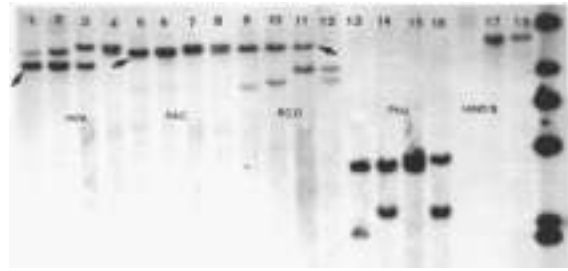


Figure 1. Autoradiograph showing Southern blots of DNA digested with *Hpa* I, *Sac* I, *Eco* RI, *Pvu* II and *Hind* III, and hybridized with the 3'HVR probe. Abnormal bands produced by hybridization to the α -globin 3'HVR probe are indicated with arrows.

King of Naples (1734-1759) and of Spain (1759-1788, known as Charles III).

Therefore, given the close relationship between these populations, this mutation probably has a common origin, either originating in Calabria and later being transported to Spain or originating in Spain and then being taken to Naples by Spanish troops during the Spanish occupation.

The fact that, at the moment, the number of reported cases of the --CAL mutation in Italy is low^{4,7} suggests that this mutation originated in Spain, perhaps Madrid, an area of great historical importance.

A population study of the provinces around Madrid is in progress in order to determine the true incidence of this mutation in the autonomous regions of Madrid and Castile.

Ana Villegas, Paloma Ropero, Fernando Ataulfo González, Eduardo Anguita, Jesús Sánchez, Evaristo Quevedo, * Isabel San Juan^o

Servicio de Hematología y Hemoterapia, Hospital Clínico San Carlos, Madrid;

*Servicio de Hematología y Hemoterapia, Hospital La Paz, Madrid;

^oServicio de Hematología y Hemoterapia, Hospital Puerta de Hierro, Madrid, Spain

Key words

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Correspondence

A. Villegas, M.D., Servicio de Hematología y Hemoterapia, Hospital Clínico San Carlos, C/ Profesor Martín Lagos s/n, 28040 Madrid, Spain. Phone: international +34-13303321 – Fax: international +34-13303322 – E-mail: avillegasm@medinet.es

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Most frequent mutations of β -thalassaemia in Rosario, Argentina

The β -thalassaemia syndromes are genetic disorders characterized by absence or decrease in β chain synthesis, producing an alteration in the α and β chain relationship.¹ From the molecular point of view β -thalassaemia is very heterogeneous. More than 100 mutations have been described.² Each ethnic group has a variety of mutations; in the Mediterranean region there are more than 30 mutations, but only 8 of them are common. Our population is composed of different ethnic groups, with immigration principally from Italy and Spain. This study was an attempt to gather information regarding the presence of the most frequent mutations in our population.

Sir,

Seventy-three non-consanguineous patients, above 1 year of age, diagnosed as having heterozygous β -thalassaemia (72 patients), and β^s/β^{Th} (1 patient), were studied at the Hospital Provincial del Centenario, Rosario, Argentina, from 1996 through 1998. Two of the 73 heterozygous β -thalassaemia patients were the parents of 4 children: 2 with Cooley's anemia, 1 with heterozygous thalassaemia, and 1 hematologically normal.

Patients with Hb A₂ between 4-6% and Hb F lower than 5% were included. Peripheral blood DNA extraction was performed using the salting out method,³ and these mutations were assessed: -codon 39C→T (β^o39), -IVS1-110 G→A (β^+), -IVS1-1 G→A (β^o), -IVS1-6 T→C (β^+), -IVS2-1 G→A (β^o) and IVS2-745 C→G (β^+) by means of a modified polymerase chain reaction technique: the Amplification Refractory Mutation System.⁴

The ethnic origin of the patients studied was: 89% Italians, 9.6% Spaniards and 1.4% Greek.

Forty patients (54.8 %) were β^o39 ; 16 (21.9 %) β^+1-110 ; 6 (8.2 %) β^o1-1 ; 3 (4.1%) β^+2-745 and 2 (2.7 %) β^o2-1 . There were no β^+1-6 mutations. Genotype could be assessed in 91.7%.

The mutation in the double heterozygote patient was β^o39 , which is the most frequent mutation in our population. The parents of the children affected by Cooley's anemia were: β^+1-110 (mother) and β^o2-1 (father), so, these patients were double heterozygous, with only one allele (β^+1-110) having a high frequency in our population. None of the six mutations under study was identified in the patient of Greek origin.

In the patients under study the prevalence of the β^o allele and the β^+ allele was 73% and 27%, respectively.

At present there is little information and very few data published about the distribution of hemoglobinopathies in Argentina.⁵⁻⁷ The frequencies of the mutations studied are similar to those reported for other regions of our country which, in their turn, are similar to those reported for the Mediterranean region.⁸⁻¹⁰ Strikingly, considering the ethnic origin, there were no patients with the β^+1-6 mutation, which reaches a frequency of 5.9% in Buenos Aires and 10.3% in Italy.

In conclusion, this study provides data for our region, for which there were no records up to now. Although there are few homozygous cases in our population, the information could be useful in cases of prenatal diagnosis.

Irma Margarita Bragós, Nélica Inés Noguera,
Lida Morisoli, Angela Cristina Milani

Cátedra y Servicio de Hematología, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Hospital Provincial del Centenario, Rosario, Santa Fe, Argentina

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β -thalassaemia mutations.

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Correspondence

Irma M. Bragós, M.D., Area Hematología - Departamento Bioquímica Clínica, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Suipacha 531-2000, Rosario, Argentina. Phone/fax: international +54-341-4804598 - E-mail: nnoguera@fbioyf.unr.edu.ar

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