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# High incidence of the CD8/9 (+G) β⁰-thalassemia mutation in Spain

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**Abstract**

***Background and Objective.*** **In Spain, as in other Mediterranean regions the most common β-thalassemia mutations are due to point mutations in gene regions that are critical for production of mRNA, such as [IVS-I-nt1 (G→A), IVS-I-nt6 (T→C), IVS-I-nt110 (G→A)] which interrupt normal RNA processing or nonsense mutations [CD39 (C→T)] which interrupt the translation of mRNA. The frameshift mutation CD8/9 (+G) is a very common allele in Asian Indians but is rare in the Mediterranean regions in which isolated alleles with this mutation have been found in Israel, Greece, Portugal and Turkey.**

***Design and Methods.*** **We performed a molecular analysis of 175 chromosomes corresponding to 233 β-thalassemia patients (221 heterozygous, 10 homozygous and 2 compound heterozygous) who belong to 169 Spanish families. The study of β-thalassemia was made by PCR-ARMS, the α genes by Southern blot, the phenotype of Hb Lepore by enzymatic amplification and the presence of –158 $\gamma^G$ C→T mutation by PCR and digestion with the restriction enzyme XmnI.**

***Results.*** **Twenty of these 233 patients showed the β-thalassemia mutation CD8/9 (+G) (17 were heterozygous, 2 homozygous and in one patient the mutation was associated with a structural variant Hb Lepore Boston).**

***Interpretation and Conclusions*****. These data reveal the heterogeneity of β-thalassemia in Spain and the relatively high frequency (8.6%) of the frameshift mutation CD8/9 (+G). It is surprising that homozygotes for β⁰-thalassemia due to this mutation with very high Hb F values (around 90%) present a phenotype of intermediate thalassemia.**


Key words: β-thalassemia, frameshift mutation CD 8/9 (+G), PCR-ARMS, Hb Lepore Boston

β-thalassemia is a diverse group of inherited human hemoglobin disorders characterized by a reduction or complete absence of β globin expression.[1] Thalassemia is most prevalent in the malarial regions of the world and more than 130 β-thalassemia mutations have been described.[2]

In Spain, as in other Mediterranean regions, these are usually point mutations in gene regions that are critical for production of the mRNA, such as [IVS-I-nt1 (G→A), IVS-I-nt6 (T→C), IVS-I-nt110 (G→A)] which interrupt the normal RNA processing or nonsense mutations [CD39 (C→T)] which suspend the translation of mRNA.[3-6] However, a large number of uncommon alleles have been observed both in Spain and in other populations. The frameshift mutation CD 8/9 (+G) is a very common allele in Asian Indians but is rare in the Mediterranean region although it has been recorded in Israel, Greece, Portugal and Turkey.[6,7] In Spain we have previously recorded this mutation in three patients.[5]

In this paper we describe 20 Spanish patients with the CD 8/9 (+G) mutation; 17 of them were heterozygous, 2 were homozygous and in another patient the mutation was associated with a structural variant Hb Lepore Boston.

## Materials and Methods

We studied, at molecular level, 175 chromosomes corresponding to 233 β-thalassemia patients (221 heterozygotes, 10 homozygotes and 2 compound heterozygotes) who belong to 169 Spanish families. The β-thalassemia was diagnosed in our laboratory by standard methodology[1,8] between January 1995 and August 1997.

The molecular study of β-thalassemia mutation was carried out using DNA obtained from peripheral leukocytes according to the technique described by Poncz *et al*.[9] One μg of DNA was used to amplify the β globin gene by the polymerase chain reaction technique (PCR-ARMS)[10] using normal and mutated oligonucleotides for the twelve most frequent mutations in our environment; six of them corresponded to point mutations (IVS-I-nt1, IVS-I-nt6, IVS-I-nt110, IVS-II-nt745, IVS-I-nt5, IVS-II-nt1), one to a nonsense mutation CD39, three to frameshifts (CD8/9, CD8, CD6, CD5) and one (–86) to the promoter region of the β gene.[5]

Hb Lepore Boston was revealed by enzymatic amplification using specific nucleotides for the allele associated with the anomaly and for the normal allele.[11] The α genes were studied by Southern Blot-

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**Table 1. Hematologic data for the CD8/9 (+G) frameshift heterozygotes, double heterozygote (β-thalassemia/Hb Lepore Boston) and homozygotes.**

| | *X (n=14) (fam. 1-12)* | *Mother (fam. 13)* | *Son (fam. 13)* | *Father (fam. 14)* | *Mother (fam. 14)* | *Daughter (fam. 14)* | *Son* (fam. 14)* |
|---|---|---|---|---|---|---|---|
| RBC ($10^{12}$/L) | 5.8±0.9 | 5.3 | 3.6 | 5.6 | 5.9 | 3.4 | 3.6 |
| Hb (g/dL) | 11.7±1.8 | 10.9 | 8.3 | 11.1 | 12.1 | 8.4 | 10.2 |
| Hct (L/L) | 35.7±5.4 | 32.4 | 25.1 | 35.2 | 37.9 | 26.2 | 30.6 |
| MCV (fL) | 62.0±3.4 | 61.2 | 69.2 | 63.2 | 64.6 | 76.5 | 85.6 |
| MCH (pg) | 20.4±1.3 | 20.6 | 22.9 | 19.9 | 20.6 | 24.6 | 28.6 |
| RDW (%) | 15.1±0.9 | 14.8 | 29.5 | 16.9 | 15.5 | 34.3 | 14.5 |
| Reticulocytes ($10^9$/L) | 11.8±5.5 | 7.0 | 12.0 | 16.0 | 23.0 | 63.0 | 8.0 |
| Hb A2 (%) | 4.3±0.4 | 5.2 | 1.7 | 5.4 | 5.3 | 2.4 | 0.8 |
| Hb F (%) | 2.4±1.0 | 5.0 | 94.0 | 9.0 | 10.0 | 90.0 | 10.0 |
| Hb Lepore (%) | 0.0 | 0.0 | 4.3 | 0.0 | 0.0 | 0.0 | 0.0 |
| $\beta^{CD\ 8/9}/\beta^{A}$ | + | + | | + | + | | |
| $\beta^{CD\ 8/9}/\beta^{Lepore\ WB}$ | | | + | | | | |
| $\beta^{CD\ 8/9}/\beta^{CD\ 8/9}$ | | | | | | + | + |

**Transfused two weeks previously.*

ting with the restriction enzymes Bam HI, Bgl II with the α and ζ probes and Nco I, Hph I with the α probe. The presence of -158 $\gamma^G$ C→T mutation was evaluated by enzymatic amplification and digestion with the restriction enzymze XmnL.[12]

## Results

Twenty patients from 14 different unrelated families (15 chromosomes) showed the β-thalassemia CD 8/9 (+G) mutation. None of the patients had any hindu or gypsy relatives.

Fourteen individuals (from 12 families) were heterozygous for the CD 8/9 (+G) mutation. Mean values and standard deviations for the hematologic data and the hemoglobin study are recorded in the first column of Table 1.

In family #13, the father was heterozygous for Hb Lepore Boston determined by electrophoresis, HPLC (data not showed) and PCR (Figure 1), the mother was a carrier of $\beta^0$-thalassemia and the son was a compound heterozygote for CD 8/9 (+G) mutation (Figure 2) and Hb Lepore Boston (Figure 1) with a phenotypic expression of intermediate thalassemia (3rd column of Table 1) with hepatosplenomegaly and peripheral erythroblasts.

There were four members in the 14th family, father, mother and two children. The parents are first cousins and are both heterozygous carriers of β-thalassemia (Figure 2) with 9 and 10% Hb F (4th and 5th columns of Table 1). The children (daughter and son) were homozygotes for the CD 8/9 (+G) mutation (Figure 2) and presented a clinical picture of intermediate thalassemia with Hb F values of 90 and 10%, respectively. The latter had been transfused a short time previously (6th and 7th columns of Table 1). All members of the family were normal for polymorphism in the -158 $\gamma^G$ (C→T). α-thalassemia deletion and α-thalassemia non deletion Nco I and Hph I associated were ruled out.

## Discussion

The molecular basis of β-thalassemia in the Spanish population is heterogenous and most cases are similar to those previously described in other countries of the Mediterranean basin where the most prevalent mutations are IVS-I-nt110 (G→A), IVS-I-nt1 (G→A), CD39 (C→T) and IVS-I-nt6 (T→C).[3-6] In our study on 175 β-thalassemia chromosomes we found 15 (8.6%) with the CD 8/9 (+G) mutation. This frameshift with

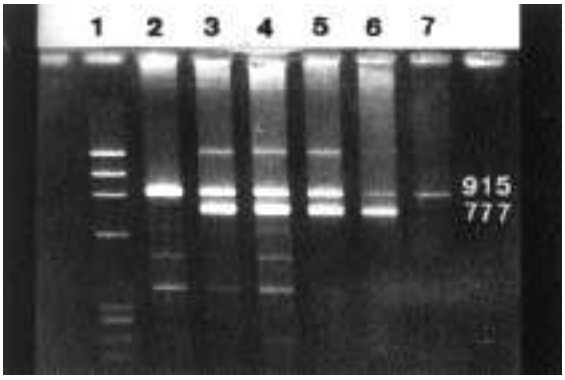


**Figure 1. Ethidium bromide-stained agarose gels illustrating the products obtained after amplification using primers $E_1$, $E_2$ and $E_3$ for Hb Lepore Boston. Lane 1 marker DNA ($\Phi_X$ Hae III). Lanes 2 and 7 no Hb Lepore (mother of family #13), lanes 3, 4 (father of family #13), 5 and 6 (son of the family 13) represent heterozygotes for Hb Lepore Boston.**

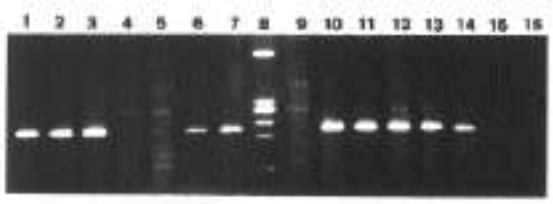


**Figure 2. Identification of the CD 8/9 (+G) frameshift mutation by PCR-ARMS. Lanes 1-7 normal assay, lanes 9-15 mutant assay. Lanes 1, 9 and 7, 15 normal subject, lanes 2, 10 mother of family #14 (heterozygote), lanes 3, 11 father of family #14 (heterozygote), lane 4, 12 son of family #14 (homozygote), lane 5, 13 son of family #13 (double heterozygote $\beta^{CD8/9}/\beta^{Lepore\ Boston}$), lane 6, 14 mother of family #13 (heterozygote) and line 16 contamination control.**

the IVS-I-nt6 mutation, represents the third most frequently found mutation in our area, behind the IVS-I-nt1 (G→A) and CD39 (C→T) mutations.

The CD8/9 (+G) mutation was phenotypically expressed in the heterozygote individual by mild symptoms, as in other cases of heterozygote $\beta^0$-thalassemia studied previously by our team.[5] The absence of $\beta^A$ globin chains and hence Hb A in the patient who was a compound heterozygote for β-thalassemia and Hb Lepore Boston confirms that the frameshift mutation CD8/9 (+G) behaves as a $\beta^0$ thalassemia with a complete lack of β chain synthesis. In the other family (the 14th) it is surprising that the children, in spite of being homozygotes for $\beta^0$-thalassemia with very high Hb F values (around 90%) present a phenotype of intermediate thalassemia. This is probably due to an associated persistent syndrome of fetal hemoglobin production (HPHF), which is at present being studied in order to define it at a molecular level.

This work once again demonstrates the heterogeneity of β-thalassemia in Spain and newly demonstrates the relatively high frequency (8.6%) of the CD 8/9 (+G) frameshift mutation which has not been reported to occurr at this high frequency in any country of the Mediterranean basin. We did not study the haplotype of our patients; it would be interesting to do this and compare it with Orkin's haplotype I[13, 14] described in Asian Indians and determine whether it could be an example of genetic migration of the mutation or whether it has arisen independently in our different ethnic group.[15]

### Contributions and Acknowledgments

*AV was the principal investigator and designed the study. PR developed and carried out the molecular studies and wrote the paper with FAG and AV. EM carried out the study of hemoglobin and globin chains by HPLC. EA studied the α genes. JMDB sent us family #14.*

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### Disclosures

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