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# β-THALASSEMIA MUTATIONS IN ROME. A HIGH FREQUENCY OF THE IVSII-745 ALLELE IN SUBJECTS OF LATIUM ORIGIN

***Adriana Massa*, Paolo Cianciulli°, Luciano Cianetti*^, Rita Iazzone°, Alessandra Cenci*, Francesco Sorrentino°, Giovanni Franco*, Giulio Pecci*, Giuseppe Papa°, Cesare Peschle*#***

**Hematology-Oncology Laboratory, Istituto Superiore di Sanità, Rome; #Thomas Jefferson University, Philadelphia, PA, USA; °Hematology Division, S. Eugenio Hospital, IInd University of Rome; ^Istituto di Medicina Sperimentale, CNR, Rome, Italy*

**ABSTRACT**

**We studied the molecular bases of β-thalassemia in Rome, a city centrally located in Latium, which is a region with a low incidence of β-carriers. People also come to Rome from other regions for specific or prenatal diagnostic assessment. Only 11 patients (20%) out of 62 characterized β-thalassemia subjects were of Latium family origin. They presented five mutations with an uncommonly high frequency of the IVSII-745 allele, that was found in homozygosis in 4 unrelated patients from a southeastern area in the province of Frosinone. These data may indicate a founder effect.**

*Key words: β-thalassemia, β-globin gene mutations, Latium β-mutations, IVSII-745 allele*



The molecular bases of β-thalassemia in the Mediterranean area are well known, and more than 50 specific defects have been classified so far.[1] In Italy extensive studies have been performed in the major islands, Sicily and Sardinia, and in some Southern areas.

Rome is centrally located in the Latium region and has a low incidence of β-thalassemia; the screening programs carried out by Bianco e al.[2] showed a 2% mean frequency. People also come to Rome from other regions for specific diagnostic assessments or prenatal diagnosis.

We performed molecular analyses in patients with Cooley's anemia and thalassemia intermedia living in Latium. The study aimed to:

1) identify the number and types of β-thalassemia alleles in Rome as a prerequisite for developing an efficient program of prenatal diagnosis;

2) characterize molecular defects in subjects of Latium family origin;

3) study the molecular bases of thalassemia intermedia.

We report here only the data for patients of Latium origin.

## *Materials and methods*

We studied 62 unrelated subjects with thalassemia major or intermedia currently being treated in the Hematology Department of S. Eugenio Hospital (II University of Rome). Patients traced their origins to six different regions (excluding Sardinia) but only eleven, less than 20%, had their family origin in Latium.

DNA was obtained from peripheral blood using standard techniques.

Direct identification of the most common β-alleles was performed on polymerase chain

*Correspondence: Dr. Adriana Massa, Istituto Superiore di Sanità, Laboratorio di Ematologia ed Oncologia, viale Regina Elena 299, 00161 Rome*
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Table 1. β-mutations in subjects of Latium family origin

| Subjects | Provinces | β/α ratio | α/non α ratio | Haplotype | β-mutations | Genotype |
|---|---|---|---|---|---|---|
| C.R. | Rm-Rm | 0.08 | 3.22 | I/VII | IVSI-110/IVSII-745 | β+/β+ |
| D.S. | Rm-Rm | 0 | 3.42 | II/II | β°39/β°39 | β°/β° |
| B.U. | Rm-Fr | 0 | 2.96 | II/II | β°39/β°39 | β°/β° |
| M.A. | Rm-Vt | 0.05 | 3.58 | I/II | β°39/IVSI-110 | β°/β+ |
| D.M. | Rm-Ri | 0,06 | 3.06 | V/VII | Fr.6/IVSII-745 | β°/β+ |
| F.S. | Rm/Vt | 0 | 2.56 | II/V | β°39/IVSI-1 | β°/β° |
| M.M.G. | Lt/Lt | 0.05 | 2.98 | I/II | β°39/IVSI-110 | β°/β+ |
| V.F. | Fr-Fr | 0.04 | 4.36 | VII/VII | IVSII-745/IVSII-745 | β+/β+ |
| C.S. | Fr-Fr | 0.03 | 3.01 | VII/VII | IVSII-745-IVSII-745 | β+/β+ |
| N.D. | Fr-Fr | 0.02 | 4.90 | VII/VII | IVSII-745-IVSII-745 | β+/β+ |
| N.M. | Fr-Fr | 0.04 | 4.40 | VII/VII | IVSII-745-IVSII-745 | β+/β+ |

reaction (PCR)-amplified DNA hybridized with 32-P-labelled specific oligoprobes. The *frameshift* 6 and IVS II-745 mutations were detected by both Southern blotting and direct band visualization on agarose gel after digestion of the amplified product with Mst II and Rsa I, respectively.

In order to examine the ζ–α gene complex, DNA was restricted with Bam HI and Bgl II endonucleases, submitted to electrophoresis and Southern blotting, and hybridized with α and ζ genomic probes.

Haplotypes of the β-globin gene cluster were determined according to Orkin et al.[3]

To identify the IVSI (G-A) defect, the appropriate DNA regions were amplified by PCR, subcloned in pGEM 4Z plasmid and sequenced according to the dideoxy chain termination method.

### Results

The data obtained from our study show the presence of 9 previously described mutations related to the Mediterranean area that have combined to give rise to 20 different genotypes; the majority of these accounted for $\beta^{\circ}/\beta^{+}$ compound heterozygosis (data not shown).

The most frequent allele was β°39, which characterized 38% of chromosomes; other common β° and $\beta^{+}$ defects identified included: IVSI-1, IVS-110, IVSI-6, IVSII-1, Fr.6, IVSII-745, and two rare β+ mutations [–87 and IVS I-5 (G-A)].

The data from patients of Latium origin are reported in Table 1.

### Discussion

β-thalassemia is the most common inherited blood disorder in Mediterranean populations

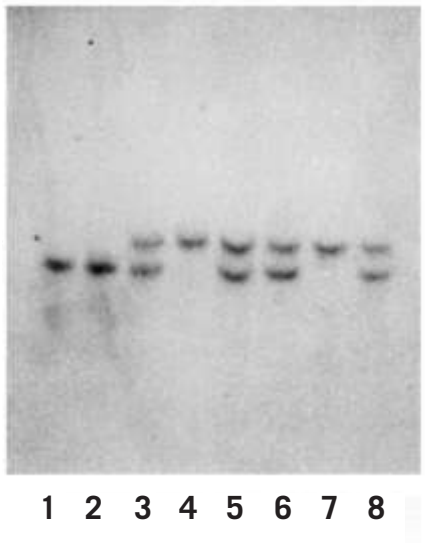


Figure 1. Analysis of DNA restriction fragment using Rsa I enzyme and a 3'β genomic probe. The 1.9 Kb band indicates the IVSII-745 mutation. Lanes 1, 2, 4, 5 represent subjects of Latium origin; lanes 3, 6, 7, 8, patients of other regions.

and represents a major public health problem in several countries. However, β-thalassemia mutations have different frequencies in the eastern and western parts of the Mediterranean basin.[4] Similar heterogeneity has been found within different areas of the same country, as in Portugal,[5] Cyprus[6] and Italy.[7]

As expected, this study confirms the presence of extreme genetic heterogeneity in Rome due to the mixed composition of people coming from different regions, especially in Southern Italy. In the subjects we studied, β° and β+ mutations each characterized 50% of chromosomes. We excluded subjects from Sardinian families, who are often homozygotes for the same mutant. Therefore we determined that the β°39 mutation accounted for the most frequent β-thalassemic allele in the region. Patients of Latium origin showed five mutations with an unusually high frequency of the IVSII-745 defect, which was found in homozygosis in 4 out of 11 unrelated patients (Figure 1) and which originated in a restricted area of the southeastern province of Frosinone.

This elevated frequency may indicate a founder effect. One possible explanation for this finding is the isolation in the past of this area due to its geographic location.

Bianco et al.[8] observed an 8% frequency of this mutation in 147 heterozygotes analyzed in their diagnostic center. In another small series,[9] an 11.5% incidence was found. Neither of these studies reported the family origin of subjects. Percent differences may be due to the composition and size of samples; however, our data indicate that the IVSII-745 mutation has a higher incidence in Latium. In fact, we found only one homozygote among the 51 patients from other regions where the mean frequency ranges from 0.4% in Sardinia to 5% in Calabria and Sicily.[9] The highest incidence (8%) is found in Campania, i.e. in a region bordering Latium. Further studies on a larger target population will be necessary to confirm these findings.

The present data on β-mutations in patients of Latium origin may contribute to the assessment of the distribution of β-thalassemia alleles in Italy.



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