β- and α-globin genotypes in Albanian patients affected by β-globin gene disorders

We investigated the molecular basis of hemoglobinopathies and restriction fragment length polymorphism (RFLP) haplotypes in 58 unrelated Albanian patients. A wide heterogeneity was detected, characterized by 11 **β**-thalassemia, 3 Hb variant and 4 **α**-globin alleles. All **β**-thalassemia and Hb variant alleles were associated with the same haplotypes described in other populations. Genotype-phenotype correlation was established.

haematologica 2002; 87:1002-1003
(*http://www.haematologica.ws/2002_09/1002.htm*)

Albania is one of the Balkan countries located in the South-East Europe on the Adriatic and Ionian Sea. The overall carrier frequency of β-thalassemia and of hemoglobin (Hb) variants is 7.1% and 4.4%, respectively.[1] Despite this high prevalence, few Albanian patients have been studied so far[2-4] and only one extensive study of 48 patients has been carried out.[5]

We studied the molecular basis of hemoglobinopathies and RFLP haplotypes associated with the mutant alleles in 58 unrelated Albanian patients and 40 relatives from districts of South-West Albania, an area with a higher prevalence of hemoglobin disorders. The heterogeneity of molecular genotypes was greater than that previously reported.[5] In fact, we found 27 genotypes characterized by 11 β-thalassemia alleles in 87 chromosomes, 3 Hb variants in 27 and 4 α-globin alleles in 7 chromosomes (Table 1). Out of the defects, the α_2 3' UTR +832 G→A was a new mutation; the alleles β-IVS-II-745, β-codon 82-83, β-codon 37, $\alpha\alpha\alpha^{anti3.7}$, -(α)$^{20.5}$, Hb C (2 families) and Hb D Los Angeles (1 family) were detected for the first time in Albania. This large heterogeneity suggests that a panel of advanced molecular techniques is necessary to diagnose all patients with hemoglobinopathies.

Twenty-two patients were receiving regular transfusions, 36 were either sporadically or never transfused. The genotype-phenotype correlation was not strong in a first group of 25/58 patients (Table 1) (group A). In fact, we observed that the same genotype led to different transfusion requirements and there were no molecular data which could explain this different behavior. In the case of β^s patients regular transfusions were required because of worsening clinical conditions due to sickling crises. In contrast, in the remaining 33/58 patients (groups B, C, and D) there was a strong genotype-phenotype correlation. Thirteen patients were compound heterozygotes for severe defects and had severe disease requiring regular transfusions (group B). Fifteen patients showed a broad spectrum of clinical features requiring only occasional blood transfusions (group C). The thalassemia intermedia phenotype of these patients can be explained by different molecular mechanisms which make the molecular and cellular alterations less severe: high residual synthesis of β-thalassemia alleles (β-IVS-I-6 or β-poly A) or increased Hb F level (β-IVS-II-1 associated with $^G\gamma$ -158 C→T). In one case the phenotype of homozygosis for β-codon 39 was improved by the associated novel substitution α_2 +832 G→A that could reduce the pool of not assembled α-chains. This new substitution is located in the 3'UTR, 13 bp downstream to the polyadenylation signal and only 2 bases upstream of the cleavage site *CA*; consequently, it could disturb the polyadenylation process and reduce the production of mature mRNA. This hypothesis needs to be confirmed by molecular and phenotypic characterization of carriers not available in this study. Finally, 5 patients showed mild clinical and hematologic alterations and had never been transfused (group D). In two patients the phenotype was due to double heterozygosis for β-thalassemia and $\alpha\alpha\alpha^{anti3.7}$, which worsens the β/α chain imbalance of the β-thalassemia carriers and causes chronic hemolytic anemia. The remaining 3 patients were double heterozygotes for β-thalassemia and the β^C or β^D alleles, which do not cause hematologic alterations in the carriers.

Comparison of the results obtained in this study with those reported in other Mediterranean countries[6] sheds light on the origin and spread of mutations in Albania (Table 2). The prevalences of β-IVS-I-110 and β-IVS-I-6 were similar to those found in the surrounding Balkan and Mediterranean areas (Greece, Macedonia, Lebanon, Cyprus, former Yugoslavia, Turkey).[6] In contrast, the relative frequency of β-codon 39 was similar to that found in Greece (16.95%),[7] but higher than that found in the other countries (≤5%).[6] The β-IVS-I-1, β-IVS-II-1 and β-IVS-II-745, found in a few Albanian families, have a low relative frequency also in other Mediterranean populations.[6] The remaining 5 alleles were from East Europe [poly A, β-codon 5, codon 82-83(-G)] or Mediterranean Asia [β-codon 44(-C), codon

Table 1. β- and α-globin genotypes of 58 Albanian patients.

	β	β and α globin genotypes α	Patients no. and age (yrs)	Transfusions
A	β-IVS-I-110 homozygosis	αα/αα	5 (18m, 3)	regular
			3 (14, 3)	sporadic
	β cod 39/β-IVS-II-1	αα/αα	1 (17)	sporadic
			1	regular
	β^s homozygosis	αα/αα	2 (14, 6)	sporadic
			1	regular
	β cod 39/β^s	αα/αα	3 (19, 15, 7)	sporadic
			1 (10)	regular
	β-IVS-I-110/β^s	αα/αα	7 (24, 27, 10, 8)	sporadic
			1 (8)	regular
B	β-IVS-I-110/β cod 39	αα/αα	4	regular
	β-IVS-I-110/β cod 44	αα/αα	2	regular
	β-IVS-I-110 homozygosis	$\alpha\alpha\alpha^{anti3.7}$/αα	1	regular
	β-cod 39 homozygosis	αα/αα	1	regular
	β-IVS-I-110/β cod 82-83	αα/αα	1	regular
	β cod 39/β cod 5	αα/αα	1	regular
	β cod 39/β-IVS-I-1	αα/αα	1	regular
	β-IVS-II-745/β-IVS-I-1	αα/αα	1	regular
	β-IVS-I-6/β cod 37	αα/αα	1	regular
C	β-IVS-I-6/β IVS-I-110	αα/αα	5 (6, 13, 15, 3)	sporadic
	β-IVS-I-6 homozygosis	αα/αα	2 (7, 43)	sporadic
	β poly A/ β IVS-I-110	αα/αα	2	sporadic
	β-IVS-I-6/β cod 39	αα/αα	1 (15)	sporadic
	β-IVS-I-6/β cod 44	αα/αα	1 (4)	sporadic
	β-cod 39 homozygosis	$\alpha^{+832\,G\to A}$α/αα	1 (10)	sporadic
	β^s homozygosis	-α$^{3.7}$/αα	2 (23, 24)	sporadic
	β IVS-I-1/β^s	αα/αα	1	sporadic
D	β cod 39/β^A	$\alpha\alpha\alpha^{anti3.7}$/αα	1 (9)	none
	β cod 44/β^A	$\alpha\alpha\alpha^{anti3.7}$/αα	1 (45)	none
	β^s/β^D	αα/αα	1 (2)	none
	β IVS-I-110/β^C	αα/αα	1	none
	β IVS-II-1/β^C	αα/αα	1	none

The α_2 +832 G→A is a novel mutation. Patients were subdivided into groups on the basis of the genotype-phenotype correlation. β-globin gene alleles were detected by ARMS-PCR[8] or by DNA sequencing. α-thalassemia defects were identified by Southern blot analysis[9] or by DNA sequencing. Age of patients — when available — has been reported in brackets; m= months.

Table 2. β-globin gene alleles and haplotypes detected in 58 Albanian patients; populations in which the alleles have been reported.

Alleles	*Haplotypes*	*Chromosomes n°*	*Populations*
IVS-I-110 (G→A)	I	42	Mediterranean
Codon 39 (C→T)	II	17	Mediterranean
IVS-I-6 (T→C)	VI	12	Mediterranean
Codon 44 (-C)	I	4	**Kurdish, Jewish (30%),** Mediterranean
IVS-I-1 (G→A)	V	3	Mediterranean
IVS-II-1(G→A)	III	2	Mediterranean
IVS-II-1(G→A)	I	1	Druze and Yemenite Jews
Poly A (AATAAA→AATGAA)	II	2	Balkan, **Yugoslavian (4%)**, *Turkish*
IVS-II-745 (C→G)	VII	1	Mediterranean
Codon 5 (-CT)	V	1	Mediterranean, ***Bulgarian (5%)***, Balkan
Codon 82-83 (-G)	III	1	*Arzebaijan, Czechoslovakian*, Croatian, *United Arabia Emirates*
Codon 37 (G→A)	I	1	***Jordanian (9%)***, **Israeli Arab (5%)**, Saudi Arabian, Egyptian, *United Arab Emirates, Syrian*, *Turkish*, Iberian
β^S	Benin type	24	Mediterranean, Central West Africa
β^C	Benin type	2	Mediterranean, Central West Africa
β^D	I	1	Mediterranean, *Indian Asian*
Total of chromosomes		114	

RFLP haplotype analysis was carried out as previously reported.[9] The RFLPs analyzed were Hind III/$^G\gamma$, Hind III/$^A\gamma$, Hinc II/$\psi\beta$ and 3' $\psi\beta$, Ava II/β and Bam HI/3'β. The haplotypes were classified according to Orkin.[10] In italics: populations in which the haplotype has not yet been characterized. In bold: populations in which rare mutations have a relatively high allelic frequency (frequencies are reported in brackets).

37(G→A), codon 82-83(-G)].

All the β-thal and the Hb variant alleles were found to be associated with the same haplotypes described in other populations[6] (Table 2). This indicates that mutations did not have an independent origin and suggests that the origin and spread of β-thal mutations and Hb variants in Albania is in keeping with the historical relationships between Albania and neighboring or more distant populations (Greeks, Romans, Slavs and Arabs) and that Albania has experienced a discrete genetic flow.

Maria De Angioletti, Giuseppina Lacerra,* Enis Boletini,° Francesca Di Noce,* Gennaro Musollino,* Clementina Carestia**

**Istituto di Genetica e Biofisica "Adriano Buzzati Traverso", Naples, Italy; °Center of Hemoglobinophathies, Department of Clinical Biochemistry, Institute of Pediatrics, Tirana, Albania*

Funding: the project was supported by grants from the Ministero dell'Università e della Ricerca Scientifica e Tecnologica, Legge 488/92, Cluster C02, Progetto Esecutivo 2.

Acknowledgments: we thank the patients for their co-operation and Romeo Prezioso, from IGB, for his precious collaboration in collecting experimental, hematological and clinical data into an electronic data-base.

Key words: β-thalassemia, HB variants; α-globin Albania.

Correspondence: Clementina Carestia, MD, Istituto di Genetica e Biofisica Adriano Buzzati Traverso, CNR, via G. Marconi 10-12, 80125, Naples, Italy. Phone: international +39.081.7257244. Fax: international +39.081.7257243. E-mail: carestia@iigb.na.cnr.it

Manuscript processing
This manuscript was peer-reviewed by two external referees and by Professor Mario Cazzola, Editor-in-Chief. The final decision to accept this paper for publication was taken jointly by Professor Cazzola and the Editors. Manuscript received May 2, 2002; accepted July 10, 2002.

References

1. Boletini E. The haemoglobinopathies in Albania. Meeting of the Mediterranean Blood Club, Milan, Italy; 1991.
2. Schwartz E. The silent carrier of β thalassemia. N Engl J Med 1969; 281:1327-33.
3. Zisovski N, Mladenovski B, Muratovska O, Glamocanin S, Efremov GD. Sickle cell anemia in an Albanian family in Yugoslavia. Hemoglobin 1987; 11:383-7.
4. Lacerra G, Fioretti G, Hani A, Duka D, De Angioletti M, Pagano L, et al. Hb O-Arab [β 121(GH4)Glu→Lys]: association with DNA polymorphisms of African ancestry in two Mediterranean families. Hemoglobin 1993; 17:523-35.
5. Boletini E, Svobodova M, Divoky V, Baysal E, Curuk MA, Dimovski AJ, et al. Sickle cell anemia, sickle cell β-thalassemia, and thalassemia major in Albania: characterization of mutations. Hum Genet 1994; 93:182-7.
6. Huisman TH, Carver MF, Baysal E. A syllabus of thalassemia mutations. Published by The Sickle Cell Anemia Foundation, Augusta, GA, USA; p. 17. 1997.
7. Kattamis C, Hu H, Cheng G, Reese AL, Gonzalez-Redondo JM, Kutlar A, et al. Molecular characterization of β-thalassaemia in 174 Greek patients with thalassaemia major. Br J Haematol 1990; 74:342-6.
8. Old JM, Varawalla NY, Weatherall DJ. Rapid detection and prenatal diagnosis of β-thalassaemia: studies in Indian and Cypriot populations in the UK. Lancet 1990; 336:834-7.
9. Carestia C, Pagano L, Fioretti G, Mastrobuoni A. β-thalassaemia in Campania: DNA polymorphism analysis in β A and β thal chromosomes and its usefulness in prenatal diagnosis. Br J Haematol 1987; 67:231-4.
10. Orkin SH, Kazazian HH Jr, Antonarakis SE, Goff SC, Boehm CD, Sexton JP, et al. Linkage of β-thalassaemia mutations and β-globin gene polymorphisms with DNA polymorphisms in human β-globin gene cluster. Nature 1982; 296:627-31.

Late response to donor lymphocyte infusions in patients with chronic myeloid leukemia relapsing after allogeneic stem cell transplantation

Donor lymphocyte infusions were given to 13 consecutive chronic myeloid leukemia patients in relapse after allogeneic stem cell transplantation. Of the 13 patients, 11 achieved a molecular remission and 2 a cytogenetic remission. The median time (range) in months to achieve a hematologic, cytogenetic and molecular remission was 2 (1-2), 5 (1-42) and 5 (1-30), respectively. After a median follow-up of 24 months, the median response duration is 16 months (range 1-36), and no patient shows evidence of relapse.

haematologica 2002; 87:1003-1005
(*http://www.haematologica.ws/2002_09/1003.htm*)

Thirteen consecutive chronic meyloid leukemia (CML) patients relapsing after allogeneic stem cell transplant (allo-SCT) from an HLA-identical sibling were treated with donor lymphocyte infu-