THALASSEMIAS AND OTHER HEMOGLOBINOPATHIES IN FORMER YUGOSLAVIA

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ABSTRACT

This review summarizes our results on the epidemiology and molecular basis of thalassemias and other hemoglobinopathies in the republics and provinces of the Former Yugoslavia. Over the past 40 years, surveys of more than 37,000 school children and more than 1,600 adults, from all over Former Yugoslavia, except Slovenia, have shown an average incidence of β -thalassemia (β -thal) trait of 1.2%, ranging from 2.9% in the south (Macedonia) to 0.8% in the northwest (Croatia). The frequency of $\delta\beta$ -thal was 0.2%, while that of Swiss type hereditary persistence of fetal hemoglobin (HPFH) was 0.4%. Screening of 12,680 newborns has shown that the frequency of α -thal trait was 1.5%. The molecular basis of the thalassemias in the populations of Former Yugoslavia has been completely defined. More than 700 β -thal chromosomes have been studied and their molecular defect was determined. In the Macedonian population, 16 different β -thal mutations were detected, four of which (IVS-I-110, G→A; IVS-I-6, T>C; IVS-I-1, G>A and codon 39, C>T) accounted for 85% of all β -thal chromosomes. In the Croatian population, 18 different β -thal alleles were detected. Four new mutations [nucleotide (nt) -87, C>A; IVS-II-850, G>C; initiation

codon mutation T>C; polyadenylation signal (poly A), AATAAA>AATGAA)] and one new deletion (1605 bp), were characterized. Molecular analyses of DNA from over 50 unrelated cases with $\delta\beta$ -thal have shown that this condition was mainly caused by a 13 kb deletion (Sicilian type); in one family, a deletion of >18 to 23 kb (Macedonian-Turkish type), and in another, a deletion of 148 kb (Yugoslavian type of $\epsilon\gamma\delta\beta$ -thal) of the β -globin gene complex, were discovered. Molecular analyses of α -thal from Former Yugoslavia revealed the following defects: the -20.5, -17.5 and -3.7 kb deletions, a 5 nt deletion, and Hb Icaria [α 142, Term→Lys (TAA>TCA in α 2)]. The incidence of abnormal hemoglobins (Hbs) in Former Yugoslavia was 0.3%. Five different α chain variants in 16 families, 16 different β chain variants in 61 families, one δ chain variant in one family, two types of Hb Lepore in 122 families and two γ chain variants, have been characterized.

Keywords: Thalassemias; Hemoglobinopathies in Former Yugoslavia

INTRODUCTION

The thalassemias and other hemoglobinopathies are the most common genetic disorders worldwide. It has been estimated that ~3% of the world's population carry a β -thalassemia (β -thal) gene [1]. It is believed that malaria has exerted selective pressure for the propagation of the thalassemia genes, although the precise scientific basis for the proposed protection of the thalassemia heterozygotes against

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malaria is unknown [2]. β -Thal has a high incidence in a broad tropical belt extending from the Mediterranean Basin through the Middle and Far East [3]. Migration has resulted in a notable increase of β -thal in Western European countries [4].

The hemoglobinopathies, also known as abnormal hemoglobins (Hbs), are classified according to the mutated gene (α , β , γ , δ and fusion genes), according to the clinical expression (Hb S syndrome, the unstable Hbs, variants with abnormal functions, Hb M variants, hyperunstable Hbs), and according to the location of the mutation (at the surface of the molecule, inside the molecule, between the heme and the globin chain, at the $\alpha 1\beta 1$ contact and at the $\alpha 1\beta 2$ contact). More than 900 different Hb variants have been characterized [5]. Here, I summarize our results on the epidemiology and molecular basis of thalassemias and other hemoglobinopathies in the populations of Former Yugoslavia.

SUBJECTS AND METHODS

Population surveys were conducted on school children, adults and newborns. A total of 37,159 school children, age 8-17 years, both male and female, 1,629 adults, and 12,680 newborns were screened between 1966 and 1994. In addition, 4,564 patients with signs of hemolysis and/or low red blood cell (RBC) indices have been examined for the presence of thalassemias and/or abnormal Hbs.

The methods for the detection and characterization of thalassemias and abnormal Hbs were those universally accepted [6,7], and consisted of the determination of RBC indices and morphology, osmotic fragility test [8], quantitation of Hb A₂ by column chromatography on DEAE-Sephadex [6] in the earlier studies, and microcolumn chromatography [9] in the later studies, quantitation of Hb F by an alkali denaturation procedure [6], and starch gel electrophoresis [10]. In addition, whenever indicated, specific studies such as tests for unstable Hbs, isoelectric focusing (IEF), structural characterization of variant Hbs, and *in vitro* biosynthesis studies [2,6,7], were performed. Gene mapping analysis, dot-blot hybridization of polymerase chain reaction (PCR), amplified DNA with specific probes, and sequencing of the amplified DNA followed methodology previously described [11-14].

RESULTS AND DISCUSSION

Population Surveys. Before the National Reference Laboratory for Hemoglobinopathies (NRLH) was established in 1970, the frequency and distribution of various thalassemia types and other hemoglobinopathies in the Republic of Macedonia were determined by the Department of Pediatrics, Faculty of Medicine at Skopje. From 1965 to 1967, blood samples from 2,861 apparently healthy school children, aged 8 to 18 years, from 32 different parts of the country, and 561 cord blood samples, obtained through the services of the Department of Obstetrics, Faculty of Medicine, Skopje, Republic of Macedonia, were studied [15]. The gene for β -thal was found to be distributed through most of the country, with a frequency from 1 to 20% (mean value 4.7%). We also detected six cases (0.21%) with three different abnormal Hbs that were later characterized as Hb J-Paris-I [$\alpha 12(A10)Ala \rightarrow Asp$, $GCC \rightarrow GAC$ ($\alpha 1$ and $\alpha 2$)], Hb Beograd [$\beta 121(GH4) Glu \rightarrow Val$, $GAA \rightarrow GTA$] and the Lepore Hbs [16,17].

After 1970, we screened 2,201 school children from the region of Gevgelija and Bitola [18] and 2,853 children from Strumica, Radovis and Stip [19]. The incidence of β -thal and other hemoglobinopathies in these regions was 9.5, 2.4, 6.0, 5.3 and 3.5%, respectively. Between 1980 and 1982, we screened 2,498 school children of both sexes living in Skopje [20]. Skopje was selected because its population had increased four-fold in the previous 30 years. In fact, one quarter of the Macedonian population live in this city. Eighty-one children were carriers of β - and $\delta\beta$ -thal trait, Swiss type of hereditary persistence of fetal Hb (HPFH), and Hb S [$\beta 6(A3) Glu \rightarrow Val$, $GAG \rightarrow GTG$], Hb O-Arab [$\beta 121(GH4) Glu \rightarrow Lys$, $GAA \rightarrow AAA$], Hb D-Punjab [$\beta 121(GH4) Glu \rightarrow Gln$, $GAA \rightarrow CAA$], Hb Strumica [$\alpha 112(G19) His \rightarrow Arg$ ($CAC \rightarrow CGC$) ($\alpha 2$ or $\alpha 1$)] and Hb Lepore-Washington-Boston (Lepore-WB; $\delta 87Gln \rightarrow \beta 116His$). The incidence of β -thal was 2.4%, and that of the observed types of hemoglobinopathies was 3.2%. This incidence seemed to be the most likely one for the Macedonian population [17,20].

Between 1992 and 1994, 3,482 school children from the northern and west-northern parts of Macedonia were screened. Heterozygous β -thal was detected in 30 children (0.9%), $\delta\beta$ -thal in eight (0.2%), α -thal in 26 (0.7%), Swiss type of HPFH in eight

(0.2%), and four carriers of abnormal Hbs (0.1%). The overall incidence of all hemoglobinopathies was 2.2%.

The incidence of α -thal and the organization of the γ -globin genes was studied in cord blood samples from 3,232 newborns [21], received from the Department of Obstetrics and Gynecology, Faculty of Medicine, Skopje, and Hospital for Obstetrics and Gynecology "Cair", Skopje, Republic of Macedonia. Twenty-six newborns were found to be carriers of α -thal (0.8%), of whom 25 were carriers of α -thal-2 and one of α -thal-1. In addition, eight babies were carriers of an abnormal Hb, three with Hb O-Arab, and one each with Hb Hamilton [β 11(A8) Val→Ile, GTT>ATT], Hb Strumica and the newly described γ chain variants: Hb F-Macedonia-I [$^{\Delta}\gamma$ 2(NA2) His→Gln, CAT>CAG] and Hb F-Macedonia-II [$^{\Delta}\gamma$ 104 (G6)Lys→Asn, AAG>AAC] [22,23]. These five population surveys on a total number of 22,136 subjects, gave an overall incidence of β -thal and other hemoglobinopathies in the Republic of

Macedonia of 3.4%, varying between 0.7% and 12.3%.

Screening of 2,700 individuals from Serbia, 1,200 from Vojvodina, 1,611 school children from Kosovo, 7,601 individuals (most of whom exhibited signs of hemolysis and/or low RBC indices) from Croatia, 2,780 school children from Bosnia and Hercegovina, and 860 school children from Monte Negro, gave an incidence of β -thal and other hemoglobinopathies in these territories of the Former Yugoslavia of 2.3, 2.0, 2.7, 4.4, 2.7, and 2.6%, respectively [24]. The frequency of β -thal in Former Yugoslavia varies from 1.2% in the north (Vojvodina) and 2.9% in the south (Macedonia) [24]. The frequency of β -thal in Serbia is 1.8%, in Bosnia and Hercegovina 1.5% [24,25], 1.9% in Monte Negro, and 2.0% in Kosovo [24]. Figure 1 shows a map of the Former Yugoslavia with the number of subjects examined and frequency of β -thal trait, and combined frequency of β -thal trait and other hemoglobinopathies, in the different republics and provinces.

Figure 1. Map of former Yugoslavia showing the number of screened school children and adults (38,888) and frequency of β -thal and β -thal and other hemoglobinopathies (in parentheses). Most of the subjects from Croatia were patients with signs of hemolysis and/or low RBC indices. N.D. = not determined

Hematological and Biochemical Characteristics of β -Thalassemia. The hematological and biochemical changes seen in the β -thal heterozygotes vary: some carriers are practically free of complaints with or without a slight anemia, while others have

a moderate anemia. Microcytosis and hypochromia with low MCV and MCH values are, however, always present, and the resistance of the erythrocyte to saline solutions is increased. Figure 2 illustrates the MCV and MCH values in β -thal heterozygotes

Figure 2. Distribution of MCV and MCH values for 1,500 β -thal heterozygotes and 2,175 normal individuals.

and normal controls studied in our laboratory. The MCV value, independent of the type of mutation, averaged 68.2 fL in comparison to 92.9 fL in normal individuals. The average MCH value was 21.0 pg in β -thal heterozygotes and 30.9 pg in normal individuals. Relatively large variations in the MCV and MCH values exist (56.0-77.0 fL and 17.0-27.0 pg, respectively) which is likely to be due to individual variations and to the type of mutation. Table 1 gives the results of some of our hematological and Hb analysis data for β -thal heterozygotes with 15 different mutations.

The resistance to saline solutions of the erythrocytes from heterozygotes for β -thal was increased. Our results on a one-tube osmotic fragility method that used three buffered solutions (0.32% saline, 0.36% saline and Tyrode solution) as a screening test for β -thal trait, published in 1981 [8], showed that 0.36% saline was the most sensitive and most effective percentage as it detected 96-100% of heterozygotes with β -thal compared to about 80% with both 0.32% and Tyrode solution.

Analysis of the *in vitro* Hb synthesis in the

reticulocytes of β -thal homozygotes and heterozygotes was very useful in estimating the globin chain imbalance due to decreased or absent β chain production. Table 2 summarizes our results for the globin chain synthesis of 47 β -thal patients homozygous for 15 different mutations.

In β -thal heterozygotes, the synthesis of the β chains was about half that of the α chains. The degree of the β chain deficiency was related to the type of mutation, and it varied from 0.44 in heterozygotes for the codon 39 (C>T) mutation to 0.65 in heterozygotes for the nt -30 (C>T) mutation (see Table 1).

Forms of Thalassemia and Related Conditions. The biochemical expressions of the homozygous and double heterozygous states of β -thal and related conditions were studied simultaneously with the population surveys. The homozygous state of β -thal was first recognized in the Republic of Macedonia in 1952 [26]. From then until 1974, 95 patients with Cooley's Anemia were registered at the Department of Pediatrics, Faculty of Medicine, Skopje, Republic of Macedonia [27]. A patient with

Table 1 Hematological and hemoglobin composition data for β -thalassemia heterozygotes with 14 different mutations (average values only)

Mutation	n	Hb g/dl	RBC $10^{12}/L$	MCV fl	MCH pg	Hb A ₂ * %	Hb F** %	β/α ratio
-101, C→T	7	13.4	4.70	87.3	28.3	3.8	1.3	0.65
- 87, C→G	6	12.4	5.40	78.4	23.6	4.7	1.2	0.60
- 30, T→A	14	12.5	5.70	69.1	20.5	4.8	1.5	0.63
Codon 5, -CT	79	11.3	5.30	67.3	21.2	4.9	1.3	0.53 (n=11)
Codon 6, -A	61	11.3	5.40	65.2	20.8	4.7	2.0	0.51 (n=17)
Codon 8, -AA	54	10.2	5.00	67.5	20.5	4.6	1.0	0.57 (n=10)
Codon 8/9, +G	9	13.1	6.17	66.0	22.0	5.5	1.6	0.54 (n=5)
Codon 39, C→T	328	10.8	5.30	63.2	20.6	4.9	1.5	0.44 (n=33)
IVS I-1, G→A	199	10.8	5.20	64.5	20.8	4.8	1.2	0.51 (n=24)
IVS I-6, T→C	155	12.7	5.70	71.6	22.4	4.3	1.0	0.63 (n=28)
IVS I-110, G→A	397	11.2	5.50	65.2	21.1	4.9	1.2	0.52 (n=41)
IVS II-1, G→A	7	11.4	5.89	61.0	19.0	5.8	1.8	0.49
IVS II-745, C→G	58	11.0	5.30	64.5	20.9	4.9	0.9	0.52 (n=18)
Poly A, -A- →G	6	13.6	5.60	78.2	24.2	4.0	0.5	0.60

* Determined by microcolumn chromatography; ** Determined by alkali denaturation

Table 2 In vitro Chain Synthesis Data for β -Thalassemia Homozygotes and Compound Heterozygotes with Different Mutations*

Genotype	n	Age (years)	Hb F %	β/α Ratio	$(\beta+\gamma)/\alpha$ Ratio
IVS I-1(G $\rightarrow$ A/-30(T $\rightarrow$ A))	1	9	9.0	0.16	0.44
IVS I-1(G $\rightarrow$ A/codon 6(-A))	2	9	54.0	0	0.33
		(5-13)	(32-76)		
IVS I-1(G $\rightarrow$ A/Poly A (A $\rightarrow$ G))	1	8	19.0	0.16	0.36
IVS I-6(T $\rightarrow$ C)/IVS I-6(T $\rightarrow$ C)	5	31	20.1	0.30	0.37
		(23-37)	(12-35)	(0.20-0.38)	(0.31-0.43)
IVS I-6(T $\rightarrow$ C)/IVS I-110(G $\rightarrow$ A)	6	13	30.0	0.20	0.33
		(2-30)	(2-60)	(0.15-0.28)	(0.27-0.40)
IVS I-6(T $\rightarrow$ C)/codon 6(-A)	2	9	52.0	0.23	0.36
		(7-11)	(40-84)	(0.21-0.25)	(0.34-0.38)
IVS I-6(T $\rightarrow$ C)/codon 8(-AA)	3	12	24.0	0.22	0.33
		(7-15)	(3-65)	(0.22-0.23)	(0.28-0.38)
IVS I-6(T $\rightarrow$ C)/Poly A (A $\rightarrow$ G)	1	20	10.0	0.33	0.44
IVS I-6(T $\rightarrow$ C)/-44 bp(-C)	1	13	42.0	0.26	0.35
IVS I-110(G $\rightarrow$ A)/IVS I-110(G $\rightarrow$ A)	8	18	31.6	0.10	0.31
		(1-37)	(6-85)	(0.07-0.17)	(0.23-0.38)
IVS I-110(G $\rightarrow$ A)/IVS II-745(C $\rightarrow$ G)	1	17	8.9	0.08	0.28
IVS I-110(G $\rightarrow$ A)/codon 6(-A)	3	22	56.0	0.06	0.34
		(9-30)	(11-95)	(0.05-0.07)	(0.27-0.39)
IVS I-110(G $\rightarrow$ A)/Poly A (A $\rightarrow$ G)	1	11	13.0	0.17	0.30
Codon 39(C $\rightarrow$ T)/codon 39(C $\rightarrow$ T)	3	11	35.0	0	0.27
		(7-15)	(5-86)		(0.18-0.37)
Codon 8(-AA)/codon 8(-AA)	2	12	70.0	0	0.30
		(8-16)	(62-78)		(0.26-0.34)

* Most of the patients were on a regular blood transfusion regimen. Analyses were performed at least 30 days after the last transfusion. Values between parenthesis identify ranges or normal values.

Hb H disease [28], nine families with Hb Lepore [29], and two families with different expressions of homozygous β -thal [30], were also described. As of 2008, 157 cases of thalassemias major have been registered at the Department of Pediatrics, Faculty of Medicine, Skopje, Republic of Macedonia.

Since 1970, when the NRLH was established, studies of patients with hemolytic anemia and cyanosis included patients from all the territories of Former Yugoslavia. Table 3 summarizes the types of thalassemia and related conditions detected and characterized through studies of patients with signs of hemolysis and/or low RBC indices. Two-thirds

of the patients were from the Republic of Macedonia and Croatia, while one-third were from the other territories of Former Yugoslavia. Twenty-three types of thalassemia and related conditions were detected. The most common type was heterozygous β -thal, observed in 1,044 patients (74.1%) of all the cases with hemoglobinopathies. The 11 different types of thalassemia major and the five different types of thalassemia intermedia were characterized in 132 and 15 patients, respectively. Heterozygous $\delta\beta$ -thal was detected in 162 individuals, Hb Lepore in 149 individuals, and the Swiss type of HPFH in 131 persons.

Table 3 Forms of Thalassemia and Related Conditions Detected in Former Yugoslavia (December 2007)

Form of Thalassemia	Hospital	Survey	Population	Survey	Total	
	n	%	n	%	n	%
Heterozygous β -thal	1044	74.1	684	74.8	1.728	74.4
β^0/β^0 -thal	8	0.6			8	0.3
β^0/β^+ -thal	22	1.6			22	0.9
β^+/β^+ -thal	60	4.3			60	2.6
$\beta^+/\beta^{\text{silent}}$ -thal	6	0.4			6	0.3
$\beta^{\text{silent}}/\beta^{\text{silent}}$ -thal	2	0.1			2	0.1
$\beta^0/(\delta\beta)^0$ -thal	3	0.2			3	0.1
$\beta^+/(\delta\beta)^0$ -thal	1	0.1			1	0.1
β^0 -thal/Hb Lepore	5	0.4			5	0.2
β^+ -thal/Hb Lepore	14	1.0			14	0.6
β^0 -thal/Hb Beograd	2	0.1			2	0.1
β^0 -thal/Hb O-Arabia	1	0.1			1	0.1
Heterozygous $(\delta\beta)^0$ -thal	111	7.9	51	5.6	162	7.0
Homozygous $(\delta\beta)^0$ -thal	3	0.2			3	0.1
$(\delta\beta)^0$ -thal/HbA ₂ -Zagreb	1	0.1			1	0.1
$(\delta\beta)^0$ -thal/Hb Lepore-Baltimore	1	0.1			1	0.1
Homozygous Hb Lepore-Washington	9	0.6			9	0.4
Heterozygous Hb Lepore-Washington	94	6.7	55	6.0	149	6.4
Hb Lepore-Washington/Hb Beograd	1	0.1			1	0.1
Hb H disease	8	0.6			8	0.3
Hb Icaria/Hb H disease	1	0.1			1	0.1
Heterozygous α -thal	4	0.3			4	0.2
Swiss HPFH	7	0.5	124	13.6	131	5.6
Total	1,408	100.0	914	100.0	2,322	100.0
Total number screened	13,612	10.34	29,506	3.1	43,118	5.4

Our results clearly indicate that hemoglobinopathies are heterogeneously distributed in the Former Yugoslavia. The frequency of β -thal and related conditions ranges from 0.1% in the northwest (Slovenia) to 3.4% in the south (Republic of Macedonia). Although the national boundaries of the republics of Former Yugoslavia do not necessarily represent particular ethnic groups, our results indicate that hemoglobinopathies are rare in Slovenians, common in Croatians and Serbs, and very common in Macedonians.

Molecular Characterization of β -Thalassemia and Related Conditions. The molecular basis of

β -thal in Former Yugoslavia was studied in great detail. Table 4 lists the 27 different β -thal alleles that were discovered in a total of 705 β -thal chromosomes, of which 508 were from Macedonian, 46 from Serb, 53 from Croatian, 62 from Albanian, and 36 from Turkish patients. Sixteen β -thal alleles were identified in Macedonian patients, and 8, 14, 8 and 11 alleles, respectively, were identified in the Serbian, Croatian, Albanian, and Turkish patients. In each of the ethnic groups, 5 to 7 different alleles account for over 85% of all the characterized alleles. The most common alleles are IVS-I-110 (G>A), IVS-I-6

Table 4 Frequencies of the different β -thalassemia alleles among Macedonian, Serbian, Croatian, Albanian, Turks identified in NRLH-RCGEB-MASA

MUTATION (β -thal allele)	ETHNIC GROUP				
	MK (508)*	SR (46)*	CR (53)*	AL (62)*	TU (36)*
nt -101 (C→T)**	1 (0.2)	-	-	3 (5.5)	1 (2.8)
nt - 87 (C→G)	2 (0.4)	-	-	-	-
nt - 87 (C→A)	-	-	1 (1.9)	-	-
nt - 30 (T→A)**	2 (0.4)	-	-	10 (18.2)	-
5'UTR;+22(G→A)**	-	-	-	-	-
In. cd. ATG→ACG**	-	-	1 (1.9)	-	-
Cd 5 (-CT)	11 (2.4)	-	-	-	1 (2.8)
Cd 6 (-A)**	18 (3.5)	3 (6.5)	-	-	2 (5.6)
Cd 8 (-AA)	10 (2.2)	1 (2.2)	4 (7.5)	2(3.6)	6 (16.7)
Cd 8/9 (+G)	-	-	-	-	9 (25.0)
Cd 29 (C→T)	2 (0.4)	-	-	-	-
Cd 30 (G→C)	-	-	-	1(2.8)	-
Cd 37 (G→A)	-	-	2 (3.8)	-	-
Cd 39 (C→)	64 (12.6)	5 (10.9)	6 (11.3)	26 (41.9)	5 (13.9)
Cd 82/83 (-G)**	-	-	1 (1.9)	-	-
IVS I-1 (G→A)	81 (15.9)	7 (15.2)	7 (13.2)	3 (5.5)	1 (2.8)
IVS I-6 (T→C)	86 (16.9)	8 (17.4)	6 (11.3)	10 (16.1)	4 (11.1)
IVS I-110 (G→A)	192 (37.8)	16 (34.8)	15 (28.3)	7 (12.7)	5 (13.9)
IVS II-1 (G→A)	8 (1.6)	1 (2.2)	4 (7.5)	-	1 (2.8)
IVS II-745 (C→G)	25 (4.9)	5 (10.9)	3 (5.7)	1 (1.8)	1 (2.8)
IVS II-848 (C→G)	1 (0.2)	-	1 (1.9)	-	-
IVS II-850 (G→C)**	-	-	1 (1.9)	-	-
PolyA(AATAAA→ AATGAA**	4 (0.9)	-	-	-	-
-44 bp deletion	1 (0.2)	-	-	-	-
1.6 kb deletion**	-	-	1 (1.9)	-	-
No. mutations>85%	5/16	5/8	7/14	6/8	6/12

MK=Macedonian; SR=Serbian; CR=Croatian; AL=Albanian living in Macedonia and Kosovo; TU=Turks living in Macedonia.

*No. of chromosomes studied; **New mutation identified in the RCGEB – MASA, Skopje, R. Macedonia

(T>C), IVS-I-1 (G>A) and codon 39 (C>T) in the Macedonian, Serb and Croatian patients. The most common alleles in the Albanian patients were codon 39, nt -30 (T>A), IVS-I-6 and IVS-I-110, and in the Turkish patients, codons 8/9 (+G), codon 8 (-AA), codon 39, IVS-I-110 and IVS-I-6.

The number of the different β -thal alleles was much smaller in the Serbian and Albanian patients. Nine of the 27 β -thal mutations, were discovered for the first time: nt -101 (C>T) in a Bulgarian patient [13], nt -30 (T>A) in a Macedonian β^+ -thal/Hb Lepore patient [31], G>A at position +22 3' to the Cap site in Bulgarian and Turkish patients [32], initiation codon mutation ATG>ACG in a Macedonian heterozygote [33], codon 6 (-A) in a Bulgarian thalassemia major patient [14], codons 82/83 (-G) in a β -thal heterozygote from Croatia [34], IVS-II-850 (G>C) in a Croatian family heterozygous for β -thal [35], polyadenylation signal (poly A) (AATAAA>AATGAA) in a Bulgarian patient with thalassemia major and one Macedonian patient with thalassemia intermedia [36], and a 1.6 kb deletion of the β -globin gene in a Croatian β -thal heterozygote [37].

Molecular characterization of the mutation in patients with thalassemia major, intermedia and minor, resulted in the identification of deletional types of β -thal. In 1986, we described a new type of deletional $\delta\beta$ -thal, named the Macedonian $\delta\beta$ -thal [38], a new type of $\epsilon\gamma\delta\beta$ -thal, named Croatian $\epsilon\gamma\delta\beta$ -thal [12], a deletional type of β -thal, named Croatian β^0 -thal [37], and a new type of γ -globin gene triplication [11].

$\delta\beta$ -Thalassemia. $\delta\beta$ -Thal results from simultaneous deficiency in synthesis of δ - and β -globin chains from an anomaly in the closely linked δ - and β -globin genes. It is characterized by normal levels of Hb A₂ and increased levels of Hb F that is unevenly distributed in the red cells. The hematological and clinical findings in $\delta\beta$ -thal heterozygotes are similar to those observed in classical β -thal heterozygotes.

Although classical β -thal is present in moderately high frequencies in the Macedonian population, no cases of $\delta\beta$ -thal were reported until 1973. In a survey of 5,500 school children from southern parts of the Republic of Macedonia, only one heterozygote for $\delta\beta$ -thal was detected [39]. In 1975, we described the first two Macedonian families with

$\delta\beta$ -thal, in which two members were heterozygous for both β - and $\delta\beta$ -thal [40], and soon thereafter, we described a Serbian family with $\delta\beta$ -thal interacting with Hb Lepore-Baltimore ($\delta 68 \rightarrow \beta 84$) [41]. The results of hematological and biochemical analyses of two patients homozygous for $\delta\beta$ -thal and 219 patients heterozygous for $\delta\beta$ -thal were presented at a meeting [42] but never published. The two $\delta\beta$ -thal homozygotes were members of Croatian families originating from Rijeka and Sisak, Croatia. The first was a 30-year-old woman, and the second patient was a worker, age 28. Both had hemolytic anemia and hepatosplenomegaly. The hematological analysis for the woman gave the following results: RBC $4.48 \times 10^{12}/L$, Hb 11.5 g/dL, PCV 0.346 L/L, MCV 77 fL, MCH 26 pg, moderate hypochromia, poikilocytosis and anisocytosis. Starch gel, cellulose acetate and agar gel electrophoreses showed the presence of Hb F only. *In vitro* biosynthetic experiments showed the presence of γ - and α chain only. The relative radioactivity of γ to α chain was 1.91, and the specific activity 1.67. The results of hematological and Hb analyses of over 300 heterozygotes for $\delta\beta$ -thal are presented in Table 5.

The Lepore Hemoglobinopathies. This was the most common hemoglobinopathy in Former Yugoslavia. The first nine unrelated families with Hb Lepore were described in 1968 [29], both in homozygous and double heterozygous states, interacting with β -thal states, and characterized as Hb Lepore-Washington [16,17,24]. Hematological and biochemical analysis of blood samples from a patient at the Department of Internal Medicine, Faculty of Medicine, Belgrade, Serbia, and her parents and relatives, disclosed the presence of Hb Lepore-Baltimore interacting with $\delta\beta$ -thal [41]. During the past 40 years, we have studied 163 unrelated patients or families with Hb Lepore-Washington-Boston, *i.e.*, nine homozygotes, 17 compound heterozygotes, and 137 heterozygotes. The symptoms and blood films in heterozygotes and homozygotes do not differ greatly from those found in equivalent β -thal states.

The homozygous state for Hb Lepore was observed in six Macedonian, two Serb and one Croatian patients. All suffered from severe hemolytic anemia; eight died between the ages of 5 and 14 years, and one at 24 years. The clinical expression of the compound heterozygotes for Hb Lepore and

Table 5. Hematological and Hb analysis data for heterozygotes for $\delta\beta$ -thalassemia

	$\delta\beta$ -heterozygotes		Healthy persons	
	Male (n=164)	Female (n=144)	Male (n=966)	Female (n=1262)
RBC ($\times 10^{12}/l$)	5.78 $\pm$ 0.64 (4.4-7.2)	5.37 $\pm$ 0.41 (4.7-6.2)	5.03 $\pm$ 0.53 (4.3-5.7)	4.47 $\pm$.31 (3.9-5.1)
Hb (gm/dl)	12.9 $\pm$ 1.3 (10.1-15.8)	12.0 $\pm$ 1.0 (9.9-14.5)	15.7 $\pm$ 1.0 (13.8-17.5)	13.8 $\pm$ 1.0 (11.9-15.6)
PCV (l/l)	0.41 $\pm$ 0.04 (0.29-0.49)	0.39 $\pm$ 0.04 (0.30-0.49)	0.47 $\pm$ 0.03 (0.41-0.53)	0.41 $\pm$ 0.03 (0.36-0.47)
MCV (fl)	71.6 $\pm$ 6.1 (60-89)		92.9 $\pm$ 4.5 (84-102)	
MCH (pg)	22.4 $\pm$ 1.9 (19-29)		30.9 $\pm$ 1.6 (28-34)	
HbA ₂ (%)	2.4 $\pm$ 1.9 (1.4-3.0)		2.5 $\pm$ 0.3 (2.0-3.3)	
HbF _{AD}	9.4 $\pm$ 3.0 (3.5-19.0)		< 1.0	
α/β ratio	1.73 $\pm$ 0.21		1.00 $\pm$ 0.15	

β -thal varies from very severe to moderate anemia, and is determined by the type of the β -thal mutation [43]. The clinical findings for the homozygotes are given in Table 6, and their hematological and Hb data in Table 7. In the heterozygotes, about 10% of the Hb is Hb Lepore, the level of Hb A₂ is normal or slightly decreased, and that of Hb F is slightly increased in about two-thirds of subjects.

Hereditary Persistence of Fetal Hemoglobin. Hereditary persistence of fetal Hb was first reported in Former Yugoslavia in 1969 [15]. Of the 2,861 subjects screened, 35 (1.2%) had elevated levels of Hb F (mean 3.7%, range 1.5-8.6%) and a normal level of Hb A₂, normal RBC indices and normal osmotic fragility. A second type of HPFH was characterized by a high $G\gamma$ level in the Hb F and by triplication of γ -globin genes ($G\gamma.G\gamma.A\gamma/G\gamma.A\gamma$) [12]. Many other cases appeared to have the Swiss type of HPFH (1.1%), which seems to be present mostly in Macedonians [23]. DNA haplotyping of the β -globin gene cluster from 23 unrelated families with the Swiss type of HPFH showed it to be associ-

ated with a haplotype identical to that of the Senegal (#3 [- + + - + + + +]) type [12].

α -Thalassemia. The first survey for α -thal, undertaken on 561 cord blood samples, showed an incidence of 3.8%. Studies of a further 1,140 newborn babies performed in 1980-81, showed an incidence of α -thal-1 of 0.8%, and for α -thal-2 of 2.4% [44]. A recent survey of 3,326 cord blood samples from Macedonian, 1,546 Croatian, 1,043 Albanian, 373 Gypsy and 99 Turkish newborns, gave incidences for α -thal-1 of 0.42, 0.39, 0.10, 0.0, 0.0 and 0.30, respectively, and for α -thal-2 of 1.89, 1.03, 0.10, 0.0, 0.0 and 1.24, respectively (unpublished data). Molecular characterization resulted in the identification of four different types of α -thal: $-\alpha^{3.7}/$, $\alpha^{MED1}\alpha/$ (-17.5 kb), $-\alpha^{20.5}/$ and Hb Icaria [$\alpha 142$, Term $\rightarrow$ Ser (TAA>TCA in $\alpha 2$)] [45-47].

Abnormal Hemoglobins. Table 8 lists the Hb variants detected in Former Yugoslavia. Twenty-eight different variants, in 253 unrelated individuals or families, were characterized. Of these, four were α chains, 18 β chains, two γ chains, one δ chain, one

Table 6 Clinical findings in nine homozygotes for Hb Lepore

Case	Age/year when detected	Age at time of death/year	Sex	Anemia	Transfusion requirements	Enlargement at the time of detection (cm)		Facies	Bone changes
						Liver	Spleen		
P.M.	2.5	9	F	+++	regular	5	5	++	++
K.M.	3.5	5	F	+++	regular	3	5	+++	+++
K.I.	0.5	7	M	+++	regular	3	4	+	+
D.R.	1.5	8	M	+++	regular	3	4	+	+
P.J.	1	24	M	+++	regular	splenectomized		++	+++
J.S. *	5	17	M	++	irregular	3	4	+	+
S.G.**	2	14	M	+++	irregular	splenectomized		++	++
V.Z.**	5	9	F	+++	regular	4	5	+++	+++
S.R.	1	11	F	+++	regular	3	5	++	+++

+ = mild; ++ = moderate; +++ = severe; *patient from Zagreb; ** patient from Beograd

Table 7 Hematological and hemoglobin composition data in nine homozygotes for Hb Lepore.

Case	Age (yr)	Hb (g/dl)	RBC ($\times 10^{12}/l$)	PCV (l/l)	MCH (pg)	MCV (fl)	Abn RBC morph.	Hb F %	HbLepore %	γ +Lepore/ α ratio
P.M.	2.5	4.1	1.6	15	26	94	+++	91.4	8.6	0.19
K.M.	3.5	4.2	1.5	15	28	100	+++	87.3	12.7	n.d.
K.I.	0.5	4.0	1.9	17	21	89	+++	92.6	7.4	0.22
D.R.	1.5	6.5	3.1	23	21	74	+++	84.9	15.1	0.17
P.J.	1	6.6	2.5	24	26	96	+++	84.7	15.3	0.21
J.S.	5	6.9	3.0	24	23	80	+++	76.0*	5.6	0.30
S.G.	9	10.2	3.3	31	30	94	+++	84.3	15.7	0.23
V.Z.	5	6.3	2.7	25	23	93	+++	88.0	12.0	0.18
S.R.	1.5	5.3	1.9	19	28	100	+++	85.0	15.0	0.20

* Tested one month after last transfusion

extended chain and two fusion chain variants. Five were unstable and two were hyperunstable variants causing a severe hemolytic anemia, two were metHb variants causing methemoglobinemia, and two were fusion chain variants causing anemia similar to that found in the thalassemias. As already mentioned, the most common variant is Hb Lepore (Lepore-WB) that is distributed throughout Former Yugoslavia and has been observed in 163 unrelated individuals or families. Hb O-Arab is the second most com-

mon variant, being found mainly in the Republic of Macedonia, where it has been observed in 22 Gypsy and six Albanian families. This variant has been observed in three instances in the homozygous state [24,48], and in two instances in association with β -thal [24]. Hb Beograd [49,50], and Hb Strumica [51], seem to occur only in Yugoslavia, and have also been found in Yugoslavian families living in Australia and New Zealand [52], and in Turkish families who emigrated from Yugoslavia [53]. Seven vari-

Table 8 Abnormal Hemoglobind Detected in Former Yugoslavia

Abnormal Hemoglobin	Codon	Substitution	No. unrelated cases		Origin	Total
			Hospital survey (n=13.540)	Population survey (n=34.648)		
α Chain variants						
J-Paris-I	12;GCC→GAC	Ala→Asp		2	Macedonia	3
				1	Croatia	
J-Oxford	15;GGT→GAT	Gly→Asp		1	Macedonia	1
Savaria	49;AGC→AGA	Ser→Arg	4	2	Croatia	6
Strumica	112;CAC→CGC	His→Arg	2	3	Macedonia	9
				2	Croatia	
				2	Vojvodina	
β Chain variants						
S	6;GAG→GTG	Glu→Val	2	1	Macedonia	5
				1	Serbia	
				1	Croatia	
C	6;GAG→AAG	Glu→Lys		1	Macedonia	1
Lieden*	6/7;not reported	Glu→0	1		Croatia	1
Hamilton	11;GTT→ATT	Val→Ile		2	Macedonia	2
Volga*	27;GCC→GAC	Ala→Asp	1		BiH	1
Yokohama*	31;CTG→CCG	Leu→Pro	1		Serbia	1
Hoshida	43;GAG→CAG	Glu→Gln		1	Vojvodina	1
M-Saskatoon	63;CAT→TAT	His→Tyr	1		Vojvodina	1
Bushwick*	74;GGC→GTC	Glu→Val	1		Serbia	1
Sabine*	91;CTG→CCG	Leu→Pro	1		Serbia	1
M-Hyde Park	92;CAC→TAC	His→Tyr	1		BiH	1
Koln*	98;GTG→ATG	Val→Met	1 + 1*		Serbia	5
			1 + 1*		Croatia	
			1		Macedonia	
Beth Israel*	102;AAC→AGC	Asn→Ser	1		Serbia	1
O-Arab	121;GAA→GTA	Glu→Lys	6	18	Macedonia	28
				2	Serbia	
			1	1	Croatia	
Beograd	121;GAA→GTA	Glu→Val	1	3	Croatia	12
			1	3	Serbia	
			1	3	Macedonia	
D-Punjab	121;GAA→CAA	Glu→Gln		2	Macedonia	2
γ Chain Variants						
F-Macedonia I	^A γ2;CAT→CAG	His→Gln		1	Macedonia	1
F-Macedonia II	^G γ104;AAG→AAC	Lys→Asn		1	Macedonia	1
δ Chain Variants						
A ₂ -Zagreb	125;CAA→GAA	Gln→Glu	1		Croatia	1
Extended Chain Variants						
Icaria	(α141+31 residues)		1		Macedonia	1
Fusion Chain Variants						
Lepore-Washington	δ87-β116		110	53	F. Yu**	163
Lepore-Baltimore	δ50-β86		1		Serbia	1
TOTAL			145	106		251

* De novo mutation; ** In all republics and provinces of former Yugoslavia

ants, *i.e.*, Hb Leiden [$\beta 6(A3)$ or $\beta 7(A4)$ Glu $\rightarrow$ 0], Hb Yokohama [$\beta 31(B13)$ Leu $\rightarrow$ Pro, CTG>CCG], Hb Sabine [$\beta 91(F7)$ Leu $\rightarrow$ Pro, CTG>CCG], Hb Koln [$\beta 98(FG5)$ Val $\rightarrow$ Met, GTG>ATG], Hb Beth Israel [$\beta 102(G4)$ Asn $\rightarrow$ Ser (AAC>AGC)] and Hb Bushwick [$\beta 74(E18)$ Gly $\rightarrow$ Val, GGC>GTC], that were found in Former Yugoslavia, have arisen as *de novo* mutations.

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