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HbA2-Partinico or δ (A2)Pro $\rightarrow$ Thr, a new genetic variation in the δ -globin gene *in cis* to the β^+ thal IVS-I-110 G>A, and the heterogeneity of δ -globin alleles in double heterozygotes for β - and δ -globin gene defects

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Abstract The study of the alleles of the δ -globin gene is relevant to the prevention of β -thalassemia homozygosis; in fact, the increase of the HbA2 is an invaluable hematological marker of the β -thalassemia heterozygosis and the double heterozygosis for alleles of δ - and β -globin genes can cause the decrease of the HbA2 up to normal or borderline values. We carried out the characterization of alleles of the δ - and β -globin genes, restriction fragment

length polymorphism (RFLP) haplotype background, and hematologic phenotype in 23 double heterozygotes belonging to 18 unrelated families. A wide heterogeneity of the δ -globin alleles was detected; seven known alleles *in trans* to the β -globin gene defects were revealed in 17 out of 18 families, while a new allele *in cis* to a β -thalassemia allele was detected in one family. Moreover, the relative frequency of the δ -mutants was quite different from that found among heterozygotes. The new allele δ -cod 5 CCT>ACT, *in cis* to the allele β^+ thal IVS-I-110 G>A, was found in five carriers of a Sicilian family. The new variant δ 5(A2)Pro $\rightarrow$ Thr, named HbA2-Partinico upon the origin of the family, was detected with high-performance liquid chromatography; it overlapped the HbA2 peak which was partially split. The double *in cis* heterozygotes had increased percentage of normal and variant HbA2 of comparable size. The variant originated most likely from a new mutational event because it was associated with RFLP haplotype I, commonly found with the β^+ thal IVS-I-110 G>A, even if crossing over or gene conversion cannot be excluded.

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Introduction

The human hemoglobin A2 (HbA2) is a minor hemoglobin, made of two α -globin and two δ -globin chains ($\alpha_2\delta_2$); its range is 2.1–3.3% of the total hemoglobin in the postnatal life [1]. The gene for the δ -globin chain is located on the

cluster of the β -like globin genes at 7,373 bp 5' of the β -globin gene on chromosome 11 [2]. Seventy point mutations or microdeletions causing decreased synthesis of the δ -chains (δ -thalassemia) or HbA2 variants have been reported [3]. In the Mediterranean area, the δ^+ thal cod 27 GCC>TCC or HbA2-Yialousa has the relative frequency of about 70%; several other alleles account for the remaining 30% [1]. The increase of the HbA2 over the normal range, associated with microcythemia, is an invaluable marker for the laboratory screening of β -thalassemia carriers [2]. The coinheritance of δ - with β -globin mutated alleles can lower the HbA2 into the normal range and, consequently, it can hinder the diagnosis of β -thalassemia carriers and the prevention of Cooley's anemia. The δ - with β -globin mutated alleles can be coinherited *in trans* or, most rarely, *in cis*. Genotype and phenotype characterization of double heterozygotes with mutations *in trans* have been carried out in some Mediterranean countries. In Sicily, among 7,153 subjects screened for β -thalassemia, 18 were found to be double heterozygotes for β - and δ -thalassemia [4]. Out of them, 14 out of 18 showed the HbA2-Yialousa mutation, three out of 18 the δ^+ thal IVS II nt 897 A>G, and one out of 18 the δ -cod 12 AAT>AAA or HbA2-NYU. In a study of Sicilian β -thalassemia carriers with HbA2 borderline percentages, 11 double heterozygotes subjects were reported; nine out of 11 had the HbA2-Yialousa mutation and two out of 11 the δ^+ thal IVS II nt 897 A>G [5]. In the Portuguese population, 51 β -thalassemia carriers with normal or borderline HbA2 level were selected. Out of them, eight out of 51 were double heterozygotes for the β - and δ -thalassemia, and all had the HbA2-Yialousa mutation [6]. In the Greek population, the Corfù deletion (nine families), the HbA2-Yialousa (two families), the δ^0 thal cod 59 (-A) (two families), and the δ -cod 141 CTG>CCG or HbA2-Pelendri have been reported *in cis* or *in trans* to different β -thalassemia mutations [7, 8]. In the Mediterranean area, a limited number of subjects have been shown to be double heterozygotes of the δ -globin gene mutations *in cis* to β -thalassemia defects [3]; out of them, two families had new δ -globin alleles. They were the HbA2-Fogo identified in a Portuguese family and the δ -Poly A +69 (G>A) identified in a Sardinian family [6, 9]. In this paper, we report the study of 23 double heterozygotes for β - and δ -thalassemia defects or variants from Southern Italy; four were unrelated single carriers, 19 belonged to 14 unrelated families. A wide molecular heterogeneity was found; in fact, eight δ -globin and seven β -globin alleles were detected. All the alleles were associated with restriction fragment length polymorphism (RFLP) haplotype background described in the Mediterranean area. Out of the families, ten out of 18 (about 55%) had the δ allele HbA2-Yialousa; one family had a new allele δ -cod 5 CCT>ACT *in cis* to the known allele β^+ thal IVS-I-110 G>A. The new

HbA2 variant δ -cod 5 Pro>Thr was detected with high-performance liquid chromatography (HPLC) as a split peak eluting just behind the HbA2 in five double heterozygotes belonging to three generations; the grandmother descent was from Partinico (Sicily).

Design and materials

Families and criteria of the selection

Fourteen unrelated families and four unrelated single carriers, originating from Southern Italy (East Sicily, Puglia, Basilicata, and Campania), were selected by the local thalassemia centers among subjects referred to them for hematological diagnosis; they were collected according to the Italian Personal Data Protection Code. The probands showed microcythemia associated with HbA2 percentage in the normal range or borderline values but other family members were carriers of the δ - or β -thalassemia trait. The four single carriers were selected because they were negative for the most common α -thalassemia mutations. Genotyping of the α - and/or β - and δ -globin gene mutations was carried out in accordance with the hematologic alterations.

Hematological data and Hb analyses

The hematological data, ferritin, serum iron, or ZPP values were determined with standard methods in the hospitals. Hb analyses were carried out by cation-exchange HPLC. All the laboratories used the VariantTM II System (Bio-Rad Laboratories, Richmond, CA, USA). Only one family was analyzed with the HA-8160 ADAMS (Menarini, Firenze, Italy). The hemoglobins were eluted in the order: HbF, glycated HbA, HbA, HbA2, while the variant's position was determined by their characteristics.

Alleles of the δ -, β -, and α -globin genes

The δ - and β -globin alleles were analyzed with the ARMS method or gap-polymerase chain reaction (gap-PCR) or DNA sequencing [1, 10]. The α -thalassemia deletions “ $-\alpha 3.7$ ”, “ $-(\alpha)20.5$,” and “ $--Med$ ” were tested with gap-PCR [11]; point mutations were analyzed with multiplex ARMS or DNA sequencing [12, 13]. Sequencing was carried out in all cases with Automated Cycle Sequencing (3100 Genetic analyzer, Applied Biosystems).

RFLP haplotypes of the β -like globin gene cluster

The analysis of the RFLPs present along the β -like globin gene cluster was carried out on PCR-amplified DNA fragments [1, 14–16]. RFLP haplotypes associated with

mutated δ -globin alleles were determined with family segregation study; when parents or relatives of the probands were not available and carriers were not homozygous, the haplotypes were assembled—if possible—according to those already reported for each allele [17, 18].

Results

Results of hematological, genotype, and RFLP haplotype characterization are reported in Tables 1 and 2. The probands showed microcythemia associated with HbA2 in the range 2.3–3% with the exclusion of families 2, 8, and 11 who had 3.4, 3.6, and 3.8, respectively. Seven known δ -globin alleles (four δ -thalassemia and three HbA2 variants) and a new δ -globin allele leading to a new HbA2 variant were detected. Seven β -globin gene alleles were also diagnosed; five with β -thalassemia mutations and two Hb variants, the Hb Neapolis and the Hb S. Only one α -thalassemia allele was found; it was the α^0 thal “--Med” deletion in the proband of family 8. RFLP haplotypes associated with the δ -globin alleles and the pattern of segregation of mutated alleles were defined in all families but not in the four single carriers because they were not RFLP homozygous and other family members were not available. Haplotypes were the same described for each allele in the Mediterranean countries. Segregation study indicated that, in 13 out of 14 families, the δ - and β -mutated alleles were coinherited *in trans*; on the contrary, the new δ -globin allele was coinherited *in cis*.

The new allele δ -cod 5 CCT>ACT was detected in a young woman, 30 years old, who came to our observation during an iron therapy (Fig. 1a, II.2). At the first hematological observation, she had extremely low Hb level (7.4%), severe microcythemia (MCV 58.3 fl), reduction of both MCH and MCHC (17 pg; 29 g/dL); serum iron was at lower limit (49 μ g/dL), while the ferritin (64 ng/mL) and total (0.69 mg/dL) and indirect bilirubin (0.54 mg/dL) were in the normal range (Table 2). At a second observation, after a cycle of iron therapy, she still showed microcythemia (MCV 66.1 fl), but Hb increased up to 10.5 g/dL. The HPLC revealed an asymmetric partially split peak of HbA2 because of a new Hb eluted just behind the HbA2 (Fig. 1b, 1). The anomalous HbA2 peak associated with microcythemia were detected in the mother (I.2), in the two children (III.1 and III.2), and in the stepsister (II.6) of the proband (Fig. 1a; Table 2). The asymmetric partially split HbA2 peak was evaluated by the HPLC as a unique peak which was 4.5% in the proband and $\geq 5\%$ in the other carriers (Table 2). The sister (II.4) had not inherited the variant but only the microcythemia associated with increased HbA2, which was 5.7% (Fig. 1a; Table 2). In order to confirm the identification of normal and new peaks, blood of a normal subject was added to that of the

carrier II.6 and the mixed sample analyzed with HPLC. This gave an increase of only the normal HbA2 peak eluted with a lower retention time and confirmed the variant elution pattern. In order to evaluate the percentage of the HbA2 and of the new peak, a blood sample of the carrier II.6 was analyzed with HPLC by diluting the hemolysate with the buffer in the ratio 1:1 (Fig. 1b, 2). In this condition, the software recognized two areas, and the ratio between the percentages of the two areas HbA2/HbA2-Partinico was about 1. DNA sequencing revealed that the anomalous peak was due to a new single base substitution in the δ -globin gene, δ -cod 5 CCT>ACT (Fig. 1c), which was detected in the five carriers of the HbA2 variant, and in all carriers, this mutation was coinherited with the β^+ thal IVS-I-110 G>A allele. On the contrary, the sister (II.4) of the proband with microcythemia and increased HbA2 but no variant was heterozygote for the β^0 thal cod 39 C>T, most likely inherited from the father (I.1; not available for the study) and showed no defects of the δ -globin gene. RFLPs family study indicated that the alleles HbA2-Partinico and β^+ thal IVS-I-110 G>A were associated with haplotype I (Fig. 1d). The new HbA2 variant was named HbA2-Partinico upon the origin of the proband's mother (Fig. 1a, I.2).

Discussion

HbA2-Partinico

The new HbA2-Partinico or $\delta 5(A2)$ Pro→Thr is the first variant due to a mutation at codon 5 CCT>ACT of the δ -globin gene. The residue 5(A2) of the δ -globin chain is in the alpha helix region and no variation of the secondary structure was predicted by the SOPMA software for the Pro→Thr substitution in this position (<http://www.expasy.org>) and, consequently, no functional alteration [19]. In the β -globin chain, three substitutions of the amino acid “proline” in the same position have been described with no functional alteration: the Hb Gorwihl or $\beta(\alpha 2)$ Pro→Ala, the Hb Tyne or $\beta(\alpha 2)$ Pro→Ser, and the Hb Warwickshire or $\beta(\alpha 2)$ Pro→Arg [20–22]. Most likely also in the case of the new HbA2-Partinico, the amino acid substitution does not cause any functional alteration. In fact, in the double *in cis* heterozygotes for the HbA2-Partinico and β^+ thal IVS-I-110 G>A, the values of the partially split peak of HbA2 + HbA2-Partinico were increased at $\geq 5\%$, a level expected in the heterozygotes for the β -thalassemia allele; the proband showed a lower percentage (4.5%) probably due to the iron deficiency anemia. Most likely, the normal and the variant alleles were expressed at a comparable rate considering that the ratio HbA2/HbA2-Partinico of the HPLC areas was about 1. This confirm that the β^+ thal alleles cause

Table 1 Hematological data and RFLP haplotypes observed in 17 families with δ - and β -globin gene mutations

Families (no./family relationship)	Sex/age (years)	δ -globin gene mutations	β -globin gene mutations	RFLP haplotypes	RBC ($10^{12}/L$)	Hb (g/dL)	Ht (%)	MCV (fL)	MCH (pg)	HbA2 (%)	HbA2-Var (%)	HbF (%)
δ^+ thalassemia mutations												
1	Proband F/24	δ^+ -31 A>G	β^+ IVS-I-110 (G>A)	I/I	5.50	12.6	40.2	70.0	23.0	2.7	=	0.2
	Mother F/51	δ^+ -31 A>G		nd	4.71	13.9	42.6	90.0	29.5	1.8	=	0.1
2	Proband M/34	δ^+ Poly A	β^0 cod 39 (C>T)	IX/II	6.25	11.9	36.0	57.0	19.0	3.4	=	2.5
	Child F/07	δ^+ Poly A		IX/V	4.69	12.8	36.0	77.0	27.0	1.7	=	1.7
	Wife F/34		β^{++} IVS-I-6 (T>C)	VI/IV	5.31	11.9	36.0	68.0	22.0	3.9	=	0.5
	Child F/04		β^0 cod 39 (C>T)	nd	5.33	9.7	30.0	57.0	18.0	5.4	=	1.0
δ^0 thalassemia mutations												
3	Proband M/38	δ^0 cod 15 TGG>TAG	β^0 cod 39 (C>T)	IX/I	6.84	12.0	39.6	57.8	17.5	2.6	=	0.8
	Father M/69		β^0 cod 39 (C>T)	I/I	6.91	13.0	42.6	61.7	18.6	4.8	=	0.4
4	Proband F/12	Corfu-type deletion	β^0 cod 44 (-C)	Corfu/II	6.18	11.9	37.7	61.0	19.3	2.4	=	3.8
	Sister F/17	Corfu-type deletion	β^0 cod 44 (-C)	Corfu/II	6.16	12.1	39.8	64.7	19.6	2.6	=	4.7
	Mother F/45	Corfu-type deletion		Corfu/I	4.65	14.7	43.0	92.5	31.5	1.3	=	3.6
HbA2 variants												
5	Proband F/33	HbA2-NYU	β^+ IVS-I-110 (G>A)	nd	5.75	11.0	36.3	63.1	19.1	3.0	3.4	1.8
6	Proband F/45	HbA2-NYU	β^+ IVS-I-110 (G>A)	I/I	4.93	9.9	32.6	66.1	20.1	2.3	nd	0.3
	Father M/75	HbA2-NYU		nd	4.70	14.1	42.4	90.1	29.9	1.3	nd	0.3
7	Proband F/47	HbA2-Coburg	β^{++} IVS-I-6 (T>C)	IX/VI	5.38	11.6	35.7	66.0	21.5	1.0	a	0.2
	Son M/29		β^{++} IVS-I-6 (T>C)	nd	6.31	41.8	13.6	66.0	21.5	4.2	=	0.2
HbA2-Yialousa												
8	Proband F/41	HbA2-Yialousa	β^+ IVS-II-745 (C>G)	IX/VII	5.38	12.2	36.4	67.7	22.7	3.6	b	0.6
	Child M/5	HbA2-Yialousa		IX/I	4.88	13.0	35.9	73.6	26.6	2.0	b	0.2
9	Proband F/13	HbA2-Yialousa	β^+ IVS-I-110 (G>A)	IX/VI	5.95	11.5	37.0	62.2	19.3	3.0	b	0.5
10	Proband F/33	HbA2-Yialousa	β^+ IVS-I-110 (G>A)	IX/I	4.89	10.4	31.3	64.0	21.2	3.0	b	0.2
	Father M/74	HbA2-Yialousa		IX/I	5.64	17.0	48.8	87.0	30.1	1.6	b	0.3
11	Proband F/29	HbA2-Yialousa	β^+ IVS-I-110 (G>A)	IV/I	5.55	11.6	37.4	67.0	20.9	3.8	b	0.5
	Child M/04	HbA2-Yialousa		IV/V	4.92	12.7	39.7	81.0	25.8	2.0	b	0.3
12	Proband M/27	HbA2-Yialousa	β^{++} IVS-I-6 (T>C)	IX/VI	5.22	12.6	37.5	71.8	24.1	2.7	b	0.7
13	Proband F/35	HbA2-Yialousa	β^{++} IVS-I-6 (T>C)	IX/VI	5.25	12.4	38.0	72.0	24.0	2.8	b	1.1
	Father M/68	HbA2-Yialousa		IX/I	4.72	15.2	42.0	88.0	32.0	2.0	b	2.4
14	Proband M/35	HbA2-Yialousa	β^{++} IVS-I-6 (T>C)	IX/VI	5.75	13.0	40.9	71.1	22.6	2.8	b	0.5
	Mother F/60	HbA2-Yialousa		IX/IX	4.55	13.2	39.4	86.6	29.0	1.4	b	0.5
15	Proband F/36	HbA2-Yialousa	β^{++} IVS-I-6 (T>C)	IX/VI	4.50	11.0	31.2	69.3	24.4	2.6	b	1.4
16	Proband M/54	HbA2-Yialousa	Hb Neapolis	IX/V	5.34	14.1	42.9	80.3	26.4	2.6	b	0.5
	Child M/19	HbA2-Yialousa		IX/I	5.83	16.8	48.9	83.9	28.8	2.1	b	0.5
17	Proband F/25	HbA2-Yialousa	HbS	IX/Benin	5.01	12.2	38.2	76.0	24.3	3.0	b	1.2
	Father M/56	HbA2-Yialousa		IX/I	5.58	16.9	50.0	90.0	30.3	1.7	b	0.4

The segregation pattern indicated that the δ - and β -globin alleles were *in trans* in 12 out of 17 probands; in the remaining four probands—who were single carriers—the allele segregation study was not possible. In these four carriers the haplotypes assigned to the δ -globin alleles were assembled according to those already reported. The Corfu haplotype was characterized by the haplotype V with the deletion of the Hinc II/3' ($\psi\beta$ (- -del/+ -); see the “[Design and materials](#)” section)

a HbA2 variant coeluting with HbA on HPLC, b HbA2 variant coeluting with HbA2

Table 2 Hematology, δ - and β -globin genotypes and RFLP haplotypes of the members of the family with the new HbA2-Partinico or δ -cod 5 CCT>ACT

Relations	Normal range Men	Normal range Women	I.2	II.1		II.2		II.4	II.6	III.1	III.2
				Mother	Husband	Proband	Sister				
Sex/age (years)			F/49	M/34	F/30	F/31	F/19	F/12	M/11	F/06	
RBC (10 ¹² /L)	4.0–5.7	3.8–5.3	5.69	5.48	4.3	4.99	6.0	5.54	5.88	5.92	
Hb (g/dL)	13.0–17.5	12–16	12.3	16.0	7.4	10.5	11	11.1	11.6	11.5	
Ht (%)	38–54	36–48	37.0	46.6	25.0	33.0	34.3	33.6	34.5	34.1	
MCV (fL)	80–99	80–99	65.0	85.1	58.3	66.1	57.2	60.5	58.7	57.7	
MCH (pg)	27–34	27–34	21.6	29.2	17.2	21.0	18.4	20.0	19.8	19.5	
MCHC (g/dL)	32–36	32–36	33.2	34.4	29.6	31.8	32.1	33	33.6	33.8	
Anisocytosis	absent		+		nd	+	+	+			
Micro cells	absent		+++		nd	+++	+++	+++	+++	+++	
Anisochromia	absent				nd	+	+		+	+	
Ipochromia	absent				nd	+++	+++	+		+	
Eritrocyte fragments	absent				nd	+	+			+	
HbA2 + HbA2-Var (%)			5.6		4.5	nd		5.0	5.7	5.6	
HbA2 (%)	1.8–3.2			2.8			5.7				
HbF (%)	0.0–2.0		1.3	2.1	2.2	nd	1.5	0.8	1.9	2.8	
Serum Iron ug/dL	49–151		82	122	49	nd	99	41	109	152	
Ferritin ng/mL	10–200		nd	nd	64	nd	nd	nd	nd	nd	
Transferrin (mg/dL)	200–336		nd	nd	382	nd	nd	nd	nd	nd	
Erythropoietin (mU/mL)	2.4–33.4		nd	nd	40.6	nd	nd	nd	nd	nd	
Total bilirubin (mg/dL)	0.2–1.2		nd	nd	0.69	nd	nd	nd	nd	nd	
Indirect bilirubin (mg/dL)	0.1–0.9		nd	nd	0.54	nd	nd	nd	nd	nd	
β-globin genotype			β IVS-1-110/βA	βA/βA	β IVS-1-110/βA	β IVS-1-110/βA	β cod 39/βA	β IVS-1-110/βA	β IVS-1-110/βA	β IVS-1-110/βA	
δ-globin genotype			δPartinico/δA	δA/δA	δPartinico/δA	δPartinico/δA	δA/δA	δPartinico/δA	δPartinico/δA	δPartinico/δA	
RFLP haplotype			I/VII	nd	I/I	I/VII	I/VII	I/II	nd	nd	

For the pedigree, see Fig. 1a
 nd not determined

Fig. 1 **a** Pedigree of the family with the new HbA2-Partinico or $\delta 5(\text{A}2)\text{Pro} \rightarrow \text{Thr}$. The mutation $\delta\text{-cod}5 \text{ CCT} \rightarrow \text{ACT}$ was *in cis* to the β^+ IVS-I-110 G>A. The two husbands of the mother of the proband (I.2) were not available for the study. The arrow indicates the proband (II.2). **b** 1 Hb-HPLC of the carrier II.6; the HbA2 peak, 5.0%, was asymmetric and partially split in two components; 2 HPLC of diluted hemolysate of the II.6 carrier; it allowed to determine the ratio between the areas HbA2/HbA2-Partinico which was about 1. **c** Sequencing of the δ -globin gene from codon 3 to codon 6 showing that the proband was heterozygote for the new mutation $\delta\text{-cod}5 \text{ CCT} \rightarrow \text{ACT}$. **d** A scheme of the β -globin gene cluster with the position of the two mutations *in cis* ($\delta\text{-cod}5 \text{ CCT} \rightarrow \text{ACT}$ and β^+ thal IVS-I-110 G>A) and with the associated RFLP haplotype

increased synthesis of δ -chains by δ -globin alleles *in cis* and *in trans* [2]. The alleles HbA2-Partinico and β^+ thal IVS-I-110 G>A were associated with RFLP haplotype I, which has been found in the majority of cases of the β^+ thal IVS-I-110 G>A. This finding is in favor of the hypothesis that the HbA2-Partinico was due to a new mutational event taking place on the chromosome carrying the allele β^+ thal IVS-I-110 G>A. But, gene conversion or crossing over events cannot be excluded considering that the RFLP 5'-haplotype “- - -” is one of the most frequent in normal subjects and that the δ - and β -globin genes are separated by a region with high recombination rate (Fig. 1d). Furthermore, it must be considered that the detection of the HbA2-Partinico *in cis* to β^+ thal IVS-I-110—instead of the HbA2-Partinico heterozygosis—is favored by the fact that Hb-HPLC is carried out mostly in subjects with microcythemia, which is absent in the HbA2 variant carriers, and that the β^+ thal IVS-I-110 G>A chromosome has about 40% of relative frequency in the Mediterranean area [18].

Epidemiologic aspects

In the present study, eight δ -globin alleles were detected; out of them, the allele HbA2-Yialousa had the relative frequency of about 55% (ten out of 18 chromosomes), while the other seven alleles were 45%. This molecular heterogeneity has not been reported in other studies on double heterozygotes [4–6]. In Sicilian double heterozygotes, only three δ -globin alleles have been detected and the relative frequency of the HbA2-Yialousa was 78%; in Portuguese double heterozygotes, only two alleles have been reported and HbA2-Yialousa was 90%. The higher heterogeneity we found could correlate with differences in the carriers selection or with different origins of the southern Italy population originated by different colonization and migration.

The relative frequency of the β -globin gene alleles showed some interesting aspects. The alleles β^+ thal cod 39 C>T, one of the most frequent in the Mediterranean area,

was detected only in two out of 18 families; the β^+ thal IVS-I-110 G>A in seven out of 18; and the β^{++} thal IVS-I-6 T>C in six out of 18 (about 30%) families. Indeed, in the Mediterranean area as well as in Southern Italy, the β^0 39 and β^+ IVS-I-110 mutations showed similar relative frequencies, i.e., 26.7% and 31.3%, respectively [16], while the β^{++} thal IVS-I-6 was rare in carriers, but most frequent in mild thalassemia intermedia. These two observations most likely indicate that double heterozygosis causes HbA2 “in the normal range” preferentially in the presence of β^+ or β^{++} thal alleles or δ^0 thal/variant (accounting for severe reduction of the HbA2). Thus, a consistent number of double heterozygotes with β^0 thal and mild δ alleles can escape the diagnosis because the decrease of HbA2 due to the δ -thal alleles is not sufficient to compensate the increase due to the β^0 thal alleles.

Hematology

The double heterozygote for HbA2-Coburg (comigrating with HbA on the HPLC) and the β^{++} thal IVS-I-6 showed a HbA2 percentage of 1.0% associated with normal ferritin values (18 ng/dL). A similar value has already been reported in a heterozygote [4]. The mechanism causing this decrease is unknown. In three cases, the HbA2 was 3.4%, 3.6%, and 3.8%. The type of β -thalassemia mutations (β^0 or β^+) and the individual phenotype variability could account for these high HbA2 values. In this study, we reported the hematologic phenotype of the carriers of two δ -globin gene alleles recently described by us (Table 1, families 2 and 3) [1]. The δ -thal mutation AAT4AA>AAT-TAA is the first mutation in the polyA sequence identified in the δ -globin gene. It was detected in a family from Sicily and gave a δ^+ thalassemic defect; the HbA2 level was 1.7% in the carrier and 3.4% in the father, double heterozygote for β^0 thal cod 39 C>T. The δ^0 thal cod 15 TGC>TAG is the second nonsense mutation identified in the δ -globin gene. It was detected in a family from Puglia in south-eastern Italy. The double heterozygote (new mutation and β^0 thal cod 39 C>T) had microcythemia associated with a normal level of HbA2 (2.6%), synthesized only by the wild-type allele (Table 1).

Clinical applications

It is important to stress the point that advanced biochemical tools are also important for the detection of minimal differences. The instrument we used gave the separation, even if minimal, leading to the HbA2-Partinico identification with DNA sequencing; indeed, a HPLC system with improved performance characteristics, allowing to separate hemoglobins which had similar retention time of the HbA2, is now under evaluation [23]. This is important because, in

an ideal flowchart, HPLC abnormalities represent a good indication for DNA sequencing. The inheritance of δ - or β -globin gene alleles cause most heterogeneous phenotypes, but in the majority of cases, the segregation patterns are of great help. This suggest to carry out the hematological and molecular studies of the families in order to avoid diagnostic mistakes. For prevention, the results we obtained indicate that the analysis of the presence of the HbA2-Yialousa mutation is not sufficient to exclude the presence of other δ -globin gene mutations. Considering the molecular heterogeneity detected and that new or rare δ -globin gene mutations we have observed, the sequencing of the δ -globin gene is irreplaceable for the clarification of any ambiguity and in order to avoid the misdiagnosis of β -thalassemia.

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