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Complexity of the alpha-globin genotypes identified with thalassemia screening in Sardinia

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ABSTRACT

α-Thalassemia commonly results from deletions or point mutations in one or both α-globin genes located on chromosome 16p13.3 giving rise to complex and variable genotypes and phenotypes. Rarely, unusual non-deletion defects or atypical deletions down-regulate the expression of the α-globin gene. In the last decade of the program for β-thalassemia carrier screening and genetic counseling in Sardinia, the association of new techniques of molecular biology such as gene sequencing and Multiplex Ligation-dependent Probe Amplification (MLPA) to conventional methods has allowed to better define several thalassemic genotypes and the complex variability of the α-cluster with its flanking regions, with a high frequency of different genotypes and compound heterozygosity for two α mutations even in the same family.

The exact molecular definition of the genotypes resulting from the interactions among the large number of α-thalassemia determinants and with β-thalassemia, is important for a correct correlation of genotype–phenotype and to prevent underdiagnosis of carrier status which could hamper the effectiveness of a screening program particularly in those regions where a high frequency of hemoglobinopathies is present.

Introduction

α-Thalassemia, affecting 5% of the world's population, is probably the most common of all single gene disorders [1]. It most frequently results from deletion of one or both α-globin genes located on the short arm of chromosome 16 and, less frequently, from different non-deletional mutations in canonical sequences that reduce the α-gene expression [2]. Rarely, down-regulation of the α-globin synthesis is caused by unusual defects as recently reviewed by Higgs [3].

The clinical phenotype of α-thalassemia varies according to the number of affected genes but the concomitant inheritance of β-thalassemia or structural variants may trouble the hematological picture. Similar to other Mediterranean previous malaria endemic areas, a high frequency of α-thalassemia (about 38%) has been found in the Sardinian population [4,5]. Migrations and travels are contributing to slightly modify the spectrum of mutations considered typical of this population. Moreover, the chromosome with the anti 3.7 α-globin gene triplication ($\alpha\alpha\alpha^{\text{anti } 3.7}$) is not rare in Sardinia and it is found during routine analysis, either alone or associated with β-thalassemia.

A routine use of the new techniques of molecular biology such as gene sequencing and Multiplex Ligation-dependent Probe Amplification (MLPA) may allow to better define several thalassemic genotypes, leading diagnostic centers to develop an effective genetic counseling of couples and a correct differential diagnosis of microcytic anemia in childhood.

The aim of this communication is to highlight the heterogeneity of the α-globin gene determinants and the complexity of genotypes that results from their interactions through the analysis of all the α-globin genotypes detected in the last decade of the program for β-thalassemia carrier screening and genetic counseling in Cagliari, Italy, associating molecular biology to conventional methods.

Patients and methods

From March 2002 to December 2012, 3594 subjects have been investigated for α-thalassemia defects in the Laboratory of Hematology of the Ospedale Regionale per le Microcitemie (Cagliari—Sardinia) for the following reasons:

- Microcytosis with normal HbA_2 and HbF, with or without anemia (suspected α-thalassemia trait) after exclusion of iron deficiency (68%).
- Suspected clinical diagnosis of HbH disease (4%).
- Heterozygous β-thalassemia with MCV ≥ 72 fl (4%).
- β-Thalassemia intermedia patients whose phenotypes could not be explained by their β-globin mutations (4%).
- Individuals with borderline HbA_2 3.3–3.9% (5%).
- Individuals with abnormal peaks on HPLC suggestive of α-chain variants (3%).

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- Relatives of probands (5%)
- Others (7%)

In addition to conventional hematological and molecular methods, the direct α-globin gene sequencing was used to identify point mutations in the coding regions of HBA1 and HBA2 and when an α-globin deletion was not identified and suspicion for α-thalassemia was high. MLPA with a set of 25 probes, covering a region of ~170 kb in the α-cluster on the short arm of the chromosome 16 allowed to characterize unsolved α-globin gene rearrangements [6,7].

Results and discussion

In 1093 individuals (31%) who had a partner with suspected α-thalassemia trait, the molecular analysis was limited to the screening of the — —Med deletion to exclude the risk of hemoglobin Bart's hydrops fetalis in genetic counseling. In 655 subjects (18%) the detection of the normal αα/αα genotype made further molecular investigations unnecessary.

The genotypes of the remaining 1846 subjects (51%) are depicted below.

1642 subjects (89%) were identified to be carriers of one or two of the most common −α3.7, — —Med I, α2 HphI, α2 NcoI, α1 NcoI thalassemic alleles, with 17 different genotypes detected, as reported in Table 1. The fetal hydrops was confirmed to be extraordinarily rare in the Sardinian population, with only one fetus, product of a consanguineous marriage, shown to be affected by chorionic villi DNA analysis. In 204 subjects (11%), less frequent α-thalassemia deletions, point mutations or micro-deletions resulting in structural α-globin chain variants and rearrangements producing additional α-globin genes were detected, with 45 different genotypes identified, as reported in Tables 2, 3 and 4.

The −α4.2 deletion, detected in 26 subjects, was prevalently found in association with other α-thalassemic determinants likely because, in the heterozygous state, as for the −α3.7, it may be asymptomatic (Table 2). The α^0 deletions [— — or −(α)] were subdivided in those that lie entirely within the α-globin gene cluster and those that extend beyond the cluster enclosing its flanking and telomeric regions. The former group included four patients belonging to three families with the $-(\alpha)^{20.5}$, one patient with the $--^{SEA}$, two siblings with the $--^{Cal}$ and three subjects from the same family with a — — deletion which removes a region of 9209 nt involving both α-globin genes and part of the first exon of the θ-gene [8]. In the group that includes flanking and telomeric regions, two relatives had a telomeric deletion involving the α-globin gene cluster and all regulatory elements with a 3′ breakpoint between the exon IV of the NME4 gene and the IVS II of the DECR2 gene [8]. Deletions removing only the regulatory region located 30 to 70 Kb upstream of the α-globin cluster, but leaving all α-globin genes intact [(αα)] were detected in six subjects. Four of them belonged to a previously reported family [9] in which the proband with a severe form of HbH disease inherited in trans two different deletions: a short deletion (S) of 3361 bp, described from other authors in patients of Portuguese origin [10,11], removing the MCS-R2 element and an extended deletion (E) removing the MCS-R1,R2,R3 elements. Two other unrelated subjects revealed a deletion involving the MCS-R elements, but the breakpoints of their deletions have not yet been elucidated (Table 2).

Among the 94 individuals showing the presence of a structural Hb α-variant, 70 had Hb J-Sardegna, 13 had Hb G-Philadelphia, 2 had Hb J-Meerut, 2 had Hb Sassari, while Hb J-Oxford, Hb J-Cape Town, Hb Stanleyville, Hb Setif, Hb I, Hb Taybe and Hb Constant Spring were all identified in single cases. Hb J-Sardegna was confirmed to be the most frequent Sardinian variant [12]. Two different mutations at codon 68 were observed in Hb-G-Philadelphia heterozygotes: the AAC > AAA mutation was found only on the α2-globin gene, whereas the AAC > AAG mutation was found on the α2-globin gene and on the α2α1 hybrid gene, suggesting that it may derive from a mechanism of gene conversion.

With the exception of Hb Taybe and Hb Constant Spring, all the Hb variants were clinically asymptomatic. Hb Taybe, an unstable α-chain, produced a phenotype of chronic anemia in a child compound heterozygote with the α2 NcoI defect. As we have already reported [13], this variant was not evident at hemoglobin analysis with cellulose acetate electrophoresis and HPLC and was detected only by direct sequencing of the α-globin genes. The single patient compound heterozygous for the Hb Constant Spring and the $--^{SEA}$ deletion showed a phenotype of HbH disease of moderate severity. HbJ-Sardegna, Hb Sassari and

Table 1
α-Globin genotypes of subjects carrier of one or two α-thalassemia common alleles (n = 1642).

α-Genotype	Subjects (n)	Phenotype
$-\alpha^{3.7}/\alpha\alpha$	627	Silent, α-thal trait or β-thal trait with high MCV and MCH in association with β-thal defect
$-\alpha^{3.7}/-\alpha^{3.7}$	403	α-Thal trait or β-thal trait with high MCV and MCH in association with β-thal defect
$\alpha^{NcoI}\ \alpha/\alpha\alpha$	205	
$\alpha^{NcoI}\ \alpha/\alpha^{NcoI}\ \alpha$	9	
$-\alpha^{3.7}/\alpha^{NcoI}\alpha$	110	
$\alpha\ \alpha/\alpha\alpha^{NcoI*}$	6	
$-\alpha^{3.7}/\alpha\alpha^{NcoI*}$	5	
$\alpha^{HphI}\alpha/\alpha\alpha$	57	
$\alpha^{HphI}\alpha/\alpha^{HphI}\alpha$	2	
$-\alpha^{3.7}/\alpha^{HphI}\alpha$	46	
$\alpha^{HphI}\alpha/\alpha^{NcoI}\ \alpha$	5	
$\alpha^{HphI}\alpha/\alpha\ \alpha^{NcoI}$	1	
$--^{Med}/\alpha\alpha$	60	
$--^{Med}/-\alpha^{3.7}$	86	HbH disease or severe carrier in association with β-Thal defect
$--^{Med}/\alpha^{NcoI}\alpha$	11	
$--^{Med}/\alpha^{HphI}\alpha$	8	
$--^{Med}/--^{Med}$	1	Hb Bart's hydrops fetalis

The initiation codon mutation in the α1 globin gene has been routinely included in the screening program since 2010.

Table 2
Less frequent α-thalassemia deletions (44 subjects).

Allele (MLPA deleted probes)	α-Genotype	Subjects (n)	Phenotype
$-\alpha^{4.2}$ (9–17)	$-\alpha^{4.2}/\alpha\alpha$	3	Silent or α-thal trait
	$-\alpha^{4.2}/-\alpha^{3.7}$	20	α-Thal trait or β-thal trait with high MCV and MCH
	$-\alpha^{4.2}/-\alpha^{4.2}$	1	in association with β-gene defect
	$-\alpha^{4.2}/\alpha^{NcoI}\alpha$	1	
	$-\alpha^{4.2}/--^{MED}$	1	HbH disease
$-(\alpha)^{20.5}$ (6–21)	$-(\alpha)^{20.5}/\alpha\alpha$	2	α-Thal trait
	$-(\alpha)^{20.5}/-\alpha^{3.7}$	2	HbH disease
$--^{SEA}$ (8–25)	$--/\alpha^{C\ S}\alpha$	1	HbH disease
$--^{CAL}$ (5–24)	$--/\alpha^{HphI}\alpha$	2	HbH disease
$--^{9209}$ (12–24)	$--/\alpha^{NcoI}\alpha$	2	HbH disease
	$--/\alpha\alpha$	1	α-Thal trait
— —tel del (1–25)	$--/-\alpha^{3.7}$	1	HbH disease
	$--/\alpha\alpha$	1	α-Thal trait
$(\alpha\alpha)^{MCS\text{-}R2\ del\ 3361}$ (S) (2–3)	$(\alpha\alpha)^{S}/\alpha\alpha$	1	α-Thal trait
$(\alpha\alpha)^{MCS\text{-}R1,R2,R3\ tel\ del}$ (E) (1–3)	$(\alpha\alpha)^{E}/\alpha\alpha$	2	α-Thal trait
	$(\alpha\alpha)^{S}/(\alpha\alpha)^{E}$	1	HbH disease
$(\alpha\alpha)^{at\ least\ MCS\text{-}R2\ del}$ (2–3)	$(\alpha\alpha)^{MCS\text{-}R}/\alpha\alpha$	1	α-Thal trait
$(\alpha\alpha)^{at\ least\ MCS\text{-}R1,R2\ tel\ del}$ (1–3)	$(\alpha\alpha)^{MCS\text{-}R}/\alpha\alpha$	1	α-Thal trait

The number of probes reported in the tables is corresponding to the DNA positions indicated in the manufacturer instructions (MRC-Holland, Amsterdam, Netherlands, P140B3 HBA probemix).

Table 3
Point mutations or micro-deletions resulting in structural Hb α-globin chain variants (94 subjects).

Variant (gene affected)	Mutation	α-Genotype	Subjects (n)	Phenotype	Origin
J-Sardegna (α2)	cd50 CAG > AAC	αα/αα	53	Silent or mild decrease HbA2. Found also in association with β-thal defect	Italy
		$-\alpha^{3.7}/\alpha\alpha$	16		
		$\alpha^{NcoI}\alpha/\alpha\alpha$	1		
J-Meerut (α1)	cd120 GCG > GAG	αα/αα	1	Found only in association with β^{E} defect	Bangladesh
		ααα/αα	1		
J-Oxford or I-Interlaken (α1)	cd15 GGT > GAT	αα/αα	1	Found only in association with $\beta^{IVS\text{-}I\ 6}$ defect	Italy
J-Cape-Town (α1 or $-\alpha^{3.7}$)	cd92 CGG > CAG	$-\alpha^{3.7}/\alpha\alpha$	1	Mild decrease of MCH	Greece
Sassari (α1)	cd126 GAC > CAC	αα/αα	2	Silent. Found also in association with β-thal defect	Italy
G-Philadelphia (α^2 or $-\alpha^{3.7}$)	cd68 AAC > AAA	αα/αα	3	Silent. Found also in association with β-thal defect	Italy and Niger
	AAC > AAG	αα/αα	6		
	AAC > AAG	$-\alpha^{3.7}/\alpha^{G\text{-}Ph}\alpha$	2		
	AAC > AAG	$-\alpha^{3.7}/-\alpha^{3.7}$	2		
Stanleyville II (α2)	cd78 AAC > AAA	$-\alpha^{3.7}/\alpha\alpha$	1	Found only in association with $\beta^{0}39$ defect	Italy
Setif (α2)	cd94 GAC > TAC	αα/αα	1	Mild decrease of MCH	Unknown
I (α1)	cd16 AAG > GAG	αα/αα	1	Silent	Italy
Taybe (α1)	del cd39-ACC	$\alpha\alpha/\alpha^{NcoI}\alpha$	1	Chronic hemolytic anemia	Italy
Constant Spring (α2)	cd 142 TAA > CAA	$--^{SEA}/\alpha\alpha$	1	HbH disease	China

HbG-Philadelphia were found both in normal and in β-thal heterozygous patients. HbJ-Meerut, Hb J-Oxford and Hb-Stanleyville II were detected respectively in association with β^{E}, β^{+}IVS I-6 and $\beta^{0}39$ mutations. Hb J-Cape-Town, Hb Setif and Hb I were not associated with a β-thalassemic defect (Table 3).

A chromosome with a triple α-globin loci, counterpart of the $-\alpha3.7$ deletion ($\alpha\alpha\alpha^{anti\ 3.7}$), was identified in 42 subjects. Not consistent abnormal hematologic characteristics were present in the simple heterozygotes, while a hematologic phenotype similar to that of heterozygous β-thalassemia or a mild clinical and hematologic picture of thalassemia intermedia was detected in combination with heterozygous β-thalassemia. In 4 patients, the $\alpha\alpha\alpha^{anti\ 3.7}$ allele seemed to counterbalance the expression of the α-globin genes when inherited with a thalassemic allele ($-\alpha3.7$ or α2 NcoI). The identification of this allele, however, remains important for a correct genetic counseling.

A rare α-triplication allele generated from mispaired X-boxes (αααanti 4.2), was identified in a man and in his daughter, both heterozygous for β^{0}-thalassemia with a thalassemia intermedia phenotype. A duplication of the α-globin gene cluster was present in 22 individuals belonging to eight families. In three of these families, the presence of an unknown molecular defect interacting with the heterozygous β-thalassemia, but unlinked to the β-cluster, had already been postulated many years before the diagnosis became finally possible with the advent of MLPA [14]. All duplications, although of different size, spanned at least a 66 Kb DNA region that includes the α-globin genes with all regulatory elements. Among subjects with the α-gene cluster duplication in association with β-thalassemia, all but one showed a thalassemia intermedia phenotype of widely variable severity ranging from the need of blood transfusions to the picture of a severe β-thalassemia carrier. The patient who had a coexistent α2 NcoI mutation, known to be more severe than the $-\alpha3.7$ deletion, and therefore able to further reduce the α/β imbalance, resulted a simple β-thal carrier. Three subjects with the α-globin cluster duplication without β-thalassemia showed a silent hematologic phenotype, but imbalanced α/β ratio (Table 4).

In addition, the α2 5′UTR polymorphism +14 (C>G) [15,16] was occasionally detected in our population. Moreover, the African polymorphism HBA2:c.301-24del-GinsCTCGGCCC and c.301-88 T>G [16,10] was identified in a subject from Algeria and the $-\alpha3.7$ hybrid gene 3′UTR +46 variation (C>A) [17,18] in a subject from Morocco.

Our study confirms therefore the complex variability of the α-cluster with its flanking regions, due to the presence of homologous regions and Alu repeats that favor deletions, insertions and duplications of DNA with a high frequency of the compound heterozygosity and of

Table 4
Rare rearrangements of the α-globin gene cluster causing additional α-globin genes (66 subjects).

Allele (MLPA duplicated probes)	α-Genotype	Subjects (n)	Phenotype
	α Triplication		
$\alpha\alpha\alpha^{anti\ 3.7}$	$\alpha\alpha\alpha^{anti\ 3.7}/\alpha\alpha$	38	Silent or slight HbA2 increase in normal. β-Thal carrier or mild T.I. in association with β-thal defect
	$\alpha\alpha\alpha^{anti\ 3.7}/-\alpha^{3.7}$	3	Silent. In one patient in association with β-thal major
	$\alpha\alpha\alpha^{anti\ 3.7}/\alpha^{NcoI}\alpha$	1	Silent in association with Hb D-Los Angeles
$\alpha\alpha\alpha^{anti\ 4.2}$ (10–18)	ααα/αα	2	T.I. in association with β-thal defect
	α Quadruplication		
ααααα (1–25)	ααααα/αα	12	T.I. in association with β-thal defect
	ααααα/−α	2	T.I. in association with β-gene defect. Silent with slight α/β ratio increase in normal
	$\alpha\alpha\alpha\alpha\alpha/\alpha^{NcoI}\alpha$	1	β-Thal carrier in association with β-thal defect
ααααα (1–24)	ααααα/αα	3	T.I. in association with β-thal defect. Silent with α/β ratio increase in normal
	ααααα/−α	2	T.I. in association with β-thal defect
ααααα (2–24)	ααααα/αα	1	T.I. in association with β-thal defect
ααααα (2–25)	ααααα/αα	1	T.I. in association with β-thal defect

The number of probes reported in the tables is corresponding to the DNA positions indicated in the manufacturer instructions (MRC-Holland, Amsterdam, Netherlands, P140B3 HBA probemix).
T.I. = thalassemia intermedia.

different α-genotypes even in the same family. Just because also members of the same families were studied, this study does not have an epidemiologic perspective but the purpose of underlining the importance of full genotyping and the need to include MLPA and direct sequencing of globin genes methods in the extended screening program for thalassemia.

The exact molecular definition of the genotypes resulting from the interactions among the large number of α-thalassemia determinants and with β-thalassemia, is important for a correct correlation of genotype–phenotype and for an adequate family screening and genetic counseling, particularly in those regions where a high frequency of hemoglobinopathies is present.

Conflict of interest statement

The authors have no conflicts of interest to declare.

Contributions

R.O. and M.E.P. designed the research, analyzed data and wrote the paper. M.C.S. M.F.D and D.L performed the molecular analysis. S.B collected the hematological data. R.G. coordinated the study and approved the final manuscript.

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References

[1] E. Vichinsky, Complexity of alpha thalassemia: growing health problem with new approaches to screening, diagnosis, and therapy, Ann. N. Y. Acad. Sci. 1202 (2010) 180–187, http://dx.doi.org/10.1111/j.1749-6632.2010.05572.x.
[2] C.L. Harteveld, D.R. Higgs, Alpha-thalassaemia, Orphanet J. Rare Dis. 5 (2010) 13, http://dx.doi.org/10.1186/1750-1172-5-13.
[3] D.R. Higgs, The molecular basis of α-thalassemia, Cold Spring Harb. Perspect. Med. 3 (2013), http://dx.doi.org/10.1101/cshperspect.a011718.
[4] R. Galanello, C. Sollaino, E. Paglietti, S. Barella, C. Perra, I. Doneddu, M.G. Pirroni, L. Maccioni, A. Cao, Alpha-thalassemia carrier identification by DNA analysis in the screening for thalassemia, Am. J. Hematol. 59 (1998) 273–278.
[5] A. Cao, R. Congiu, M.C. Sollaino, M.F. Desogus, F.R. Demartis, D. Loi, M. Cau, R. Galanello, Thalassaemia and glucose-6-phosphate dehydrogenase screening in 13- to 14-year-old students of the Sardinian population: preliminary findings, Community Genet. 11 (2008) 121–128, http://dx.doi.org/10.1159/000113873.
[6] C.L. Harteveld, A. Voskamp, M. Phylipsen, N. Akkermans, J.T. den Dunnen, S.J. White, P.C. Giordano, Nine unknown rearrangements in 16p13.3 and 11p15.4 causing alpha- and beta-thalassaemia characterised by high resolution multiplex ligation-dependent probe amplification, J. Med. Genet. 42 (2005) 922–931.
[7] A. Colosimo, V. Gatta, V. Guida, E. Leodori, E. Foglietta, S. Rinaldi, M.P. Cappabianca, A. Amato, L. Stuppia, B. Dallapiccola, Application of MLPA assay to characterize unsolved α-globin gene rearrangements, Blood Cells Mol. Dis. 46 (2011) 139–144, http://dx.doi.org/10.1016/j.bcmd.2010.11.006, (Feb 15).
[8] M.E. Paglietti, M.C. Sollaino, D. Loi, F. Sarra, E. Zaccheddu, R. Galanello, Two atypical forms of HbH disease in Sardinia, Haematologica 96 (2011) 1733–1734, http://dx.doi.org/10.3324/haematol.2011.048900.
[9] M.C. Sollaino, M.E. Paglietti, D. Loi, R. Congiu, R. Podda, R. Galanello, Homozygous deletion of the major alpha-globin regulatory element (MCS-R2) responsible for a severe case of hemoglobin H disease, Blood 116 (2010) 2193–2194, http://dx.doi.org/10.1182/blood-2010-04-281345.
[10] M. Phylipsen, J.F. Prior, E. Lim, N. Lingam, I.P. Vogelaar, P.C. Giordano, J. Finlayson, C.L. Harteveld, Thalassemia in Western Australia: 11 novel deletions characterized by multiplex ligation-dependent probe amplification, Blood Cells Mol. Dis. 44 (2012) (2010) 146–151, http://dx.doi.org/10.1016/j.bcmd.2009.12.011.
[11] A. Coelho, I. Picanço, F. Seuanes, M.T. Seixas, P. Faustino, Novel large deletions in the human alpha-globin gene cluster: clarifying the HS-40 long-range regulatory role in the native chromosome environment, Blood Cells Mol. Dis. 45 (2010) 147–153, http://dx.doi.org/10.1016/j.bcmd.2010.05.010.
[12] P. Pistidda, L. Guiso, L. Frogheri, M. Longinotti, The homozygous state of Hb J Sardegna, Hematol. J. 3 (2002) 176–178.
[13] M.E. Paglietti, M.C. Sollaino, D. Loi, S. Barella, M.F. Desogus, R. Galanello, First detection of Hb Taybe [α38(C3) or α39(C4) Thr → 0 (α1)] in an Italian child, Hemoglobin 36 (2012) 299–304, http://dx.doi.org/10.3109/03630269.2012.659780.
[14] M.C. Sollaino, M.E. Paglietti, L. Perseu, N. Giagu, D. Loi, R. Galanello, Association of α globin gene quadruplication and heterozygous β thalassemia in patients with thalassemia intermedia, Haematologica 94 (2009) 1445–1448, http://dx.doi.org/10.3324/haematol.2009.005728.
[15] C.L. Harteveld, A.J. Heister, P.C. Giordano, M. Losekoot, L.F. Bernini, Rapid detection of point mutations and polymorphisms of the alpha-globin genes by DGGE and SSCA, Hum. Mutat. 7 (1996) 114–122.
[16] A. Zorai, C.L. Harteveld, A. Bakir, P. Van Delft, A. Falfoul, K. Dellagi, S. Abbes, P.C. Giordano, Molecular spectrum of alpha-thalassemia in Tunisia: epidemiology and detection at birth, Hemoglobin 26 (2002) 353–362.
[17] V. Guida, A. Colosimo, M. Fichera, T. Lombardo, L. Rigoli, B. Dallapiccola, Hematologic and molecular characterization of a Sicilian cohort of alpha thalassemia carriers, Haematologica 91 (2006) 409–410.
[18] A. Tamaddoni, V. Hadavi, N.H. Nejad, A. Khosh-Ain, R. Siami, J. Aghai-Meibodi, N. Almadani, C. Oberkanins, H.Y. Law, H. Najmabadi, Alpha-thalassemia mutation analyses in Mazandaran province, North Iran, Hemoglobin 33 (2009) 115–123, http://dx.doi.org/10.1080/03630260902817297.