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α-Thalassemia in Premature Newborns

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ABSTRACT. In this study we have carried out α-globin gene mapping, hemoglobin (Hb) Bart's quantitation serum bilirubin, and red blood cell indices determination in a group of Sardinian appropriate for gestational age premature infants (from 32 to 35 wk gestation) in order to define the incidence in this population of the different α-thalassemia syndromes, their expression rate, and the correlation between the α-globin genotype and phenotype at this developmental stage. The gene frequencies of deletion (-α) and nondeletion ($\alpha\alpha^{th}$) α-thalassemia were 0.29 and 0.04, respectively, and thus not different from those found in full-term newborns from the same population. The majority of premature newborns with a single α-globin gene deletion [(-α/αα) genotype] were hematologically silent. Those who manifested increased Hb Bart's (1.2 to 3.4%) had slightly reduced Hb levels (17.4 ± 2.6 g/dl), mean corpuscular volume (102.6 ± 6.3 fl), and mean corpuscular Hb (34.8 ± 2.0 pg) values. Those infants with the deletion of two α-globin structural genes (-α/-α) showed without exception moderate amount of Hb Bart's in the 3.5–8.1% range and an obvious decrease of Hb levels (16.1 ± 1.6 g/dl) mean corpuscular Hb (30.6 ± 3.5 pg), and mean corpuscular volume (88.5 ± 11.5 fl) values. The only infant with the deletion of 3 α-globin structural genes had 25% Hb Bart's associated with a moderate microcytic anemia at birth and developed the clinical picture of Hb H disease. Carriers of nondeletion α-thalassemia ($\alpha\alpha/\alpha\alpha^{th}$) showed variable amount of Hb Bart's always associated with thalassemia-like red cell indices. Higher Hb Bart's levels were observed in those subjects carrying the initiation codon mutation of the α_2 gene as compared with carriers of other nondeletion α-thalassemia defects. (*Pediatr Res* 20: 1077–1081, 1986)

Abbreviations

Hb, hemoglobin
AGA, appropriate for gestational age
MCV, mean corpuscular volume
MCH, mean corpuscular Hb

The two α-globin structural genes lie on chromosome 16 linked to a ζ-globin structural gene, a ψζ and a ψα genes in the order 5′-ζ-ψζ-ψα-α_2-α_1-3′ (1, 2). Different genetic lesions in this gene cluster produce the α-thalassemia, a heterogeneous group of genetic disorders characterized by deficient (α^+-thalassemia) or absent (α^0-thalassemia) output of the α-globin chains (3). Four clinical phenotypes of increasing severity are presently recognized, *i.e.* the silent carrier state, heterozygous α-thalassemia, Hb H disease, and hydrops fetalis (4). Most commonly these conditions are caused by the deletion of one (-α/αα), two (-α/-α or --/αα), three (-α/--) or all four (--/--) α-globin structural genes, respectively (2, 5–7). However, different genetic defects, which leave both α-globin structural genes intact (nondeletion α-thalassemia) ($\alpha\alpha^{th}$), may also produce α-thalassemia (8–13).

In the neonatal period, a minority (40–50%) of those infants with a single α-globin gene deletion (-α/αα) express phenotypically with Hb Bart's in the 1–3% range, associated frequently with minimal microcytosis (4, 14). However, the majority is completely silent. Those infants with the deletion of two α-globin structural genes (-α/-α and --/αα genotypes) show consistently increased Hb Bart's in the 3–8% range and thalassemia-like hematological manifestations (4, 14, 16). Infants with Hb H disease (-α/--) have 25% Hb Bart's and moderate microcytic anemia (4). Infants, heterozygous for a nondeletion lesion (initiation codon mutation of the α_2 gene) (13), showed Hb Bart's levels within the range found in infants with the (-α/αα) genotype (14).

The clinical phenotypes at 32–35 wk gestation resulting from the different α-thalassemia genotypes and their expression rate at this gestational age have not yet been defined. Sardinians are an ideal population for studying the hematological manifestations of α-thalassemia in premature newborns because the different types of this disorder occur with a high frequency in this population (14).

In the present study, we carried out α-globin gene mapping, Hb Bart's quantitation, serum bilirubin, and red cell indices determination in a group of Sardinian AGA premature infants (from 32 to 35 wk gestation), in order to define the incidence in this population of the different α-thalassemia syndromes, their expression rate, and the correlation between the α-globin genotype and phenotype at this developmental stage. The study regarding the incidence of α-thalassemia syndromes and the expression rate of the α-thalassemia silent carrier state was carried out in a random group of premature infants consecutively born in the Obstetric Department of Cagliari University (consecutive sample). The investigation on the genotype-phenotype correlations in addition to this group of infants included a series of premature infants with Hb Bart's >1% identified by screening for abnormal Hb (selected sample).

SUBJECTS

Selected sample. On the basis of visual inspection of cellulose acetate electrophoretic strips from Sardinian AGA premature infants screened at birth for abnormal Hb, we selected 10 subjects with Hb Bart's levels higher than 1%. In these infants we carried

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out red cell indices and serum bilirubin determination, Hb Bart's quantitation, and restriction endonuclease analysis of the α-like globin gene complex. All these infants were then prospectively followed untill 5–8 wk from birth.

Consecutive sample. This part of the study included 51 AGA premature infants consecutively born in the Obstetric Department of Cagliari University. In all of them we carried out red cell indices analysis, serum bilirubin determination, Hb Bart's quantitation, and α-globin gene mapping.

METHODS

Red blood cell indices were measured with the Coulter Counter model S regularly calibrated with a commercial standard (4C, Coulter Counter Cell Control, Coulter Diagnostic, Hialeah, FL). Bilirubin was measured according to Maloy and Evelyn (17). Globin chain synthesis analysis on peripheral blood reticulocytes was performed according to Kan *et al.* (18). The relative amounts of Hb Bart's were estimated in duplicate by elution from cellulose acetate strips following electrophoresis in a phosphate buffer system (19).

Restriction endonuclease analysis of the α-globin gene cluster was carried out either by digestion of the DNA with Bgl II and hybridization with the ζ-globin specific probe or by digestion with Bam HI and hybridization with the α-globin specific probe. Since the most common nondeletion defect in Sardinians is the initiation codon mutation (ATG→ACG) of the α_2 gene (13, 20) which abolishes a Nco I site ($\alpha^{Nco}\alpha/\alpha\alpha$), we carried out also Nco I mapping in those subjects with high Hb Bart's and a normal α-globin genotype. DNA was prepared from white blood cells by phenol-chloroform isoamyl-alcohol extraction and ethanol precipitation (21). Ten μg of DNA were digested with 30 U of the restriction endonucleases Bgl II, Bam HI (Boehringer, Mannheim, West Germany), or Nco I (BRL, Bethesda, MD) for 24 h under conditions recommended by the manufacturers. The restricted DNA was electrophoresed in 0.8% agarose, transferred to nitrocellulose filters (22), and hybridized either with a specific ^{32}P-labeled α-globin gene probe prepared by nick-translation as described by Maniatis *et al.* (23), from α-globin cDNA cloned in the plasmid JW-101 (24) or with a ζ-globin specific probe prepared by nick-translation of a Hinf I fragment, isolated from the pBRζ plasmid, which is complementary to the whole gene excluding the 5′ part of the first exon (1, 25). The statistical significance of the results was evaluated with the Student's *t* test.

RESULTS

α-Globin gene mapping (including both the selected and consecutive blood sample). Figure 1 shows representative Bgl II and Bam HI maps and Table 1 summarizes the overall results. All subjects with the deletion of a single α-globin gene (-α/αα) had the rightward deletion lesion.

The ζ-specific fragment spanning from the ζ to the ψζ gene varied in size between 10 and 11.5 Kb. The majority of the infants investigated had the 11.0 Kb fragment, one infant showed the 10.0 Kb fragment and three the 11.5 Kb fragment. DNA from three subjects showed the presence of the polymorphic Bgl II site located between the ψζ and ψα genes. In one subject both

Table 1. *α-Globin gene mapping in AGA premature infants*

		Fragment lengths		
		Bam HI	Bgl II	
	n	α-Globin probe	ζ-Globin probe	α-Globin genotype
Consecutive samples	21	14	11–12	αα/αα
	4*	14	11–12	αα/ααth
	22	10.5–14	11–12–16	−α/αα
	4	10.5	11–16	−α/−α
Selected samples	1*	14	11–12	αα/ααth
	5	10.5–14	11–12–16	−α/αα
	3	10.5	11–16	−α/−α
	1	10.5	11–14–16	−−/−α

* Nco I mapping showed in two of these carriers the initiation codon mutation (ATG→ACG) of the α_2 gene.

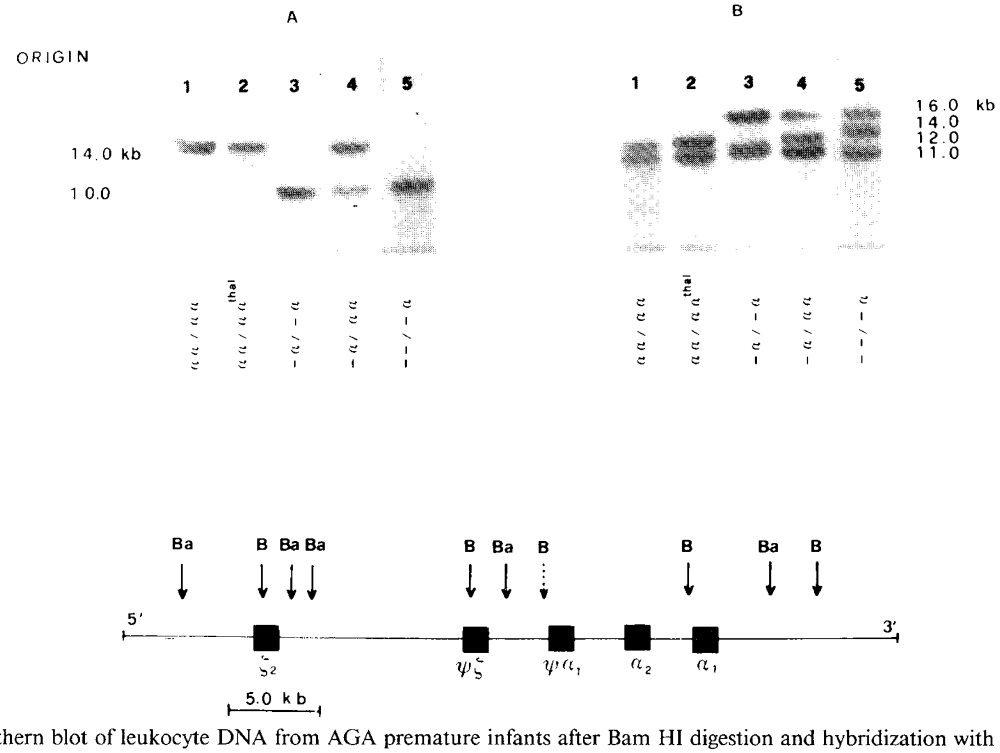

Fig. 1. *Top*, southern blot of leukocyte DNA from AGA premature infants after Bam HI digestion and hybridization with α-specific probe (*A*) or Bgl II digestion and hybridization with ζ-specific probe (*B*). *Bottom*, diagram of the α-like globin gene complex. *B*, Bgl II; *Ba*, Bam HI.

chromosomes showed the presence of this polymorphic site. This site was never observed in a chromosome containing the single α-globin gene (-α).

α-Thalassemia incidence and phenotype expression rate of the α-thalassemia genotypes (consecutive sample). α-Globin gene mapping in the group of 51 consecutively born premature infants showed a normal complement of four α-globin structural genes (αα/αα) in 25 subjects (49.0%), a single α-globin gene deletion (-α/αα) in 22 (43.1%), and the deletion of two α-globin genes (-α/-α) in 4 (7.8%) (Table 2). The gene frequency of the (-α) haplotype calculated from these results is 0.29.

Of those infants with the (-α/αα) globin genotype, 10 of 22 (45%) expressed phenotypically at birth; of these seven had either increased Hb Bart's (2) or mycrocytosis (5) and three had both these phenotypic manifestations.

All the infants with the (-α/-α) genotype had increased Hb Bart's levels and thalassemia-like red cell indices. In the group of infants with a normal α-globin genotype four subjects (7.8%) had detectable Hb Bart's and were classified as carriers of non-deletion α-thalassemia. The gene frequency of this determinant is 0.04.

Genotype-Phenotype Correlations (Including both the Selected and Consecutive Samples). Hb Bart's levels (Fig. 2). Infants with deletion of two α-globin structural genes (-α/-α) had mean Hb Bart's levels of 6.1 ± 1.8% (3.5–8.1 range) while those with a single α-globin gene deletion had mean levels of 2.9 ± 0.39 (1.2 to 3.4% range). Two subjects with the (-α/αα) genotype had 8.1 and 10.5% Hb Bart's, which are definitively outside the range associated with the above genotype. The only infant with the (-α/--) genotype had 25% Hb Bart's and developed the clinical and hematological manifestations of Hb H disease.

At follow-up all infants with high Hb Bart's levels and a normal α-globin genotype had thalassemia-like hematological manifestations, normal Hb A_2 and HbF levels, and an α/β globin chain synthesis ratio within the α-thalassemia carrier range (data not shown). One of their parents had similar haematological features

Table 2. *Incidence of α-thalassemia in AGA premature infants*

n	α-Globin genotype	%
21	αα/αα	41.2
22	−α/αα	43.1
4	−α/−α	7.8
4	αα/ααth	7.8
Gene frequency	αα: 0.67; −α: 0.29; ααth: 0.04	

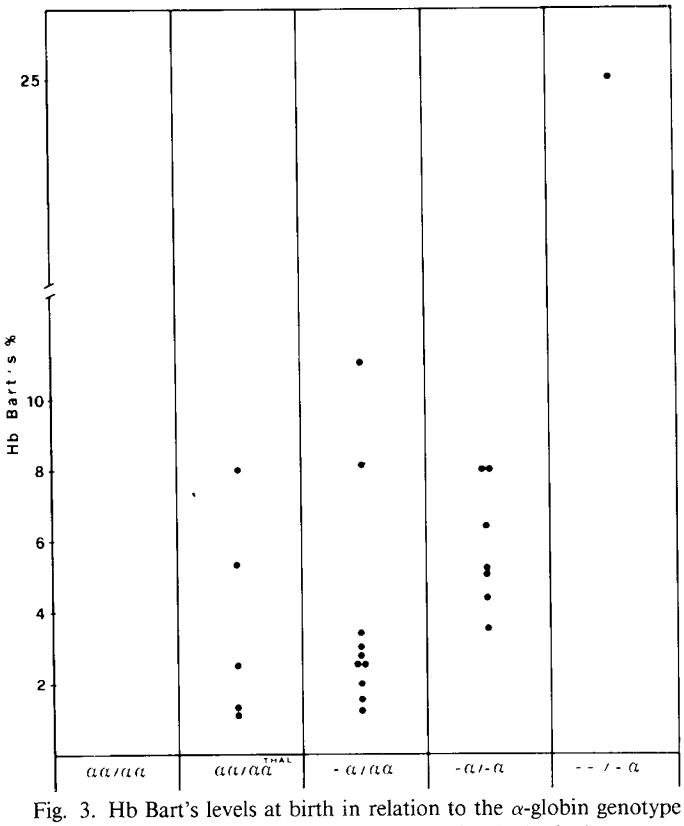

Fig. 3. Hb Bart's levels at birth in relation to the α-globin genotype in AGA premature infants. Only those subjects with the (-α/αα) genotype and increased Hb Bart's are reported herein.

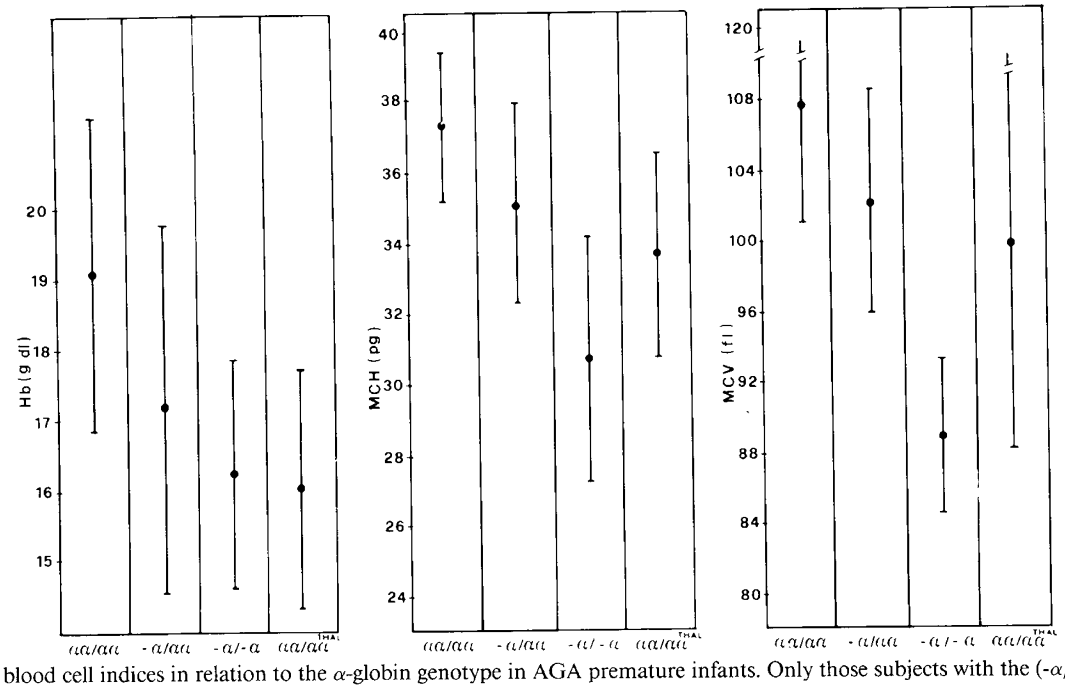

Fig. 2. Red blood cell indices in relation to the α-globin genotype in AGA premature infants. Only those subjects with the (-α/αα) genotype and increased Hb Bart's are reported herein.

associated with a normal α-globin genotype. These infants are, therefore, carriers of a nondeletion α-thalassemia defect. In two of them Nco I mapping showed the initiation codon mutation of the α_2 gene ($\alpha^{Ncol}\alpha/\alpha\alpha$) (data not shown). In these infants with the initiation codon mutation ($\alpha^{Ncol}\alpha/\alpha\alpha$) Hb Bart's levels were 5.3 and 8% while in those carrying a nondeletion defect, not yet defined at molecular level, Hb Bart's levels were 1.1, 1.3, and 2.5%.

Red blood cell indices. Premature infants with the ($-\alpha/-\alpha$) genotype had significantly reduced mean Hb ($p < 0.05$), MCV ($p < 0.001$, and MCH ($p < 0.001$) values as compared to those infants with a full complement of four α-globin structural genes ($\alpha\alpha/\alpha\alpha$) with practically no overlap between the two groups (Fig. 3). MCV ($p < 0.005$) and MCH ($p < 0.02$) values from premature infants with the ($-\alpha/-\alpha$) genotype were also significantly reduced as compared to premature infants with the ($-\alpha/\alpha\alpha$) genotype. Those infants with the ($-\alpha/\alpha\alpha$) genotype, who manifested increased Hb Bart's levels at birth, showed significantly lower Hb ($p < 0.05$), MCV ($p < 0.02$), and MCH ($p < 0.005$) values than normal controls with a full complement of four α-globin structural genes. However, there was an extensive overlap on one hand with the normal values and on the other hand with those associated with the ($-\alpha/-\alpha$) genotype. As predicted, the only infants with the ($-\alpha/--$) genotype had a moderate microcytic anemia. Those premature infants with nondeletion defects had Hb levels ($p < 0.02$), MCV ($p < 0.05$), and MCH ($p < 0.01$) values significantly reduced as compared with those of premature infants with a normal α-globin genotype.

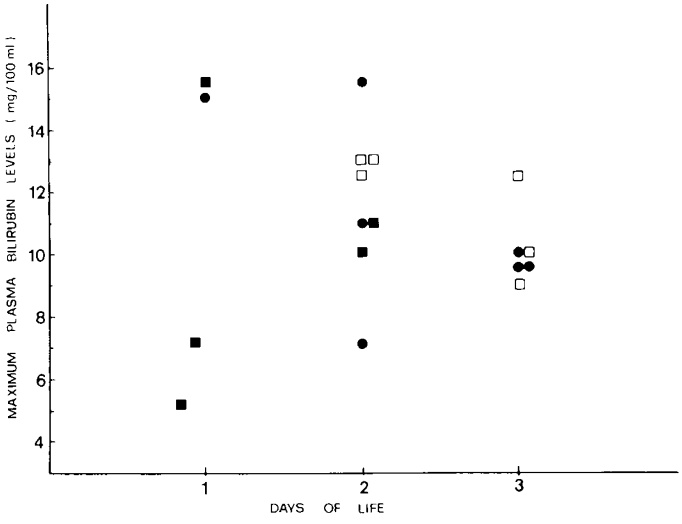

Fig. 4. Bilirubin levels according to the α-globin genotype in AGA premature infants. ■, $\alpha\alpha/\alpha\alpha^{th}$; ●, $-\alpha/\alpha\alpha$; □, $-\alpha/-\alpha$.

Longitudinal studies. This part of the study includes 51 infants, prospectively followed until 5–8 wk after birth. There were no significant differences in mean bilirubin levels between infants with the ($-\alpha/-\alpha$) genotype and normal controls. The only infant who developed maximum bilirubin levels above 13 mg/dl had also the G6PD deficiency of the Mediterranean type (Fig. 4).

Within the first 5–8 wk, there was a similar trend in the decline of Hb levels, red blood cell count, and MCV and MCH values in infants with a normal α-globin genotype ($\alpha\alpha/\alpha\alpha$) as compared with those with the ($-\alpha/\alpha\alpha$) and ($-\alpha/-\alpha$) genotypes (Fig. 5). However, in this time interval Hb Bart's levels fell only slightly in infants with the ($-\alpha/-\alpha$) globin genotype while no modifications were seen in those with the ($-\alpha/\alpha\alpha$) genotype. The infant with 25% Hb Bart's developed hematological and clinical manifestations consistent with HbH disease with an α/β globin chain synthesis ratio of 0.42.

DISCUSSION

This study shows that in Mediterranean AGA premature infants Hb Bart's levels >1% indicate without exception the presence of α-thalassemia, either of the deletion or nondeletion variety. Those subjects, with elevated Hb Bart's, who showed a normal α-globin map, were in fact all carriers of a nondeletion α-thalassemia defect as indicated by the reduced α/β globin chain synthesis ratio, the development of a thalassemia-like phenotype with normal Hb A_2 and F levels at follow-up, and the finding of the α-thalassemia carrier state phenotype in at least one of their parents. Moreover, Nco I mapping demonstrated in two of them the presence of the initiation codon mutation (ATG→ACG) of the α_2 gene ($\alpha^{Nco}\alpha/\alpha\alpha$), (13) which is the most common non-

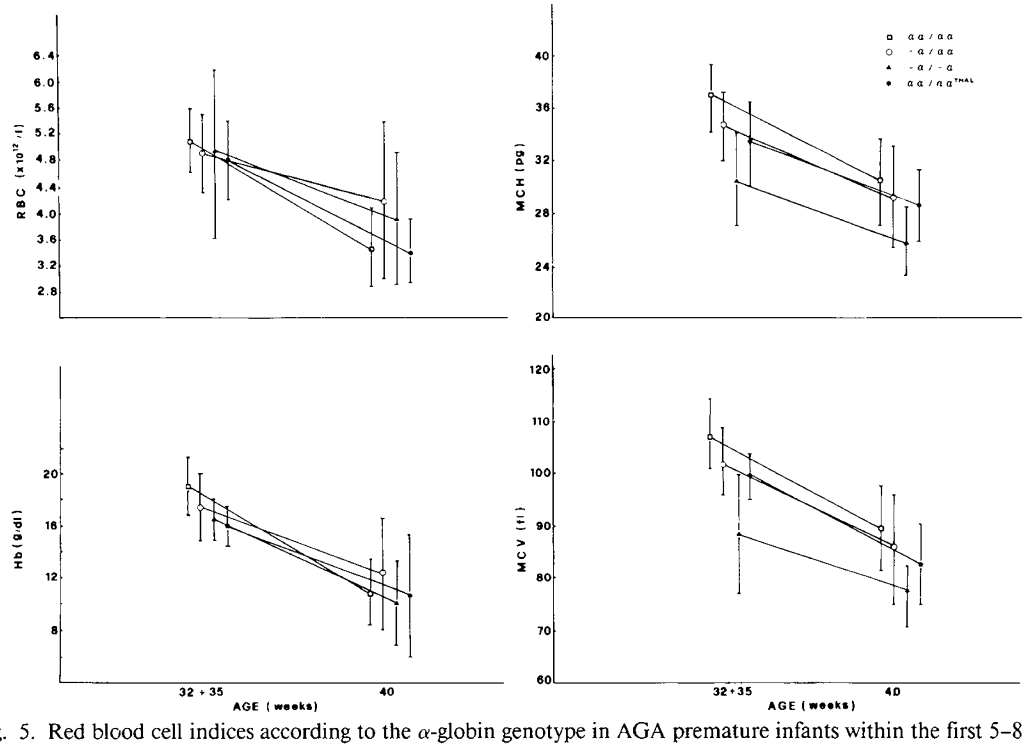

Fig. 5. Red blood cell indices according to the α-globin genotype in AGA premature infants within the first 5–8 wk.

deletion α-thalassemia defect in Sardinians (20). A similar conclusion was drawn in previous studies in full-term newborns from Asian as well as Mediterranean populations (14, 15). The incidence of the different α-thalassemia determinants found in prematurely born infants in this study is similar to that detected in full-term newborns from the same population (14) suggesting that α-thalassemia in the form of the defect of one, two, or three α-globin structural genes has no detrimental effect on the outcome of the pregnancy unlike the deletion of four α-globin genes which produces fetal hydrops and maternal toxemia (3).

The deletion of a single α-globin gene (-α) can be produced by two different cross-over mechanisms which are referred to as rightward and leftward deletion (6). In this study, all chromosomes with a single α-globin gene deletion (-α/αα) resulted from the rightward cross-over, which is the most common lesion accounting for the (-α) haplotype in the Mediterranean populations (6).

As has previously been found in other Mediterranean populations (26) in this study the DNA segment spanning from the ζ to the ψζ gene varied in size because of the presence in this region of a hypervariable region containing a variable number of DNA repeats. In a few cases the polymorphic Bgl II site located between the ψα and ψζ gene (27) was also detected.

The (-α/αα) genotype appeared silent in the majority of the infants investigated. Screening by Hb Bart's levels in premature infants, thus underestimates the prevalence of this genotype. A similar phenotypic expression rate of the (-α/αα) genotype has already been found in full-term newborns from the same (14) as well as from the Jamaican population (28).

In this study, we found a striking correlation between the number of functional α-globin genes and the hematological phenotype. Those subjects with the (-α/-α) genotype, who presented phenotypical manifestations at birth, had low Hb Bart's level in the range of 1–3% associated frequently with slightly reduced Hb levels, moderate microcytosis, and reduced Hb content per cell (MCH), those infants with the deletion of two α-globin structural genes (-α/-α) showed without exception moderate amount of Hb Bart's in the 3–8% range associated with an obvious decrease of Hb, MCV, and MCH values. The infant with the deletion of 3 α-globin structural genes had 25% Hb Bart's and moderate microcytic anemia. The only exception to this pattern regards two infants with a single α-globin gene deletion (-α/αα) and Hb Bart's levels of 10.5 and 8.1%, respectively. The best explanation for this finding is that these infants may have also inherited a nondeletion α-thalassemia determinant and therefore actually have the (-α/α^{th}α) genotype, which in a previous study was seen to be associated with Hb Bart's levels higher than those resulting from the (-α/-α) genotype (29).

The phenotypic manifestations of nondeletion α-thalassemia in premature infants were quite heterogeneous. Those infants with the initiation codon mutation of the α_2 gene (α^{Nco}α/αα) had higher Hb Bart's levels as compared with either infants with other nondeletion defects not yet characterized at molecular level or with infants with a single α-globin gene deletion. Accordingly, previous studies in the same Sardinian population (20) have shown that a chromosome containing the initiation codon mutation of the α_2 gene produces a more severe defect than that resulting from a chromosome with a single α-globin gene deletion. Hb H disease caused by the combination of the (α^{Nco}α) haplotype with the (--) haplotype is, in fact, clinically and hematologically more severe than that produced by the common deletion mechanism (-α/--) (20, 30). Moreover, the homozygous state for the initiation codon mutation of the α_2 gene (α^{Nco}α/α^{Nco}α) produces Hb H disease while the deletion of two α-globin genes (-α/-α) results in the α-thalassemia carrier state (20).

However, thalassemia-like hematologic manifestations were seen in all the carriers of nondeletion lesions regardless of the type of molecular defect. In this series, none of the premature infants with α-thalassemia showed hyperbilirubinemia. This finding excludes that α-thalassemia may cause unexplained hyperbilirubinemia in premature infants. From these findings, we may conclude that at 32–35 wk gestation the different α-thalassemia genotypes produce an Hb pattern and hematologic features like those observed in full-term newborns with the same genotypes.

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REFERENCES

1. Lauer J, Shen JCK, Maniatis T 1980 The chromosomal arrangement of human α-like globin genes: sequence homology and α-globin gene deletions. Cell 20:119–130
2. Pressley L, Higgs DR, Clegg JB, Weatherall DJ 1980 Gene deletions in α-thalassemia prove that the 5′ ζ-locus is functional. Proc Natl Acad Sci USA 77:3586–3589
3. Weatherall DJ, Clegg JB 1981 The thalassaemia syndromes, 2nd ed. Blackwell Scientific Publications, Oxford, pp 508–612
4. Higgs DR, Weatherall DJ 1983 α-Thalassemias. Curr Topics Haematol 4:37–97
5. Orkin SH 1978 The duplicated human α-globin genes lie close together in cellular DNA. Proc Natl Acad Sci USA 75:5950–5954
6. Embury SH, Miller JA, Dozy AM, Kan YW, Chang V, Todd D 1980 Two different molecular organizations account for the single α-globin gene of α-thalassemia-2 genotype. J Clin Invest 66:1319–1325
7. Pressley L, Higgs DR, Aldridge B, Metaxatou-Mavromati A, Clegg JB, Weatherall DJ 1980 Characterization of a new α-thalassemia-1 defect due to a partial deletion of the α-globin gene complex. Nucleic Acids Res 8:4889–4898
8. Kan YW, Dozy AM, Trecartin RF, Todd M 1977 Identification of a nondeletion defect in α-thalassemia. N Engl J Med 297:1081–1084
9. Orkin SH, Old J, Lazarus H, Altay C, Gurgey A, Weatherall DJ, Nathan DG 1979 The molecular basis of α-thalassemia: Frequent occurrence of dysfunctional α-loci detected among non-Asian with HbH disease. Cell 17:33–42
10. Higgs DR, Pressley L, Aldridge B, Clegg JB, Weatherall DJ, Cao A, Hadjiminas MG, Kattamis C, Metaxatou-Mavromati A, Rachmilewitz EA, Sophocleous T 1981 Genetic and molecular diversity in nondeletion HbH disease. Proc Natl Acad Sci USA 78:5833–5837
11. Orkin SH, Goff SC 1981 The duplicated human α-globin genes: their relative expression as measured by RNA analysis. Cell 24:345–351
12. Goossens M, Lee KY, Liebhaber SA, Kan YW 1982 Globin structural mutant $\alpha^{Leu\text{-}Pro}$ is a novel cause of α-thalassemia. Nature 296:864–865
13. Pirastu M, Saglio G, Chang JC, Cao A, Kan YW 1984 Initiation codon mutation as a cause of α-thalassemia. J Biol Chem 259:12315–12317
14. Galanello R, Maccioni L, Ruggeri R, Perseu L, Cao A 1984 α-Thalassemia in Sardinian newborns. Br J Haematol 58:361–368
15. Lie-Injo LE, Solai A, Herrera AR, Nicolaisen L, Kan YW, Wasi WP, Hasan K 1982 Hb Bart's level in cord blood and deletions of α-globin genes. Blood 59:370–376
16. Ohene-Frempong K, Rappaport E, Atwater J, Schwartz E, Surrey S 1980 α-Gene deletions in black newborn infants with Hb Bart's. Blood 55:931–933
17. Maloy HT, Evelyn KA 1937 The determination of bilirubin with the photoelectric colorimeter. J Biol Chem 119:481–490
18. Kan YW, Schwartz F, Nathan DG 1968 Globin chain synthesis in the α-thalassemia sindromes. J Clin Invest 47:2515–2522
19. Wood WG 1983 Hemoglobin analysis. In: Weatherall DJ, ed. Methods in hematology: the thalassemias. Churchill-Livingstone, Edinburgh, pp 31–53
20. Paglietti E, Galanello R, Moi P, Pirastu M, Cao A Molecular pathology of hemoglobin H disease in Sardinia. Br J Haematol (in press)
21. Goossens M, Kan YW 1981 DNA analysis in the diagnosis of hemoglobin disorders. Methods Enzymol 76:805–807
22. Southern EM 1975 Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 98:503–517
23. Maniatis T, Jeffrey A, Kleid DG 1975 Nucleotide sequence of the rightward operation of phage λ. Proc Natl Acad Sci USA 72:1184–1188
24. Wilson JT, Wilson LB, De Riel JK, Villa-Komaroff L, Efstratiadis A, Forget BG, Weissman SM 1978 Insertion of synthetic copies of human globin genes into bacterial plasmids. Nucleic Acids Res 5:563–581
25. Proudfoot NJ, Gil A, Maniatis T 1982 The structure of the human ζ globin gene and a closely linked nearly identical pseudogene. Cell 31:553–563
26. Higgs DR, Goodbourn SEY, Wainscoat JS, Clegg JB, Weatherall DJ 1981 Highly variable regions of DNA flank the human α-globin genes. Nucleic Acids Res 9:4213–4224
27. Wainscoat JS, Higgs DR, Kanavakis E, Cao A, Georgiou D, Clegg JB, Weatherall DJ 1983 Association of two DNA polymorphism in the α-globin gene cluster: implications for genetic analysis. Am J Hum Genet 35:1086
28. Higgs DR, Pressley L, Clegg JB, Weatherall DJ, Serjeant G 1980 Detection of α-thalassemia in Negro infants. Br J Haematol 46:39–46
29. Galanello R, Melis MA, Maccioni L, Pirastu M, Cao A 1984 Longitudinal study of a newborn with a combination of deletion and nondeletion α-thalassemia-2. Pediatr Res 18:158–162
30. Galanello R, Piratsu M, Melis MA, Paglietti E, Moi P, Cao A 1983 Phenotype-genotype correlation in hemoglobin H disease in childhood. J Med Genet 20:425–429