Am J Hum Genet 39:631–639, 1986

Molecular Basis for HbH Disease in Italy: Geographical Distribution of Deletional and Nondeletional α-Thalassemia Haplotypes

A. Di Rienzo,[1] A. Novelletto,[2] M. C. Aliquò,[3] I. Bianco,[3] A. Tagarelli,[4] C. Brancati,[4] B. Colombo,[1] and L. Felicetti[1]

SUMMARY

We have investigated the molecular basis for HbH disease in 16 patients from Sardinia, and central and southern Italy. We have shown that HbH disease is produced by the interaction of at least 10 different deletional or nondeletional α-thalassemia haplotypes, some of which have been already described in the Mediterranean area ($--^{Med}$,$-(\alpha)^{20.5}$, $-\alpha^{3.7}$ type I,$-\alpha^{3.7}$ type II, $\alpha_2{}^{NcoI}\alpha_1$, $\alpha_2{}^{HphI}\alpha_1$). Among the new mutations found in the course of our study, there is a complete deletion of the ζ-α cluster and three nondeletional determinants ($\alpha\alpha^T$), affecting to various extents α-globin gene expression.

The different α-thalassemia haplotypes are not evenly distributed throughout the country. Two α^0 determinants [$-(\alpha)^{20.5}$ and the complete deletion of the ζ-α cluster] and four α^+ determinants ($-\alpha^{3.7}$ type II, three nondeletional $\alpha\alpha^T$ mutations) are found exclusively in southern Italy.

INTRODUCTION

The human α-like globin genes are clustered on chromosome 16 [1] in the order ζ_2-ζ_1-$\psi\alpha_1$-α_2-α_1 [2, 3]. Genetic defects that result in a reduced or absent expression of the α-genes are called α^+- and α^0-thalassemias, respectively [4]. Molecular analysis has revealed a great heterogeneity of α-thalassemia muta-

Received February 25, 1986; revised June 13, 1986.

This work was partially supported by Progetto Finalizzato "Ingegneria Genetica e Basi Molecolari delle Malattie Ereditarie" del Consiglio Nazionale delle Ricerche.

[1] Istituto di Biologia Cellulare, C.N.R., Via G. D. Romagnosi 18/A, 00196 Rome, Italy.

[2] Dipartimento di Biologia, Università di Roma, Tor Vergata, Italy.

[3] Centro di Studi della Microcitemia, Rome, Italy.

[4] Istituto per lo Studio delle Malattie Ereditarie e Carenziali, Cosenza, Italy.

© 1986 by the American Society of Human Genetics. All rights reserved. 0002-9297/86/3905-0008$02.00

tions, and population studies have demonstrated a great variability in their relative frequencies in different regions [5]. The most frequent α-thalassemia mutations are due to the deletion of one or both α genes. In the first case, the resulting α^+ determinant is produced by two types of unequal crossing-over events, one giving rise to a 3.7-kilobase (kb) deletion ($-\alpha^{3.7}$ haplotype or rightward deletion), the other giving rise to a 4.2-kb deletion ($-\alpha^{4.2}$ haplotype or leftward deletion). The rightward deletion is the most frequent, and it is found in all populations [5]. The α^0 determinants usually result from the deletion of both α-genes and differ as to the extent of the deletion. The two most frequent types are the Southeast Asia deletion ($--^{SEA}$), which includes the ψα and leaves the ζ_1-gene intact and the Mediterranean deletion ($--^{Med}$) that removes also the ζ_1-gene.

Nondeletional α-thalassemia mutations, which usually produce α^+ determinants, have also been described. They include different mutations in the termination codon of the α_2-gene producing elongated chains [5], a 5-base pair (bp) deletion at the IVS1 splice junction of the α_2-gene [6], a mutation at the initiation codon of the α_2- and α_1-genes [7, 8], a single nucleotide deletion within the codon 14 of the α_1-gene, and a polyadenylation signal mutation in the α_2-gene [9].

Population studies demonstrated that usually when α-thalassemia is frequent, only a given α^+ and/or α^0 haplotype reaches high frequencies, whereas other mutations, if any, are rare. For example, in Southeast Asia, the most frequent α^+ is the $-\alpha^{3.7}$ and the predominant α^0 is the $--^{SEA}$ deletion, both showing high frequencies [10]; in the Mediterranean area, the $-\alpha^{3.7}$ determinant is the most common α^+ haplotype and the $--^{Med}$ represents the great majority of the α^0 haplotypes, but in this case, while the former reaches frequencies up to 0.18 [11], the latter is rare [12].

Few data are available on the frequencies and type distribution of α-thalassemia haplotypes in southern and central Italy. Previous reports, based on mass screening tests [13] and on Hb Bart's level in newborns [14], suggest that α-thalassemia is not very frequent in this area.

However, sporadic cases of HbH disease have been detected in these populations. We report here restriction mapping analysis of 16 patients with HbH disease from Sardinia and central and southern Italy, carried out in the aim of obtaining information on the relative frequencies of the α-thalassemia haplotypes present in this area.

MATERIALS AND METHODS

Sixteen HbH patients were diagnosed by standard hematological methods and studied together with their families, when available, at the Centro di Studi della Microcitemia (Rome) and the Istituto per lo Studio delle Malattie Ereditarie e Carenziali (Cosenza).

The places of origin, ascertained up to the second generation, were: 13 from central and southern Italy (Latium, Campania, Calabria, and Apulia) and three from Sardinia.

Hematological studies and hemoglobin analysis were performed by standard techniques [15]. Routine hematological parameters were obtained with a Coulter Counter, model S. DNA was extracted from peripheral blood and analyzed by the Southern blot technique as described [11].

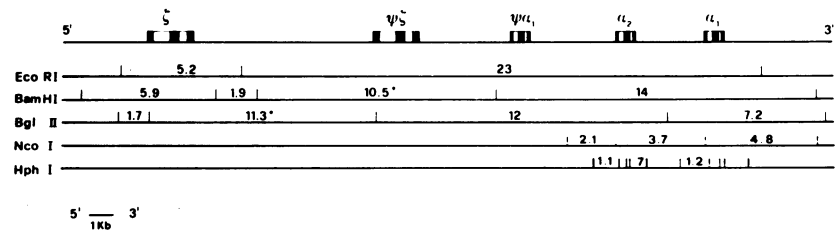

FIG. 1.—Restriction map of the α-globin gene cluster. In the case of *Nco*I and *Hph*I, only the fragments containing the α-globin genes have been indicated.

DNA samples from patients carrying the $-\alpha^{3.7}$ type II mutation were digested with *Pst*I and submitted to agar gel electrophoresis. The gel, dried under vacuum, was then hybridized with the synthetic 19-meric olygonucleotide 5'-AGCACCA TGGGGTTCTCTC-3' in a medium containing 0.9 M NaCl, 3 mM EDTA, 0.15 M Tris, pH 8.0, 5× Denhardt's solution, 0.5% Nonidet P40, and 10% dextran sulphate for 2 hrs at 50°C. The gel was then washed thoroughly at room temperature in 6× SSC and finally for 3 min at 61°C in 6× SSC.

RESULTS

Diagnosis of HbH disease was based on the presence of hypochromic-microcytic anemia, HbH, and inclusion bodies in the peripheral blood. In all cases except families 8, 9, and 13, the hematological data confirmed the presence of α^0-thalassemia in one parent and of α^+-thalassemia in the other.

Restriction endonuclease mapping was carried out by DNA digestion with *Apa*I, *Bam*HI, *Bgl*II, *Eco*RI, *Hph*I, and *Nco*I restriction enzymes, followed by hybridization with an α- and/or ζ-specific probe. Figure 1 shows the restriction map of the α-globin gene cluster obtained with these enzymes.

Normal DNA digested with *Eco*RI and *Bam*HI, and hybridized with an α-specific probe, produces 22- and 14-kb fragments, respectively, while α-specific fragments of 19 and 10 kb are characteristic of a single gene deletion (-α). Ten subjects carried out the -α deletion, which was shown to be of the rightward type by digestion with *Bgl*II and hybridization with an α-specific probe. In fact, this deletion produces a 15.8-kb band, whereas the $-\alpha^{4.2}$ deletion gives rise only to a fragment 7.4 kb long.

This mutation can be further characterized by digestion with *Apa*I, which produces 2.7-, 1.7-, and 0.89-kb bands with normal DNA, 2.5- and 0.89-kb fragments with the $-\alpha^{3.7}$ type I, a single 3.5-kb band with the $-\alpha^{3.7}$ type II, and 2.7- and 0.89-kb fragments with $-\alpha^{3.7}$ type III [16]. Eight subjects (families 1, 4, 5, 10, 11, 12, 14, and 15) carried the type I deletion, whereas type II was found in two subjects (families 9 and 13).

The $-\alpha^{3.7}$ type I mutation was associated with the $--^{Med}$ in five cases (families 1, 4, 5, 14, and 15) and with a deletion of the whole α-cluster in two cases (families 11 and 12). In fact, in each of the first five cases, digestion of the DNAs of the propositus and one of his parents with *Bgl*II and hybridization with a ζ-specific probe gave rise, in addition to the 15.8-kb fragment, to a 13.9-kb fragment, typical of the $--^{Med}$ mutation; in the other two cases, this latter

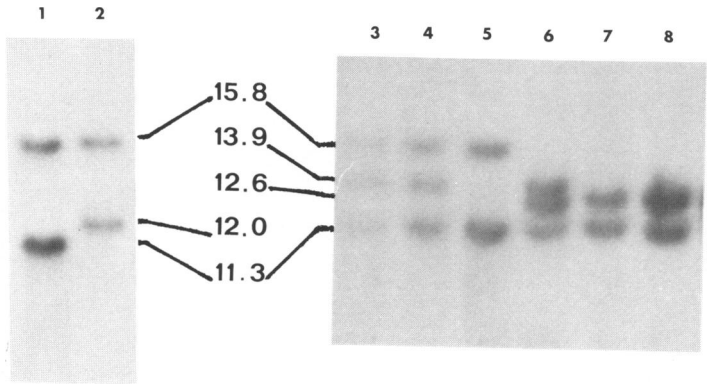

FIG. 2.—ζ-Globin gene specific fragments (in kb) after *Bgl*II digestion of DNA from selected HbH patients. *Lane 1:* Patient no. 9 ($-\alpha^{3.7}$II/$-\alpha^{3.7}$II). *Lane 2:* Patient no. 12 (--/$-\alpha^{3.7}$I). *Lane 3:* Patient no. 4 ($--^{Med}/-\alpha^{3.7}$I). *Lane 4:* Patient no. 1 ($--^{Med}/-\alpha^{3.7}$I). *Lane 5:* Patient no. 11 (--/$-\alpha^{3.7}$I). *Lane 6:* Patient no. 3 ($--^{Med}/\alpha_2{}^{NcoI}\alpha_1$). *Lane 7:* Patient no. 7 ($\alpha\alpha^T/\alpha_2{}^{NcoI}\alpha_1$). *Lane 8:* Patient no. 8 ($\alpha\alpha^T/\alpha\alpha^T$). The genotypes of patients nos. 3, 7, and 8 were determined by restriction with enzymes other than *Bgl*II, as shown in table 1 and discussed in RESULTS.

fragment was not observed (fig. 2). It can be excluded that the propositi 11 and 12 were homozygotes for a $-\alpha^{3.7}$ haplotype with a reduced expression, since this haplotype was present only in one parent while the other showed the normal 14- and 22-kb fragments after digestion with *Bam*HI and *Eco*RI, respectively. The $-\alpha^{3.7}$ haplotype was associated in these two patients with two different polymorphic fragments containing the inter ζ hypervariable region (fig. 2 and table 1). These results can be explained either by a complete deletion of the α-globin cluster, or by two different deletions, each producing a *Bgl*II ζ-specific fragment comigrating with the 11.3- and 12-kb polymorphic fragments present in the homologous chromosome. Since the latter possibility seems very unlikely, we favor the existence in Calabria of a deletion of the whole cluster. The size of this deletion(s) is not known. Subject 10 had the $-\alpha^{3.7}$ type I deletion associated with the large deletion that ends at codon 56 of the α_1-gene [17]; this mutation produces a 2.6-kb fragment after *Eco*RI digestion and, according to the size of the deleted segment, has been referred to as the $-(\alpha)^{20.5}$ haplotype [18].

The $-\alpha^{3.7}$ type II deletion was found in two cases (families 9 and 13); both patients showed only the 3.5-kb fragment after *Apa*I digestion and hybridization with an α-specific probe. In family 9, the parents had the same 3.5-kb band as well as the 2.7- and 1.7-kb normal fragments (fig. 3). Therefore, HbH disease appears to be the product of the homozygous state for a $-\alpha^{3.7}$ type II deletion. In family 13, parents were not available; therefore, it is not possible to exclude that this subject carries a chromosome with the complete deletion of the α-globin cluster.

Since the homozygous state for this haplotype produces the HbH phenotype, it is likely that the residual gene carries another mutation that reduces its output

TABLE 1

RESTRICTION ANALYSIS OF DNA FROM 16 HbH PATIENTS: SIZE OF α- AND ζ-SPECIFIC GLOBIN GENE FRAGMENTS (kb) AND INFERRED GENOTYPES

Patient no.	Place of origin	*Eco*RI α	*Bam*HI α	*Bgl*II α	*Apa*I α	*Hph*I α	*Nco*I α	*Bgl*II ζ	Genotype	HbH %
1	Central Italy	19	10	15.8	2.5	...	...	15.8, 13.9, 11.3	$--^{Med}/-\alpha^{3.7}I$	12
2	Southern Italy	23, 2.6	14	12.6, 7.4	...	1.4, 1.2, 0.7	4.8, 3.7, 2.1	...	$-(\alpha)^{20.5}/\alpha_2{}^{HphI}\alpha_1$	20
3	Sardinia	23	14	12.6, 7.4	...	...	5.8, 4.8	13.9, 12.6, 11.3	$--^{Med}/\alpha_2{}^{NcoI}\alpha_1$	18
4	Sardinia	19	10	15.8	2.5	...	...	15.8, 13.9, 11.3	$--^{Med}/-\alpha^{3.7}I$	5
5	Central Italy	19	10	15.8	2.5	...	...	15.8, 13.9, 11.3	$--^{Med}/-\alpha^{3.7}I$	4
6	Southern Italy	23, 2.6	14	...	...	1.4, 1.2, 0.7	4.8, 3.7, 2.1	...	$-(\alpha)^{20.5}/\alpha_2{}^{HphI}\alpha_1$	...
7	Southern Italy	23	14	...	...	1.2, 1.1, 0.7	5.8, 4.8, 3.7, 2.1	12.6, 11.3	$\alpha_2{}^{NcoI}\alpha_1/\alpha\alpha^T$	12
8	Southern Italy	23	14	12.6, 7.4	...	1.2, 1.1, 0.7	4.8, 3.7, 2.1	12.6, 11.3	$\alpha\alpha^T/\alpha\alpha^T$	5
9	Southern Italy	19	10	15.8	3.5	...	...	...	$-\alpha^{3.7}II/-\alpha^{3.7}II$	8
10	Southern Italy	19, 2.6	10	15.8	2.5	...	...	...	$-(\alpha)^{20.5}/-\alpha^{3.7}I$	9
11	Southern Italy	19	10	...	2.5	...	...	15.8, 11.3	$--/-\alpha^{3.7}I$	10
12	Southern Italy	19	10	...	2.5	...	...	15.8, 12.0	$--/-\alpha^{3.7}I$	5
13	Southern Italy	19	10	...	3.5	...	...	15.8, 11.3	$-\alpha^{3.7}II/-\alpha^{3.7}II$	16
14	Central Italy	19	10	...	2.5	...	...	15.8, 13.9, 11.3	$--^{Med}/-\alpha^{3.7}I$	4
15	Sardinia	19	10	...	2.5	...	...	15.8, 13.9, 11.3	$--^{Med}/-\alpha^{3.7}I$	8
16	Southern Italy	23, 2.6	14	...	...	1.2, 1,1, 0.7	4.8, 3.7, 2.1	...	$-(\alpha)^{20.5}/\alpha\alpha^T$	8

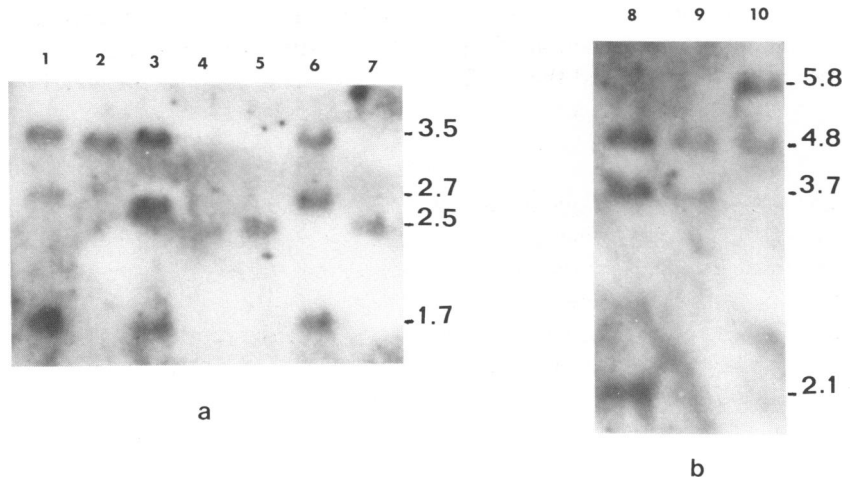

FIG. 3.—α-Globin gene specific fragments (in kb) after *Apa*I (*a*) and *Nco*I (*b*) digestion. *Lane 1:* Patient no. 9 mother ($-\alpha^{3.7}$II/αα). *Lane 2:* Patient no. 9 ($-\alpha^{3.7}$II/$-\alpha^{3.7}$II). *Lane 3:* Patient no. 9 father ($-\alpha^{3.7}$II/αα). *Lane 5:* Patient no. 1 ($--^{Med}/-\alpha^{3.7}$I). *Lane 6:* Patient no. 13 daughter ($-\alpha^{3.7}$II/αα). *Lane 7:* Patient no. 14 ($--^{Med}/-\alpha^{3.7}$I). *Lane 8:* Patient no. 8 ($\alpha\alpha^{T}/\alpha\alpha^{T}$). *Lane 9:* Patient no. 16 [$-(\alpha)^{20.5}/\alpha\alpha^{T}$]. *Lane 10:* Patient no. 3 ($--^{Med}/\alpha_2{}^{NcoI}\alpha_1$).

to approximately ½ of the classical hybrid gene. A deletion of two nucleotides at position −2 and −3 preceding the AUG codon was demonstrated in an Algerian family with the same phenotype and pattern of $-\alpha^{3.7}$ type II inheritance [19]. The hybridization with a 19-meric synthetic olygonucleotide complementary to the mutated sequence described by these authors showed that in families 9 and 13 at least one chromosome carries this mutation.

Several types of compound heterozygotes between nondeletional α^{+} determinants and α^{0} mutations were identified. Two subjects (families 3 and 7) carried the mutation in the initiation codon (ATG to ACG) of the α_2-gene, which can be detected by restriction analysis with the enzyme *Nco*I [7]. In both cases, a 5.8-kb fragment was present. In subject 3, this mutation was associated with the $--^{Med}$ determinant, as demonstrated by the presence in the propositus and in his father of the 13.9-kb *Bgl*II ζ-specific fragment (fig. 2). In subject 7, the mutation at the initiation codon of the α_2-gene was associated with a nondeletion determinant, as demonstrated by the presence of additional normal *Nco*I fragments (3.7 kb and 2.1 kb); normal fragments were also obtained with *Eco*RI, *Bam*HI, *Bgl*II, and *Hph*I.

In two subjects (families 2 and 6), digestion with *Hph*I revealed the altered 1.4-kb fragment associated with the normal 1.2- and 0.7-kb fragments [6]. The counterpart chromosome in both these patients was shown to have the $-(\alpha)^{20.5}$ deletion as detected by *Eco*RI digestion.

Another patient (family 16) showed the same pattern with *Eco*RI but neither *Nco*I nor *Hph*I analysis gave rise to abnormal fragments; therefore, a new uncharacterized nondeletional haplotype must be present in this patient.

Finally, patient 8 showed normal α- and ζ-specific restriction fragments with all the enzymes mentioned above (fig. 2). The similarity of the hematological picture of the parents and their referred consanguinity suggested that the proband is homozygous for a nondeletional $\alpha\alpha^T$ haplotype.

DISCUSSION

The results of hematological studies and restriction endonuclease mapping of 16 subjects with HbH disease indicate that in central and southern Italy this condition is produced by the interaction of a great variety of molecular defects in the α-globin gene cluster.

Hematological and clinical findings are not correlated with the molecular genotypes; however, the presence of a nondeletional α-thalassemia mutation appears to be associated with higher levels of HbH (table 1).

α^+ *Deletions*

The most common α^+ deletional determinant is the rightward deletion as in other populations of the Mediterranean area [20, 21]. Most of these deletions are of type I, which has been found to be the only α^+ lesion present in Sardinia [11]. On the contrary, type II crossover, which appears to be in linkage disequilibrium with the dinucleotide deletion described by Morlé et al. [19] is exclusively present in southern Italy (table 1).

α^0 *Deletions*

The most common types of α^0 deletional determinants present in southern Italy are the $--^{Med}$ and the $-(\alpha)^{20.5}$ (table 1). In addition, we have strong evidence for the existence of two chromosomes carrying a complete deletion of the α-globin cluster, whose length remains to be determined. These appear to be the first cases of ζα-thalassemia found in the Mediterranean area, while deletions of the same type have been reported from other areas [10, 22–24].

Nondeletional Determinants

We found five different nondeletional determinants. The first is the mutation removing five nucleotides at the IVS1 splice junction of the α_2-gene. The second is produced by a mutation in the initiation codon of the α_2-globin gene (ATG → ACG) and is detectable by *Nco*I restriction analysis. This mutation has been found in Sardinia, where it seems to be the most common nondeletional α^+ mutation [8], but it has not yet been reported in other Mediterranean populations. Finally, we found three chromosomes carrying an unknown mutation not detectable by Southern analysis. While in one case (subject 16), one must assume that this is clearly an α^+ mutation, in the other (subject 7), the coexistence of this determinant with an α^+ mutation (*Nco*I mutation) suggests that its output is close to 0 or at least well below that of one α-gene. In fact, Paglietti et al. [8] recently described an HbH patient who was homozygous for the $\alpha_2\,{}^{NcoI}\alpha_1$ haplotype, suggesting that two α^+ determinants can actually give rise to the HbH phenotype even if only the two α_2-genes are inactive.

Several data on α-thalassemia in the Mediterranean area are available, but

most of them cannot be used for a detailed micromapping because the different mutations have not been precisely localized within the different geographical regions and the size of the samples is relatively small [5, 20, 21].

We can compare our data in Continental Italy only with the results obtained in the extensive studies on α-thalassemia in Sardinia [11, 12, 20]. Recently, Paglietti et al. [8] analyzed the molecular basis for HbH disease in Sardinians and found that the only α^0 mutation present is the $--^{Med}$ and that the $-\alpha^{3.7}$ haplotype accounts for the 76% of all the α^+ mutations.

As to the α^0 mutations, we found the $--^{Med}$ deletion in patients from central Italy and Sardinia, while the complete deletion of the cluster and the $-(\alpha)^{20.5}$ haplotype were present only in southern Italy; the latter haplotype has been previously described in Greek, Cypriot, and Turkish patients [18, 20, 21].

As to the α^+ haplotypes, the $-\alpha^{3.7}$ type I deletion is spread all over the country; however, in the subset of patients from southern Italy, the relative frequency of other α^+ mutations, either deletional or nondeletional, seems to be higher than that found in Sardinia.

The higher degree of heterogeneity of α-thalassemia mutations in southern Italy as compared to Sardinia can be explained by: (1) the relative isolation of Sardinian populations [25]; (2) the higher prevalence of *P. falciparum* malaria in Sardinia [26]; and (3) the higher exposure of southern Italy to migratory flows from other Mediterranean countries, mainly Greece and North Africa, due to its geographical location.

The last argument is strongly supported by the presence in our sample of α-thalassemia mutations found in Greece, Turkey, and Algeria as well as by the relative high frequency of HbS in southern Italy as compared to other Italian regions [27].

ACKNOWLEDGMENTS

We thank A. Di Luzio for technical assistance and A. Sebastiano for typing the manuscript.

REFERENCES

1. Deisseroth A, Nienhuis A, Turner P, et al.: Localization of the human α-globin structural gene to chromosome 16 in somatic cell hybrids by molecular hybridization assay. *Cell* 12:205–218, 1977
2. Lauer J, Shen C-KJ, Maniatis T: The chromosomal arrangement of human α-like globin genes: sequence homology and α-globin gene deletions. *Cell* 20:119–130, 1980
3. Proudfoot NJ, Maniatis T: The structure of a human α-globin pseudogene and its relationship to α-globin gene duplication. *Cell* 21:537–544, 1980
4. Higgs DR, Weatherall DJ: Alpha-thalassemia. *Curr Top Hematol* 4:37–97, 1983
5. Weatherall DJ, Clegg JB: *The Thalassemia Syndromes,* 3rd ed. Oxford, England, Blackwell, 1981
6. Orkin SH, Goff SC, Hechtman RL: Mutation in an intervening sequence splice junction in man. *Proc Natl Acad Sci USA* 78:5041–5045, 1981
7. Pirastu M, Saglio G, Chang JC, Cao A, Kan YW: Initiation codon mutation as a cause of α-thalassemia. *J Biol Chem* 259:12315–12317, 1984
8. Paglietti E, Galanello R, Pirastu M, Cao A: Molecular pathology of Hemoglobin H disease in Sardinians. In press, 1986

9. HIGGS DR, GOODBOURN SEY, LAMB J, CLEGG JB, WEATHERALL DJ, PROUDFOOT NJ: α-Thalassemia caused by a polyadenylation signal mutation. *Nature* 306:398–400, 1983
10. WINICHAGOON P, HIGGS DR, GOODBOURN SEY, CLEGG JB, WEATHERALL DJ, WASI P: The molecular basis of α-thalassemia in Thailand. *EMBO J* 3:1813–1818, 1984
11. DI RIENZO A, FELICETTI L, NOVELLETTO A, FORTELEONI G, COLOMBO B: Frequency and types of deletional α^+ thalassemia in northern Sardinia. *Hum Genet* 71:147–149, 1985
12. GALANELLO R, MACCIONI L, RUGGERI R, PERSEU L, CAO A: Alpha thalassemia in Sardinian newborns. *Br J Haematol* 58:361–368, 1984
13. BIANCO I, GRAZIANI B, LERONE M, ET AL.: A screening programme for the prospective prevention of Mediterranean anemia in Latium: results of seven years work. *J Med Genet* 21:268–271, 1984
14. BRANCATI C, PUCCETTI G: Microcitemie ed emoglobine abnormi in Calabria. *Prog Medico* 20:632–638, 1964
15. GRAZIANI B, BIANCO I, CARBONI C, ET AL.: Tecniche e strategie di screenings di massa delle microcitemie. Atti del VI Congresso Internazionale: La prevenzione delle malattie microcitemiche, April 1980, Rome, Edizioni Minerva Medica
16. HIGGS DR, HILL AVS, BOWDEN DK, WEATHERALL DJ, CLEGG JB: Independent recombination events between the duplicated human α-globin genes; implications for their concerted evolution. *Nucleic Acid Res* 12:6965–6977, 1984
17. ORKIN SH, MICHELSON A: Partial deletion of the α-globin structural gene in human α-thalassemia. *Nature* 286:538–540, 1980
18. NICHOLLS RD, HIGGS DR, CLEGG JB, WEATHERALL DJ: α^0-Thalassemia due to recombination between the α_1-globin gene and an Alu I repeat. *Blood* 65:1434–1438, 1985
19. MORLÉ F, LOPEZ B, HENNI T, GODET J: α-Thalassemia associated with the deletion of two nucleotides at position −2 and −3 preceding the AUG codon. *EMBO J* 4:1245–1250, 1985
20. PIRASTU M, LEE KY, DOZY AM, ET AL.: Alpha-thalassemia in two Mediterranean populations. *Blood* 60:509–512, 1982
21. ORKIN SH, OLD J, LAZARUS H, ET AL.: The molecular basis of α-thalassemias: frequent occurrence of dysfunctional α-loci among non-Asians with HbH disease. *Cell* 17:33–42, 1979
22. WEATHERALL DJ, HIGGS DR, BUNCH C, ET AL.: Hemoglobin H disease and mental retardation. *N Engl J Med* 305:607–612, 1981
23. FELICE AE, CLEEK MP, MCKIE K, MCKIE V, HUISMAN THJ: The rare α-thalassemia-1 of blacks is a ζα-thalassemia-1 associated with deletion of all α- and ζ-globin genes. *Blood* 63:1253–1257, 1984
24. CHAN V, CHAN TK, LIANG ST, GHOSH A, KAN YW, TODD D: Hydrops fetails due to an unusual form of HbH disease. The International Conference on Thalassemia, July 1985, Bangkok, Thailand
25. MODIANO G, TERRENATO L, SCOZZARI R, ET AL.: Population genetics in Sardinia. *Memoria Acc Naz Lincei.* In press, 1986
26. MISSIROLI A: *Lezioni sulla Epidemiologia e Profilassi della Malaria.* Rome, Armani, 1934
27. BRANCATI C, TAGARELLI A: Thalassemia types in Calabria (south Italy), in *Thalassemia: Recent Advances in Detection and Treatment,* vol 18, New York, Alan R. Liss, 1982, pp 147–155