An Initiation Codon Mutation (AUG → GUG) of the Human α1-Globin Gene

Structural Characterization and Evidence for a Mild Thalassemic Phenotype

Paolo Moi,* Faith E. Cash,‡ Stephen A. Liebhaber,‡ Antonio Cao,§ and Mario Pirastu||

**Ospedale Regionale per le Microcitemie USL21, Cagliari; ‡Howard Hughes Medical Institute, and the Departments of Human Genetics and Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104; §Istituto di Clinica e Biologia Dell'Eta Evolutiva University Degli Studi Di Cagliari; ||Istituto di Ricerca Sulle Talassemie ed Anemie Mediterranee, CNR, Cagliari, Sardegna, Italy*

Abstract

α-globin is encoded by two adjacent genes, α1 and α2. Recent evidence suggests that these genes are not equally expressed and that the α2-globin gene encodes the majority of α-globin. This finding would predict that a thalassemic mutation of the α2-globin gene would result in a more severe loss of α-chain synthesis than a similar mutation in the α1-globin gene. In a previous study we described a nondeletion α-thalassemia defect in the α2-globin gene resulting from an AUG → ACG initiation codon mutation. In the present study we describe a different initiation codon mutation, AUG → GUG, present in the α1-globin gene. The α1- and α2-globin gene initiation codon mutations result in similarly lowered levels of encoded mRNA. Despite the similarity of these two mutations, the α2 mutant results in a more severe loss of α-globin synthesis and a more severe clinical α-thalassemia phenotype than the corresponding α1-globin gene mutation. This difference reflects the dominant role of α2-globin gene in overall α-globin synthesis.

Introduction

The α-globin gene cluster is located on the short arm of chromosome 16 (1). The genes in this cluster are arranged in the order 5′-ξ-ψξ-ψα-α2-α1-3′ (2–3). A variety of mutations within this cluster result in deficient or absent synthesis of α-globin and the consequent group of genetic disorders known as the α-thalassemias (4). Most commonly, α-thalassemia results from the deletion of one or both of the α-globin genes (5). Less commonly, α-globin gene function is altered by a defect that does not produce a gross gene deletion (6–8). Several such nondeletion α-thalassemias have now been defined at a molecular level. These include: termination codon mutations such as Hb Constant Spring (9), a splicing defect caused by a 5-basepair (bp) deletion of the first intervening sequence (10), Hb Quong Sze, an extremely unstable α-globin structural variant (11, 12), a single nucleotide substitution of the polyadenylation site (13), a two nucleotide deletion at position −1 and −2 in the 5′ untranslated region preceding the AUG codon (14), an initiation codon mutation (AUG → ACG) (15) and a nonsense mutation (α116 GAG → UAG) (16). Apart from the −1, −2 deletion that occurs in a single α-globin gene chromosome, each of these mutations affects the α2-globin gene.

Address reprint requests to Dr. Pirastu, Ospedale Regionale, Microcitemico, via Jenner 5N, 09100 Cagliari, Sardegna, Italy.

Received for publication 7 April 1987 and in revised form 29 June 1987.

J. Clin. Invest.

0021-9738/87/11/1416/06 $2.00
Volume 80, November 1987, 1416–1421

Hemoglobin H (HbH)[1] disease, the most severe form of α-thalassemia compatible with life, commonly results from the deletion of three of the four α-globin genes (17–23). Less commonly, HbH disease can also result from the deletion of the two α-globin genes on one chromosome combined with a nondeletion defect affecting one of the two α-globin genes on the other chromosome. In Sardinians, the most common nondeletion α-thalassemia defect is the AUG → ACG mutation in the initiation codon of the α2-globin gene (15, 24–26). This mutation destroys a recognition site of the enzyme NcoI. While screening a group of nondeletion HbH disease patients ($--/\alpha^{Th}\alpha$) for this mutations by Southern blot analysis, we found two siblings in whom the NcoI map suggested a mutation at the initation codon of the α1 rather than the α2 locus. The severity of α-thalassemia in these individuals was significantly less than in those with the NcoI mutation in the α2-globin gene. In the present study, we define the structure of this α1-globin gene mutation and characterize its impact upon α-globin mRNA and protein synthesis.

Methods

Hematologic analysis. Hematologic measurements were made with a Coulter Counter (model S, Coulter Electronics, Hialeah, FL). Electrophoresis of hemoglobin was carried out on Titan III cellulose acetate plates, pH 8.6 (Helena Laboratories, Beaumont, TX). Hb A_2 was determined by DE-52 microchromatography (27). Globin chain synthesis analysis was carried out according to the method of Kan et al. (28).

DNA analysis. DNA was extracted from peripheral leukocytes as previously described (29). The α-globin cluster was mapped by Southern blot analysis using the restriction endonucleases BglII, HphI, NcoI, and HindIII in single and double digestion. The ζ-globin gene probe was a 1.8-kb HinfI genomic fragment containing the entire pseudozeta gene excluding the 5′ part of the first exon (30); the α-globin gene probe was a 1.5-kb PstI genomic fragment spanning the entire α-globin gene.

Gene cloning and sequence analysis. Total genomic DNA isolated from individual III-2 (Fig. 1) was digested to completion with the restriction enzyme HindIII. The fraction containing 3.7 Kb fragments was collected by density sedimentation through a continuous sucrose gradient, ligated to Charon 28 vector in the HindIII site and packaged in vitro. The recombinant phages were propagated in *Escherichia coli.* Three phage clones containing the 3.7-kb α-globin gene fragment were identified out of 75,000 recombinant phages screened with a ^{32}P-labeled α-globin specific probe. The 3.7-kb fragment, which spans from the HindIII site at codon 90-91 of the α2-globin gene to the HindIII site at the same position within the α1-globin gene, was subcloned in the HindIII site of the pSP64 plasmid (New England Nuclear, Boston, MA). Sequencing was performed by the primer extension method directly on supercoiled plasmid (31, 32) using as a primer for the Klenow fragment of DNA polymerase a 20-mer oligonucleotide (5′ CCT TGA CGT TGG TCT TGT CG 3′) complementary to the α-glo-

1. *Abbreviations used in this paper:* HbH, hemoglobin H.

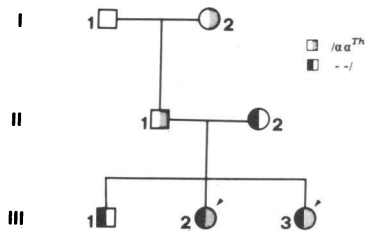

Figure 1. Pedigree of the index family. The segregation of the deletion and nondeletion α-thalassemic mutations is shown.

bin DNA sequence from nucleotide +13 to +32 (the *A* of the AUG initiation codon being designated as +1).

RNA isolation and analysis. The RNA was isolated from peripheral reticulocytes as previously detailed (33) and used for subsequent studies without further purification. 1 μg of total RNA was translated in micrococcal nuclease treated rabbit reticulocyte lysate prepared from New Zealand white rabbits as previously described (34). Translations were done at 30°C in the presence of [^{35}S]methionine exactly as previously detailed (34). The labeled protein products were resolved on a Triton-acid-urea slab gel (35, 36) and visualized by autoradiography. The ratio of α2- to α1-globin mRNA was established by primer extension analysis as previously detailed (33) with the following exception: we have substituted for the previously described 31 nucleotide cDNA primer, a 20 nucleotide synthetic oligonucleotide (3′-CACCCGCCGTTTTTTTTTTT-5′) that is complementary to the terminal 9 nucleotides of both α1- and α2-globin mRNAs and to 11 adenosines of the contiguous poly-A tail. This oligomer was 5′ end-labeled with γ-(^{32}P)ATP (5,000 Ci/mM; Amersham Corp., Arlington Heights, IL) and polynucleotide kinase and purified by a single precipitation with three volumes of ethanol in the presence of 50 μg/ml tRNA carrier and 0.2 M Na acetate. 1 ng of the end-labeled oligonucleotide primer was added to 0.5 μg of reticulocyte RNA in a reverse transcription reaction containing 1 μl of AMV reverse transcriptase (Life Science Associates, Bayport, NY, 15 U/μl). The subsequent HaeIII restriction nuclease digest of the 5′ end-labeled single strand cDNA, and the 8% acrylamide/8 M urea gel analysis of the digest products were all carried out exactly as previously detailed (33). Band intensities on autoradiographs of the in vitro translation gels and the primer extension analysis gels were quantitated in the linear range by densitometry using a Zeineh soft laser scanner (model SL-504-XL; Biomed Instruments, Fullerton, CA).

Results

Family studies. Two sisters (III-2, III-3, indicated by the arrows in Fig. 1) with HbH disease and their family were studied at the hematology laboratory of the Ospedale Regionale per le Microcitemie. The two patients presented with a similar history of chronic pallor and anemia. The spleen was barely palpable, the liver was slightly enlarged, and the children were of normal stature for their ages. There was no history of acute hemolytic crises. The results of the hematological evaluation of the family are shown in Table I.

α-Globin gene mapping. The α-globin genotype of each member of the family was determined by Southern blot analysis. The presence of a double α-globin gene deletion [(−−)chromosome] was inferred by digesting the genomic DNA with BglII and using both the α-globin and ζ-globin probes (8) (data not shown). The presence of the nondeletion mutation was detected by the loss of an NcoI restriction site. When normal DNA is cleaved with NcoI, three α-globin specific fragments of 4.8, 3.7, and 2.1 kb are produced (Fig. 2). The 4.8 and 3.7 fragments contain the α1- and the α2-globin genes, respectively. The 2.1-kb fragment spans from the initiation codon of the α2-globin gene to a NcoI site located in the 5′ flanking region. DNA from the patients investigated (III-2 and III-3) yielded the normal 2.1-kb fragment associated with a new 8.5-kb fragment that replaces the normal 3.7- and 4.8-kb fragments (Fig. 2). This pattern suggests the loss of the NcoI restriction site located in the α1-globin gene. This interpretation was confirmed by double digestion of the DNA with NcoI and HindIII (data not shown). These findings indicate that our patient has a mutation in the α1-globin gene that abolishes the NcoI site normally located in the 5′ part of the gene. NcoI digestion of DNA isolated form the father and paternal grandmother yielded the 8.5-kb abnormal fragment in addition to the normal 4.8-, 3.7-, and 2.1-kb fragments which indicated the presence of the nondeletion defect in one chromosome and a normal α-globin gene complement in the other chromosome. A summary of the α-thalassemia inheritance in this family is presented in Fig. 1.

Sequence analysis of the α1-globin gene initiation codon mutation. The absence of the normal NcoI site in the α1-globin gene raised the possibility that the previously reported ATG → ACT initiation codon mutation found in the α2-globin gene of a Sardinian patient with HbH disease (15) may have been introduced at a homologous position in the α1 gene by a gene conversion event (37). To determine the exact nature of the α1-globin gene mutation that destroys the NcoI site, the

Table I. Hematologic Evaluation of a Family with Nondeletion HbH Disease

	I-1	I-2	II-1	II-2	III-1	III-2	III-3
Genotype	αα/αα	αα/ααTh	αα/ααTh	−−/αα	−−/αα	−−/ααTh	−−/ααTh
Age (*yr*)	86	84	58	53	22	12	10
RBC (×10^{12}/*liter*)	4.42	4.62	5.28	5.21	6.11	5.92	6.64
Hb (*g/dl*)	13.1	11.3	14.2	11.5	14.5	9.5	10.1
Hct (%)	39	35.5	40.2	34.7	44	30	31.7
MCV (*fl*)	87	75	77	67	72	52	49
MCH (*pg*)	29.5	24.4	28	23	24	16	15
MCHC (*g/dl*)	32.3	30.7	36	34	31	31	31
Electrophoresis	A+A_2	A+A_2	A+A_2	A+A_2	A+A_2	H (3%)+A+A_2	H(1.5%)+A+A_2
Hb A_2 (%)	2.92	2.84	2.96	2.62	2.38	1.45	1.60
Reticulocytes (%)	8	3	5	6	8	26	22
RBC with HbH inclusion bodies per 1000	Absent	2	0.19	0.04	0.8	140	60

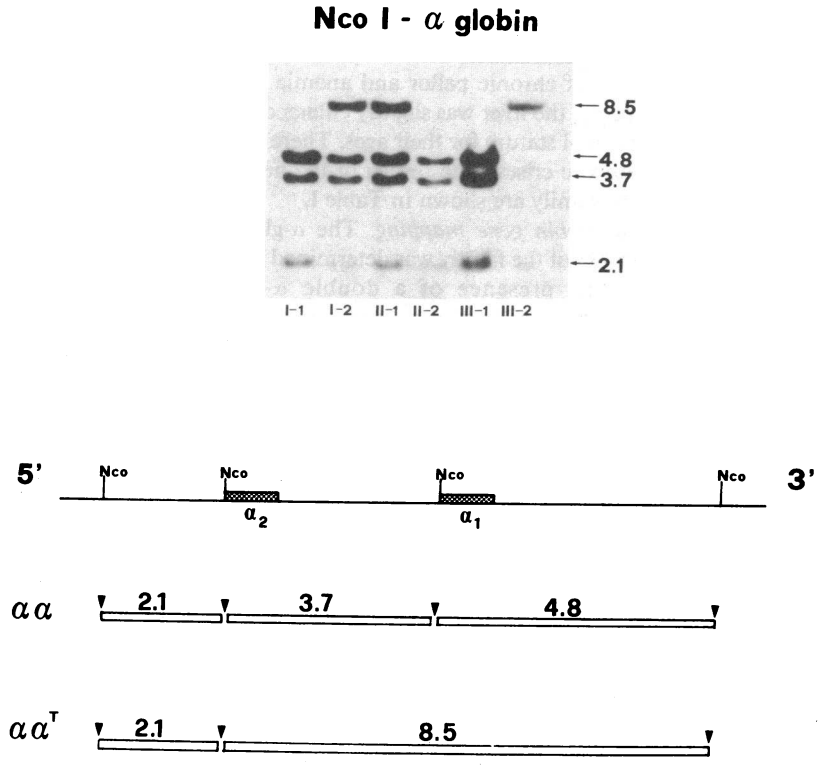

Figure 2. Southern blot analysis of the α-globin genes in the index family using the restriction enzyme NcoI and the α-globin gene as a probe. At the bottom of the figure the NcoI map of the normal chromosome ($\alpha\alpha$) and of the nondeletion thalassemic chromosome ($\alpha\alpha^{Th}$) is schematically represented. The result for sample III-3 (not shown in this figure) is identical to that of III-2.

mutant α1-globin gene was cloned from individual III-2 and the 5′ end from nucleotides −35 to +14 was sequenced. This sequence analysis demonstrated a single nucleotide substitution at the initiation codon:ATG → GTG (Fig. 3).

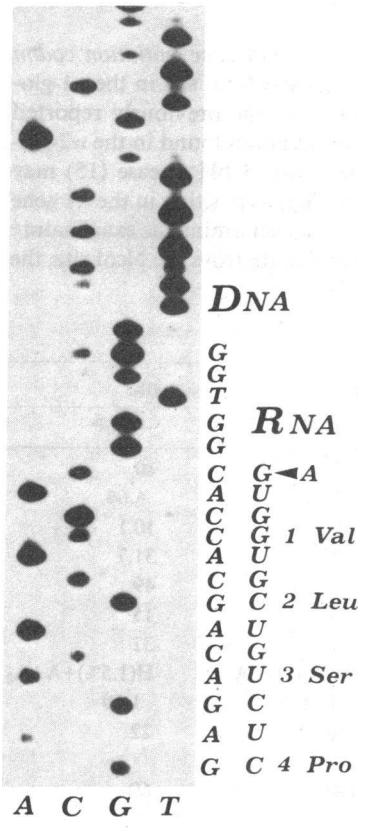

Figure 3. Sequence analysis of the region of the mutant α1-globin gene surrounding the initiation AUG codon. The arrow indicates the position of the A → G mutation.

mRNA analysis. The relative levels of α1- and α2-globin mRNA in total reticulocyte RNA was measured by primer extension mapping. The results of studies on a normal $\alpha\alpha/\alpha\alpha$, a previously reported $--/\alpha^{Th}\alpha$ individual, and one of the $--/\alpha\alpha^{Th}$ individuals (III-2) are shown in Fig. 4. In normal reticulocytes the α2:α1 mRNA ratio is 2.6 (33, 38). In four unrelated Sardinian patients with nondeletion hemoglobin H disease in whom the nondeletion defect was the T → C substitution in the ATG initiation codon of the α2 gene ($--/\alpha^{Th}\alpha$) the average ratio was 0.9 (range 0.75–1.3) indicating that the level of steady state α2-globin mRNA was decreased to approximately one-third of its normal level (Fig. 4 and Table II). This value is consistent with the previously reported value of 1.0 (15). In the two sisters III-2 and III-3 with the ATG → GTG mutation in the α1-globin gene ($--/\alpha\alpha^{Th}$) the α2/α1 ratios of 12 and 14, respectively, indicated a reduction in the steady-state level of α1 mRNA to approximately one-fourth of its normal level.

In vitro translation. Unfractionated mRNA was translated in vitro and the labeled translation products were resolved and quantitated by electrophoresis and densitometry. The results of this analysis are shown in Fig. 5 and summarized in Table II.

In vitro translation of normal reticulocyte mRNA gives an α/β globin synthesis ratio (corrected for methionine content of two in α and one in β) of 1.5 while in four unrelated individuals with HbH disease and the nondeletion (ATG → ACG) defect in the α2-globin gene ($--/\alpha^{Th}\alpha$) had an average ratio of 0.095 (range 0.04–0.16; Table II). The α/β globin ratios of III-2 and III-3 ($--/\alpha\alpha^{Th}$) were 0.22 and 0.27, respectively.

In a parallel set of experiments α/β synthesis was measured in intact reticulocytes from each of these individuals. As in the in vitro translations the level of α-globin synthesized by those with the α1 mutation ($--/\alpha\alpha^{Th}$) was greater than those with

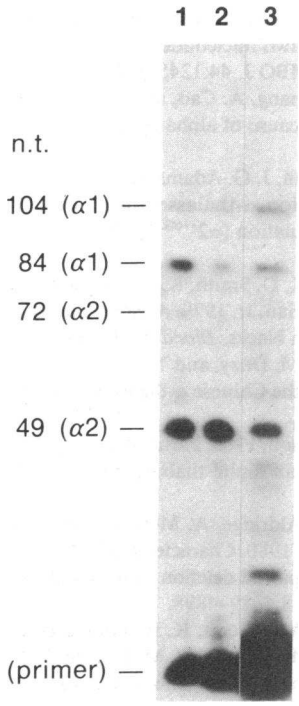

Figure 4. Primer extension analysis of the α2:α1 globin mRNA ratio in reticulocyte RNA. Samples of reticulocyte RNA were isolated and cDNA was synthesized by extending a ^{32}P end-labeled synthetic oligonucleotide primer complimentary to the 3′ terminus of α-globin mRNA. The cDNA reverse transcripts were digested with HaeIII and the products analyzed on a denaturing gel. The position of the primer and of the α1- and α2-globin specific bands are indicated to the left of the autoradiograph and the size of each band is noted in nucleotides (*n.t.*). The additional bands above the primer appear to result from reverse transcription of mRNA degredation fragments as they comigrate with short end-labeled DNAs noted on gel analysis of the sample before HaeIII digestion (data not shown). The RNA originated from individuals with the following α-globin genotypes: lane *1*, αα/αα; lane *2*, $--/\alpha\alpha^{Th}$; lane *3*, $--/\alpha^{Th}\alpha$.

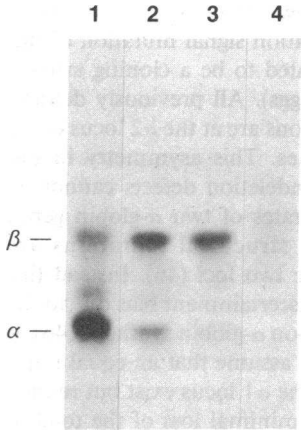

Figure 5. In vitro translation of reticulocyte RNA. Total reticulocyte RNA isolated from each of three individuals was translated in a rabbit reticulocyte lysate in the presence of [^{35}S]methionine and resolved on a Triton-acid-urea gel. The positions of β- and α-globin are indicated to the left of the autoradiograph. The RNA originated from individuals with the following α-globin genotypes: lane *1*, αα/αα; lane *2*, $--/\alpha\alpha^{Th}$; lane *3*, $--/\alpha^{Th}\alpha$. Lane *4* is a control translation to which no exogenous RNA was added.

the α2 mutation ($--/\alpha^{Th}\alpha$). The uniformly lower α/β ratios measured by in vitro translation as compared to the reticulocyte incubations may reflect the ability of proteolytic systems in the intact cell to partially compensate for the chain imbalance. Such discrepancies between the α/β synthetic ratios obtained in vitro and during the labeling of intact reticulocytes are observed in a wide variety of deletion and nondeletion α-thalassemias (S. A. Liebhaber and F. E. Cash, unpublished data).

Discussion

In this study we have characterized a newly discovered mutation, ATG → GTG, in the initiation codon of the α1-globin gene that, when combined with deletion of both α-globin genes on the sister chromosome, results in the clinical phenotype of α-thalassemia. In higher eucaryotes the ATG serves as the only functional initiation codon (39, 40). The only exception may be the use of ACG in certain viral systems (41). Whether the ATG → GTG mutation in the α1-globin gene, or the previously described ATG → ACG mutation in the α2-globin gene completely abolishes α-globin mRNA translation has not been directly determined. However, the clinical phenotypes of the HbH disease in both categories of patients (25; this study) suggest a functional loss of the affected α-globin gene. The initiation codon mutations in the α1 and α2 loci are associated with similar three- to fourfold decreases in the steady state level of encoded mRNA. This comparable effect suggests that the two mutations may decrease the steady state level of their encoded mRNAs by similar mechanisms. While effects upon transcription or RNA processing cannot be ruled out at present, mRNA instability linked to the block in translation would appear to be likely cause.

Table II. Synthesis of α- and β-Globin in Individuals with HbH Disease

		α/β Protein Synthesis		
Patient	Genotype	*In vitro*	Reticulocytes	α2:α1 mRNA
Normal	αα/αα	1.5	1.00	2.6
C.C. (III-2)	$--/\alpha\alpha^{Th}$	0.22	0.59	12.0
R.C. (III-3)	$--/\alpha\alpha^{Th}$	0.22	0.75	14.0
F.M.	$--/\alpha^{Th}\alpha$	0.04	0.49	1.3
V.A.	$--/\alpha^{Th}\alpha$	0.14	0.47	0.91
A.L.	$--/\alpha^{Th}\alpha$	0.04	0.22	0.75
G.A.	$--/\alpha^{Th}\alpha$	0.16	0.46	0.85

$\alpha^{Th}\alpha$, AUG→ACG mutation at the initiation codon of the α2-globin gene. $\alpha\alpha^{Th}$, AUG→GUG mutation at the initiation codon of the α1-globin gene.

The severity of the α-thalassemia phenotype appears to relate both to the specific α-globin gene lost (α1 or α2) and the manner in which it is lost (deletion versus nondeletion). α-globin production during in vitro translation of reticulocyte RNA and during labeling of intact reticulocytes was less significantly depressed in the $--/\alpha\alpha^{Th}$ patients (III-2 and III-3), than in the patients with a similar initiation codon mutation in the α2-globin gene ($--/\alpha^{Th}\alpha$) (Fig. 5 and Table II). The mild phenotype noted in these two patients with the $--/\alpha\alpha^{Th}$ genotype is very similar to that manifested by patients with the deletional form of HbH disease ($--/-\alpha$), and is significantly less severe than patients with HbH disease resulting from nondeletion defects (ATG → ACG, Hb Constant Spring) in the α2 gene (25, 42, 43). Since the α2-globin gene normally encodes two- to threefold higher steady state level of mRNA (33, 38, 44) and produces two- to threefold more α-globin (45, 46) than the α1-globin gene, a nondeletion defect in the α2-globin gene would be expected to result in the loss of two to three times more α-globin synthesis than a comparable mutation in the α1 gene. The rightward type α-thalassemia deletion ($-\alpha^{3.7}$) would be predicted to yield a mild phenotype comparable to an α1 nondeletion mutation since the loss of the α2-globin gene is associated with a 1.8-fold compensatory increase in the expression of the remaining α1-globin gene (38).

The initiation codon mutation described herein is the first α-thalassemia mutation to date identified in the α1-globin

gene; the previously reported frameshift in the α1-globin gene associated in *Cis* to a polyadenylation signal mutation of the α2-globin gene is now demonstrated to be a cloning artifact (personal communication, D. Higgs). All previously defined nondeletion α-thalassemia mutations are at the α2 locus or on single α-globin gene chromosomes. This asymmetry in the distribution of α-globin gene nondeletion defects cannot be attributed to unequal mutation rates of two α-globin genes since nonthalassemic α-globin structural mutations are equally distributed between these two loci (46). Instead this distribution probably reflects an ascertainment bias due to the greater impact of α2 mutations upon α-globin synthesis (46). It is therefore entirely reasonable to assume that an equal number of thalassemia mutations of the α1 locus exist but remain phenotypically silent due to the minimal loss of the total α-globin synthetic capacity. It is only when the α1 mutation is associated with other α-thalassemic mutations in the genome that the small incremental loss of α1-globin gene function can be appreciated.

Acknowledgments

Supported in part by grant 1-Ro1-AM-33975 from the National Institutes of Health and Progetto Finalizzato 833 from Assessorato Igiene e Sanita Regione Autonoma della Sardegna.

References

1. Deisseroth, A., A. Nienhuis, P. Turner, R. Valez, W. F. Anderson, F. Ruddle, J. Lawrence, R. Creagan, and R. Kucherlapati. 1977. Localization of the human α-globin gene to chromosome 16 in somatic cell hybrids by molecular hybridization assay. *Cell.* 12:205–211.

2. Lauer, J., C. KJ. Shen, and T. Maniatis. 1980. The chromosomal arrangement of human α-like globin genes. Sequence homology and α-globin gene deletions. *Cell.* 20:119–130.

3. Proudfoot, N. J., and T. Maniatis. 1980. The structure of a human α-globin pseudogene and its relationship to α-globin gene duplication. *Cell.* 21:537–44.

4. Weatherall, D. J., and J. B. Clegg. 1981. The Thalassaemia Syndromes. Boston, Blackwell Scientific Publications, Boston, MA.

5. Higgs, D. R., and D. J. Weatherall. 1983. α-Thalassemia. *Curr. Top. Haematol.* 4:37–97.

6. Kan, Y. W., A. M. Dozy, R. Trecartin, and D. Todd. 1977. Identification of a nondeletion defect in α-thalassemia. *N. Engl. J. Med.* 297:1081–1084.

7. Orkin, S. H., J. Old, H. Lazarus, C. Altay, A. Gurgey, D. J. Weatherall, and D. G. Nathan. 1979. The molecular basis of α-thalassemia; Frequent occurrence of dysfunctional α-loci among non-Asians with HbH disease. *Cell.* 17:33–42.

8. Higgs, D. R., L. Pressley, B. Aldridge, J. B. Clegg, D. J. Weatherall, A. Cao, M. G. Hadjiminas, C. Kattamis, A. Metaxatou-Mavromati, E. A. Rachmilewitz, and T. Sophocleous. 1981. Genetic and molecular diversity in nondeletion HbH disease. *Proc. Natl. Acad. Sci. USA.* 78:5833–5837.

9. Clegg, J. B., D. J. Weatherall, and P. F. Milner. 1971. Hemoglobin Costant Spring—a chain termination mutation. *Nature (Lond.).* 234:337–339.

10. Orkin, S. H., S. C. Goff, and R. L. Hechtman. 1981. Mutation in an intervening sequence splice junction in man. *Proc. Natl. Acad. Sci. USA.* 78:5041–5045.

11. Goossens, M., K. Y. Lee, S. A. Liebhaber, and Y. W. Kan. 1982. Globin structural mutant α^{125} Leu → Pro is a novel cause of α-thalassemia. *Nature (Lond.).* 296:864–866.

12. Liebhaber, S. A., and Y. W. Kan. 1983. α-Thalassemia Caused by an Unstable α-Globin Mutant. *J. Clin. Invest.* 71:461–466.

13. Higgs, D. R., S. E. Y. Goodbourn, J. Lamb, J. B. Clegg, D. J. Weatherall, and N. J. Proudfoot. 1983. α-thalassaemia caused by a polyadenylation signal mutation. *Nature (Lond.).* 306:398–400.

14. Morle', F., B. Lopez, T. Henni, and J. Godet. 1985. α-Thalassaemia associated with the deletion of two nucleotides at position −2 and −3 preceding the AUG codon. EMBO J. 44:1245–1250.

15. Pirastu, M., G. Saglio, J. C. Chang, A. Cao, and Y. W. Kan. 1984. Initiation codon mutation as a cause of alpha thalassaemia. *J. Biol. Chem.* 259:12315–12317.

16. Liebhaber, S. A., M. B. Coleman, J. G. Adams, III, F. E. Cash, and M. Steinberg. 1987. Non-deletion α-thalassemia in a black kindred resulting from a nonsense mutation ($\alpha 2^{116GAG \rightarrow UAG}$) *J. Clin. Invest.* 80:154–159.

17. Phillips, J. A., III, A. F. Scott, K. D. Smith, K. E. Young, K. L. Lightbody, R. M. Jiji, and H. H. Kazazian, Jr. 1979. A molecular basis for hemoglobin H disease in American blacks. *Blood.* 54:1439–1445.

18. Embury, S. H., R. V. Lebo, A. M. Dozy, and Y. W. Kan. 1979. Organization of the α-globin genes in the Chinese α-thalassaemia syndromes. *J. Clin. Invest.* 63:1307–1310.

19. Orkin, S. H., and A. M. Michelson. 1980. Partial deletion of the alpha-globin structural gene in human alpha-thalassaemia. *Nature (Lond.).* 286:538–540.

20. Pressley, L., D. R. Higgs, B. Aldridge, A. Metaxatou-Mavromati, J. B. Clegg, and D. J. Weatherall. 1980. Characterization of a new alpha-thalassaemia-1 defect due to a partial deletion of the alpha globin gene complex. *Nucleic Acids Res.* 8:4889–4898.

21. Philips, J. A., III, T. A. Vik, A. F. Scott, K. E. Young, H. H. Kazazian, Jr., K. D. Smith, V. F. Fairbanks, and H. M. Koenig. 1980. Unequal crossing-over. A common basis of single α-globin genes in Asians and American blacks with hemoglobin H disease. *Blood.* 55:1066–1069.

22. Sophocleous, T., D. R. Higgs, B. Aldridge, R. J. Trent, L. Pressley, J. B. Clegg, and D. J. Weatherall. 1981. The molecular basis for the haemoglobin Bart's hydrops fetalis syndrome in Cyprus. *Br. J. Haematol.* 47:153–156.

23. Nicholls, R. D., D. R. Higgs, J. B. Clegg, and D. J. Weatherall. 1985. Alpha°-Thalassemia due to recombination between the alpha-globin gene and an Alu repeat. *Blood.* 65:1434–1439.

24. Pirastu, M., K. Y. Lee, A. M. Dozy, Y. W. Kan, G. Stamatoyannopoulos, M. G. Hadjiminas, Z. Zachariades, A. Angius, M. Furbetta, C. Rosatelli, and A. Cao. 1982. Alpha-thalassemia in two Mediterranean populations. *Blood.* 60:509–515.

25. Paglietti, E., R. Galanello, P. Moi, M. Pirastu, and A. Cao. 1986. Molecular pathology of hemoglobin H disease in Sardinians. *Br. J. Haematol.* 63:485–496.

26. Kan, Y. W., A. M. Dozy, G. Stamatoyannopoulos, M. G. Hadjiminias, Z. Zachariades, M. Furgetta, and A. Cao. 1979. Molecular basis of hemoglobin-H disease in the Mediterranean population. *Blood.* 54:1434–1438.

27. Huisman, T. H. J., W. A. Schroeder, A. N. Brodie, S. M. Mayson, and J. Jakway. 1975. Microchromatography of hemoglobins. III. A simplified method for the determination of hemoglobin A_2. *J. Lab Clin. Med.* 86:700–703.

28. Kan, Y. W., E. Schwartz, and D. G. Nathan. 1968. Globin chain synthesis in the alpha thalassemia syndromes. *J. Clin. Invest.* 47:2515–2522.

29. Goossens, M., and Y. W. Kan. 1981. DNA analysis in the diagnosis of hemoglobin disorders. *Methods Enzymol.* 76:805–817.

30. Proudfoot, N. J., A. Gil, and T. Maniatis. 1982. The structure of the human zeta-globin gene and a closely linked, nearly identical pseudogene. *Cell.* 1:553–563.

31. Sanger, Fr., S. Micklen, and A. R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA.* 74:5463–5467.

32. Chen, E. J., and P. H. Seeburg. 1985. Supercoil sequencing: a fast simple method for sequencing plasmid DNA. *DNA.* 4:165–170.

33. Liebhaber, S. A., and Y. W. Kan. 1981. Differentiation of the

mRNA transcripts originating for the α1- and α2-globin loci in normals and α-thalassemics. *J. Clin. Invest.* 68:439–446.

34. Liebhaber, S. A., F. E. Cash, and S. H. Shakin. 1984. Translationally associated helix-destabilizing activity in rabbit reticulocyte lysate. *J. Biol. Chem.* 259:15597–15602.

35. Alter, B. 1979. The Gγ:Aγ composition of fetal hemoglobin in fetuses and newborns. *Blood.* 54:1158–1163.

36. Rovera, G., C. Magarian, and T. W. Borun. 1978. Resolution of hemoglobin subunits by electrophoresis in acid urea polyacrylamide gels containing Triton X-100. *Anal. Biochem.* 85:506–518.

37. Liebhaber, S. A., E. F. Rappaport, F. E. Cash, S. K. Ballas, F. F. Schwartz, and S. Surrey. 1984. Hemoglobin I mutation encoded at both α-globin loci on the same chromosome. Concerted evolution in the human genome. *Science (Wash. DC).* 226:1949–1951.

38. Liebhaber, S. A., F. E. Cash, and D. M. Main. 1985. Compensatory increase in α1-globin gene expression in individuals heterozygous for α-thalassemia-2 deletion. *J. Clin. Invest.* 76:1057–1064.

39. Kozak, M. 1983. Comparison of initiation of protein synthesis in procaryotes, eucaryotes, and organelles. *Microbiol. Rev.* 47:1&45.

40. Brown, J. C., and A. E. Smith. 1970. Initiator codon in eukaryotes. *Nature (Lond.).* 226:610–612.

41. Becerra, S. P., J. A. Rose, M. Hardy, B. M. Bardudy, and C. V. Anderson. 1985. Direct mapping of associated virus capsid proteins B and C′ a possible ACG initiation codon. *Proc. Natl. Acad. Sci. USA.* 82:7919–7923.

42. Pongsamart, S., S. Pootrakul, P. Wasl, and S. Nanakorn. 1975. Hemoglobin Costant Spring: hemoglobin synthesis in heterozygous and homozygous states. *Biochem. Biophys. Res. Commun.* 63:681–686.

43. Winchagoon, P., P. Adironjnanon, and P. Wasl. 1980. Levels of haemoglobin H and proportion of red cells with inclusion bodies in two types of haemoglobin H disease. *Br. J. Haematol.* 46:507–509.

44. Orkin, S. H., and S. C. Goff. 1981. The duplicated human α-globin genes: Their relative expression as measured by RNA analysis. *Cell.* 24:345–352.

45. Shakin, S. H., and S. A. Liebhaber. 1986. Translational profiles of α1, α2, and β-globin messenger ribonucleic acids in human reticulocytes. *J. Clin. Invest.* 78:1125–1129.

46. Liebhaber, S. A., F. E. Cash, and S. K. Ballas. 1986. Human α-globin gene expression. The dominant role of the α2-locus in mRNA and protein synthesis. *J. Biol. Chem.* 261:15327–15333.