bjh research paper

Hb Southern Italy: coexistence of two missence mutations (the Hb Sun Prairie α_2 130 Ala $\rightarrow$ Pro and Hb Caserta α_2 26 Ala $\rightarrow$ Thr) in a single *HBA2* gene

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Summary

This study describes a new molecular condition in the α_2-globin gene (*HBA2*) found in six unrelated families from Southern Italy (Campania and Sicily). This new double mutant form of haemoglobin is called Hb Southern Italy and originated from the coexistence of two known mutations occurring in the same globin gene, *HBA2* 26 G $\rightarrow$ A (Hb Caserta) and *HBA2* 130 G $\rightarrow$ C (Hb Sun Prairie). Hb Sun Prairie was originally observed in Indian patients in either the homozygous state, with severe hemolytic anemia, and in the heterozygous state with microcytosis, or in asymptomatic cases as an α-thalassemia carrier phenotype. Hb Caserta was observed for the first time in a Casertian family (South Italy) that displayed a slowmigrating haemoglobin upon investigation. We report the clinical phenotype and molecular study of this new double mutant form of haemoglobin in heterozygous and homozygous subjects, as well as in association with α° delectional thalassemia.

Keywords: α-thalassemia, Hb Sun Prairie, Hb Caserta, co-inheritance of globin gene mutations, HbH.

α-Thalassemia is the most common human single gene disorder worldwide (Bernini & Harteveld, 1998; Weatherall *et al*, 2001). In Southern Italy, β-thalassemia is the most common form of thalassemia, though α-thalassemia is also prevalent. In Sicily, α-thalassemia does not constitute a public or clinical health problem because α^+-thalassemia is the predominant form (Fichera *et al*, 1997; Guida *et al*, 2006). However, an accurate diagnosis is important in order to provide genetic counseling for the prevention of severe forms of α-thalassemia that range from mortal Hb Bart's hydrops fetalis to severe HbH disease. Moreover, a proper diagnosis is also needed to differentiate between the phenotypes of heterozygous α-thalassemia and cases that phenotypically resemble α-thalassemia, such as co-inherited δ and β-thalassemia (Giambona *et al*, 2006).

This report describes a new double mutant form of haemoglobin, which has been named Hb Southern Italy. This double mutation originates from the co-inheritance of two known point mutations occurring in the same gene (*HBA2*), namely the codon 26 alteration from GCG to ACG, Ala $\rightarrow$ Thr observed in Hb Caserta (unpublished observation), and the codon 130 alteration GCT $\rightarrow$ CCT, Ala $\rightarrow$ Pro found in Hb Sun Prairie (Harkness *et al*, 1990). Only a few Hb Caserta observations have been reported to date. This variant was identified for the first time in a Casertain family (Ventruto *et al*, 1964), and successively as an unstable hemoglobin in a family from the same region where an α-thalassemia phenotype was present (Unpublished observation).

The Sun Prairie haemoglobin variant was first reported in the homozygous state (Harkness *et al*, 1990) in a young Indian patient with severe hemolytic anemia, microytosis and hypochromia. Subsequently, the same abnormal hemoglobin was described in the heterozygous state in an Asian Indian family with an α thalassemia carrier phenotype (Ho *et al*, 1996).

Materials and methods

Routine examinations

After written informed consents were obtained, fresh blood samples from different members of six unrelated families were collected in Na_2EDTA and analyzed using standard methods.

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The red cell indices were measured on an automated blood cell counter (Beckman ACT-diff; Coulter Corporation Miami Florida, USA). Serum ferritin levels were measured using a Kriptor immunoflorescent assay (Brahams, Hennigsdorf/Berlin, Germany).

Red cell lysates were analyzed by electrophoresis, using an alkaline pH on cellulose acetate (pH 9·0) an acidic pH on agarose citrate (pH 6·0–8·0), and by isoelectric focusing.

HbA, HbA_2, HbF and Hb variants were identified and measured by cation exchange high performance liquid chromatography (HPLC) on a Variant II system (Bio-Rad Laboratories, Richmond, CA, USA) using the β-Thalassemia Short Program provided by the manufacturer. Hb Bart's and HbH were measured using a previously described method, with modifications (Papassotiriou *et al*, 1999).

Heat stability and isopropanol precipitation tests were carried out as indicated by Husman and Jonxis (1977).

Molecular studies

Genomic DNA was isolated from white blood cells using a salting out extraction method, as described by Miller *et al*, 1988.

HBB was analyzed by directly sequencing from the – 130 nt CAP site to the 150 nt 3′ Poly A site. GAP-polymerase chain reaction (PCR) was performed to allow the detection of the most common Mediterranean deletion defects ($-\alpha^{3.7}$, $-\alpha^{4.2}$, α^{-Med}, $\alpha^{-20.5}$, α^{-CAL}), using previously described primers (Foglietta *et al*, 1996; Fichera *et al*, 1997).

HBA1 and *HBA2* were amplified from the – 36 nt CAP site to + 76 nt 3′ Poly-A using forward and reverse primers (Foglietta *et al*, 1996). Direct sequencing was performed with forward (5′-CTG AGC GAC CTG CAC GCG CAC-3′) and reverse (5′-AAG GCG CCA TCT CGC CCC TC-3′) primers using an ABI PRISM 3130xl DNA Analyzer (PE BioSystems, Foster City, CA, USA) and the ABI PRISM Big Dye Terminator v3.1 Cycle Sequencing Kit (PE BioSystems).

Results

Electrophoretic investigations and isoelectric focusing analyses did not reveal any abnormal haemoglobin bands that separated from the normal adult haemoglobin. Analysis of the haematological and electrophoretic Hb characteristics showed a marked homogeneity in all analyzed cases. An α-thalassemia-like phenotype with reduced mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH), and a normal or low level of HbA_2 was observed in all the carrier subjects (Table I).

Case report: clinical and haematological findings

Case A. Proband, a 35-year-old male from Naples (Campania, South Italy), was investigated because his wife (II.2) was an α°-thalassemia (–Med) carrier. The propositus (II.1) had a normal Hb level (149 g/l), but displayed the typical haematological parameters of the α^{+}-thalassemia trait (Table I).

Table I. Haematological and Hb data of the family members.

Subjects	RBC (10^{12}/l)	Hb (g/l)	MCV (fl)	MCH (pg)	HbA_2 (%)	HbF (%)	HbH (%)	SerumIron (μmol/l)	Ferritin (μg/l)	Isopropanol test	Heinz bodies	α-Thal mutation
I.1-A	5·7	139	75·0	26·2	2·6	0·3	–	$11{\cdot}6 \times 10^3$	41	+	n.d.	$\alpha^{Hb\ S.I.**}\alpha/\alpha\alpha$
II.1-A	5·9	149	74·2	25·1	2·4	0·9	–	$15{\cdot}6 \times 10^3$	112	+	n.d.	$\alpha^{Hb\ S.I.}\alpha/\alpha\alpha$
II.2-A	4·7	125	64·1	23·7	2·5	1·2	–	$15{\cdot}2 \times 10^3$	18	n.d.	n.d.	$\alpha^{-MED/}\alpha\alpha$
I.1-B	5·6	149	77·0	26·0	2·3	0·8	–	$23{\cdot}1 \times 10^3$	34	+	–	$\alpha^{Hb\ S.I.}\alpha/\alpha\alpha$
I.2-B	5·1	105	63·0	21·0	2·4	0·9	–	$10{\cdot}9 \times 10^3$	6	n.d.	n.d.	$-\alpha^{20.5}/\alpha\alpha$
II.1-B	5·3	123	68·0	23·0	2·1	0·7	–	$14{\cdot}0 \times 10^3$	8	n.d.	n.d.	$-\alpha^{20.5}/\alpha\alpha$
II.3-B*	3·6	84	78·0	21·0	0·8	1·0	8	$44{\cdot}8 \times 10^3$	680	+	+	$-\alpha^{20.5}/\alpha^{Hb\ S.I.}\alpha$
II.4-B	5·1	126	73·0	24·0	2·3	1·0	–	$22{\cdot}6 \times 10^3$	26	+	n.d.	$\alpha^{Hb\ S.I.}\alpha/\alpha\alpha$
III.1-B	5·9	127	61·0	21·0	2·2	0·9	–	$11{\cdot}3 \times 10^3$	19	n.d.	n.d.	$-\alpha^{20.5}/\alpha\alpha$
III.2-B	6·4	142	66·0	22·0	2·6	0·8	–	$11{\cdot}1 \times 10^3$	41	n.d.	n.d.	$-\alpha^{20.5}/\alpha\alpha$
I.1-C	5·9	147	76·0	25·1	2·8	0·1	–	$21{\cdot}5 \times 10^3$	54	n.d.	n.d	$\alpha^{Hb\ S.I.}\alpha/\alpha\alpha$
I.2-C	5·4	135	73·0	23·7	2·6	0·2	–	$14{\cdot}3 \times 10^3$	45	n.d.	n.d.	$\alpha^{Hb\ S.I.}\alpha/\alpha\alpha$
II.1-C†	3·4	73	69·6	23·7	1·7	0·7	–	$13{\cdot}6 \times 10^3$	–	+	–	$\alpha^{Hb\ S.I.}\alpha/\alpha^{Hb\ S.I.}\alpha$
I.1-D	5·4	142	75·0	26·1	2·8	0·1	–	$16{\cdot}1 \times 10^3$	45	n.d.	n.d.	$\alpha^{Hb\ S.I.}\alpha/\alpha\alpha$
I.2-D	4·9	132	74·1	25·3	2·6	0·3	–	$13{\cdot}4 \times 10^3$	36	n.d.	n.d.	$\alpha^{Hb\ S.I.}\alpha/\alpha\alpha$
II.1-D*	3·8	93	78·5	24·3	1·9	0·6	–	–	113·9	+	–	$\alpha^{Hb\ S.I.}\alpha/\alpha^{Hb\ S.I.}\alpha$
I.1-E	5·5	131	74·3	23·8	2·4	0·3	–	–	99·0	+	n.d.	$\alpha^{Hb\ S.I.}\alpha/\alpha\alpha$
I.1-F	5·9	154	76·1	26·2	2·4	0·9	–	$18{\cdot}3 \times 10^3$	286	+	n.d.	$\alpha^{Hb\ S.I.}\alpha/\alpha\alpha$
II.1-F	6·1	157	74·4	25·9	2·4	0·8	–	$16{\cdot}1 \times 10^3$	170	+	n.d.	$\alpha^{Hb\ S.I.}\alpha/\alpha\alpha$

*Transfused.

**S.I. = Southern Italy.

†Not transfused.

Molecular analysis of *HBB* did not reveal any defect, while direct individual sequencing of the amplified *HBA2* and *HBA1* revealed the presence of two single point mutations, a G → A transition at codon 26 (GCG → ACG) and a G → C base substitution at codon 130 (GCT → CCT). Familial analysis showed that both base substitutions were inherited from the father (I.1) and they were present in the same *HBA2* gene. This composite allele was also associated in cis with the *HBA2* +861 G → A polymorphic site in the downstream untranslated region.

Prenatal diagnosis for this α-thalassemia at risk couple was required.

Case B. Seven members belonging to three generations of a Neapolitan family were examined (Fig 1, Table I). The propositus, a 49-year-old woman (II.3) affected by α-thalassemia with HbH, who had been subject to transfusion since the age of three years, was referred to the Microcitemia Unit Cardarelli Hospital in Naples. HPLC analysis showed 8% HbH and Heinz-bodies were clearly visible. The father (I.1) had normal levels of haemoglobin, slightly reduced red cell indices while HbA, HbA_2 and HbF were normal without any pathological bands. The mother (I.2) showed an α°-thalassemia-like phenotype.

Molecular analyses were performed on the three subjects, and extended to other members of the family. *HBA* mapping demonstrated abnormal *HBA* arrangements in all analyzed subjects. The father carried two point mutations at codon 26 and at codon 130, and the +861 G → A polymorphic site in *HBA2* (Fig 2). The mother was a carrier of the Mediterranean deletion $-\alpha^{20.5}$, while the affected propositus had one allele containing the $-\alpha^{20.5}$ deletion and the other allele contained the same two mutations in *HBA2* observed in the father, but in the homozygous state (Fig 2).

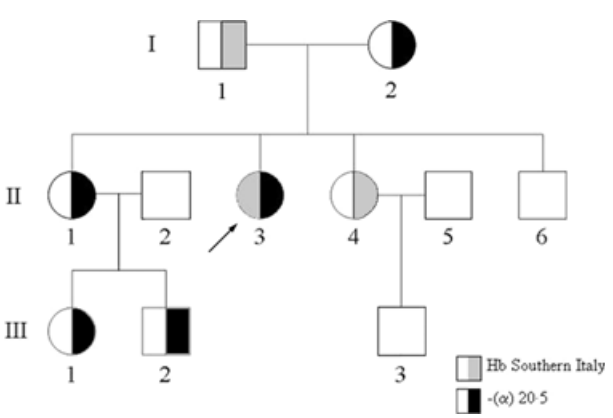

Fig 1. Pedigree of the three generation members of family B showing the segregation of the codon 26, codon 130 mutations and polymorphic site at position *HBA2* +861. Half solid symbols indicate members heterozygous for Hb Southern Italy or for –α (20·5) deletion, solid symbol indicates the affected propositus carrier of Hb Southern Italy and –α (20·5). The propositus is indicated by an arrow.

Two other sons (II.1 and II.4) were also examined: one was a carrier of the same α°-thalassemia characteristic shown by the mother, whereas the other subject displayed the fathers double *HBA2* mutation in the heterozygous state. Subject II.1 married a normal partner and generated carrier children with similar phenotypes (Fig 1).

Case C. Proband (II.1), a 50-year-old female from Alcamo village (Western, Sicily), was screened for thalassemia at the Haematology Unit of Cervello Hospital in Palermo. The patient had been diagnosed with an unclassified thalassemic phenotype (growth retardation, pallor, severe anemia, microcytosis and marked splenomegaly) from the age of three in different hospitals. Haematological analysis of the proband revealed that her Hb values were in the range of 80–100 g/l with a median of 92 g/l, and she had previously received blood transfusions during acute infection. At an older age, the patient's hemoglobin level was lower (5·0–8·0 g/dl), and she presently refuses further blood transfusions.

Molecular analysis by directly sequencing of *HBA2* revealed the occurrence of the two single point mutations that are characteristic of Hb Caserta and Hb Sun Prairie, and the +861 G → A polymorphic site in the homozygous state (Fig 2). Her parents (I.1, I.2) were cousins and molecular analysis confirmed that both were carriers of the new Hb variant (Table I). This is the first report of the Hb Southern Italy mutation in the homozygous state.

Case D. A 22-year-old male (II.1) from Calatafimi (Trapani-Western Sicily) showed the typical features of thalassemia intermedia, with low levels of Hb (about 90 g/l). Before splenectomy at the age of 20 years, the patient's Hb level was about around 70 g/l. His father and mother (I.1, I.2) originated from the same country, and had typical α^{+}-thalassemia carrier phenotypes. Molecular analysis of the propositus revealed the presence of the Hb Southern Italy mutation as well as the *HBA2* +861 G → A polymorphic site in the homozygous state. Both defects were inherited from his parents who were heterozygotes for the new variant (Table I).

Three additional heterozygotes for Hb Southern Italy, in association with the *HBA2* +861 G → A polymorphic site, were identified in two independent families: a 28-year-old Sicilian man from Alcamo (I.1-E), and two subjects (father and son, (I.1-F and II.1-F) from Naples (Table I).

Discussion

Hb variants with more than one point mutation in the same polypeptide chain are rare; so far 29 such variants have been described in the beta chain, while none has been identified in the alpha chain (database of Human Hemoglobin Variants http://globin.cse.psu.edu; Blackwell *et al*, 1972).

Hb Southern Italy is the first variant haemoglobin of *HBA2* that originated from an unusual combination of two known molecular defects, GCG → ACG at codon 26 that was

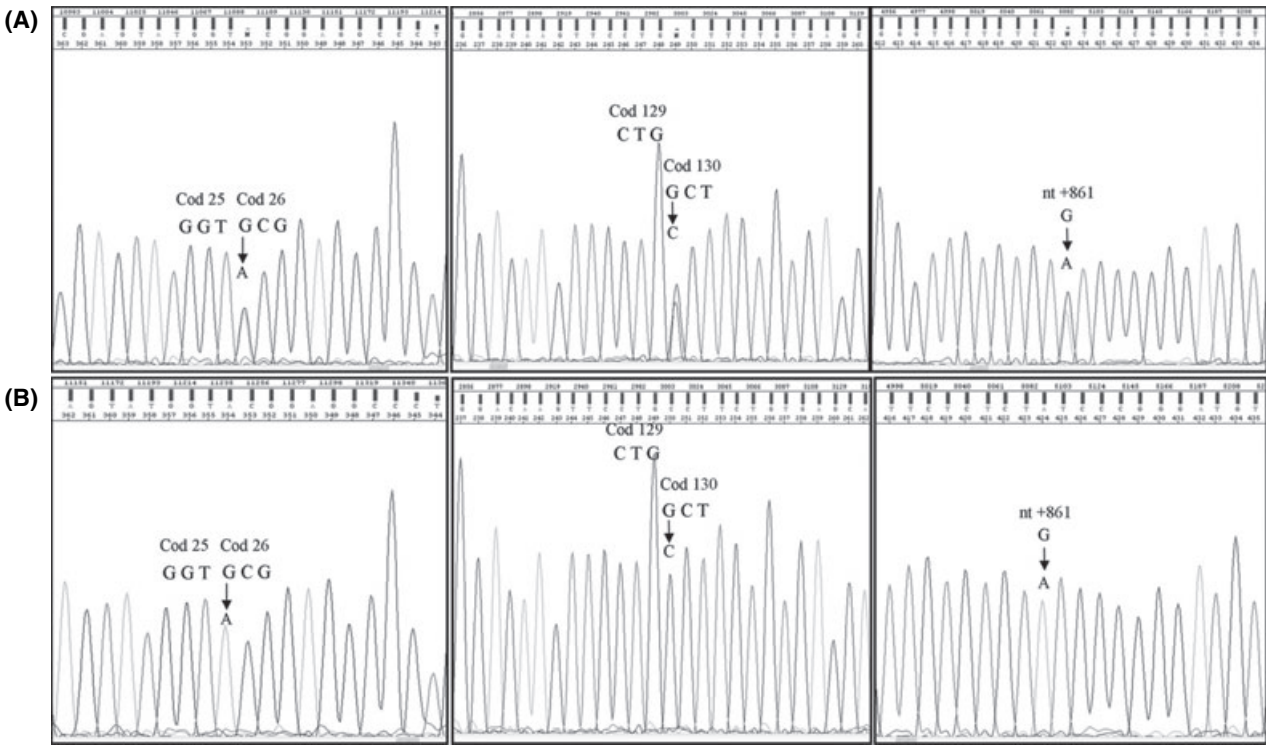

Fig 2. Sequencing of the DNA from carrier for Hb Southern Italy on the upper panel (a). The analysis shows codon (Cod) 26 GCG → ACG mutation, codon 130 GCT → CCT and polymorphic site at position *HBA2* +861 G → A in heterozygous state. The lower panel (b) shows the DNA sequencing of an affected propositus. The codon mutations and the polymorphic site are found in the homozygous state.

previously found in Hb Caserta, and GCT → CCT at codon 130, already described in Hb Sun Prairie.

Hb Caserta was described for the first time in a family from Caserta (Southern Italy) as a slow-migrating form of haemoglobin, and then as a variant haemoglobin with reduced haematological parameters (MCV and MCH) and normal HbA_2. More recently, Hb Caserta was described to be in association with an α-thalassemia-like phenotype, and it was suggested that there was a decreased production of normal mRNA due to the activation of a cryptic splicing site at codons 25–27 (unpublished observations). These reports did not mention any other alterations (mutations or polymorphisms) in *HBA2*, either because they were absent or because *HBA2* was not completely analyzed by sequencing methods. The results presented here suggest that when new or rare mutations are observed and little information is available, entire *HBA1*, *HBA2* and *HBB* genes should be completely analyzed using sequencing method.

In Hb Caserta, a conservative replacement of an alanine residue with threonine occurs within the B helix, buried in the tertiary structure of the α-globin chain and not involved in the α/β contacts or in the heme globin linkage. Taking these factors into consideration, it may be suggested that this mutation should not significantly affect Hb function.

Two other Hb variants at codon 26 of *HBA2* have previously been described, Hb Campinas C → T, Ala → Val (Wenning *et al*, 2000) and Hb Shenyang C → A, Ala → Glu (Zeng *et al*, 1982). The first variant was found to be functionally normal, while the latter abnormal Hb was slightly unstable.

Hb Sun Prairie was also associated with another molecular defect in a patient of Indian origin (Sarkar *et al*, 2005). The authors described Hb Sun Prairie in association with a C → T transition in the 5′ untranslated region (UTR) of the same *HBA2* gene. Molecular modelling studies did not display large distortions in the Hb molecule, suggesting that the severe clinical picture of the patients carrying these mutations might have been related to the co-inheritance of the mutation in the 5′-UTR of the same *HBA2* gene containing the Sun Prairie substitution. The authors then suggested that the co-occurrence of these two defects would lead to an additional depression of *HBA2* expression.

We have also investigated a 1-year-old girl of Philippine origin who displayed thalassemia with splenomegaly and hepatomegaly. Her Hb level was lower than 83 g/l and she was regularly treated with blood transfusions. Molecular analysis of the proband and her parents showed that she had inherited Hb Sun Prairie from her father and a deletional α^0-allele from her mother (–SEA). Sequencing analysis of the propositus and her father did not indicate the presence of any further mutations, either at the 5′UTR or at codon 26, and the polymorphic site *HBA2* +861 was also absent.

Our molecular analysis provided evidences that the mutation at codon 130 present in the Hb Sun Prairie variant (identified in the Indian population and in Eastern Indians) and in the Hb Southern Italy double variant, has different origins. In the new Hb Southern Italy variant, mutation at codon 130 is always associated with mutation at codon 26 and with the polymorphic site *HBA2* +861.

Two possible mechanisms may have contributed to the formation of Hb Southern Italy allele. First, a crossover between a gene encoding Hb Caserta and a gene encoding Hb Sun Prairie. The Mediterranean basin was the crossroads of the world for thousands of years and was traversed by several races, each of which spread their ideas and cultures. The geographical position of Sicily and Campania made them a natural seaport point on these travels. Large numbers of Greeks, Byzantines, Saracens, and Normans came to Campania to settle, and not just to rule and exploit the region. Each of these people contributed to the gene pool and left an imprint on the genetic composition of the Campanian people.

Alternatively, the variant allele might have been originated by the occurrence of two separate point mutational events, perhaps sequentially, although the specific family in which one of the point mutations existed in a parent and both in a child, could not be found.

The founder effect would explain the unexpected frequency of this rare type of mutation in a restricted area like South of Italy allele.

The phenotypes of both homozygote and heterozygote subjects for Hb Sun Prairie described by other authors were very similar to those of our patients carrying the Hb Southern Italy variant in the homozygous and heterozygous states. Both groups of patients showed α-thalassemia-like carrier phenotypes with microcytosis and normal HbA_2 levels in the heterozygous state, while chronic hemolytic anemia was observed in homozygotes. However, the instability of the Hb Southern Italy variant, which is rapidly catabolized and therefore not detected by routine analysis of hemolysate, was slightly higher than Hb Sun Prairie. The variant alpha globin from Hb Sun Prairie, but not that from Hb Southern Italy could be detected by liquid chromatography mass spectrometry analysis (LC-MS) (data not shown), which suggests that the mutation at codon 26 might somehow contribute to the instability of Hb Southern Italy. However, the reduction of *HBA2* expression and the consequent severity of the clinical picture in patients with the Hb Southern Italy variant should essentially be associated with the mutation in codon 130.

It is important to note that, although, in the homozygous state Hb Southern Italy presents as a severe anemia, as in the Hb Sun Prairie homozygous state, this does not necessarily require transfusion. However, the combination of Hb Southern Italy with an α^0-thalassemia deletional determinant leads to the presentation of a severe HbH disease, which is blood transfusion-dependent.

References

Bernini, L.F. & Harteveld, C.L. (1998) Alpha-thalassaemia. *Bailliéres Clin Haematol*, **11,** 53–90.

Blackwell, R.Q., Boon, W.H., Liu, C.S. & Weng, M.I. (1972) Hemoglobin J Singapore: alpha 78 Asn–Asp; alpha 79 Ala–Gly. *Biochimica et Biophysica Acta*, **278,** 482–490.

Fichera, M., Spalletta, A., Fiorenza, F., Lombardo, T., Schilirò, G., Tamouza, R., Lapoumeroulie, C., Labie, D. & Ragusa, A. (1997) Molecular basis of thalassemia in Sicily. *Human Genetics*, **99,** 381–386.

Foglietta, E., Deidda, G., Graziani, B., Modiano, G. & Bianco, I. (1996) Detection of α-globin gene disorders by a simple PCR methodology. *Haematologica*, **81,** 387–396.

Giambona, A., Passarello, C., Ruggeri, G., Renda, D., Teresi, P., Anzà, M. & Maggio, A. (2006) Analysis of delta-globin gene alleles in the Sicilian population: identification of five new mutations. *Haematologica*, **91,** 1681–1684.

Guida, V., Colorino, A., Fichera, M., Lombardo, T., Rigoli, L. & Dalla piccola, B. (2006) Hematologic and molecular characterizaion of a Sicilian cohort of alpha-thalassemia carriers. *Haematologica*, **91,** 409–410.

Harkness, M., Harkness, D.R., Kutlar, F., Kutlar, A., Wilson, J.B., Webber, B.B., Codrington, J.F. & Huisman, T.H. (1990) Hb Sun Prairie or alpha(2)130(H13)Ala——Pro beta 2, a new unstable variant occurring in low quantities. *Hemoglobin*, **14,** 491–497.

Ho, P.J., Rochette, J., Rees, D.C., Fisher, C.A., Huehns, E.R., Will, A.M. & Thein, S.L. (1996) Hb Sun Prairie: diagnostic pitfalls in thalassemic hemoglobinophathies. *Hemoglobin*, **20,** 103–112.

Husman, T.H.J. & Jonxis, J.H.P. (1977) *Techiniques of Identification. Clinical and Biochemical Analysis*. Vol. **6,** Marcel Dekker. Inc., New York.

Miller, S.A., Dyles, D.D. & Polesky, H.F. (1988) A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acid Research*, **16,** 1215.

Papassotiriou, I., Traeger-Synodinos, J., Vlachou, C., Karagiorga, M., Metaxotou, A., Kanavakis, E. & Stamoulakatou, A. (1999) Rapid and accurate quantitation of hemoglobins Bart's and HbH using weak cation-exchange high pressure liquid chromatography: correlation with the alpha thalassemia genotype. *Hemoglobin*, **23,** 203–211.

Sarkar, A.A., Mukhopadhyay, C., Chandra, S., Banerjee, S., Das, M.K. & Dasgupta, U.M. (2005) Co-inheritance of Hb Sun Prairie mutation with point mutation at 5′-UTR in the eastern Indian population. *British Journal of Haematology*, **129,** 282–286.

Ventruto, V., Derosa, L., Bianchi, P., Ricchi, L. & Quattrini, N. (1964) Description of a new slow hemoglobin in a Casertian family (Hb Caserta). *Il Progresso Medico*, **20,** 157–162.

Weatherall, D.J., Clegg, J.B., Higgs, D.R. & Wood, W.H. (2001) The hemoglobinopathies. In: *The Metabolic and Molecular Bases of Inherited Disease* (ed. by C.R. Scriver), pp. 4571–4636. McGraw-Hill, New York.

Wenning, M.R., Silva, N.M., Jorge, S.B., Kimura, E.M., Costa, F.F., Torsoni, F., Ogo, S.H. & Sonati, M.F. (2000) Hb Campinas [alpha26(B7)Ala –>Val]: a novel, electrophoretically silent, variant. *Hemoglobin*, **24,** 143–148.

Zeng, Y.T., Huang, S.Z., Zhou, X.D., Qiu, X.K., Dong, Q.Y., Li, M.Y. & Bai, J.H. (1982) Hb Shenyang (alpha 26 (B7) Ala replaced by Glu): a new unstable variant found in China. *Hemoglobin*, **6,** 625–628.

