

The homozygous state of Hb J Sardegna

Paola Pistidda¹, Luciana Guiso¹, Laura Frogheri¹ and Maurizio Longinotti^{*1}

¹Istituto CNR Patologia del Sangue, Sassari Italy; ²Istituto di Ematologia, Università di Sassari, Sassari, Italy

Hb J Sardegna is a well known innocent Hb variant which is widespread in Sardinia. As yet, homozygosity for Hb J Sardegna has not been documented. This report deals with the homozygous state for Hb J which we demonstrate by molecular analysis in two Sardinian siblings in which β -thalassemia coexists. The Hb J specific mutation was determined both by enzyme digestion and by sequencing specific segments of PCR amplified α -globin genes. A pregnant girl showed mild non-sideropenic microcytic anemia, normal Hb A₂ levels (2.4%) on DE-52 microchromatography, 50% of Hb variant on HPLC and 2.1 α/β globin chain biosynthetic ratio. She proved to be a carrier of the $\beta^{\circ}6(-A)$ thalassemia determinant. The α -globin gene mapping did not reveal α -thalassemia. Btg I restriction analysis of both α_2 -globin genes showed a recognition site defect for this enzyme in both chromosomes, which resulted to be the C→A point mutation in homozygosity at the first nt of α_2 -globin gene 50th codon by sequencing. This defect, typical of Hb J Sardegna, was also present in her brother. From a practical point of view, this study demonstrates that the association of β -thalassemia with Hb J, may show falsely reduced Hb A₂ levels on routine Hb A₂ quantitation techniques, such as DE-52 microchromatography. This possibility implies that identification methods such as simple Hb electrophoresis, which permit visualization of Hb A₁² should be used in thalassemia screening involving populations in which Hb J and β -thalassemia coexist.

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The clinically innocent hemoglobin J Sardegna (Hb J) is the most common α -globin structural mutant in Northern Sardinia where it occurs with an incidence of ~0.25%.^{1,2}

The molecular base of Hb J is a single C→A substitution in the first position of codon 50 of the α_2 -globin gene.³ This mutation, removing a recognition site for the DsaI restriction enzyme can be quickly recognized by PCR-amplified α -globin gene DNA digestion. It should be emphasized that Paleari *et al.*,³ demonstrating that a posttranslational deamidation occurs in this human Hb variant also defined its actual structural formula, that is Hb J [α 50(CD8)His→Asn→Asp].

To the best of our knowledge, the homozygous state for Hb J has not yet been found even during the largest mass hemoglobinopathy screening in Sardinia^{2,4,5} (Galanello R., personal communication, June 2001). As we have recently had this chance, that is of 1:640 000

cases on the basis of ~0.25% Hb J frequency, we describe here a Sardinian family in which two siblings, carriers of the rare $\beta^{\circ}6(-A)$ thalassemia mutation, resulted, on molecular analysis, homozygotes for Hb J Sardegna.

Case report

During a voluntary thalassemia screening, a pregnant girl (the proband, II-1, Table 1) displayed mild, non-sideropenic, microcytic anemia, hypochromia and normal Hb A₂ levels (2.4%) at DE-52 microchromatography. On visual inspection of Hb cellulose-acetate electrophoresis, pH 8.9, a ~50% J Sardegna-like band was present as well as a ~2% Hb A₁²-like band moving between the Hb A and the Hb A₂ (data not shown). Hematological, hemoglobin and biosynthetic data were obtained by standard methods.⁵ The family study (Table 1) demonstrated that the proband's brother (II-2) had identical hemoglobin and hematological features. Electrophoresis showed both parents to have ~25% Hb J as well as faint Hb A₁²-like bands. The imbalanced α/β ratio of 2.1 in the proband proved the presence of β thalassemia: the frameshift $\beta^{\circ}6(-A)$ mutation, rare in Sardinia, was in fact demonstrated in members I-2, II-1 and II-

*Correspondence: M Longinotti, Istituto di Ematologia, Università di Sassari, Viale San Pietro 12-07100 Sassari, Italy;
Tel: 39-079-228280; Fax: 39-079-228282;
E-mail: M.Longinotti@ipsoc.ss.cnr.it
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Table 1 Hematological phenotype and α - and β -globin genotypes of the family with Hb J-Sardegna homozygosity and β -thalassemia.

Hb	MCV (fL)	HbA ₂ (%)	Hb-J (%)	α/β ratio	α -globin genotype	β -globin genotype
I-1	14.7	88	1.6 ^b	25	n.d.	β^A/β^A
I-2	10.1	62	3.3 ^b	25	n.d.	$\beta^{6(-A)}/\beta^A$
II-1 ^a	11.1	65	2.4 ^b	50	$\alpha\alpha/\alpha\alpha$	$\beta^{6(-A)}/\beta^A$
II-2	12.2	61	2.8 ^b	50	n.d.	$\beta^{6(-A)}/\beta^A$

^aSubject II-1: Proband. ^bMean of repeated determinations by DE 52 microchromatography.

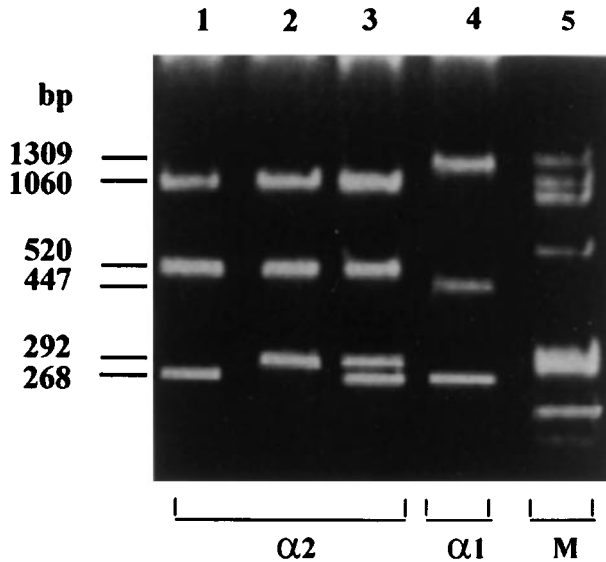


Figure 1 BtgI digestion of PCR amplified α_2 (1907 bp) and α_1 (2162 bp) gene fragments. Lines 1-2-3, α_2 genes: line 1 normal subject (268 bp); line 2 proband homozygous for Hb J Sardegna (292 bp); line 3 mother heterozygous for Hb J Sardegna (268 + 292 bp); Line 4, α_1 genes proband (normal 268 bp fragment); Line 5: ϕ x174 Hae III DNA marker.

2 by the Amplification Refractory Mutation System (ARMS).

At this stage, these data and the family hemoglobin pattern distinctly pointed to homozygosity for Hb J interacting with $\beta^{6(-A)}$ thalassemia. Therefore we performed the Hb J mutation investigation following the described original approach³ with minor modifications. We chose to amplify first the α_2 and α_1 globin gene segments from position -1024 to +883 and from position -1273 to +889 to CAP sites respectively, by the common upstream primer 5'-GATGCACC-CACTGGACTCCT-3' and by the downstream primers 5'-CCATTGTTGGCACATTCCGG-3' (α_2) and 5'-CCATGCTGGCACGTTTCTGA-3' (α_1).

The $-\alpha^{3.7}/\alpha\alpha$, $-/\alpha\alpha$, $\alpha\alpha/\alpha^{NcoI}\alpha$ and $\alpha\alpha/\alpha^{Hpb1}\alpha$ thalassemias were excluded on these PCR products by standard α -globin gene mapping. The α -globin gene fragments were then digested by BtgI which recognizes the sequence C ↓ C (A or G) (T or C) GG. Finally these α_2 and α_1 globin gene PCR products were sequenced by the Sanger dideoxy chain termination method on an automated analyser (ALF-Pharmacia)

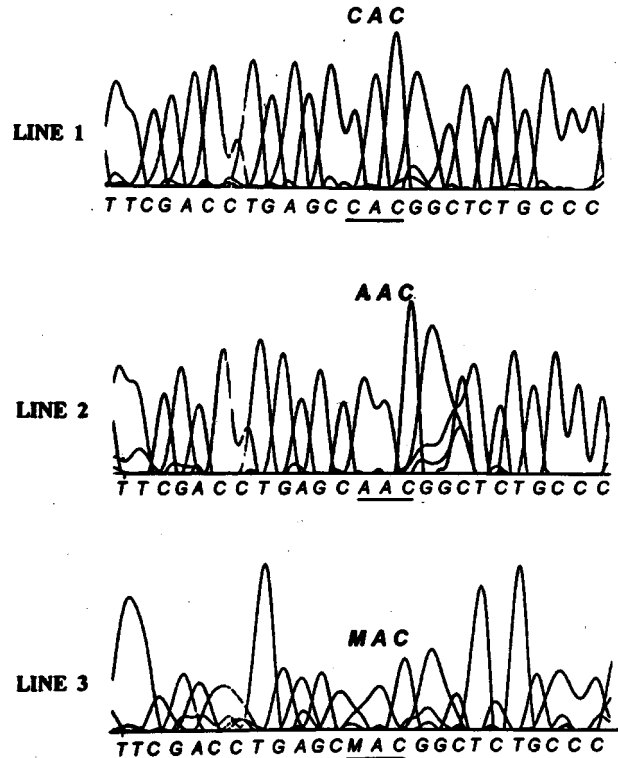


Figure 2 Sequencing of α -genes: the 50th codon is underlined. Lines 1 and 2 (proband) show the homozygosity for normal CAC codon (line 1, α_1 genes) and mutated AAC codon (line 2, α_2 genes). Line 3 (mother) shows an ambiguity C,A indicated by letter M, in the first nt of the 50th codon of the α_2 globin genes which is to be expected in heterozygosity for Hb J.

using a cycle sequencing kit (Thermo Sequenase fluorescent cycle sequencing kit, Amersham Pharmacia Biotech) and a common fluorescent reverse primer located at position +476 to 493 from Cap sites in IVS II regions.

The BtgI restriction enzyme analysis pattern (Figure 1) demonstrated that the parents and their children were heterozygotes and homozygotes respectively for a defect near codon 50, very likely the C→A mutation in the first nt, of the selectively amplified α_2 -globin gene. This evidence was confirmed by analysing the relevant segment of α_2 and α_1 -globin genes. Direct sequencing of DNA of the proband II-1 detected the CAC→AAC single substitution at the first position of codon 50 in both α_2 genes while the normal triplet CAC was present at the same position in the α_1 genes (Figure 2).

Discussion

Our conclusions that subjects II-1 and II-2 are Hb J homozygotes, suspected as a result of the pattern of inheritance as well as of the electrophoretic mobility of their Hb variant levels, are based on the following molecular findings: (1) BtgI digestion pattern of amplified DNA in the family carriers of 25% Hb variant which shows the presence of both mutated 292

and normal 268 bp fragments; while the proband II-1 with 50% Hb variant shows the mutated 292 bp fragment only (Figure 1). This newly generated 292 bp fragment is in fact compatible with the C→A mutation at codon 50 in the α_2 globin gene which removes the BtgI recognition site; (2) direct sequencing of DNA of separately amplified α_2 and α_1 globin genes which detects the same C→A nucleotide substitution in the first position of codon 50 in both α_2 globin genes of the proband. As expected, sequencing of α_2 genes of the heterozygous mother shows a C,A ambiguity at the same position. Finally, both the proband's α_1 genes had the normal α^A -globin sequence (Figure 2). In conclusion, C→A single point mutation at codon 50 found in both α_2 genes of the proband can be exclusively ascribed to the homozygous state of Hb J Sardegna.

From a practical point of view, these Hb J homozygous subjects are healthy, their phenotypic

alteration being a very mild microcytic anemia quite identical to that shown by β^0 -thalassemia carriers and by β^0 -thal/Hb J compound heterozygotes.⁶ In this respect, it is worth noting that the 3.3% borderline Hb A₂ value in the Hb J heterozygous mother was further reduced (2.4 and 2.8%) in the homozygous offspring. As anecdotally reported,⁷ these false-normal Hb A₂ determinations could probably be ascribed to the additional negative charges of mutated Hb A₂($\alpha^J_2\delta_2$) which elutes separately from the normal Hb A₂ whether on DE-52 microchromatography or on cation exchange HPLC. In practice, neither of the two methods, routinely employed in mass thalassemia screening, visualize the Hb A₂ fraction, unlike the classic and inexpensive Hb electrophoresis or isoelectric focusing. This misleading phenomenon must be borne in mind when analyzing members of at-risk couples in which Hb J and β -thalassemia might coexist.

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