Haematologica 2000; 85:899-901
original paper

Red Cells & Iron

A new polymorphism (G→A) in the ψζ1 globin gene

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ABSTRACT

***Background and Objectives*. α-globin cluster polymorphisms are obtained with specific restriction enzymes (Xba I, Eco RI, Sac I, Apa I, Bgl II, etc) that can also have implications for genetic analysis.**

***Design and Methods*. We studied three unrelated patients; one from Argentina, one from Spain and one from Australia but of Polish origin. Genomic DNA was digested with several different restriction enzymes and probes, amplified and sequenced with an ABI Prism 310 sequencer.**

***Results*. Three patients had an abnormal 26 kb band when their DNA was studied with restriction enzyme Bgl II and ζ probe. A fragment of 944 bp was amplified with primers that cover from -280 to +714 bp of the recognition sequence of Bgl II enzyme (AGATCT) localized 5' from pseudogene ζ1. After digestion of this PCR product with Bgl II, two fragments of 714 and 280 bp were produced in normal controls, whereas in patient #1 the PCR fragment was undigested and in patients #2 and #3 both undigested and digested fragments were observed. Sequencing of the PCR fragment showed that in all three patients it was the same polymorphism (G→A) at nucleotide 153171 of the 16 p sequence found in the Bgl II recognition site that changed to AAATCT.**

***Interpretation and Conclusions*. We describe a new polymorphism in the ψζ1 first exon Bgl II restriction site (G→A). The polymorphism is associated in cis with haplotype -α3.7. The fragment obtained by PCR enabled us to corroborate the presence of the polymorphism quickly without having to use complicated sequencing techniques.**

Key words: thalassemia, Hb H disease, polymorphism, Bgl II enzyme

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The two structural genes for the α-globin chain are located on the short arm of chromosome 16, in band 13.3. The α-globin gene cluster is arranged in the following order: 5'-ζ2-ψζ1-ψα2-ψα1-α2-α1-θ-3'.[1,2] α-thalassemias are usually produced by deletion of one, two, three or four α genes. The loss of two α genes within the same chromosome produces an α^0-thalassemia, in which there is complete absence of α-chain synthesis. These deletions can affect from 5.2 to more than 100 kb of DNA, in the latter case affecting the complete α-like gene complex.[3]

We describe a new polymorphism in the ψζ1 first exon *Bgl* II restriction site (G→6A) in three unrelated families from different countries.

Design and Methods

The first patient is Argentinian of Spanish origin. We also studied her son and her sister. The second is Spanish, her son was also analyzed. The third is Australian of Polish origin.

Hematologic data were obtained with a Coulter STKS.

DNA was isolated from the white blood cells, digested with several different restriction enzymes and hybridized with the following probes: a 1.8 kb *Sac* I ζ fragment, a 1.5 kb *Pst* I α fragment, a 1.1 kb *Alu* I IZHVR fragment and a 4.0 kb *Hinf* I 3'HVR fragment.[4]

Up to 200 ng of DNA was amplified with the primers 5'-AAGGGGAGACAGAAGCGATAGG-3' and 5'-GATGGTTGTGCAACTCCTAATCGT-3'. Following amplification, 450 μL of the product were electrophoresed in a 1.5% agarose gel and the 994 bp fragment produced by PCR was extracted and purified and directly sequenced with the previously cited primers and in one patient was also cloned in pGEMTM-T vector (Promega, Madison, WI, USA) and was sequenced with the primers 5'-GTAAAACGACGGCCAGT-3' and 5'-TTCACACAGGAAACAG-3'.

Sequencing was done in a ABI PRISM 310 sequencer (Perkin-Elmer Cetus, Norwalk, CT, USA).

Results

Three unrelated patients with α-thalassemia were characterized by Southern blot.

Hematologic data are listed in Table 1.

Table 1. Hematologic data.

Patient	*#1*	*#2*	*#3*
Sex/age (years)	F/34	F/40	F/20
Genotype	--/-$\alpha^{3.7}$	-$\alpha^{3.7}$/-$\alpha^{3.7}$	-$\alpha^{3.7}$/$\alpha\alpha$
RBC (10^{12}/L)	5.0	4.9	5.2
Hb (g/dL)	8.3	9.9	12.6
MCV (fL)	58.4	6.37	7.4
CHM (pg)	16.7	2.01	24.5
MCHC (g/dL)	29.4	3.15	32.7
Hb F (%)	2.0	2.0	2.0
Hb A2 (%)	1.3	1.55	2.9
Inclusion bodies	(+++)	(–)	(–)

Table 2. Family I: α-globin gene fragments identified with several enzymes and probes (I).

Probe	*Enzyme*	I_1	I_2	II_1
α	*Bam* HI	10.5	14	14
	Bgl II	26	12;7.4	12; 7.4
	Eco RI	19	23	23
ζ	*Bam* HI	10.5; 5.4	10.5; 5.4	10.5; 5.4
	Bgl II	26; 13.9	12; 11	13.9; 12; 11
	Eco RI	19; 4.6	23; 4.6	23; 4.6
	Hind III	16; 13	16;13	16;13
IZHVR	*Bam* HI	10.5; 8	10.5	10.5; 8
	Bgl II	26; 13.9	11	13.9; 9
	Eco RI	19; 15	23	23; 15
	Hpa I	13; 11	11	13; 11
	Hind III	16; 8	16	16; 8
	Acc I	8; 5	8.5; 7	8; 5
3' HVR	*Bam* HI	11	11; 10.9	11
	Bgl II	6.6	6.6; 6.4	-
	Eco RI	15; 10	10; 9.9	-
	Hpa I	15; 13	14	14; 13
	Sac I	15; 10	14	14; 10
	Puv II	4.1	4; 3.8	4.1; 3.9

Patient #1 (I_1), her sister (I_2) and her son (II_1).

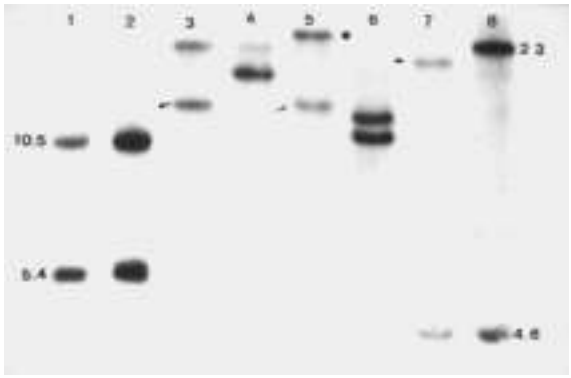

Figure 1. DNA analysis with the ζ probe. Lanes 1, 3, 5 and 7 correspond to patient #1. Lanes 2, 4, 6 and 8 correspond to a control (her sister). Lanes 1 and 2: Bam HI; lanes 3 and 4: Xba I; lanes 5 and 6: Bgl II; lanes 7 and 8: Eco RI. The comma corresponds to the-$\alpha^{3.7}$ deletion, the arrow to the – – MED deletion and * to the Bgl II polymorphism.

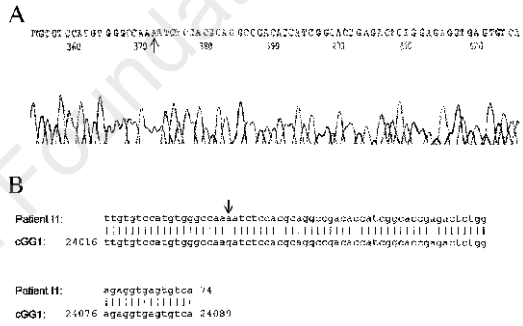

Figure 2. A. DNA sequencing of patient #1 shows the guanine to adenine substitution, above the arrow, at the level of the recognition sequence of the Bgl II enzyme. B. Comparision of patient #1's sequence (top) and the cosmid cGG1 (bottom).

Patient #1. The Southern blot study with DNA digested with the restriction enzyme *Bgl* II and hybridized with the ζ probe showed abnormal bands of about 26 and 13.9 kb. The 26 kb band was detected with α, ζ and IZHVR probes in *Bgl* II digested DNA, but not with the probe 3'HVR (Table 2). The patient's son and sister were also analyzed, but the 26 band was not detected.

Digestion of DNA with *Bam* HI, *Eco* RI, *Hpa* I, *Hind* III and *Acc* I and hybridization with IZHVR probe showed, in both the mother and the son the abnormal fragments corresponding to the --MED mutation (Figure 1).

Patient #2. The second patient showed a homozygous $\alpha^{3.7}$ deletion. When this DNA was studied with the ζ probe, bands of 16 and 11 kb appeared, corresponding to the former deletion, but the 26 kb band was again detected. The patient's son had 12, 11 and 26 kb bands after digestion and hybridization with with *Bgl* II-ζ, consistent with the same deletion and polymorphism.

Patient #3. Southern blot analysis showed bands of 14 and 10.5 kb with the *Bam* HI-ζ probe; and 12, 11 and 26 kb bands with *Bgl* II-ζ.

By using PCR of the DNA from the three patients, a 994 bp fragment was obtained with primers spanning -280 and +714 bp to the recognition sequence of *Bgl* II enzyme (AGATCT) localized in the first exon of the $\psi\zeta_1$ gene. After digestion of this PCR product with *Bgl* II, two fragments of 714 and 280 bp were produced in normal controls, whereas in patient #1 the PCR fragment was undigested and in patients #2 and #3 both undigested and digested fragments were observed. Sequencing of the PCR fragment showed that in all three patients the same polymorphism (G→A) at nucleotide 153171 of the 16p sequence localized in the *Bgl* II recognition site had changed to AAATCT (Figure 2).

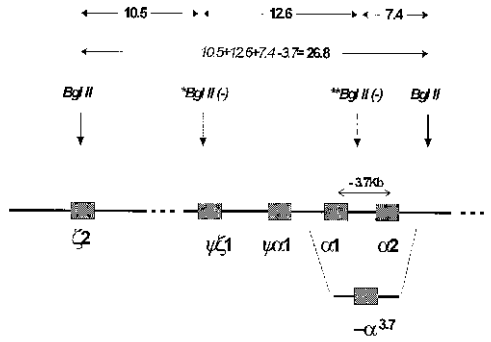

Figure 3. Map of the α-globin gene complex. Distances between Bgl II sites are given in Kb. The polymorphic Bgl II restriction site is indicated by a broken arrow (*). The loss of the Bgl II restriction site due to the 3.7 deletion is indicated by a broken arrow (**).

Discussion

The α-globin cluster is comprised of 4 genes (α1, α2, θ, ζ_2), 3 pseudogenes ($\psi\zeta_2$, $\psi\alpha_1$, $\psi\alpha_2$) and a series of hypervariable regions of different length which provide a useful genetic marker for the α gene cluster.[5] In addition to these variable regions, polymorphisms are obtained with specific restriction enzymes (*Xba* I, *Eco* RI, *Sac* I, *Apa* I, *Bgl* II, etc.)[6] that can also have implications for genetic analysis.[7]

Two percent of the Spanish population present a kind of polymorphism described in other Mediterranean populations (Greek, Cypriot, Sardinian),[7] in Mexicans[8] and in Polynesians,[9] that consists of the presence of a cut-off point with the *Bgl* II enzyme between pseudogenes $\psi\zeta_1$ and thel $\psi\zeta_1$ (5' to $\psi\zeta_1$) that produces two specific fragments of 10.5 and 5.2 kb that are detected after hybridization with the ζ probe.

However, the cases studied here present an unusual restriction fragment length polymorphism (RFLP) associated with the $\psi\zeta_1$ gene. As far as we are aware, this is the first time cases like this have been reported in the literature. In contrast to the previous case, this polymorphism is due to the absence of the cut-off point of the *Bgl* II restriction polymorphism in the first $\psi\zeta_1$ exon (153171 position), after stop codon. This polymorphism is not of biological significance but does have diagnostic importance giving rise to a long 30 kb fragment. Because of a cis association with haplotype $-\alpha^{3.7}$ this results in a 26 kb fragment (Figure 3).

The fragment of 994 bp obtained by PCR with primers covering from -280 to +714 bp is digested with the enzyme *Bgl* II. If there is polymorphism the fragment remains unchanged (994 bp) because of absence of the site of enzyme restriction, while in a normal individual two fragments, of 280 and 714 bp, appears.

This method enables us to corroborate the presence of the polymorphism quickly in patients bearing haplotype $-\alpha^{3.7}$/ who show a fragment of 26 kb instead of the expected 16 kb (enzyme *Bgl* II, probe ζ). This gives us a quick result without having to use complicated sequencing techniques.

Contributions and Acknowledgments

AV is the chief of the Service, contributed to the conception of the study and wrote the paper. EA and PR performed the cloning and sequencing studies. NN and ACM sent us the case from Argentine. FA and JS designed the study and wrote the paper. DB sent us case #3. DE and DH gave critical comments and approved the final version of the manuscript.

Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous publications.

Manuscript processing

Manuscript received May 3, 2000; accepted July 18, 2000.

Potential implications for clinical practice

- We describe a new polymorphism in the ψζ1, first exon *Bgl*II restriction site (G-A). The polymorphism is associated in cis with haplotype –α3.7, creating a fragment of about 26kb with *Bgl*II and ζ probe.

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