γ-Globin gene triplication and quadruplication in Japanese newborns

Evidence for a decreased in vivo expression of the 3′-$^{A}\gamma$-globin gene

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Received 8 July 1985

Cord blood samples of 113 Japanese babies from Kurashiki, Japan, were analyzed for the presence of possible modifications in γ-globin gene arrangements and for changes in the relative quantities of the γ chains $^{G}\gamma$, $^{A}\gamma$, and its variant $^{A}\gamma^{T}$. As many as 9 babies had the γ-globin gene triplication, one a γ-globin gene quadruplication, and two a γ-globin gene deletion. The triplication and quadruplication involved cross-overs within the $^{G}\gamma$- and $^{A}\gamma$-globin genes resulting in a hybrid gene with the 5′-segment being derived from the $^{A}\gamma$ and the 3′-segment from the $^{G}\gamma$-globin gene (-$^{AG}\gamma$-). The $^{G}\gamma$ value of the 9 babies with the -$^{G}\gamma$-$^{AG}\gamma$-$^{A}\gamma$-globin gene arrangement averaged ~80% and that of the baby with -$^{G}\gamma$-$^{AG}\gamma$-$^{AG}\gamma$-$^{A}\gamma$- was 78%. Four of the 9 babies with the triplication and the baby with the quadruplication had an $^{A}\gamma^{T}$ mutation in trans; in these babies, the $^{A}\gamma$ chain level (from the $^{A}\gamma$-globin gene in 3′-position in the tri- and quadruplication arrangements) was decreased to about one-third and one-sixth of the level observed in the simple $^{A}\gamma^{T}$ heterozygote. This observation supports the suggestion that transcription beginning at the 5′-γ-globin gene interferes with that of the 3′-γ-globin gene.

γ-Globin gene triplication *γ-Globin gene quadruplication* *Hybrid gene* *$^{G}\gamma$ level* *$^{A}\gamma$ level* *$^{A}\gamma^{T}$ level* *Decreased transcription*

1. INTRODUCTION

Human fetal hemoglobin (Hb F) is heterogeneous because its γ chain is a mixture of two types: the $^{G}\gamma$ chain with glycine at position γ136 and the $^{A}\gamma$ chain with alanine at that position [1]. These γ chains are the products of two nonallelic genes, located on the short arm of chromosome no. 11 in an -5′-ε-$^{G}\gamma$-$^{A}\gamma$-ψβ-δ-β- arrangement [2,3]. The -$^{G}\gamma$-$^{A}\gamma$-globin gene arrangement of the normal newborn expresses itself in a $^{G}\gamma$ to $^{A}\gamma$ chain ratio of 70 to 30 [4,5]. Some babies have modified arrangements, such as -$^{G}\gamma$-$^{G}\gamma$- and -$^{A}\gamma$-$^{A}\gamma$-, and their $^{G}\gamma$ to $^{A}\gamma$ chain ratios differ accordingly [5,6]. Another modification concerns deletion of a γ-globin gene through a cross-over within the $^{G}\gamma$ and $^{A}\gamma$ genes resulting in a -$^{GA}\gamma$- hybrid gene [7–9]. Two types of γ-globin gene triplication have also been discovered; in one, a corresponding -$^{AG}\gamma$- hybrid gene is flanked 5′ by a $^{G}\gamma$- and 3′ by an $^{A}\gamma$-globin gene (-$^{G}\gamma$-$^{AG}\gamma$-$^{A}\gamma$-), while in the other the crossing over occurred between the $^{G}\gamma$- and the $^{A}\gamma$-globin genes resulting in a -$^{G}\gamma$-$^{G}\gamma$-$^{A}\gamma$- arrangement. The first type is mainly observed among Chinese and Japanese [10,11] and the second has been found in two families from Yugoslavia [12].

The present study is a continuation of a larger study evaluating γ-globin gene arrangements in Japanese babies [11]. The frequencies of the $^{A}\gamma$ mutant, $^{A}\gamma^{T}$, and of the γ-globin gene triplication in babies who were from families living in a more restricted area were rather high, while one baby had a γ-globin gene quadruplication on one chromosome. Since the $^{A}\gamma^{T}$ mutation occurred on

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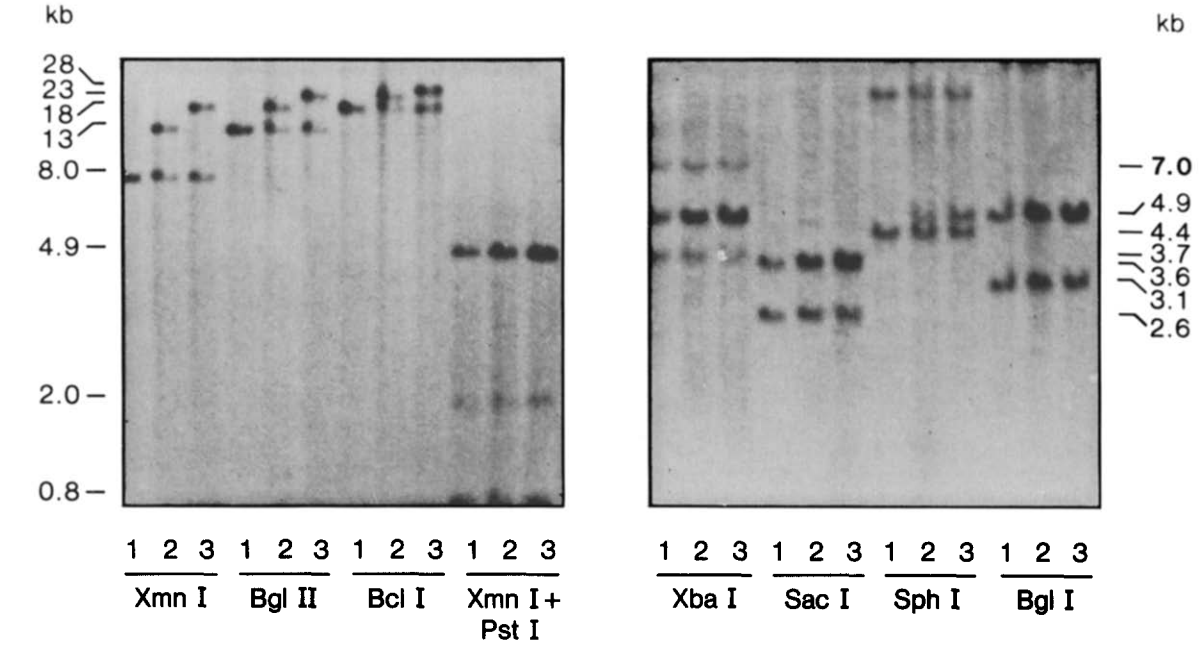
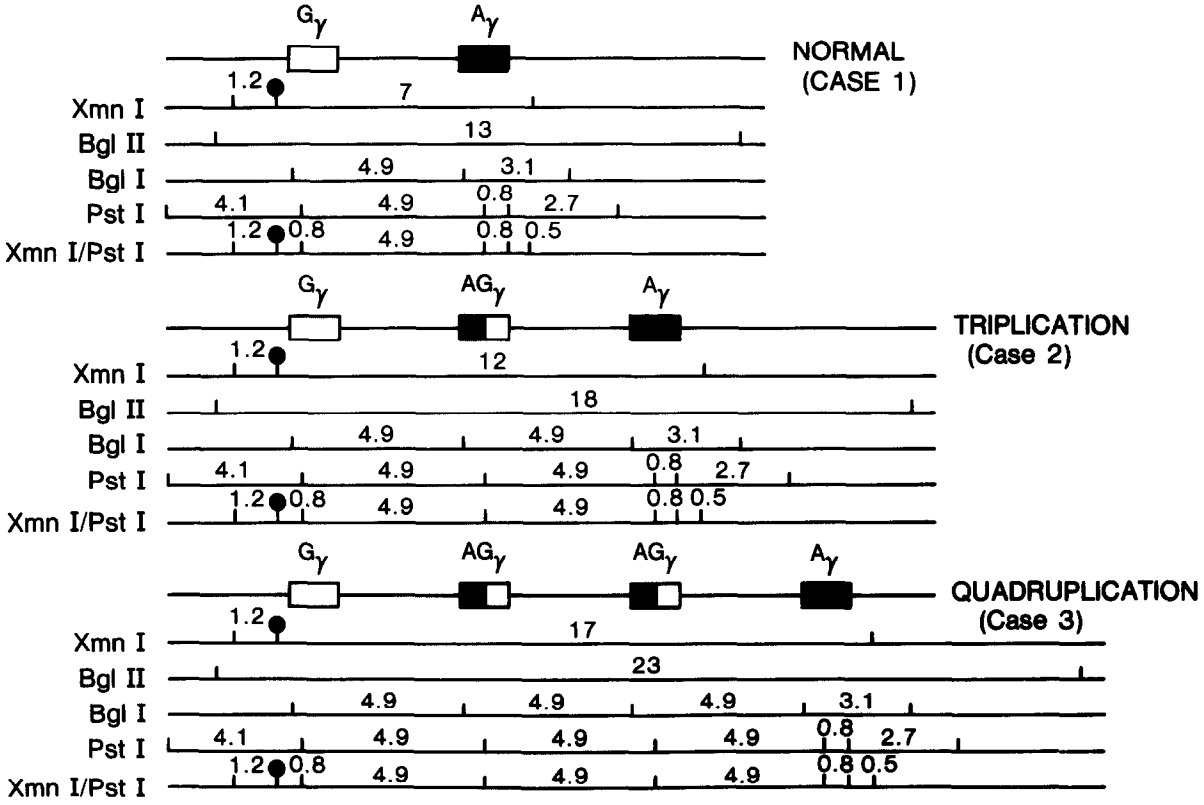
00145793/85/$3.30





Sizes in kb; γIVS-II probe; Xmn I polymorphic site

the normal chromosome in 4 babies with a γ-globin gene triplication and in the one baby with the quadruplication, the effect of these rearrangements on the expression of the $^{A}\gamma$ gene in 3'-position could be evaluated.

2. MATERIALS AND METHODS

Blood samples from the umbilical cord were collected with EDTA as anticoagulant and processed at the local institution. DNA was isolated from white cells using the procedure of Poncz et al. [13] and red cell lysates were prepared from washed red cells. Batches of 9–20 DNA samples with corresponding red cell lysates were transported in ice, airmail, special delivery to Augusta, GA. The time of transport was less than 5 days.

DNA was digested with the following enzymes: *Xmn*I, *Bgl*II, *Bcl*I, *Hin*dIII, *Bgl*I, *Sph*I, *Sac*I, *Xba*I, and *Pst*I. Hybridization was with the γIVS-II probe. Details about the methodology and the probe are given elsewhere [14,15]. In some instances, the X-ray film was scanned with a Quick Scan Auto Scanner (Helena Laboratories, Beaumont, TX) to evaluate the relative intensities of some of the bands.

Red cell lysates were analyzed by cellulose acetate electrophoresis [16] to evaluate the possible presence of an abnormal hemoglobin (Hb) (none were found) and by reverse phase high performance liquid chromatography (HPLC) using a (4.6×250 mm) Vydac C_4 column following the directions of Shelton et al. [17]. This procedure allows an accurate quantitation of the two types of γ chain, $^{G}\gamma$ and $^{A}\gamma$, and the mutant $^{A}\gamma$ chain, the $^{A}\gamma^{T}$ chain.

3. RESULTS AND DISCUSSION

3.1. *Subjects*

All 113 newborn babies were from a relatively selected area and were delivered at the Kurashiki Central Hospital, Kurashiki, Japan. None of the babies had hematological abnormalities.

3.2. *DNA analysis*

As in a previous study [11], the analyses were directed towards identifying specific anomalies of γ-globin gene arrangements, such as a triplication [1–12], or a deletion [7–9] of those genes. Routinely, DNA was digested with *Xmn*I and *Bgl*II, and hybridized with the γIVS-II probe. Fig.1 gives 3 examples. Sample 1 is from a normal control as shown by the presence of an 8 kb *Xmn*I fragment and a 13 kb *Bgl*II fragment; as many as 101 of the 113 newborn babies had this normal $\text{-}^{G}\gamma\text{-}^{A}\gamma\text{-}$ arrangement. Sample 2 represents 9 other newborns, all having a γ-globin gene triplication because of the presence of a 13 (or 12) kb *Xmn*I fragment and an 18 kb *Bgl*II fragment which are both ~5 kb larger than normally seen. Sample 3 is most unusual, and the presence of an 18 kb *Xmn*I fragment and a 23 Kb *Bgl*II fragment suggests the presence of two extra γ-globin genes, i.e. a γ-globin gene quadruplication. DNA from two additional newborns contained a 3 kb *Xmn*I fragment and an 8 kb *Bgl*II fragment, characteristic for a γ-globin gene deletion [12].

The DNA's of the 3 babies were analyzed with several additional enzymes; some of the results are illustrated in the photographs of fig.1, while quantitative data are listed in table 1. The increased sizes of the *Bcl*I fragments supported the presence of one additional γ-globin gene in case 2 and of two genes in case 3. No abnormal fragments were observed with any of the other enzymes (except with *Sph*I) but a distinct increase in the relative intensity of a specific fragment was noted (fig.1). Relative ratios were determined by densitometric scanning and data obtained for *Bgl*I, *Sac*I, and *Xba*I digests and for the *Xmn*I/*Pst*I double digest confirm that the relative ratios listed in the legend to table 1 approached the expected 1:1.5:2.0 for babies with a normal $\text{-}^{G}\gamma\text{-}^{A}\gamma$-globin gene arrangement, and for those with a γ-globin gene triplica-

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Fig.1. (Top) Photographs of X-ray films illustrating restriction fragments in the DNA from a normal baby ($\text{-}^{G}\gamma\text{-}^{A}\gamma\text{-}$, case 1), a baby with a γ-globin gene triplication on one chromosome ($\text{-}^{G}\gamma\text{-}^{AG}\gamma\text{-}^{G}\gamma\text{-}$, case 2) and a baby with a γ-globin gene quadruplication on one chromosome ($\text{-}^{G}\gamma\text{-}^{AG}\gamma\text{-}^{AG}\gamma\text{-}^{G}\gamma\text{-}$, case 3). (Bottom) Schematic presentation of the γ-globin gene arrangements in the three babies, and the expected sizes of fragments with different restriction enzymes. See text for further details.

Table 1

Sizes (in kb) of fragments in enzymatic digests of DNA from a normal baby and two babies each with a normal chromosome and one with a γ-globin gene triplication (case 2) or with a γ-globin gene quadruplication (case 3) (IVS-II probe)

Enzyme	Case 1	Case 2	Case 3
*Xmn*I	8	13	18
*Bgl*II	13	18	23
*Bcl*I	18	23	28
*Bgl*I	3.1; 4.9	3.1; 4.9*	3.1; 4.9*
*Sph*I	4.4	4.4;5.0	4.4;5.0*
*Sac*I	2.6;3.6	2.6;3.6*	2.6; 3.6*
*Xba*I	3.7; 5.0; 7.0	3.7; 5.0*; 7.0	3.7; 5.0*; 7.0
*Pst*I	0.8; 4.1; 4.9	0.8; 4.1; 4.9*	0.8; 4.1; 4.9*
*Xmn*I/*Pst*I	0.8; 2.0; 4.9	0.8; 2.0; 4.9*	0.8; 2.0; 4.9*
γ-Globin genes	$-^{G}\gamma\text{-}^{A}\gamma-$	$-^{G}\gamma\text{-}^{AG}\gamma\text{-}^{A}\gamma-$	$-^{G}\gamma\text{-}^{AG}\gamma\text{-}^{AG}\gamma\text{-}^{A}\gamma-$

Fragments marked with an asterisk were observed as having increased intensity. Densitometric scanning gave the following relative ratios for cases, 1, 2, and 3, respectively: *Bgl*I 4.9/3.1, 1:1.6:2.0; *Sac*I 3.6/2.6, 1:1.5:2.1; *Xba*I 5.0/7.0, 1:1.6:2.0; 5.0/3.7, 1:1.8:2.3; *Xmn*I/*Pst*I 4.9/2.0; 1:1.7:2.2. Expected relative ratio for heterozygotes is 1:1.5:2.0

tion and quadruplication, respectively (see also the scheme given in fig.1).

Two types of γ-globin gene triplication have been described thus far; most common is the one due to a crossing over within the $-^{G}\gamma-$ and $-^{A}\gamma-$ globin genes [10,11], resulting in a $-^{G}\gamma-^{AG}\gamma-^{A}\gamma-$ globin gene arrangement (the $-^{GA}\gamma-$ hybrid gene observed in babies with a γ-globin gene deletion is the counterpart of the $-^{AG}\gamma-$ hybrid gene of this type of triplication). The second type has been observed in two Yugoslavian families [12] and is due to a crossing over between the $-^{G}\gamma-$ and $-^{A}\gamma-$ globin genes, resulting in a $-^{G}\gamma-^{G}\gamma-^{A}\gamma-$ globin gene arrangement. The two types can be differentiated through analyses of their *Xmn*I digests (a 13 kb in the first type and 5 kb and 7 kb fragments in the Yugoslavian type) and of their *Xmn*I/*Pst*I double digests (the presence of an extra 4.1 kb fragment in the Yugoslavian type) [12]. All 9 babies with the γ-globin gene triplication were found to have the $-^{G}\gamma-^{AG}\gamma-^{A}\gamma-$ type which is most commonly seen in Chinese and Japanese newborns [10,11]. Comparable data for the baby with the quadruplication (table 1 and fig.1) made us conclude that two hybrid $-^{AG}\gamma-$ genes were present as a $-^{G}\gamma-^{AG}\gamma-^{AG}\gamma-^{A}\gamma-$ globin gene arrangement. Such an unusual situation probably occurred through an unequal crossing over within the $-^{G}\gamma-$ and $-^{A}\gamma-$ globin genes between mismatched chromosomes, one with a normal $-^{G}\gamma-^{A}\gamma-$ globin gene arrangement and a second with the Japanese type of triplication, i.e. $-^{G}\gamma-^{AG}\gamma-^{A}\gamma-$.

3.3. *Hemoglobin analysis*

Quantitation of the percentages of the different γ chains in the Hb F of the 113 newborn babies showed that 41 were heterozygous for the $^{A}\gamma^{T}$ chain; none were homozygotes. This corresponds to a frequency of 0.181 which is somewhat higher than the frequency ($f = 0.156$) in newborns from the Tokyo area [11]. The average $^{G}\gamma$ values (67.8%

Table 2

The percentages of the three types of γ chain in the Hb F of Japanese newborn babies with different γ-globin gene arrangements

Globin genes	*n*	$^{A}\gamma^{T}$	$^{G}\gamma$	$^{A}\gamma$
$-^{G}\gamma-^{A}\gamma-/-^{G}\gamma-^{A}\gamma-$	65	0	67.8 ± 2.2	32.2 ± 2.2
$-^{G}\gamma-^{A}\gamma-/-^{G}\gamma-^{AG}\gamma-^{A}\gamma-$	5	0	80.0 ± 1.0	20.0 ± 1.0
$-^{G}\gamma-^{A}\gamma^{T}-/-^{G}\gamma-^{A}\gamma-$	36	17.7 ± 1.8	67.3 ± 2.1	15.0 ± 1.8
$-^{G}\gamma-^{A}\gamma^{T}-/-^{G}\gamma-^{AG}\gamma-^{A}\gamma-$	4	15.4 ± 2.6	79.6 ± 2.7	5.0 ± 0.5
$-^{G}\gamma-^{A}\gamma^{T}-/-^{G}\gamma-^{AG}\gamma-^{AG}\gamma-^{A}\gamma-$	1	19.4	78.0	2.6
$-^{G}\gamma-^{A}\gamma-/-^{GA}\gamma-$	2	0	35.0 – 43.9	65.0 – 56.1

Average values and SD; $n = 113$

for normal babies and 69.3% for $^{A}\gamma^{T}$ heterozygotes) were as expected [18] (table 2). The 9 babies with γ-globin gene triplications fell into two groups. Five babies were $^{A}\gamma^{T}$ negative and had an average $^{G}\gamma$ value of 80%. Four were $^{A}\gamma^{T}$ heterozygotes with the $^{A}\gamma^{T}$ mutation on the normal chromosome; their $^{G}\gamma$ values averaged 79.6%. The baby with the γ-globin gene quadruplication had a similar $^{A}\gamma^{T}$ heterozygosity and a $^{G}\gamma$ value of 78%. Of major interest were the levels of the $^{A}\gamma$ chain: 15% in the simple $^{A}\gamma^{T}$ heterozygotes, 5% in the $^{A}\gamma^{T}$ heterozygotes with a triplication of γ-globin genes, and only 2.6% in the heterozygote with a quadruplication of γ-globin genes on the second chromosome. This reduction to values about 30% and 15% of those observed for simple $^{A}\gamma^{T}$ heterozygotes suggests an interference of transcription which begins at the 5′-gene with that concerning the gene at the 3′ position. The mechanism responsible for this interference is not understood but might involve a decreased binding of a transcription factor at specific enhancer sites.

ACKNOWLEDGEMENTS

The authors appreciate the assistance of Dr T. Ukita in the collection of the blood samples and the helpful comments from Dr J.G. Gilman. This work was supported by USPHS Research Grant HLB-05168. This is contribution no. 0908 of the Department of Cell and Molecular Biology at the Medical College of Georgia in Augusta, GA.

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