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The in vivo expression of the globin genes of the β cistron in γ-, δ-, and δβ-thalassemia heterozygotes

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Abstract. There is considerable evidence suggesting that the switch from γ to δ and β chain production after birth is due, in part, to silencing of the γ genes by stage-specific factors which bind to their promoters and to the competition from the adult (δ and β) genes for a common enhancer element located in the locus control region. As a consequence one can expect that the increased Hb F production in adults with hereditary persistence of fetal hemoglobin or δβ-thalassemia is directed mainly by γ-globin genes in cis to the deletion(s) responsible for these conditions. Here we review data on heterozygotes with γ-, δ- or δβ-thalassemia, who also had an $^{A}\gamma^{T}$ mutation, in cis or in trans, which was used as a marker of γ gene expression. The results show that a deletion affecting adult β genes favors the expression of γ genes in cis, while the deletion of a single γ gene does not affect the expression of the β gene in cis but leads to a faster γ → β switch postnatally.
Key words. In vivo; cistron; thalassemia; heterozygotes; enhancer; locus control region; in cis; in trans.

Introduction

The human β-like globin genes are located on the short arm of chromosome 11 and are arranged in a cluster as shown in figure 1. Two switches are observed in the expression of these genes during human development; ε → γ in the embryonic to fetal period and γ → β postnatally. This hemoglobin (Hb) switching is believed to be directed by the locus control region (LCR) sequences 5′ to the ε gene. Different suggestions have been made to explain the modulating factors of this process; one involves the competition of the adult and fetal globin genes for interaction with the LCR which may be the major factor in suppressing the γ-globin genes, and another involves the silencing of these genes by stage-specific negative factors which bind to their promoters. These processes may depend on the position of the particular gene in the cistron and on the presence of other active genes between it and the LCR[1–3]. This would suggest that in the adults the deletion of the adult globin genes (δ and β) results in an increased expression of the two fetal ($^{G}\gamma$- and $^{A}\gamma$-) globin genes from the same chromosome, while the deletion of the fetal globin genes would not affect the production of the remaining adult globin genes in cis.

Here we review data on patients with a heterozygosity for one of a number of deletions in the β-globin gene cluster who also have a mutation in the 75th codon of the fetal $^{A}\gamma$-globin gene that leads to an Ile → Thr amino acid substitution (i.e. γ75(E19)Ile → Thr in $^{A}\gamma$ (ref. 4)). The resulting variant $^{A}\gamma$ chain, named $^{A}\gamma^{T}$, can be easily separated and quantitated from the wild type $^{A}\gamma$ chain, and thus provides an excellent marker for the relative contribution of a pair of γ genes from each chromosome. The deletions of γ, δ, δ and β, or $^{A}\gamma$, δ, and β genes are further referred to as γ-, δ-, δβ-, or $^{G}\gamma(^{A}\gamma\delta\beta)$-thalassemias, respectively, as generally accepted. The listed information comes mainly from reports published during the past 20 years (references in the table) and from some unpublished data.

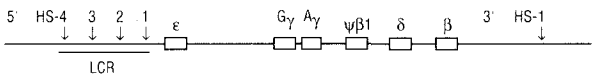

Figure 1. Arrangement of the human β-like globin genes on chromosome 11.

Hb A_2 was quantified by microcolumn chromatography[5] or by cation exchange high performance liquid chromatography (HPLC)[6,7], and Hb F by alkali denaturation[8] or by cation exchange HPLC[6,7]; the latter two procedures give comparable data in the Hb F ranges of 5–20%[7]. The relative quantities of the $^{G}\gamma$, $^{A}\gamma$, and $^{A}\gamma^{T}$ chains were determined by reversed phase HPLC[9–11]. Identification of the deletion was by gene mapping analyses as described before[12–16]. The presence of the $^{A}\gamma^{T}$ mutation was confirmed in many cases by dot-blot analysis involving hybridization of amplified DNA with ^{32}P-labeled specific probes (methodology in ref. 17). The presence of the $^{A}\gamma^{T}$ mutation in cis or in trans to a particular deletion was evaluated by lineage analyses from extensive family studies.

$^{A}\gamma^{T}$ heterozygotes without deletion(s) in the β-globin gene cluster

In a recent survey[18], we reviewed over 1,000 newborn babies with AA, AS, AC, SS, SC, and CC, who all had

Table. Hb A_2, Hb F, and γ chain composition data for $^{A}\gamma^{T}$ heterozygotes[a]

Condition	n	Hb A_2 %	Hb F %	$^{G}\gamma$ %	$^{A}\gamma^{T}$ %	$^{A}\gamma$ %	$^{A}\gamma^{T}$:$^{A}\gamma$[b] Ratio	Ref.
Newborn	1088	-	-	67.7	16.7	15.6	52:48	18
SS Adults (haplotypes 17/20; 17/19)	12	2.9	7.9	40.5	23.3	36.2	39:61	19
SS Adults (haplotypes 17/3)	1	2.7	4.5	60.0	18.2	21.8	45:55	19
AA (normal adults)	8	2.6	1.0	39.4	27.9	32.7	46:54	20
AA (Fanconi's Anemia)	9	2.1	7.3	40.4	25.1	34.5	42:58	*
$^{G}\gamma(^{A}\gamma\delta\beta)^{0}$-Thal (Black)	24	2.3	11.2	93.3	0	6.7	No $^{A}\gamma^{T}$	14
$^{G}\gamma(^{A}\gamma\delta\beta)^{0}$-Thal ($\beta^{S}$ in trans; Black)	7	2.3	19.2	93.1	0	6.9	No $^{A}\gamma^{T}$	14
$^{G}\gamma^{A}\gamma(\delta\beta)^{0}$-Thal (Spanish)	11	2.5	6.2	38.4	58.7	2.9	$^{A}\gamma^{T}$ in cis	21
$^{G}\gamma^{A}\gamma(\delta\beta)^{0}$-Thal (Macedonian)	6	2.3	10.5	40.1	57.7	2.2	$^{A}\gamma^{T}$ in cis	22
$^{G}\gamma^{A}\gamma(\delta\beta)^{0}$-Thal (Sicilian)	3	2.2	8.8	30.4	9.4	60.2	$^{A}\gamma^{T}$ in trans	23*
$^{GA}\gamma$-Thal (adults)	2	2.8	0.7	37.1	14.0	48.9	$^{A}\gamma^{T}$ in trans	18
$^{GA}\gamma$-Thal (newborn)	19	-	75.3	40.7	0	59.9	No $^{A}\gamma^{T}$	18
$^{GA}\gamma$-Thal (newborn)	2	-	-	39.7	17.9	42.4	$^{A}\gamma^{T}$ in trans	18
$^{GA}\gamma$-Thal (newborn)	1	-	76.9	42.6	38.8	18.6	$^{A}\gamma^{T}$ in cis	18
δ-Thal (Corfu)	1	2.2	<1.0	33.9	4.1	62.0	$^{A}\gamma^{T}$ in trans	24

[a]Average values only; $^{A}\gamma^{T}$ was absent in patients with a few of the conditions; these are listed because calculations can be based on $\%^{G}\gamma$- or $\%^{A}\gamma$ in trans.
[b]$^{A}\gamma^{T}$:$^{A}\gamma$ ratios were only calculated in individuals without deletions in the β-globin gene cluster; for the others, see fig. 2 which shows the % of total γ contributed in cis to the deletions.
*T.H.J. Huisman, F. Kutlar and C. Altay, unpublished data.

about equal percentages of $^{A}\gamma^{T}$ and $^{A}\gamma$ chains (52 and 48% of total $^{A}\gamma$, respectively). Our data for 8 normal adults gave a slightly different ratio of 46:54% ($^{A}\gamma^{T}$:$^{A}\gamma$) which are similar to that seen in SS patients with haplotypes 17/19 or 17/20 or with haplotypes 17/3 (haplotypes #3, #17, #19, and #20 refer to the Senegal, Cameroon, Benin, and Central African Republic (CAR) types; β^{S} chromosomes with haplotype #17 carry the $^{A}\gamma^{T}$ mutation[19]). Thus, in all adults reviewed, a ratio of about 40 ($^{A}\gamma^{T}$) to 60 ($^{A}\gamma$) was observed, which is about the same ratio of abnormal (X) to normal Hb chain (A) as commonly found in heterozygotes for other abnormal Hbs (Hb X)[25]. Our data on nine children with Fanconi's anemia with elevated Hb F levels averaging 7.1%, who also has the $^{A}\gamma^{T}$ variant, showed a similar ratio (table).

$^{A}\gamma^{T}$ heterozygotes with various $\delta\beta$-thalassemia deletions

The table also shows data on 4 types of deletional $\delta\beta$-thal, characteristics of which are in figure 2. The average values are based on 6 or more cases except for 3 subjects with the Sicilian type of $\delta\beta$-thal. The Black $^{G}\gamma(^{A}\gamma\delta\beta)^{0}$-thal includes part of the $^{A}\gamma$-globin gene; thus, all $^{A}\gamma$ present in the Hb F of the 31 heterozygotes originates from the $^{A}\gamma$ gene in trans. The average Hb F level is 11% (19% in the seven with Hb S-$^{G}\gamma(^{A}\gamma\delta\beta)^{0}$-thal) and the contribution of the $^{G}\gamma$ gene in cis is about 90% (fig. 2). No difference is seen between the subjects with the simple heterozygosity and those with the Hb S-$^{G}\gamma(^{A}\gamma\delta\beta)^{0}$-thal condition except for a higher level of Hb F in the latter.

The 11 Spanish and 6 Macedonian $\delta\beta$-thal heterozygotes have the $^{A}\gamma^{T}$ mutation in cis to the deletion,

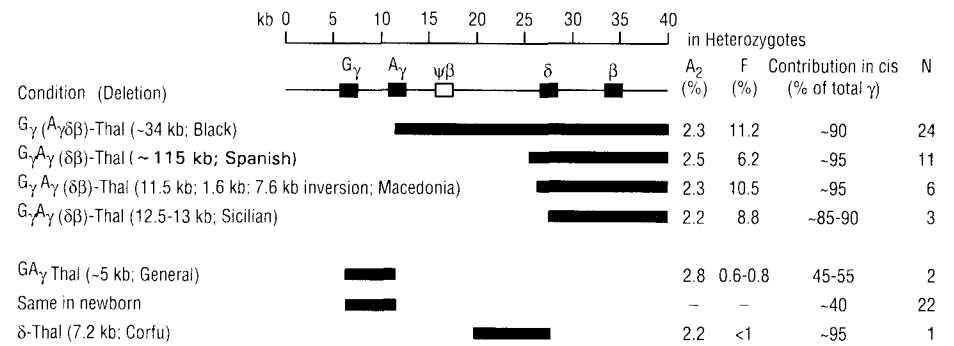

Figure 2. A comparison between the levels of Hb A_2 and Hb F in adults with a heterozygosity for deletions of different globin genes of the β cistron, and the relative contribution of the γ-globin genes in cis. For details, see text.

and the high $^{A}\gamma^{T}$ percentages (60 versus 3% for $^{A}\gamma$) indicate that the increased synthesis of Hb F is directed by the γ genes in cis. Comparable data were obtained for the 3 subjects with a heterozygosity for the Sicilian $\delta\beta$-thal who had a normal chromosome with the $^{A}\gamma^{T}$ mutation (fig. 2). The high level expression of the γ-globin genes in cis is consistent with the suggestion that the deletion of the adult β- (and δ-) globin gene promoter sequences prevents, in part, the silencing of the γ-globin genes in cis because the activation by the LCR sequences continues to be directed towards the remaining γ-globin genes. The possible differences in percentages of γ chain contribution to the total Hb F levels by the genes in cis between the 4 types of $\delta\beta$-thal might be due to the presence of only one γ-globin gene in cis (the $^{G}\gamma(^{A}\gamma\delta\beta)$-thal) and the presence (in the Silician $\delta\beta$-thal) or absence (in the Spanish and Macedonian $\delta\beta$-thal) of the δ promoter on the chromosome with the $\delta\beta$-thal deletion.

$^{A}\gamma^{T}$ heterozygotes with γ-thalassemia

γ-Thal is a deletion of a DNA segment of ~5 kb between the $^{G}\gamma$- and $^{A}\gamma$-globin genes[26]. The hybrid gene that results from the presumed crossover between $^{G}\gamma$ and $^{A}\gamma$ is indicated as a $^{GA}\gamma$ because its protein product is identical to the $^{A}\gamma$ chain, while its 5′ end, including the promoter sequence, is the same as $^{G}\gamma$. As a result, its $^{A}\gamma$ chain is produced at a rate characteristic for the $^{G}\gamma$ chain (reviewed in ref. 18). The data listed in the table concern 22 newborn babies and 2 adults with a heterozygosity for this deletion. Nineteen babies did not carry the $^{A}\gamma^{T}$ chain and had 60% $^{A}\gamma$, 2 babies with $^{A}\gamma^{T}$ in trans had ~40% $^{A}\gamma$ and ~20% $^{A}\gamma^{T}$, and one baby with $^{A}\gamma^{T}$ in cis (the $^{A}\gamma^{T}$ mutation is located within the $^{GA}\gamma$ hybrid gene) had ~20% $^{A}\gamma$ and ~40% $^{A}\gamma^{T}$. The 2 adults with $^{A}\gamma^{T}$ in trans had ~50% γ chain (37% $^{G}\gamma$ and 14% $^{A}\gamma^{T}$) from genes in trans and ~50% ($^{A}\gamma$ only) from the $^{GA}\gamma$ hybrid gene.

Newborn babies with a γ-thal heterozygosity had slightly higher levels of Hb A ($24.7 \pm 6.2\%$) than their normal counterparts ($18.9 \pm 4.9\%$)[18], while 2 newborn babies with a homozygosity for this deletion had ~55% Hb F (only $^{A}\gamma$) and ~45% Hb A at birth; the replacement of Hb F by Hb A appears to be nearly complete after 2–4 months, although the available data are far from complete[27].

$^{A}\gamma^{T}$ heterozygote with δ-thalassemia

The adult with the 7.2 kb Corfu deletion (table), which removes the entire δ-globin gene including the δ promoter (without an additional mutation in the β gene), was an Italian man who had this deletion on one chromosome and the codon 39 (C→T) β°-thal determinant on the other[24]. His wife was heterozygous for the IVS-I-110 (G→A) β-thal mutation and negative for $^{A}\gamma^{T}$, while their son with a compound heterozygosity

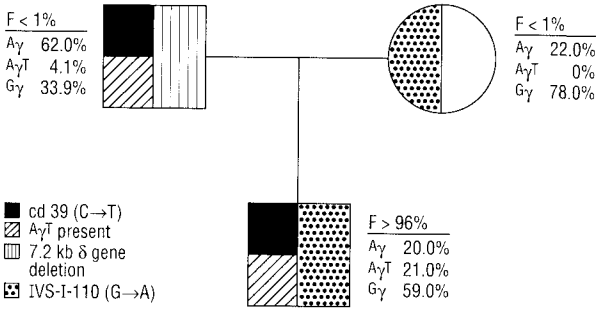

Figure 3. Pedigree of an Italian family where the father carries the δ gene (Corfu) deletion on one chromosome and the codon 39 β-thal and $^{A}\gamma^{T}$ mutation on the other. The son is also heterozygous for the $^{A}\gamma^{T}$ mutation.

for both β-thal mutations, was heterozygous for $^{A}\gamma^{T}$. In the father the Hb F level was low (<1%) with $^{A}\gamma^{T}$ of ~4% and ~60% $^{A}\gamma$, while the son had approximately equal amounts of $^{A}\gamma^{T}$ and $^{A}\gamma$(~20%) in his 96% Hb F at the age of about one year (fig. 3).

Although only this case of deletional δ-thal was available, the result might suggest that loss of the δ-globin gene promoter alone has a minimal effect on γ chain synthesis which appears to be directed towards the γ genes in cis while it has no effect on the β gene expression.

Conclusions

The data in this report show the deletions affecting the adult globin genes (β, δ, or both) appear to favor the expression of the fetal genes in cis, especially when the β-globin gene promoter is deleted. Deletion limited to only the δ-globin gene (e.g. Corfu δ-thal) is associated with a minimal effect on the γ gene expression. On the other hand, the deletion of a single γ gene does not affect the expression of the β gene in cis in adults; however, it may lead to a faster $\gamma \rightarrow \beta$ switch in the postnatal period. These data are consistent with the hypothesis that γ and β genes compete for activation by a common enhancer element, presumably located in the LCR sequences. Since in β and/or δ gene deletions the increased γ-globin gene expression in adults does not approach the level of β chain produced by an intact β gene, it would appear that the γ genes are actively silenced in adult hematopoietic cells. The factors involved in this process are not fully known but may include stage-specific regulatory (suppressors and/or activators) nuclear factors or factors involved in the organization of the chromatin structure of the β-globin gene cluster.

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