Disorders of Erythropoiesis

Influence of $^{G}\gamma$ –158 C→T and β- $(AT)_x(T)_y$ globin gene polymorphisms on HbF levels in Italian β-thalassemia carriers and wild-type subjects

Clinical manifestations of β-thalassemia (β-thal) intermedia phenotypes are influenced by the persistence of fetal hemoglobin (HbF) and by several polymorphisms located in the promoters of γ- and β-globin genes. The aim of this study was to evaluate the distribution of the -158$^{G}\gamma$ (C→T) polymorphism and of the $(AT)_x(T)_y$ configuration, as well as their eventual association with elevated levels of HbF in 188 β-thal carriers and 229 wild-type individuals of Italian descent. The -158$^{G}\gamma$T and the $(AT)_9(T)_5$ alleles were found to be associated with increased levels of HbF in β-thal carriers, but not in wild-type subjects.

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In normal adults the level of fetal hemoglobin (HbF) is lower than 1%, while subjects who have erythropoietic stress have mild expression of HbF. Individuals harboring severe β-globin mutations and concomitant high levels of HbF usually show a β-thalassemia intermedia phenotype, instead of the classical β-thalassemia major phenotype.[1] Therefore, all genetic factors capable of increasing HbF levels are clinically relevant. The co-inheritance of the -158$^{G}\gamma$ (C→T) polymorphism at the 5'UTR of the γ-globin gene, and the $(AT)_9(T)_5$ configuration at the -540 region of the β-globin gene has been shown to be involved in altered HbF levels in several populations.[1-4] In this study, we report the distribution and the relationship between the -158$^{G}\gamma$ (C→T) polymorphism and the $(AT)_x(T)_y$ repeat, as well as their effects on the increase of HbF levels, in a large cohort of Italian individuals, including 229 wild-type subjects and 188 β-thalassemia carriers.

Peripheral blood samples were taken from the 417 Italian individuals at the Centro Microcitemie of Rome (CSMR) after informed consent. Genomic DNA was isolated from the peripheral blood and all samples were characterized for red cell indices, HbA2 and HbF levels, the presence of α- and β-thalassemia mutations, the -158$^{G}\gamma$ (C→T) polymorphism and the $(AT)_x(T)_y$ configuration, as described elsewhere.[5] The $(AT)_x(T)_y$ region was amplified and sequenced using an ABI prism 3100 (Applied Biosystems). Statistical analysis was performed using the non-parametric mood median test.

All 229 wild-type subjects were negative for α- and β-thalassemia mutations and had normal HbA_2 value and HbF levels ranging from 0.2 to 5.1%. Seventeen of these subjects had HbF levels higher than 1.2%; the presence of high HbF levels in normal individuals was previously described in an Algerian population and correlated with polymorphisms located at the promoters of $^{G}\gamma$- and β-globin genes.[3] Our cohort of 188 β-thalassemia carriers did not have α-globin alleles, while they harbored 19 different mutations in the β-globin gene (Table 1). The analysis of the $(AT)_x(T)_y$ polymorphism revealed 11 different genotypes: [$(AT)_7(T)_7$/$(AT)8(T)_5$; $(AT)_9(T)_5$/$(AT)_{11}(T)_3$; $(AT)_7(T)_8$/$(AT)_{11}(T)_3$); $(AT)_7(T)_7$/$(AT)_6(T)_8$; $(AT)_8(T)_5$/$(AT)_{11}(T)_3$ $(AT)_7(T)_7$/$(AT)_7(T)_7$; $(AT)_7(T)_7$/$(AT)_{11}(T)_3$; $(AT)_7(T)_7$/$(AT)_7(T)_8$; $(AT)_7(T)_7$/$(AT)_7(T)_9$; $(AT)_7(T)_7$/$(AT)_9(T)_5$; $(AT)_9(T)_5$/$(AT)_9(T)_5$] including five novel Italian alleles [$(AT)_8(T)_5$; (AT)11$(T)_3$; $(AT)7(T)_8$; $(AT)_6(T)_8$; $(AT)_7(T)_9$]. The $(AT)_9(T)_5$ allele was less represented in β-thalassemia carriers (5/188) than in wild-type individuals (15/229) in concordance with previous studies.[6,7] Two novel alleles, $(AT)_6(T)_8$ and $(AT)_7(T)_8$, were found for the first time worldwide, being present only in heterozygous or wild-type subjects, respectively.

Table 1. β-globin mutations, -158 $^{G}\gamma$(CT) alleles, $(AT)_x(T)_y$ genotypes and HbF levels found in our cohort of β-thalassemia carriers.

β-globin gene mutations (n.)	*-540 $(AT)_x(T)_y$ Polymorphism*	*HbF>2*	*Presence of T allele -158$^{G}\gamma$*	*Mean HbF (%)*
-101 (25)	1 (AT)7(T)7/(AT)11(T)3	1	–	1.33±0.85
	19 (AT)7(T)7	5	7	
	5 (AT)7(T)7/(AT)9(T)5	–	3	
-87 (5)	3 (AT)7(T)7/(AT)9(T)5	2	2	4.68±2.67
	2 (AT)9(T)5	2	2	
-30 (2)	1 (AT)7(T)7/(AT)9(T)5	–	1	1
	1 (AT)7(T)7/(AT)7(T)9	–	–	1.3
Cd10/cd16 (1)	1 (AT)7(T)7/(AT)9(T)5	–	1	0.9
Cd 39 (65)	37 (AT)7(T)7	8	5	
	1 AT)7(T)7(AT)11(T)3	-	–	
	1 (AT)7(T)7/(AT)6(T)8	1	1	1.68±1.55
	23 (AT)7(T)7/(AT)9(T)5	8	10	
	1 (AT)8(T)5/(AT)11(T)3	–	–	
	1 (AT)9(T)5	–	–	
	1 (AT)7(T)7/(AT)7(T)9	–	–	
Cd41-42 (2)	2 (AT)7(T)7/(AT)9(T)5	–	–	0.55±0.07
Cd 5 (2)	1 (AT)7(T)7/(AT)9(T)5	–	–	0.9±0.14
	1 (AT)9(T)5	–	–	
Cd 6 (2)	2 (AT)7(T)7	–	2	1.55±0.50
Cd 59 a-t (1)	1 (AT)7(T)7	–	1	1
Cd8/9 (1)	1 (AT)7(T)7/(AT)9(T)5	–	1	1.6
IVSI-1 (8)	6 (AT)7(T)7	–	1	0.66±0.22
	1 (AT)7(T)7/(AT)9(T)5	–	1	
	1 (AT)7(T)7/(AT)7(T)8	–	–	
IVSI-110 (30)	19 (AT)7(T)7	1	4	0.96±0.47
	9 (AT)7(T)7/(AT)9(T)5	–	4	
	2 (AT)7(T)7/(AT)7(T)8	–	–	
IVSI-5 (2)	2 (AT)7(T)7	1	1	1.6±1.70
IVSI-6 (18)	14 (AT)7(T)7	–	4	0.8±0.45
	4 (AT)7(T)7/(AT)9(T)5	–	2	
IVSII-1 (9)	7 (AT)7(T)7/(AT)9(T)5	3	5	
				3.49±3.36
	1 (AT)9(T)5	–	1	
	1 (AT)7(T)7/(AT)7(T)9	–	1	
IVSII-745 (13)	9 (AT)7(T)7	3	3	
				1.95±3.43
	3 (AT)7(T)7/(AT)9(T)5	1	–	
	1 (AT)7(T)7	–	–	
IVSII-844 (1)	1 (AT)7(T)7	–	–	0.5
IVSII-848 (1)	1 (AT)7(T)7	–	–	0.7

In agreement with our personal unpublished observations, molecular analysis of the -158 $^{G}\gamma$ polymorphism showed a similar distribution of C/C, C/T and T/T genotypes in both wild-type individuals and β-thalassemia carriers (p=0.023). This finding excludes the possibility that this polymorphism may influence *per se* the HbF levels in β-thalassemia carriers. The association between the combination of the -158 $^{G}\gamma$ and $(AT)_x(T)_y$ polymorphisms and increased HbF levels has been previously demonstrated in β-thalassemia and wild-type subjects;[3,8,9] one other study did not confirm this result, probably due to the limited number of thalassemia patients analyzed.[10] Our data, obtained in a much higher number of β-thalassemia carriers and normal individuals, revealed that the $(AT)_9(T)_5$ allele was always associated with the T allele of the -158 $^{G}\gamma$ polymorphism and high levels of HbF (Figure 1B). This relationship was not found in the control population, even

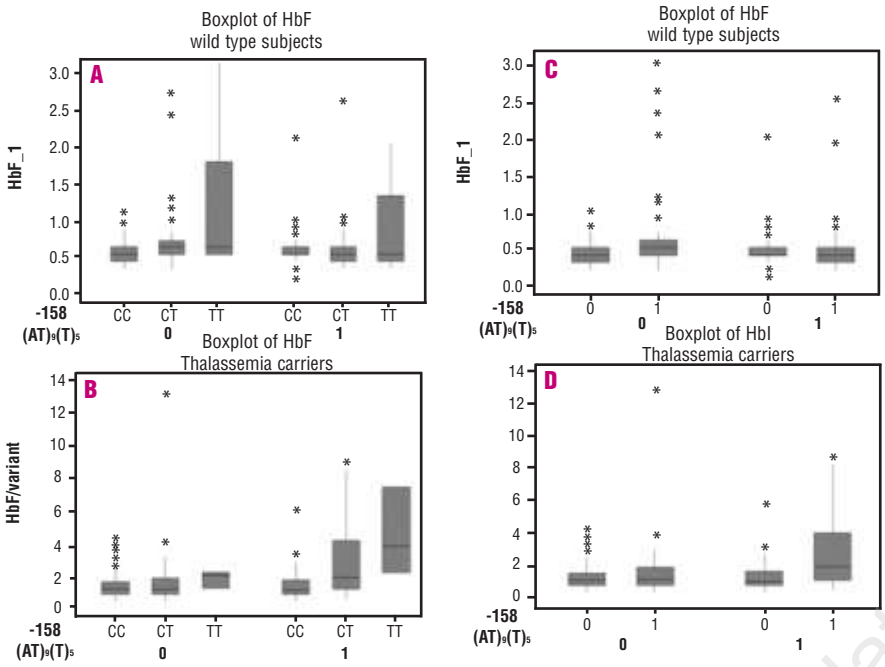

Figure 1. Absolute HbF levels in wild-type subjects and β-thalassemia carriers. (A,B) HbF levels correlated with the three genotypes (CC, CT , TT) of the -158$^{G}\gamma$ (C→T) polymorphism and the presence (1) or absence (0) of the AT_9T_5 allele. (C,D) HbF levels correlated with the presence (1) or absence (0) of the T allele of the -158$^{G}\gamma$ (C→T) polymorphism and the presence (1) or absence (0) of the AT_9T_5 allele.

when 12 wild-type subjects with higher HbF levels were included in the statistical analysis (Figure 1A).

Differently from the Algerian population,[3] seven of these 12 subjects carried the $(AT)_7(T)_7$ allele, only three of them were heterozygous carriers of the $(AT)_9(T)_5$ allele, and no $(AT)_9(T)_5$ homozygotes were found. From a statistical point of view, these 12 individuals should be considered as a different population; the discrepancy between our results and those of the Algerian study is likely to be due to the number of subjects analyzed in the two studies or to the different ethnic populations.

In β-thalassemia carriers HbF levels were increased more consistently when the $(AT)_9(T)_5$ allele was present both in a homozygous state and in association with the most common $(AT)_7(T)_7$ repeat, if compared with the $(AT)_7(T)_7$ allele *per se* (Figure 1D). This association was not found in wild-type subjects (Figure 1C). It has been suggested that the repressor protein BP1 binds the $(AT)_9(T)_5$ repeat more strongly than the other configurations, thus reducing the β-globin gene expression.[4,6] The $(AT)_9(T)_5$ allele, by binding a critical regulatory protein, could interact with other regulatory factors causing the observed discrepancy between wild-type individuals and heterozygous β-thalassemia carriers. Further functional studies should be carried out to verify this possibility.

Valentina Guida, Mara Pia Cappabianca,° Alessia Colosimo,*# Francesca Rafanelli,*@ Antonio Amato,° Bruno Dallapiccola*@*

**IRCCS-CSS, San Giovanni Rotondo and CSS-Mendel Institute, Rome, Italy; °ANMI Onlus-Centro Studi Microcitemie, Rome, Italy #Department of Comparative Biomedical Sciences, University of Teramo, Italy; @Department of Experimental Medicine and Pathology, University "La Sapienza", Rome, Italy*

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Correspondence: Valentina Guida, PhD, CSS-Mendel Institute, Viale Regina Margherita 261, 00198 Rome, Italy. Phone: international +39.06.44160530. Fax: international +39.06.44160548. E-mail: v.guida@css-mendel.it

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