Preliminary study on AHSP locus in North Sardinian β-thalassemic patients

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In β-thalassemias (β-thal), the excess α-chains aggregates in large insoluble precipitates (Heinz bodies) in red cell precursors originating mechanical damages which result in ineffective erythropoiesis and peripheral hemolysis. Factors which reduce the degree of globin chain imbalance and the magnitude of the surfeit of α-chains, have an ameliorating effect on the β-thal phenotype. In a large number of β-thal patients, the reduced disease severity is due to the co-inheritance of α-thal alleles and/or to defects in the normal switch from fetal to adult hemoglobin (HbF→HbA), which can lead to the persistence of HbF in adults. Linkage studies suggested that polymorphic configurations within the β-globin gene cluster may play a role in the regulation of HbF levels [1]. Recently, it has been shown that an abundant erythroid-expressed protein, called alpha-hemoglobin stabilizing protein (AHSP), can modulate β-thal phenotype due to its ability to bind and stabilize free α-globin [2]. Nowadays, only three missense mutations were indentified in AHSP *locus*: A**T**G→A**A**T (Met→Arg) at codon 45, A**A**C→A**T**C (Asn→Ile) at codon 75, and **C**CC→**A**CC (Pro→Thr) at codon 100. Functional studies showed that variant at codon 75 is responsible of the synthesis of a mutant AHSP with 30% less capacity of reducing ROS formation from free aHb. Seven SNPs and three alleles of a Tn-homopolymer (T14, T15, T18) are described for AHSP gene. Authors identified 18 haplotypes, grouped in two main clades. "*In vitro*" studies suggested that T18 is associated with higher AHSP expression levels with respect to the T15, whereas the 12391 G→A polymorphism reduces the AHSP synthesis [3, 4].

In this study we report preliminary data on AHSP *locus* in 15 Sardinian $\beta^0$39 homozygotes previously grouped in TI and TM patients, on the basis of their Hb composition and clinical manifestations: the four patients in group TI had never been transfused, had total Hb levels of 8.6 ± 1.5 g/dl with 96.1 ± 1.8 percent of HbF. Patients in TM group were transfusion dependent, with HbF levels between 5 and 30%. Controls have been added to the study.

Sequencing of AHSP gene coding regions showed the heterozygosity A**A**C/A**T**C at codon 75 in one TM subject. All probands were also genotyped for the seven SNPs and Tn-homopolymer: six of the seven SNPs, included the 12391 G→A polymorphism, and two alleles at the Tn-homopolymer (T18 and T15) were observed. The T18 allele was the most represented in all groups examined. By analyzing Tn and SNPs associations, five different haplotypes were determined. We are currently looking for a possible correlation between AHSP haplotype and clinical variability.

Acknowledgements

This work is supported by grants from Fondazione Banco di Sardegna.

References

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