Asymptomatic and mild $\beta$-thalassemia in homozygotes and compound heterozygotes for the IVS2+1G>A mutation: role of the $\beta$-globin gene haplotype

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Background and Objectives. We report on two families in which the $\beta^{0}$-thalassemia mutation IVS2+1G>A occurs either in the homozygous or compound heterozygous condition with other $\beta$-thalassemia determinants. In the first family the proband, homozygous for the IVS2+1 determinant, is asymptomatic and was detected by chance during a screening program for $\beta$-thalassemia. In the second family, the proband is a 43-year-old female with a very mild thalassemia intermedia due to compound heterozygosity for the IVS2+1G>A and IVS1+10G>A mutations. Her father was diagnosed as having a thalassemic disorder only during the family studies carried out because of the proband’s condition. He is a compound heterozygote for the Sicilian type $\delta^{0}$-thalassemia and the IVS2+1 mutation and has a normal level of hemoglobin.

Design and Methods. In both families, the heterozygous carriers of the IVS2+1G>A have unusually elevated levels of fetal hemoglobin (HbF), and the homozygotes showed 98% HbF, reflecting an increased production of well hemoglobinized F-cells not associated with a significant erythroid expansion.

Results. The high HbF levels co-segregate with the $\beta^{0}$-thalassemia mutation; the size and structure of both pedigrees do not allow the contribution of unlinked genes to the elevated production of HbF to be assessed.

Interpretation and Conclusions. We propose that the unusual phenotypes resulting from homozygosity and compound heterozygosity for IVS2+1 are, against the background of a polygenic quantitative control of HbF expression, principally due to elements, such as repetitive sequences or single nucleotide polymorphisms, within or closely linked to the $\beta$-gene cluster. These are potentially implicated in chromatin environment modifications, and could, therefore, be responsible for sustained HbF synthesis during development.

Key words: thalassemia intermedia, $\beta$-thalassemia mutations, fetal hemoglobin, $\beta$-globin gene cluster polymorphisms.

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were performed using standard protocols. Analysis of the propositus' DNA revealed homozygosity for the mutant IVS2+1G>A mutation which causes abnormal mRNA splicing and results in β0 thalassemia. Both parents and the other three siblings are heterozygous for the same mutation and have a typical thalassemic blood picture with microcytosis, high HbA2 and unusually elevated HbF levels ranging from 3.4 to 14.0% (Table 1 and Figure 1).

The extended haplotypes (including restriction fragment length polymorphisms and microsatellites) of all family members are presented in Table 1. The IVS2+1G>A mutation is in cis to β-globin haplotype III (here indicated as A) positive for the Xml restriction site at position -158 upstream of the Gγ gene; the other two haplotypes (E and F) are variants of haplotype I and are in cis to the normal allele βA. Recent hematologic evaluation of the propositus showed that the blood picture had not differed significantly from that observed at the moment of diagnosis (Hb 12.4 g/dL, Figure 1).

In the second family (S.A.), along with the IVS2+1G>A, two more thalassemic determinants, IVS1+110G>A and the Sicilian type δβ0 thalassemia segregate in three generations. The former mutation results invariably in a transfusion-dependent β-thalassemia major in homozygotes, whereas the latter is a 13.377 bp deletion which includes the third exon of the δ gene and the whole β-gene and is responsible for thalassemia intermedia in homozygotes and for mild anemia and elevated HbF (4-19%) in heterozygotes.

The hematologic and molecular data of the family are presented in Table 1 and Figure 1. The propositus (II-1) was a 43-year old female, affected by a very mild form of thalassemia intermedia caused by compound heterozygosity for the IVS2+1G and IVS1+110G β-globin gene mutations.

The patient's clinical condition is so mild that the diagnosis was made only when she was already 34 years old. Since then the patient has been trans-
Figure 1. Pedigree of families G.L. and S.A.
fused only twice.

The father (I-1) is a compound heterozygote for Sicilian δβ0 thalassemia and the IVS2+1 splice mutation. His genotype was first characterized during the family study carried out because of the proband’s condition. Until then he had remained asymptomatic despite a large splenomegaly. The hematologic analysis showed a Hb of 15 g/dL, consisting almost exclusively of fetal hemoglobin. The mother (I-2) is heterozygous for the IVS1+110 and shows the characteristic blood picture of thalassemic trait.

Five β-haplotypes segregate in the pedigree (Table 1). The IVS2+1 determinant (A) is associated with a haplotype III identical to that identified in family G.L., the IVS1+110 mutation (B) is located in cis to haplotype I, the Sicilian δβ0 thalassemia (G); the normal βα allele is in cis either to haplotype III (D) or haplotype I (E).

HbF levels range from 2 to 8% in the heterozygous carriers of β-thalassemia and up to 30% in the individual carrier of δβ0 thalassemia in whom 64% of F cells were found (Figure 1). The ratio Gy/Ay ratio is higher than 1 (59-63% Gy) in all members of the family.

α thalassemia, as well as deletions or single base substitutions of the β globin cluster leading to hereditary persistence of fetal hemoglobin, were excluded in both families.

Discussion

The striking aspect about the patients of these two families is the asymptomatic phenotype of homozygosity for the IVS2+1 mutation and the extremely mild thalassemia intermedia presented by the two compound heterozygotes for the same mutation and either δβ0 thalassemia or the severe IVS1+110 determinant.

The unusual clinical picture of the three individuals is clearly related to the high capacity of the bone marrow to produce a sufficient number of well hemoglobinized F-cells without major signs of ineffective erythropoiesis and erythroid expansion. Several structural changes of the γδβ-complex could account for the beneficial increase of fetal Hb levels observed in the thalassemic individuals of both pedigrees. Besides the Sicilian δβ0 mutation, we did not find any other deletion which might cause hereditary persistence of fetal hemoglobin (HPFH). Mutations of γ-genes promoters were ruled out by sequencing. It is, therefore, quite likely that the elevated production of HbF in the thalassemic individuals studied is caused by the co-inheritance of β-thalassemia and a form of HPFH. This heterogeneous group of conditions is characterized by a small or moderate increase of HbF and F-cells in the normal individual, but is responsible for a marked effect following erythropoietic stress. The capacity for elevated Hb F production has been attributed to single nucleotide or microsatellite polymorphisms in cis to the β-gene cluster as well as to the action of genes or regulatory elements located on other chromosomes. A polygenic control of fetal hemoglobin is well documented and three quantitative trait loci (QTL) have been mapped to chromosomes X, 6 and 8.

In both families presented here, the high HbF levels co-segregate with the IVS2+1 mutation in cis to the β-haplotype III, involving the presence of the restriction site XmnI site (C>T at -158 5' to the Gγ gene cap site), described as being associated with increase of HbF and F-cell levels, delayed GaY switching and a fetal type Gγ/Aγ ratio (60-70 Gγ/40-30 Aγ) in the residual fetal Hb present in the adult.

The same XmnI+ haplotype, in cis to the normal β0 gene, segregates in family S.A. and is found in the heterozygous condition in three normal individuals and in a carrier of δβ0 thalassemia. Two out of the three normal individuals show HbF percentages higher than 1%. Considering their age this finding might not be significant; the high percentage of HbF (30%) and F-cells shown by the heterozygote for δβ0 thalassemia is, however, remarkable.

To date, two other thalassemic families with IVS2+1 mutation have been reported. However, in the first case the mutation was located on haplotype I and the high fetal Hb production was limited to only one of the two homozygotes carrying the same mutation on an identical haplotype. In the second one, the mutation was in cis to different hybrid XmnI-haplotype, and the pedigree included non-thalassemic individuals with higher than normal numbers of F cells.

In the Sicilian families, polymorphic microsatellites were further identified within the haplotype carrying the IVS2+1 mutation: (AT)nNv; (AT)n in the 3' region of the β-LCR/HS2, a (TG)11 sequence in the Aγ gene IVS2, in cis with a (TG)11(CG)11 sequence in the Gγ gene IVS2, and finally (AT)nT5 motif 0.5 kb 5' to the β-gene.

All these polymorphic variants have been singly associated with an increased production of HbF but to the best of our knowledge these polymorphisms have never been found associated in a unique haplotype. The frequency of the β0-thalassemia allele IVS2+1G>A is rather low in Italy and Greece (about 2%), higher in the Middle East (7 to 47%), Azerbaijan (21%), and in the African population of Guadeloupe (15%). The mutation can be linked to various β haplotypes (I, III, IX or atypical) and results in a disease of variable severity. However, very mild to moderate phenotypes are virtually only found among individuals who are homozygotes for the mutation.
in cis to the Xmnl+ haplotype III.10-11,27 The same observation holds also for other β0 determinants such as Cd 6 (-A), Cd B (AA) and Cd 30 (G>C). These mutations, when in cis to Xmnl+, often result in a mild or moderate anemia in homozygotes, whereas Xmnl negative determinants, such as Cd 39 (C>T) or IVS1+10G>A, frequent in the Middle East, almost invariably cause severe Cooley’s anemia.

In Sardinia mild thalassemia occurs in homozygotes for Cd 39 (Xmnl−) as well as compound heterozygotes for Cd 39 and the Xmnl+ Cd 6 (-A).28 Family studies suggest the involvement of an unlinked HbF determinant in many cases.29

In Greece a measurable (1.7-9.0%) amount of HbF is found only in heterozygous carriers of the Xmnl+ haplotype IIIa.30 These findings seem to restrict the propensity to produce higher levels of HbF to a specific Xmnl + haplotype III.

Although a correlation between high HbF production and the Gγ-Xmnl site was demonstrated in 1985, the functional significance of this has never been documented so far.31

Conversely, several studies in different genomic environments, propose that multiallelic microsatellite polymorphisms play a functional role in quantitative control of gene expression and thus of phenotypic variation.32,33

In particular for the β-gene cluster, binding of a trans-acting factor (BP1) to a silencer sequence 5’ to the β-globin gene was shown in 1989 by Berg et al.34 A variation in this (AT)xTy polymorphism was later proven to correlate in AS individuals with the benign evolution of the disease in some families.47 Transcriptional de-repression has been later shown to be the binding sites for trans-acting factors.44,45

A reciprocal regulation of β and γ-globin genes has been previously documented in vitro and in vivo.44,45

Finally the tandem repeat (TG)n within the IVS2 of the Aγ-gene has been found more frequently in Sicilian individuals with thalassemia intermedia than in patients with severe Cooley’s anemia.

The fundamental question arising from these data is, therefore: besides the presence of the Xmnl-Gγ site, is the association of the other polymorphisms, in particular a β-globin haplotype, relevant to the reactivation of HbF production under erythropoietic stress?

It can be hypothesized that the combination of specific recognition sites and their interaction with transcription factors could generate chromatin structures with regulatory properties (gene activation or repression).35-37,43-46

Our observation goes a step further, since it involves the co-existence of several microsatellite polymorphisms which have been singly proven to be the binding sites for trans-acting factors.

An interaction of these factors is obviously possible, and could induce chromatin modifications. Our hypothesis is that of a possible epigenetic effect due to modification of the chromatin environment. The γ genes could then entirely compensate for the β0 thalassemic defect.

Epigenetic regulation of gene expression during development is now a well-recognized phenomenon, as is the role of DNA methylation in this feature.48 Transcriptional de-repression has been shown to be a potential cause of genetic diseases.48 Why not propose the same hypothesis for the expression of γ genes and the rescue of thalassemia phenotype?

The mildness of the β-thalassemic phenotype in both families presented here seems to correlate with intrinsic property(ies) of a particular haplotype. The study of the structural and functional properties of such haplotype(s) in the relevant populations will give insights into the mechanism of the benign evolution of the disease in some homozygous β0 thalassemias.

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Contributions
AR: conception and design, analysis and interpretation of data, drafting the article and revising it critically for important intellectual content, final approval of the version to be published; SA: conception and design of the study, analysis and interpretation of data, and drafting the article or revising it critically for important intellectual content; TL: drafting the article and revising it critically for important intellectual content; LC: conception and design, or analysis and interpretation of data; MM-R: conception and design of the study, analysis and interpretation of data; DL: drafting the article or revising it critically for important intellectual content; final approval of the version to be published; LB: drafting the article or revising it critically for important intellectual content; final approval of the version to be published. The author thank Mr. Maurizio Sturnio from the Laboratorio di Patologia Genetica, IRCCS OASI M.SS Troina, for technical assistance during preparation of the manuscript.

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In the following paragraphs, Professor Cazzola summarizes the peer-review process and its outcomes.

What is already known on this topic
The clinical presentation of individuals who are homozygotes or double heterozygotes for β-thalassemia mutations is highly variable, ranging from severe, transfusion-dependent anemia to milder conditions known as thalassemia intermedia. The ability to produce HbF is a factor capable of modulating the clinical phenotype of thalassemic patients.

What this study adds
The unusually mild thalassemic phenotypes reported in this study appear to be mainly due to the contribution of cis-acting elements to the production of HbF.