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Co-inherited Gilbert's syndrome: a factor determining hyperbilirubinemia in homozygous β-thalassemia

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ABSTRACT

*Background and Objective. Patients with thalassemia major and intermedia show a marked variability of serum indirect bilirubin levels. In this paper we tested the hypothesis related to the variability of the glucuronidation bilirubin rate which depends on the configuration of the A(TA)$_n$TAA motif of the UGT1*1 glucuronosyltransferase gene promoter.*

Design and Methods. We studied the configuration of the A(TA)$_n$TAA motif in 26 patients with thalassemia major and 34 with thalassemia intermedia.

Results. In patients with thalassemia major and in those with thalassemia intermedia significantly higher bilirubin levels were found in patients with the (TA)$_7$/(TA)$_7$ genotype, than in those with the (TA)$_7$/(TA)$_6$ or (TA)$_6$/(TA)$_6$ genotype.

Interpretation and Conclusions. These results indicate that the (TA)$_7$/(TA)$_7$ genotype, the configuration found in patients with Gilbert's syndrome, is capable of modifying the clinical phenotype of homozygous β-thalassemia. This is an example of the role played by co-inherited modifying gene(s) on the extent of clinical heterogeneity of monogenic disorders.

Key words: Gilbert's syndrome, hyperbilirubinemia, homozygous β-thalassemia

Homozygotes for β-thalassemia manifesting either transfusion-dependent thalassemia major or a milder non transfusion-dependent clinical picture (thalassemia intermedia), show a marked variability in serum unconjugated bilirubin levels. This variability may be related either to the transfused red blood cell destruction rate, to ineffective erythropoiesis, to hemolysis, or to bilirubin elimination capacity which depends partially on the bilirubin glucuronidation activity.

Gilbert's syndrome, a chronic mild form of unconjugated hyperbilirubinemia, is caused by a decreased enzymatic activity of UDP-glucuronosyltransferase (UGT), resulting either from missense mutation of the UGT1*1 gene, the isoform of UGT responsible for bilirubin glucuronidation, or to the presence of a variant promoter of UGT1*1, containing a two base-pair addition in the TATA element of the promoter, giving rise to seven rather than the more usual six repeats [A(TA)$_7$TAA instead of A(TA)$_6$TAA].[1-3] The presence of this expanded element has been shown to decrease the expression of the UGT-1A gene. Homozygosity for the A(TA)$_7$TAA promoter configuration has been recently identified as one of the co-factors determining the increase in bilirubin levels in heterozygous β-thalassemia and glucose-6-phosphate dehydrogenase deficient subjects.[4,5] In this study, we investigated whether the marked variation in the serum bilirubin levels of β-thalassemia homozygotes either with thalassemia major or intermedia is also related to the configuration of the A(TA)$_n$TAA motif of the UGT1*1 gene.

Design and Methods

Patients

We determined the configuration of the A(TA)$_n$TAA motif of the UGT-1A promoter in the following groups of patients:

- 26 β°-thalassemia major regularly transfused patients, selected on the basis of different bilirubin levels: 13 with hyperbilirubinemia (serum bilirubin higher than 45 (μmol/L) and 13 with normal serum bilirubin (lower than 17 (μmol/L);
- 34 untransfused thalassemia intermedia patients not selected for bilirubin levels.

The mean age of the patients with thalassemia major was 18.5 y (range 7-25 y) and of those with thalassemia intermedia 27.8 y (range 5-51 y).

All patients had normal liver function and normal Coombs' test. The patients were of Sardinian origin and genotypically either homozygotes for β-gene codon 39 (C-T) nonsense mutation (57 patients) or compound heterozygotes for this mutation and Sardinian δβ-thalassemia (3 patients).[6] The reasons for the mild phenotype in thalassemia intermedia patients have been previously reported.[7]

Clinical diagnosis of thalassemia intermedia was

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Table 1. Bilirubin, alanine amino-transferase and mean pretransfusional hemoglobin of β°-thalassemia major patients selected on the basis of bilirubin levels.

	N°	$(TA)_7/(TA)_7$ UDGT1*1 genotype	Bilirubin (µmol/L) Tot	Bilirubin (µmol/L) Ind	ALT* IU/dL	Pre-transfusional Hb g/dL
Increased bilirubin	13	11/13	65.4±24.0	49.6±20.1	35.8±16.6	9.8±0.3
Normal bilirubin	13	0/13	13.7±4.5	9.1±3.2	32.0±12.8	9.9±0.4

**Alanine amino-transferase.*

Table 2. Hematologic, bilirubin and ALT data from β°-thalassemia intermedia patients.

UDGT1*1 genotype	N°	Bilirubin (µmol/L) Tot	Bilirubin (µmol/L) Ind	ALT° IU/dL	Mean annual Hb g/dL	Reticulocytes %
$(TA)_6/(TA)_6$	13	39.1±19.1*	28.2±15.8*	15.2±10	8.2±1.0	3.5±3.0
$(TA)_6/(TA)_7$	14	49.5±22.1*	36.6±19.6*	28.9±14	9.0±1.0	4.0±3.0
$(TA)_7/(TA)_7$	7	117.0±53.9*	102.1±47.3*	27.4±23	9.0±1.0	2.8±1.0

**p < 0.0005 [referred to $(TA)_7/(TA)_7$ vs $(TA)_7/(TA)_6$ and $(TA)_6/(TA)_7$]. °Alanine amino-transferase.*

based on mild-to-moderately severe microcytic anemia (Hb levels > 7 g/dL) not requiring regular transfusions, nucleated red blood cells in peripheral blood, moderate-to-severe bone modification and spleen/liver enlargement. The hemoglobin electrophoresis in these patients showed only HbF (96-98%) and HbA_2 (2-4%). Twenty-nine out of 34 thalassemia intermedia and 1 out of 26 thalassemia major patients had been splenectomized.

Methods

Serum bilirubin, alanine-aminotransferase (ALT), γ-glutamyl-transpeptidase and hemoglobin level were measured by standard methods. Reticulocyte number was counted by the fully automated analyzer Sysmex R-1000. Glucose-6-phosphate dehydrogenase (G6PD) activity was determined by the differential pH technique.[8]

Genomic DNA was obtained by standard methods from peripheral blood leukocytes.[9] Analysis of the $A(TA)_nTAA$ motif, in the promoter region of the UGT1*1 gene was performed by nucleotide sequencing with the primers described by Bosma *et al*.[1] and/or radioactive PCR followed by separation of amplified products on 6% denaturing polyacrylamide gels and autoradiography.[2]

Results

Thalassemia major patients

Bilirubin, transaminases and mean transfusional hemoglobin of thalassemia major patients are reported in Table 1. Homozygosity for the expanded promoter element $A(TA)_7TAA$ was present in 11 out of 13 (85%) of the thalassemia major patients with hyperbilirubinemia, but was never found in those with normal bilirubin levels. The bilirubin levels in those patients with the $(TA)_7/(TA)_7$ genotype were in the range found in patients with Gilbert's syndrome. No relationships between bilirubin levels, pretransfusional Hb levels, and blood consumption were detected (data not shown).

Thalassemia intermedia patients

Table 2 summarizes hematologic, bilirubin and ALT data from the thalassemia intermedia patients. In patients with thalassemia intermedia there was extensive overlapping in bilirubin levels of patients with the three $(TA)_n$ genotypes. However, mean serum indirect bilirubin was significantly higher in patients with the $(TA)_7/(TA)_7$ genotype than in patients with $(TA)_7/(TA)_6$ or $(TA)_6/(TA)_6$ genotype. No significant differences in bilirubin levels were detected between splenectomized (n = 29) and non-splenectomized (n = 5) patients. In the group of patients with $(TA)_7/(TA)_7$ genotype, those with G6PD deficiency had higher bilirubin levels than patients with normal G6PD activity.

Discussion

This study shows that in patients with transfusion-dependent thalassemia major and normal liver function, the serum indirect bilirubin levels are related to the configuration of the polymorphic $A(TA)_nTAA$ motif of UGT1*1 promoter: those patients with the $(TA)_7/(TA)_7$ configuration, the sequence associated

with Gilbert's syndrome,[1,2] have levels higher than those with the $(TA)_6/(TA)_6$ or $(TA)_6/(TA)_7$ genotype. Patients with high bilirubin values and $(TA)_6$ configuration could have missense mutations in the UGT1*1 gene. These findings indicate that the variability of bilirubin levels in transfusion-dependent thalassemia major patients is mostly due to differences in the bilirubin glucuronidation rate. The other most important factor which may influence bilirubin levels in patients with thalassemia major is the red blood cell destruction rate, which was, however, not very variable in our patients, as shown by the homogeneity of the blood consumption values.

A similar trend was also detected in thalassemia intermedia, although in this group there was a marked overlap in bilirubin levels between patients with different UGT1*1 promoter genotypes. In these patients the variability of the bilirubin levels, besides being due to bilirubin conjugation, may be related to the interplay of many factors among which the degree of ineffective erythropoiesis and hemolysis could have relevant roles.[10] Moreover, G6PD activity also contributes to this variability as demonstrated by the higher serum bilirubin values in G6PD deficient patients.

The results produced in this study indicate that Gilbert's syndrome genotype is an inherited factor capable of modifying some aspects of the clinical phenotype of homozygous β-thalassemia, as has already been reported for heterozygous β-thalassemia and G6PD deficiency.[4,5] This is a clear example of the role of coinherited modifying gene(s) in the determination of clinical heterogeneity of monogenic disorders. Analysis of UGT1*1 gene promoter should be performed in thalassemia major patients with hyperbilirubinemia, normal liver function and normal blood consumption in order to evaluate the presence of associated Gilbert's syndrome genotype.

Contributions and Acknowledgments

RG conceived and designed the study and wrote the paper with AC. MDC carried out the DNA analyses, CD, NG and EL were the clinicians involved in following the patients and collected the data. We thank Valeria Siccardo for her editorial assistance and Pierluigi Schirru for the reticulocyte analyses.

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Disclosures

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