Thalassemia minor, the Gilbert mutation, and the risk of gallstones

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Background and Objectives. Gallstones are a frequent complication of hemolytic anemias. The association with the mutation of the A(TA)nTAA motif of the promoter of the bilirubin UDP-glucuronosyltransferase gene has also been reported to increase the risk of gallstones. We studied the prevalence of cholelithiasis in thalassemia minor and the role of the Gilbert mutation.

Design and Methods. A group of 143 women obligate carriers of β-thalassemia, and a control group of 170 hematologically normal women were compared. In both groups serum bilirubin, total cholesterol, and alanine-aminotransferase were measured and analysis of the mutation of the UGT-1A gene was performed. On the same occasion the women underwent ultrasonography.

Results. Total and unconjugated bilirubin were significantly higher in β-thalassemia heterozygotes. Carriers of thalassemia had a higher prevalence of gallstones (20.3% vs 10.6% OR=2.15). Among the control group, the prevalence of gallstones did not differ significantly in relation to UGT1-A1 genotype, while in women carriers of β-thalassemia it increased in an allele dose-dependent fashion. As compared to the controls, the odds ratios for the development of gallstones in thalassemic women were 1.68 (95% C.I.: 0.70-4.03) for those who had the normal UGT1-A1 genotype [(TA)6/(TA)6], 2.31 (95% C.I.: 1.06-5.02) for heterozygote carriers of the mutated genotype [(TA)7/(TA)6] and 3.88 (95% C.I.: 1.31-11.55) for those homozygous for the mutated genotype [(TA)7/(TA)7].

Interpretation and Conclusions. Thalassemia minor represents a risk factor for cholelithiasis and the Gilbert mutation further increases this risk. This is an additional example of how two genotypes can interact and modify a phenotype.

Key words: Gilbert mutation, hyperbilirubinemia, UDP-glucuronosyltransferase, thalassemia trait, gallstones.

Haematologica 2003; 88:1106-1109
http://www.haematologica.org/2003_10/1106.htm

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Thalassemia is a common genetic disorder worldwide. The heterozygous state is characterized by chronic, low-grade ineffective erythropoiesis and variable levels of serum bilirubin.

Cholelithiasis is known to be associated with red cell defects. Recently, the so-called Gilbert mutation has been reported to play a role in inducing gallstones in several hemolytic disorders.[1-4] The Gilbert mutation affects the A(TA)nTAA motif of the promoter of the bilirubin UDP-glucuronosyltransferase gene (UGT1-A1). A normal promoter contains six dinucleotide repeats (TA)6 whereas a mutated promoter contains an additional dinucleotide resulting in the (TA)7 motif. The presence of this expanded element has been shown to decrease the expression of the UGT1-A1 gene by about 30%. In 1983 we reported that women with β-thalassemia trait have a higher risk of developing gallstones than do controls.[5] However an association with the Gilbert mutation, identified only in 1995, has never been investigated.

We designed this study to confirm the increased risk of gallstones in women with thalassemia minor and to investigate any association with the Gilbert genotype.

Design and Methods

The mothers of patients with thalassemia major followed at our hospital, who are therefore obligate carriers, were asked to participate in this study. All the women were Italian and were originally from, or living in, the area around the Po River delta. Controls were enrolled from among female personnel of the hospital who knew, on the basis of a previously performed, population-based, screening test, that they were β-thalassemia carriers.

In order to avoid preferential inclusion of women who had symptoms attributable to gallstones, detailed explanations were not given to the women who were asked to participate in the research. Subsequently, after verbal acceptance, the study was explained in detail to the participating women and written acceptance was obtained. The ethical committee of the University of Ferrara approved the study. On the morning of the test, date of birth, parity, body mass index, and use of sexual hormones were recorded. Blood was drawn, after 12 hours of fasting, and serum bilirubin, total cholesterol, and serum alanine-aminotransferase were determined by the random access instrument DAX- 96 (Bayer Italia, Milan, Italy). A blood sample collected in EDTA was

Table 1. UGT1-A1 genotype pattern in β-thalassemic women and in controls. $(TA)_6/(TA)_6$ indicates patients with a normal genotype, $(TA)_6/(TA)_7$ indicates the heterozygotes and $(TA)_7/(TA)_7$ indicates the homozygotes for the Gilbert mutation.

	(TA)6/(TA)6		*(TA)6/(TA)7*		*(TA)7/(TA)7*	
	N	*%*	*N*	*%*	*N*	*%*
β-Thalassemia	54	39.1	65	47.1	19	13.8
Controls	60	37.5	70	43.8	30	18.7

immediately frozen for analysis of the Gilbert mutation. On the same occasion ultrasonography was performed. Women who had undergone cholecystectomy were considered as having gallstones.

The analysis of the A(TA)nTAA motif in the promoter region of the UGT-1A gene was performed as previously described.[1] The patients with a normal genotype were designated as (TA)6/(TA)6, the heterozygotes as (TA)6/(TA)7 and the mutated homozygotes as (TA)7/(TA)7.

Comparisons among means were performed using Student's t test or the analysis of variance. Comparisons among proportions were carried out using the χ^2 test. Logistic regression analysis was used to compare the prevalence of cholelithiasis in women with and without thalassemia minor, taking into account the effect of other selected variables. A significance level of 0.05 was used.

Results

One hundred and forty three cases (β-thalassemia heterozygous women) and 170 controls were enrolled. No significant differences were found with regard to age, body mass index, parity, use of sexual hormones, serum alanine-aminotransferase and cholesterol level. UGT1-A1 genotype was ascertained in 298 subjects (138 cases and 160 controls). There was no difference between the two groups (χ^2=1.35; p=0.508) in the distribution of the three genetic patterns, as shown in Table 1. The clinical and laboratory characteristics of women who were normal, heterozygous or homozygous for the UGT1-A1 mutation were not significantly different, with the exception of serum bilirubin levels.

The mean values (and the corresponding standard deviations) of total and unconjugated serum bilirubin are reported in Table 2. The analysis of variance, performed on the log-transformed values, revealed a significant difference between cases and controls both for total bilirubin (F-test=12.52; p<0.001) and for unconjugated bilirubin (F-test=14.14; p<0.001) with higher mean values in β-thalassemia carriers. A significant trend according to the UGT1-A1 phenotype was also found for total

Table 2. Mean levels of bilirubin (mg/dL) in carriers of β-thalassemia and in controls according to the length of the promoter of the UDP-glucuronosyltransferase gene.

	Total		*Indirect*	
	β-Thalassemia Mean±SD	*Controls Mean±SD*	*β-Thalassemia Mean±SD*	*Controls Mean ±SD*
$(TA)_6/(TA)_6$	0.51± 0.19	0.44±0.16	0.37±0.14	0.31±0.12
$(TA)_6/(TA)_7$	0.68± 0.30	0.51±0.18	0.51±0.23	0.37±0.15
$(TA)_7/(TA)_7$	1.07± 0.41	0.92±0.53	0.83±0.33	0.70±0.42

$(TA)_6/(TA)_6$ indicates patients with a normal genotype, $(TA)_6/(TA)_7$ indicates the heterozygotes and $(TA)_7/(TA)_7$ indicates the homozygotes for the Gilbert mutation. F-tests for the difference of the means between cases and controls: 12.52 (p<0.001) for total bilirubin, 14.14 (p<0.001) for unconjugated bilirubin. F-tests for the difference of the means among the three UGT1-A1 genotypes: 42.24 (p<0.001) for total bilirubin, 42.28 (p<0.001) for unconjugated bilirubin. F-tests for the interaction between thalassemic status and UGT1-A1 genotype: 0.62 (p=0.538) for total bilirubin, 0.78 (p=0.458) for unconjugated bilirubin. To convert values for serum bilirubin to micromoles per liter, multiply by 17.1.

(F-test=42.24; p<0.001) and unconjugated (F-test=42.28; p<0.001) bilirubin. These results did not differ between cases and controls (total bilirubin: F-test=0.62, p=0.538; unconjugated bilirubin: F-test=0.78, p=0.458).

The women carriers of thalassemia had a significantly higher prevalence of gallstones (21.0% vs 10.7%; χ^2=6.13; p=0.013, OR=2.24; 95% C.I.:1.12-4.57). This result was confirmed also when adjustment for age (OR=2.00; 95% C.I.: 1.03-3.86) and for body mass index was made (OR=2.07; 95% C.I.: 1.07-4.00).

Figure 1 shows the prevalence of cholelithiasis among cases and controls according to UGT1-A1 genotypes. Multivariate logistic regression showed a significant association between β-thalassemia and cholelithiasis which was independent of UGT1-A1 genotype. The adjusted odds of β-thalassemia carriers having cholelithiasis were about 2-fold greater than in controls (OR=2.23; 95% C.I.: 1.17-4.29) in the three UGT1-A1 genotypes. However, a significant interaction was found between thalassemic status and UGT1-A1 genotype (χ^2=3.96; p=0.047). In fact, while the prevalence of cholelithiasis in controls did not differ significantly in relation to the three UGT1-A1 genotypes, in β-thalassemic women, the percentage of women with gallstones increased in an allele dose-dependent fashion. Using the rate in the control subjects as the reference, the odds ratios for the development of gallstones in thalassemic women were 1.68 (95% C.I.: 0.70-4.03) -4.03) for those who carried the normal UGT1-A1 genotype $[(TA)_6/(TA)_6]$, 2.31 (95% C.I.: 1.06-5.02) for those who carried the mutated UGT1-A1 genotype $[(TA)_7/(TA)_6]$ and 3.88 (95% C.I.: 1.31-11.55) for those who were homozygous for the mutated UGT1-A1 genotype $[(TA)_7/(TA)_7]$.

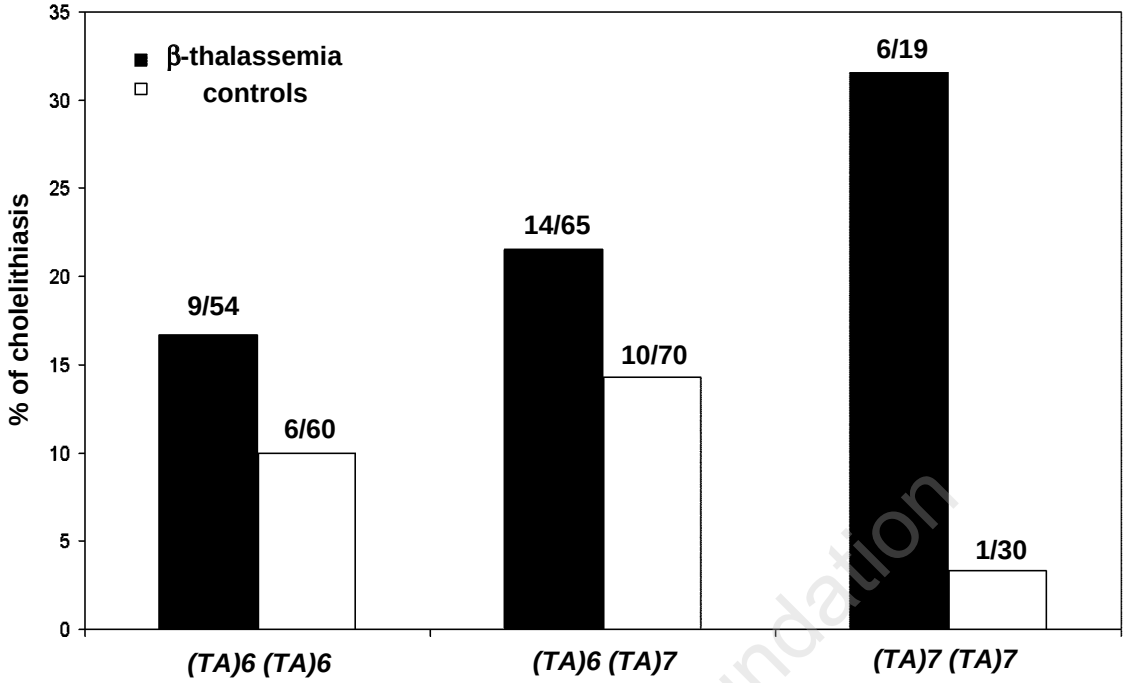

Figure 1. Prevalence of cholelithiasis in carriers of β-thalassemia and in controls, according to the length of the A(TA)nTAA element in the promoter region of the gene for UDP-glucuronosyltransferase 1.

Discussion

In this study we report that the odds of developing gallstones is twice as high in women carriers of β-thalassemia than in the normal population and that the odds increase significantly in both those heterozygous and those homozygous for the Gilbert mutation. In contrast, the presence of the mutation in the heterozygous or the homozygous state does not increase the risk of gallstones in normal women. In accordance with previous studies,[6-7] we found that total and unconjugated bilirubin levels, explained by mild hemolysis and ineffective erythropoiesis, increase in the presence of the Gilbert mutation.

Bilirubin is converted in the hepatocyte to bilirubin monoglucuronide (BMG) or diglucuronide (BDG) by several classes of the enzyme bilirubin glucuronosyltransferase. The poorly soluble biliary bilirubin monoconjugates are believed to play a pivotal role in stone formation. We do not know the composition of the stones present in the affected women; however, it has been demonstrated that a bilirubin center is also frequently present in cholesterol stones. In fact, defective conjugation could act as a trigger for gallstone initiation, regardless of the final composition of the stone[8-9] and unconjugated bilirubin has been demonstrated to enhance the cholesterol crystallization process by either a direct interaction with biliary lipids, an indirect alteration of the bile salt-micellar lipid holding capacity, or both. On the other hand, the presence of β-glucuronidase, decreasing the amount of unconjugated bilirubin, may inhibit the formation of pure cholesterol stones even in the presence of cholesterol supersaturation.[10]

An inverse correlation has been observed between total serum cholesterol and the risk of gallstones. Individuals with thalassemia minor are said to have lower cholesterol levels than control populations,[11] a finding that we did not confirm in this population of women.

In conclusion, we demonstrated that thalassemia minor, at least in women, is a risk factor for cholelithiasis, and that the Gilbert mutation both in the heterozygous and the homozygous state further increases this risk in thalassemia carriers. The results of this study are an additional example of how the phenotype of a genetic disease can be modified by interaction with a second genotype.

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Pre-publication Report & Outcomes of Peer Review

Contributions

CB-P: study design, data analysis, collection and interpretation, writing of the report; FR: data collection, analysis and interpretation, writing of the report; RC, LM: study design, data collection, analysis and interpretation; RG, RM: study design, data analysis and interpretation, critical revision of the report for intellectual content: LP: data analysis and interpretation, critical revision of the report for intellectual content.

Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

Funding

The study was supported by a grant for projects of local interest 2001 of the University of Ferrara to CBP, and by L.R. 11, April 30, 1990, Project PERF0001 Conv ICS 160 3/RF00.368 to RG. The funding sources had no influence on the study design, collection, analysis or interpretation of data, or presentation of the results.

Manuscript processing

This manuscript was peer-reviewed by two external referees and by Dr. Michael Kaplan, who acted as an Associate Editor. The final decision to accept this paper for publication was taken jointly by Dr. Kaplan and the Editors. Manuscript received June 17, 2003; accepted July 21, 2003.

In the following paragraphs, Dr. Kaplan summarizes the peer-review process and its outcomes.

What is already known on this topic

Gilbert's syndrome is associated with polymorphism for the TA nucleotide sequence in the promoter of the gene encoding the bilirubin conjugating enzyme, UDP-glucuronosyltransferase 1A1 (UGT). In and of itself, Gilbert syndrome appears to be a benign condition associated with mild jaundice or slightly elevated serum total bilirubin concentrations, in the absence of abnormal liver function tests. However, Gilbert syndrome tends to interact with additional factors including glucose-6-phosphate dehydrogenase deficiency, hetero-and homozgygous beta-thalassemia and hereditary spherocytosis, exacerbating their effects. This phenomenon has resulted in increased serum bilirubin values, intensification of neonatal hyperbilirubinemia and the development of gall stones.

What this study adds

In the present study, Drs. Borgna-Pignatti and colleagues now demonstrate an interaction between Gilbert syndrome and thalassemia minor thereby precipitating the development of gall stones. This finding adds to the list of phenotypic interactions between UGT promoter polymorphism (Gilbert syndrome) and other conditions, with exacerbation of their harmful effects.