



## Association of *UGT1A1* polymorphism with prevalence and age at onset of cholelithiasis in sickle cell anemia

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**Background and Objectives.** High levels of erythrocyte destruction in sickle cell anemia (SCA) result in chronic hyperbilirubinemia, with cholelithiasis occurring in a subset of patients. We investigated whether susceptibility to cholelithiasis in SCA was associated with the promoter polymorphism of the 5'-diphosphate-glucuronosyltransferase 1A1 (*UGT1A1*) gene encoding a key enzyme in bilirubin catabolism.

**Design and Methods.** We determined the frequencies of *UGT1A1* promoter alleles in 171 SCA children and 153 SCA adults regularly followed for a number of years at the Guadeloupe sickle cell center. These patients had undergone liver/biliary tree ultrasound scans every year. We analyzed the relationships between the various *UGT1A1* promoter alleles and hemoglobin levels, steady-state total and unconjugated bilirubin concentrations and the frequency of cholelithiasis.

**Results.** In both children and adults, (TA)<sub>6</sub> was less frequent and (TA)<sub>7</sub> more frequent in patients with cholelithiasis than in those without this condition. Total and unconjugated bilirubin levels and the frequency of cholelithiasis were significantly higher in patients with (TA)<sub>7</sub>/(TA)<sub>7</sub> and (TA)<sub>7</sub>/(TA)<sub>8</sub> genotypes than in those with other genotypes. Those homozygous for (TA)<sub>6</sub> or carrying at least one (TA)<sub>5</sub> allele had the lowest total and unconjugated bilirubin levels and were least likely to have cholelithiasis. Patients with (TA)<sub>6</sub>/(TA)<sub>7</sub> and (TA)<sub>6</sub>/(TA)<sub>8</sub> genotypes presented intermediate values. Kaplan-Meier analysis of cholelithiasis-free survival in children demonstrated an early age-at-onset for cholelithiasis in patients with (TA)<sub>7</sub>/(TA)<sub>7</sub> and (TA)<sub>7</sub>/(TA)<sub>8</sub> genotypes.

**Interpretations and Conclusions.** This study shows that the *UGT1A1* gene promoter polymorphism is a major genetic risk factor modifying the frequency and age-at-onset of cholelithiasis in SCA patients.

Key words: sickle cell anemia, *UGT1A1* polymorphism, cholelithiasis.

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High levels of erythrocyte destruction in patients with sickle cell anemia (SCA) result in chronic hyperbilirubinemia. A significant proportion of patients are prone to cholelithiasis due to the resulting high biliary concentration of unconjugated bilirubin, which tends to coprecipitate with calcium in the gall bladder lumen. Cholelithiasis, by promoting cholecystitis and choledocholithiasis, is responsible for high levels of morbidity in SCA patients<sup>1</sup> and elective cholecystectomy is therefore recommended for patients developing this complication.<sup>2</sup> The genetic basis of Gilbert's syndrome, a benign condition in which the unconjugated bilirubin concentration is slightly high, has been shown to result from promoter polymorphisms in one of the isoforms of the UDP-glucuronosyltransferase (*UGT*) gene.<sup>3,4</sup> *UGT1A* is a nested gene complex comprising nine transcriptional units, each encoding a specific isoform of the enzyme. *UGT1A1* is respon-

sible for bilirubin glucuronidation, whereas the other gene products are involved in the metabolism of a number of aromatic compounds.<sup>5</sup> The most common molecular mechanism involved in Gilbert's syndrome involves a polymorphism in the promoter of the *UGT1A1* gene.<sup>3</sup> The promoter region contains a run of thymine-adenine (TA) repeats, with four alleles described in humans. These alleles differ in the number of repeats, from 5 to 8.<sup>6,7</sup> Homozygosity of the (TA)<sub>7</sub> allele, which is also known as *UGT1A1*\*28, has been implicated in Gilbert's syndrome<sup>3</sup> and in the unusually high unconjugated bilirubinemia observed in individuals with various forms of inherited chronic hemolysis: heterozygous  $\beta$ -thalassemia,<sup>8,9</sup>  $\beta$ -thalassemia intermedia,<sup>10,11</sup> neonatal jaundice associated with glucose-6-phosphate dehydrogenase deficiency,<sup>12</sup> hereditary spherocytosis<sup>13</sup> and sickle cell anemia.<sup>14,15</sup> In this study, we investigated the clinical impact of *UGT1A1* promoter poly-

morphism on the frequency of cholelithiasis in SCA patients. We studied 171 children and 153 adults with SCA followed by the sickle cell center of Guadeloupe, a French Caribbean island. We compared (i) the *UGT1A1* statuses of patients with and without stones; and (ii) the frequency of cholelithiasis in patients with and without *risk* genotypes.

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## Design and Methods

### Subjects

We selected 324 SCA patients (171 children and 153 adults) on the basis of homozygosity for the  $\beta^s$ -globin gene. All these patients were followed at the sickle cell center in Pointe-à-Pitre, Guadeloupe. The adult sample consisted of 67 men and 86 women (mean age: 35 years, SD: 12 years), all with at least three years of follow-up. The pediatric group was taken from a longitudinally followed cohort with a mean follow-up period of  $7.8 \pm 4.5$  years. One hundred fourteen of the pediatric patients (65 boys and 49 girls) were identified during the newborn screening program and had been followed ever since (follow-up began at a mean age of two months). The remaining 57 children (33 boys and 24 girls) were cases referred to the center and diagnosed with SCA following family studies or on the basis of acute events (follow-up began at a mean age of 38 months). Liver/biliary ultrasound scans were performed annually to detect cholelithiasis only in patients over the age of three years. We therefore excluded children below this age as no ultrasound data were available for such children. Informed consent was obtained from all adult patients and from the parents of the children.

### Clinical events analyzed

Cholelithiasis was diagnosed on the basis of echodense images within the gall bladder with acoustic shadowing or gravitational change in position.

### Laboratory methods

SCA was diagnosed on the basis of isoelectrofocusing, citrate agar electrophoresis, cation-exchange high performance liquid chromatography, together with, in most cases, family studies and further confirmation by means of DNA studies. Hematologic data were obtained with an automated cell counter. Genomic DNA was extracted from peripheral blood by the standard phenol-chloroform procedure.  $\beta^s$ -globin gene restriction fragment length polymorphism (RFLP) haplotypes were determined as previously described.<sup>16</sup>

Biochemical data were averaged for each patient in steady state (at least three values). We determined total and fetal hemoglobin (Hb F) concentrations, lactate dehydrogenase (LDH) levels, and reticulocyte count.

Total, unconjugated and conjugated bilirubin concentrations in serum were determined by a standardized colorimetric procedure (COBRAS INTEGRA, Meylan, France).

### Detection of the promoter variants of the *UGT1A1* gene

We analyzed the (TA)<sub>n</sub> motif in the promoter region of the *UGT1A1* gene by polymerase chain reaction (PCR) with radioisotopes. We then separated the amplified products in denaturing 6% polyacrylamide gels and detected the amplicons by autoradiography, as previously described.<sup>14</sup> Homozygosity of five patients for the (TA)<sub>6</sub> allele and five patients for the (TA)<sub>7</sub> allele was also confirmed by DNA sequencing. The amplified fragments were purified on a column and cycle sequenced with the ABI PRISM Big Dye Termination ready reaction kit and an ABI 310 DNA sequencer (PE Applied Biosystems, Foster City, USA).

### Statistical methods

Allele frequencies were determined from the genotype data. We assess deviation from expected frequencies based on the Hardy-Weinberg equilibrium by calculating the Pearson ( $\chi^2$ ) statistic. However, as some genotypes occurred in only small numbers, the usual assumption concerning the distribution of this statistic did not apply. Observed values of the statistic were tested against a distribution of 1,000 statistics generated by random assignment of the observed numbers of alleles. Differences in hematologic data between groups of patients were assessed using the Mann-Whitney rank sum test or the Kruskal-Wallis test, as appropriate. The prevalence of cholelithiasis was assessed by calculating the Pearson statistic. Kaplan-Meier analysis was used to estimate cholelithiasis-free survival (in years) for the group of children, the dependent variable being the detection of cholelithiasis. Participation time was defined as the time from the beginning of follow-up by the sickle cell center until the date on which cholelithiasis was detected or the end point of the study (December 31, 2002). Differences in unadjusted survival curves were assessed by means of the log-rank test, using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego CA, USA).

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## Results

Children and adults were studied independently as age is an important determinant for the incidence of cholelithiasis. We compared the frequency of cholelithiasis in children and adults: there was a significant difference, with a frequency of 29.9% in children and 66.7% in adults ( $p < 0.0001$ ). This confirmed our

original assumption that the groups would be different and we therefore analyzed the two groups independently (Table 1).

### Frequencies of *UGT1A1* promoter alleles

DNA analysis demonstrated the presence of four previously described *UGT1A1* promoter alleles – (TA)<sup>5</sup>, (TA)<sup>6</sup>, (TA)<sup>7</sup> and (TA)<sup>8</sup> – in the study group. The frequencies of these alleles did not differ between children and adults ( $\chi^2 = 2.41$ , *ddl* = 3, *p* = 0.49). (TA)<sup>6</sup> was the most common allele (0.45 and 0.5 in children and adults, respectively), followed by (TA)<sup>7</sup> (0.40 and 0.36, respectively). The other two alleles, (TA)<sup>5</sup> and (TA)<sup>8</sup>, were less common (0.09/0.08 and 0.06/0.06 respectively). There was no evidence of significant deviation from the Hardy-Weinberg equilibrium in either group.

### Comparison of patients stratified according to the occurrence of cholelithiasis

Children with SCA and cholelithiasis had significantly lower Hb F and higher serum total and unconjugated bilirubin concentrations than did the children with SCA who did not have cholelithiasis (Table 1). No difference in hemoglobin concentration or reticulocyte count was observed. In the adult SCA group, patients with cholelithiasis had higher serum total and unconjugated bilirubin concentrations but no difference was found for the other hematologic data, including LDH. In children and adults, the frequencies of (TA)<sup>5</sup> and (TA)<sup>6</sup> were lower in patients with cholelithiasis than in patients without cholelithiasis, whereas the frequencies of (TA)<sup>7</sup> and (TA)<sup>8</sup> were higher. However, these differences were statistically significant only for the most frequent alleles: (TA)<sup>6</sup> and (TA)<sup>7</sup> (Table 1).  $\beta^s$ -globin gene haplotype distribution did not differ between children and adults or between patients with and without cholelithiasis. The Benin haplotype was the most frequently encountered typical haplotype (72%), followed by the Bantou (14%), Senegal (8%), Cameroon (1.8%) and Arabo-Indian (0.2%) haplotypes; 4% of the haplotypes identified were atypical.

### Comparison of patients stratified according to *UGT1A1* genotype

We investigated the hematologic effect of *UGT1A1* gene polymorphism by stratifying SCA children (Table 2) and adults (Table 3) according to genotype and comparing them. Serum total and unconjugated bilirubin concentrations were significantly different in the *UGT1A1* genotype groups for both adults and children. No difference was observed for the concentrations of conjugated bilirubin (Tables 2 and 3), hemoglobin and reticulocytes (*data not shown*), suggesting similar hemolytic rates in these groups. We compared the total bilirubin and unconjugated bilirubin concentrations of

**Table 1.** Hematological parameters of SCA patients with and without cholelithiasis.

	Children			Adults		
	Cholelithiasis	No cholelithiasis	<i>p</i>	Cholelithiasis	No cholelithiasis	<i>p</i>
N	50	121	NS	102	51	NS
Sex ratio (M/F)	31/19	67/54	NS	45/57	22/29	NS
Age (years)	13±2.9	9.2±4.7	NS	36±12	34 ± 9.1	NS
Hb (g/dL)	7.7±0.9	7.9±1.3	NS	8.4±1.5	8.6 ± 1.5	NS
Hb F (%)	7.4±4.7	11±6.8	0.007	8.8±5.5	8.4 ± 5.7	NS
LDH (IU/L)	NA	NA	–	824±350	786 ± 286	NS
Reticulocytes (10 <sup>9</sup> /L)	291±113	332±118	NS	311±123	320±105	NS
Total bilirubin (μM)	73.4±44.1	53±36	<10 <sup>-4</sup>	70.9±35.9	56±35.8	<10 <sup>-4</sup>
Unconjugated bilirubin (μM)	62.4±38.1	42±32	<10 <sup>-4</sup>	50.2±35.4	43.5±30.5	0.01
Allele frequency						
(TA) <sup>5</sup>	0.07	0.12	NS	0.06	0.11	NS
(TA) <sup>6</sup>	0.32	0.5	0.002	0.45	0.61	0.01
(TA) <sup>7</sup>	0.52	0.35	0.005	0.42	0.23	0.0015
(TA) <sup>8</sup>	0.09	0.04	NS	0.07	0.05	NS

NA: not available; NS: not significant. The hematologic values are indicated as mean ± standard deviation.

the group with the wild-type (TA)<sup>6</sup>/(TA)<sup>6</sup> promoter genotype with those of each of the other groups. No difference was observed with the patient groups carrying at least one (TA)<sup>5</sup> allele. Groups of patients heterozygous for (TA)<sup>6</sup>/(TA)<sup>7</sup> or (TA)<sup>6</sup>/(TA)<sup>8</sup> had higher values for the two parameters analyzed but the differences were statistically significant only for the (TA)<sup>6</sup>/(TA)<sup>7</sup> group. The most significant values were obtained for the groups of patients homozygous for (TA)<sup>7</sup> allele or heterozygous (TA)<sup>7</sup>/(TA)<sup>8</sup>. Such pairwise comparisons were used to bundle genotypes into three patient groups. Group 1 consisted of patients carrying at least one (TA)<sup>5</sup> allele or homozygous for (TA)<sup>6</sup>, group 2 consisted of patients heterozygous for (TA)<sup>6</sup>/(TA)<sup>7</sup> or for (TA)<sup>6</sup>/(TA)<sup>8</sup> and group 3 consisted of patients homozygous for (TA)<sup>7</sup> or heterozygous (TA)<sup>7</sup>/(TA)<sup>8</sup>. The unconjugated bilirubin concentration was 29.3±12.4 μM for group 1, 42.2±17.5 μM for group 2 and 90±40 μM for group 3 for children with SCA. For adults, unconjugated bilirubin concentration was 32±14.5 μM for group 1, 53.5±28 μM for group 2 and 94±35.5 μM for group 3. The frequency of cholelithiasis was 22%, 33% and 61% for groups 1, 2 and 3, respectively for children and 51%, 74% and 84% groups 1, 2 and 3, respectively for adults. In both children and adults, unconjugated bilirubin concentrations and the frequency of cholelithiasis differed significantly between groups 1, 2 and 3, with group 3 having the highest unconjugated bilirubin concentration and frequency of cholelithiasis (Figure 1).

**Table 2. Bilirubin-related parameters in SCA children stratified according to their UGT1A1 genotype.**

	5/5	5/6	5/7 Group 1	5/8	6/6	6/7 Group 2	6/8	7/7	7/8 Group 3	p
n	5	11	9	3	37	64	5	27	10	
Sex ratio (M/F)	2/3	5/6	6/3	3/0	18/19	41/23	3/2	17/10	3/7	NS
Total bilirubin (μM)	43±7.5	39±11	32.8±9.9	43±16.8	40±14.4	56±34.2°	59±27	101±44#	102±41#	p<10 <sup>-4</sup>
Unconjugated bilirubin (μM)	28.9±11	29±9.4	21±8.9	37±19.8	30.3±13	42±17°	48±25.7	89.5±43#	91±41#	p<10 <sup>-4</sup>
Conjugated bilirubin (μM)	14.4±4	10.5±3	12±3.9	6±3.5	10±4.6	12.8±9.5	12±3	11.2±5.5	11±3.1	NS

Overall differences in serum bilirubin concentrations expressed as mean ± standard deviation, were assessed using the Kruskal-Wallis test. Pairwise comparisons between bilirubin concentrations of each genotype were performed with the 6/6 patient group using the Mann-Whitney rank sum test: °p < 0.005, #p < 0.0004. Comparison of the sex ratio between groups was performed by Pearson's test. NS: not significant.

**Table 3. Bilirubin-related parameters in adult SCA patients stratified according to their UGT1A1 genotype.**

	5/5	5/6	5/7 Group 1	5/8	6/6	6/7 Group 2	6/8	7/7	7/8 Group 3	p
n	2	12	6	2	42	51	6	21	11	
Sex ratio (M/F)	1/1	4/8	3/3	1/1	18/24	26/25	2/4	10/11	2/9	NS
Total bilirubin (μM)	30.5±7.5	35±17.5	38±5.8	40±11.8	45±17	69±35°	60±29	112±39#	94±25#	<10 <sup>-4</sup>
Unconjugated bilirubin (μM)	24±10.5	26±15	35±5	36±12.6	34±15	55±29°	47±21	102±39#	80±24#	<10 <sup>-4</sup>
Conjugated bilirubin (μM)	5±2	8.7±3	6.4±1.7	4±2.5	10.8±5.6	14.5±10	11.9±8	15±7	13.6±5	NS

Overall differences in serum bilirubin concentrations expressed as mean ± standard deviation, were assessed using the Kruskal-Wallis test. Pairwise comparisons between bilirubin concentrations of each genotype were performed with the 6/6 patient group using the Mann-Whitney rank sum test: °p < 0.005, #p < 0.0004. Comparison of the sex ratio between groups was performed by Pearson test. NS: not significant.

### Cholelithiasis-free survival curves in SCA children

We analyzed the phenotype-genotype relationship further by plotting cholelithiasis-free survival curves for children with SCA (Figure 2). The median time to first ultrasound detection of cholelithiasis differed significantly between the three genotype groups (log rank coefficient of 12.8,  $p=0.0014$ ). The median to first ultrasound detection of cholelithiasis was shorter for group 3 patients than for the patients of group 2 (log rank coefficient = 8.2,  $p=0.0041$ ) and group 1 (log rank coefficient = 10.5,  $p=0.001$ ) risk groups. No significant difference was observed between groups 1 and 2 (log rank test of 0.88,  $p=0.4$ ).

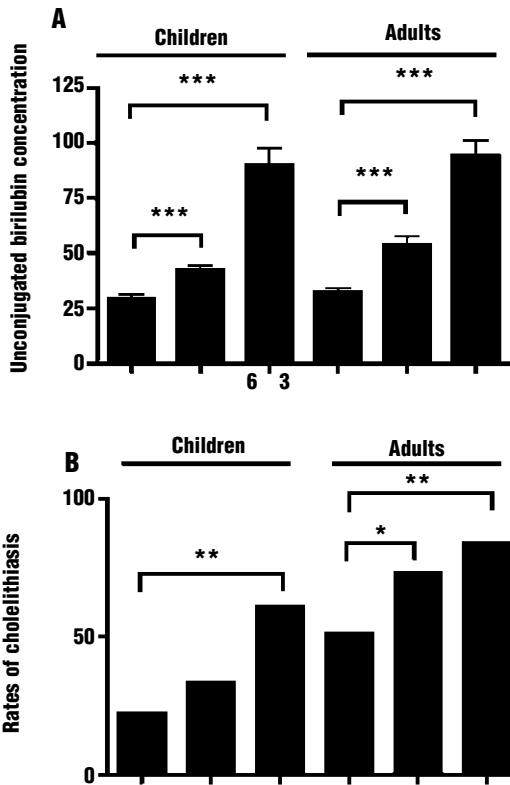
## Discussion

The sickle cell gene alone is not sufficient to account for one of the major characteristics of SCA: its wide range of phenotypic expression, even in patients with identical hemoglobin genotypes from apparently similar environments.<sup>17</sup> This strongly suggests that genes other than  $\beta^S$  play a role in the phenotypic diversity of SCA. We used longitudinally recorded clinical data and hematologic/biochemical parameters for SCA patients

followed by a single sickle cell center from 1991 onwards and, before this date, by the various departments of the University Hospital of Pointe-à-Pitre, Guadeloupe. The medical follow-up of these patients included an annual hepatobiliary ultrasound scan making it possible to assess the date of onset and frequency of cholelithiasis precisely.

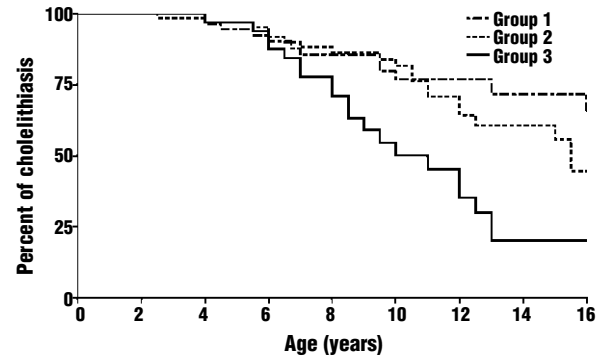
The frequency of cholelithiasis observed in SCA children in this study (29.8%) is similar to the frequencies reported for comparable groups in the United States<sup>18-21</sup> and in the Jamaican cohort study.<sup>22</sup> The high frequency of cholelithiasis (66.7%) in adults is not unexpected as age is a known risk factor.<sup>23</sup>

Several risk factors for cholelithiasis in SCA patients have been identified in previous studies, including low Hb F concentration and high reticulocyte counts,<sup>24</sup> high total<sup>19,24</sup> and unconjugated bilirubin concentrations.<sup>24,25</sup> In our study, cholelithiasis was associated with high total and unconjugated bilirubin concentrations in both children and adults with SCA and with low Hb F concentration in children with SCA only, suggesting that high Hb F concentration – known to be associated with a decrease in hemolytic rate in SCA patients – has a protective effect. However, no difference was detected between groups of patients with and without



**Figure 1.** Unconjugated bilirubin concentration and rates of cholelithiasis in SCA patients classified accordingly to the *UGT1A1* genotype. A. Unconjugated bilirubin concentration expressed as mean  $\pm$  standard deviation were compared between group 1 and the other groups using the Mann-Whitney rank sum test. B. Cholelithiasis rates were compared between group 1 and the other groups using Pearson's statistic. \*:  $p < 0.05$ ; \*\*:  $p < 10^{-2}$ ; \*\*\*:  $p < 10^{-4}$ . Group 1:  $(TA)_5/(TA)_5$ ,  $(TA)_5/(TA)_6$ ,  $(TA)_5/(TA)_7$ ,  $(TA)_5/(TA)_8$ ,  $(TA)_6/(TA)_6$ ; group 2:  $(TA)_6/(TA)_7$ ,  $(TA)_6/(TA)_8$ ; group 3:  $(TA)_7/(TA)_7$ ,  $(TA)_7/(TA)_8$ .

cholelithiasis for the other hematologic markers of hemolytic anemia severity studied. This suggests that the relationship between frequency of cholelithiasis and degree of hemolysis is not straightforward and that additional factors are involved. The degree of dyserythropoiesis and hemolysis may also be similar in the various groups. We then evaluated the contribution of *UGT1A1* genotype to unconjugated hyperbilirubinemia and to the prevalence of cholelithiasis. The four known alleles in the *UGT1A1* promoter region and nine of the ten possible genotypes were retrieved. Two global surveys showed a wide variation in the frequency of the number of  $(TA)$  repeats in the promoter region of the *UGT1A1* gene.<sup>7,26</sup>  $(TA)_6$  is the most common allele in all studied populations whereas  $(TA)_7$  is most frequent in populations of the Indian subcontinent, rarest in Chinese populations and has a highly variable frequency in European populations. In African populations, the spectrum is quite broad, ranging from five to eight  $(TA)$  repeats. The allele distribution observed in this study is



**Figure 2.** Cholelithiasis-free survival curves of the pediatric SCA patients. Group 1: genotype  $(TA)_5/(TA)_5$ ,  $(TA)_5/(TA)_6$ ,  $(TA)_5/(TA)_7$ ,  $(TA)_5/(TA)_8$ ,  $(TA)_6/(TA)_6$ . Group 2: genotype  $(TA)_6/(TA)_7$ ,  $(TA)_6/(TA)_8$ . Group 3: genotype  $(TA)_7/(TA)_7$ ,  $(TA)_7/(TA)_8$ . Kaplan-Meier analysis was used to estimate the cholelithiasis-free survival of children and differences in survival curves assessed by log-rank test.

consistent with the known history of the population of Guadeloupe, which results from the recent mixing of different ethnic groups, with West Africans making up the largest group, followed by Indians (mostly from South India) and Caucasians from mainland France and other Mediterranean countries (Syria and Lebanon). We compared patients with and without cholelithiasis and found that the frequency of *UGT1A1* alleles with smaller numbers of  $(TA)_n$  repeats ( $n=5,6$ ) was lower, and that of alleles with larger numbers of  $(TA)_n$  repeats ( $n=7,8$ ) was higher in patients with cholelithiasis. These data suggest a possible relationship between the *UGT1A1* locus and the occurrence of cholelithiasis in patients with SCA. Two previous studies have shown that homozygosity for  $(TA)_7$  is associated with high steady-state concentrations of unconjugated bilirubin in serum and is a risk factor for cholelithiasis in children with SCA.<sup>14,15</sup> The limitations of these studies included differences in the number of laboratory measurements between patients and previous cholecystectomy as an indicator of symptomatic cholelithiasis, resulting in underestimation of the true prevalence of subclinical cholelithiasis. These two previous studies focused mainly on SCA patients carrying the two most frequent alleles –  $(TA)_6$  and  $(TA)_7$  – and provided only very limited information about the other two *UGT1A1* alleles. Our study included the largest cohorts of children and adult with SCA yet studied in terms of genetic risk factors for cholelithiasis, provided information about all the *UGT1A1* genotypes identified and strongly suggested that *UGT1A1* promoter polymorphism is a significant non-globin genetic modifier in SCA. Furthermore, it was recently shown that *UGT1A1* polymorphism is an important pharmacogenomic factor modulating the hematologic response to hydroxyurea treatment in

SCA patients.<sup>27</sup> In conclusion, we show here that SCA patients (whether children or adults) can be classified into three risk groups according to *UGT1A1* genotype. Patients homozygous for (TA)<sub>7</sub> or heterozygous (TA)<sub>7</sub>/(TA)<sub>8</sub> display similar ranges of unconjugated bilirubin concentration associated with a high frequency of cholelithiasis. We have also shown that SCA children with these genotypes are likely to develop cholelithiasis at an early age. (TA)<sub>6</sub> seems to be co-dominant with (TA)<sub>7</sub> and (TA)<sub>8</sub>, as patients with the (TA)<sub>6</sub>/(TA)<sub>7</sub> and (TA)<sub>6</sub>/(TA)<sub>8</sub> genotypes displayed higher unconjugated bilirubin concentrations and frequencies of cholelithiasis than did homozygous (TA)<sub>6</sub> patients. Patients with at least one (TA)<sub>5</sub> allele and patients homozygous for (TA)<sub>6</sub> had the lowest bilirubin concentrations and frequency of cholelithiasis, suggesting that (TA)<sub>5</sub> has a dominant protective effect over other alleles. Such a genotype - phenotype association does not necessarily imply a causal role, but the inverse relation-

ship between the number of (TA) repeats in the *UGT1A1* gene promoter and the *in vitro* activity of this promoter is consistent with this locus being biologically relevant to susceptibility to cholelithiasis.<sup>6</sup> *UGT1A1* genotyping is therefore a potentially useful tool for identifying individuals with SCA at high risk of cholelithiasis and needing close clinical monitoring.

*VC, LK and RM contributed to the conception, design, interpretation of data and wrote the article with the contribution of JE and RK who revised it. JC performed the statistical analysis and contributed to the interpretation of the data. JPD and CL participated to the collection, analysis and interpretation of the data. All authors have seen and approved the final version of the article. The authors declare that they have no potential conflicts of interest.*

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