with clinical signs, possibly underestimating the presence of IRE mutations with low penetrance. Nevertheless, our data on 16,162 subjects suggest that HHCS is a relatively rare condition, even in the setting of selected ophthalmological and/or hyperferritinemic patients.

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Risk factors for hyperbilirubinemia and gallstones in Chinese patients with β thalassemia syndrome

We studied the relationship between jaundice and gallstones and Gilbert alleles (Gly71Arg: 27.8% and $(TA)_7$: 19.6%) in 94 Chinese patients with thalassemia major (TM) and 33 with thalassemia intermedia (TI). Determinants of bilirubin level included age, transfusion (TI>TM) and genetic profile ($\alpha\alpha\alpha/\beta^0$ > β^+/β^0 > β^0/β^0 in TI, β^+/β^0 > β^0/β^0 in adult TM, Gilbert homozygotes > others in TM and TI, Gilbert heterozygotes > wild type in TM). Determinants of gallstones (39%) included age, TI and Gilbert alleles. We conclude that the finding of unusually high bilirubin may indicate either homozygous Gilbert genotype or hemolytic thalassemia genotypes.

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Gilbert's syndrome is caused by recessive alleles for uridine diphosphate-glucuronosyl transferase 1 (UGT1*1) enzyme. It is a reported cause of jaundice and gallstones in thalassemia patients.[1,2] Published studies, however, involved patients with relatively homogeneous Gilbert alleles e.g. $(TA)_7$ and β thalassemia genes e.g. 39 C/T and HbE mutations. The situation in the Southern Chinese population is complicated by heterogeneous β gene defects, α thalassemia co-inheritance, and multiple Gilbert alleles, mainly $(TA)_7$ and Gly71Arg substitution.[3] We revisited the Gilbert/thalassemia interaction in 127 Chinese with clinically defined thalassemia major (TM, n=94) and thalassemia intermedia (TI, n=33) syndromes.[4] Mean unconjugated bilirubin levels were measured. Molecular methods were used to define the thalassemia gene defect,[4, 5] and to detect the two common Chinese Gilbert alleles (Figure 1).[3]

The TI cases were heterogeneous (9 HbE/β^0, 7 $\beta^0/\alpha\alpha\alpha$, 3 β^0+unknown defect, 4β^+/β^+,10 β^+/β^0), while the TM cases included 63 β^+/β^+ and 31 β^+/β^0 cases.[4] The $(TA)_7$ and Gly71Arg alleles were found in 25 (19.6%, 2 homozygous) and 34 cases (26.8%, 6 homozygous), respectively, with 3 double heterozygotes. The median age of patients with TI was higher than that of the patients with TM (32.6 vs 20.0 years; p<0.001), but there was no difference in the incidence of Gilbert alleles (p=0.684). For all patients, serum bilirubin levels increased with age (p<0.001). The median bilirubin level was higher among TI cases than among TM ones (p<0.001). Patients carrying Gilbert alleles had higher bilirubin levels (p=0.027); this effect was mainly contributed by homozygotes and double heterozygotes (p=0.002). The heterozygote effect was evident only in less jaundiced TM cases (p=0.022), but not in TI cases (p=0.68). Apart from Gilbert alleles, the thalassemia genotype was also significant. Higher bilirubin levels were found in β^+/β^0 adult TM cases (p=0.047) and $\beta^0/\alpha\alpha\alpha$ TI cases (p=0.021). Neither the transfusion frequency nor baseline hemoglobin significantly affected bilirubin levels. Gallstones were found by ultrasound or magnetic resonance imaging in 26 of 78 studied patients (median age 23, range 5-66 years), while 8 patients had already surgical stone removal. The prevalence of gallstones increased with age (p<0.001), TM (p=0.002), bilirubin level (p<0.001) and Gilbert genotype (p=0.003). On multivariate analysis, significant predictive factors of bilirubin level included age (p=0.001), Gilbert genotype (p=0.003) and TI (p<0.001). However, only age (p<0.001) and Gilbert genotype (p=0.002) were important gallstone determinants, and the importance of bilirubin level was lost (p=0.052).

Both bilirubin production (hemolysis) and clearance (glucuronidation) are genetically determined in thalassemia patients.[1,2,6] Defective glucuronidation in carriers of Gilbert alleles is known to aggravate jaundice in all hemolytic anemias, including thalassaemia, G6PD deficiency[7] and spherocytosis.[8] The genotype-phenotype relationship in thalassemia is

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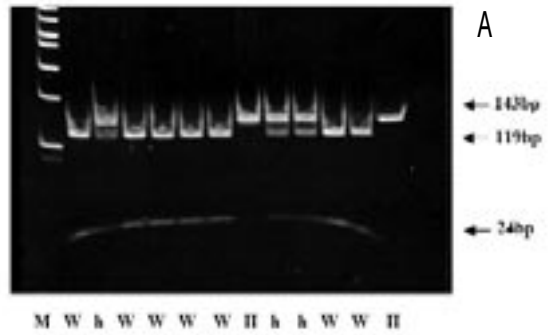

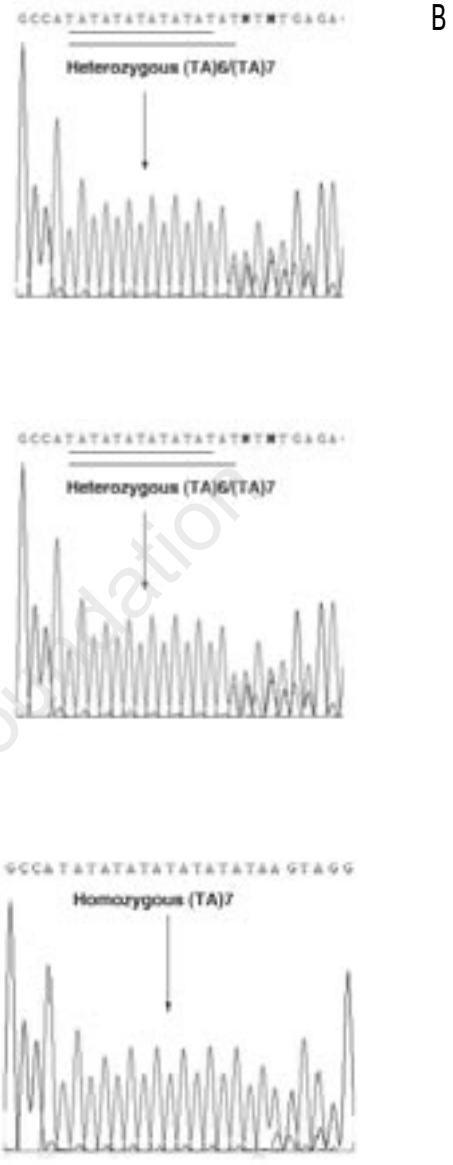

Figure 1A (above). PCR amplified UGT1*1 exon-1 (35 cycles, denaturation 94°C × 30s, annealing 53°C × 30s, extension 72°C ×60s, 5-TGA CGC CTC GTT GTA CAT CAG AGC C-3, 5-TCA CAC GCT GCA GGA AAG AA-3) was digested with Msp1 restriction enzyme (1 mg PCR mix+5U MspI at 37°C ×16 h). Abolition of the cleavage site results in an uncleaved 143bp product in Gly71Arg carriers, while wild type DNA is cleaved into 119bp and 24bp fragments. W: wild type; h: heterozygote; H: homozygote.
Figure 1B (right). PCR amplified 5' promoter region (same conditions, 5-GAG GTT CTG GAA GTA CTT TGC-3, 5-CCA AGC ATG CTC AGC CAG-3) and sequenced directly to identify wild type $(TA)_6$, and homozygous and heterozygous $(TA)_7$ repeat (left to right).

complex and worldwide allelic variations means that global data are needed. This is the first study of Gilbert interactions in Chinese thalassemics, and the first to involve multiple Gilbert alleles and a mixture of β^+ β^0 and α mutations. The incidence of Gilbert alleles was within a previously reported range in Chinese[3] and we did not confirm an increased incidence of Gilbert alleles in TI cases.[6] We showed that patients homozygous and double heterozygous for Gilbert alleles had a doubling of bilirubin levels. Heterozygosity for Gilbert alleles also gave a median increase of 10mmol in the bilirubin level in patients with TM, but the effect was diluted out by higher median bilirubin levels in TI cases. Occasional unexplained cases of jaundice may harbor rare UGT1-A1 mutations (e.g. Pro229Gln, Tyr486Asp, Pro364Leu), which occur in 2% of the Chinese population.[3] Furthermore, we showed that the thalassemia genotype, governing the rate of hemolysis, is also important. The higher bilirubin levels in TI cases are obviously expected. Interestingly however, TM cases with β^+/β^0 genotype[4] and TI cases with $\beta^0/\alpha\alpha\alpha$ genotype (both unique to Southern Chinese)[5] are associated with increased jaundice. This suggests that the absolute amount of a chain production, rather than the α/β ratio, may be more important in determining hemolysis. Finally, we investigated the determinants of gallstones and their relation with jaundice in Chinese thalassemia patients. The prevalence of gallstones in our study was 33%, compared with 20-57% in Italian and Sri Lankan studies.[2,9] This difference may be partly explained by dietary, social and climatic factors. We showed for the first time that apart from age, the Gilbert allele is the *only* significant predictor of gallstone prevalence. It remains to be seen whether enzyme-inducing drugs[10] could have a future role in ameliorating jaundice and preventing gallstones in susceptible patients.

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Partial splenectomy in children with sickle cell disease

Partial splenectomy was performed in 50 patients with sickle cell disease and acute splenic sequestration. Follow-up after surgery ranged from 2 to 14 years. During this period no recurrence of splenic sequestration crisis occurred and the quality of life of these children has improved.

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Acute splenic sequestration (ASS) is a well recognized complication in children with sickle cell disease (SCD) under 5 years old and a significant cause of morbidity and mortality.[1,2] The classical treatment of this crisis is total splenectomy but this procedure often carries the risk of fulminant septicemia.[3] For this reason we performed elective partial splenectomy in SCD patients with more than one episode of ASS.

We report the follow-up on the 50 children (Hb SS 34 and HbS/βthal 7) with SCD who underwent partial splenectomy. All had experienced more than one episode of ASS, defined as a fall in the hemoglobin (Hb) level of more than 2 g/dL from the baseline concentration, associated with an enlarged spleen and evidence of bone marrow erythroid activity.[1] Partial splenectomy was performed using a previously described technique.[4] Hematologic data were obtained in steady state according to standard methods.

Table 1. Clinical events in patients with SCD who had partial splenectomy.

Events	Before surgery Mean	Before surgery SD	After surgery Mean	After surgery SD	p
Hospitalizations	5.77	3.63	3.07	3.21	0.01
Transfusions	6.25	4.39	0.60	1.12	0.01
Infections	1.65	2.25	0.98	1.75	0.13

Table 2. Hematologic parameters in patients with SCD who had a partial splenectomy.

Tests	Before surgery N. patients	mean	SD	After surgery N. patients	mean	SD	P
Hemoglobin (g/dL)	49	6.0	1.3	50	7.7	2.0	0.01
Reticulocytes (%)	49	138.6	88.8	47	129.3	74.3	0.39
Leukocytes (10^9/L)	50	13.7	1.5	50	12.8	3.3	0.59
Platelets (10^9/L)	50	228.0	105.6	50	408.5	146.5	0.01
Pitted red cells (%)	20	4.9	1.7	33	3.7	2.9	0.07

To measure the functional capacity of the spleen, the percentage of pitted red cells was determined by the method of Pearson.[5] Splenic function was also evaluated by radionuclide scan with ^{99}Tc sulfur colloid. Student t test was used to compare clinical and hematological data before and after splenectomy. After the operation the children were followed at our clinic at intervals of one-to-three months. No prophylaxis was given prior to January 1989. Afterwards oral penicillin was given to all patients for 3 years after the operation. Pneumococcal vaccine was not used. The median age at the time of surgery was 3 years (1-9) and the median and mean duration of follow-up were 9 years (range 2-14), and 8.3 years (SD±3.7).

Immediate postoperative morbidity was limited to fever of unknown origin in 10 patients, wound infections in 5 and pneumonia in 3 children. No episode of ASS has been observed during the post-surgical follow-up. There was a significant reduction in requirements for blood transfusions and a decrease in the number of hospitalizations (Table 1). No patient died during the post-surgical follow-up. One patient developed overwhelming septicemia.[6] Hematologic data before and after surgery are shown in Table 2. The mean hemoglobin concentration and platelet counts were significantly increased after surgery.

There was not significant difference in the percentage of pitted red cells before and after the operation. The postoperative spleen scans showed the presence of the splenic remnant in 13 of 20 children. Total splenectomy exposes the patient to the threat of overwhelming septicemia.[3] The incidence of this complication is higher in younger children with SCD (those less than five years of age) and reported to be about 7% with high mortality.[7] Our data show that preservation of a part of the spleen can provide protection against serious infections. Although in SCD there is a splenic dysfunction it is reasonable not to perform total splenectomy because reticuloendothelial activity persists, as demonstrated by the return of splenic function to normal by transfusion not only in young children[5] but also in adults.[8] Pitted red cell counts are a reliable marker for splenic function. The mean pitted red cell count in our patients

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