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Gilbert's syndrome as a predisposing factor for idiopathic cholelithiasis in children

The frequency of the $(TA)_7/(TA)_7$ promoter genotype of UDP-glucuronosyltransferase gene (UGT1A1) was significantly higher ($p<0.05$) in a group of 30 children with cholelithiasis than in a control group of 40 healthy children, indicating that this genotype might be an underlying factor for gallstone initiation in otherwise healthy children.

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The reported incidence of cholelithiasis, an infrequent condition in children, has been increasing since the inclusion of ultrasound investigation of gallstones for every case with vague upper abdominal complaints.[1] Most gallstones in infants and young children are composed primarily of calcium bilirubin pigment with varying amounts of cholesterol and calcium carbonate.[1] The major risk factors for gallstone formation include hemolytic diseases, prematurity (<30th week of gestation), prolonged neonatal jaundice, liver diseases, necrotizing colitis, Crohn's disease, ileal resection, congenital anomalies of the biliary tract and total parenteral alimentation.[1,2] In the absence of the above conditions, gallstone formation has been attributed to a high proportion of unconjugated bilirubin present in the bile.[3] Reduction in hepatic glucuronidating activity has been observed in patients with Gilbert's syndrome (GS), resulting in high levels of unconjugated bilirubin and a 30% increase in bilirubin monoglucuronide excreted in the bile. The monoglucuronide is less water soluble than the normally predominating diglucuronide.[4,5] The most common genetic basis of the reduced glucuronidation expressed by low level of bilirubin UDP-glucuronosyltransferase-1 in GS is a variant promoter of the UGT1A1 gene, containing an additional TA dinucleotide to the normally existing six nucleotide repeats $[(TA)_6]$.[4]

Table 1. UGT1A1 promoter genotype A(TA)nTAA in children with cholelithiasis and in the control group.

Population studied	*N.*	*Observed frequencies of UGT1A1 promote genotypes compared to expected, calculated from respective single allele frequencies according to the Hardy-Weinberg equilibrium*		
		$(TA)_6/(TA)_6$ *observed/expected*	$(TA)_6/(TA)_7$ *observed/expected*	$(TA)_7/(TA)_7$ *observed/expected*
Children	30	9 (30%)	10(33.3%)	11(36.7%)
with choleithiasis		6.9(23%)	14.97(49.9%)	8.112(27.04%)
Control group	40	23(57.5%)	11(27.5%)	6(15%)
		16.9(42.25)	18.2(45.5)	4.9(12.25%)

Our study included 30 unrelated children, (13 boys, 17 girls) aged 12 months to 15 years (mean age 6.5±0.8) with symptomatic cholelithiasis evaluated by liver and biliary ultrasonography and mean bilirubin level of 0.77±0.2 mg/dL. The control group consisted of 40 healthy unrelated children (18 boys, 22 girls) aged 2 to 10 years (mean age 5.4±0.5). These children were unselected for bilirubin levels and had normal abdominal ultrasound. All were born after a full-term pregnancy and none had a history of major risk factors considered to be implicated in gallstone formation.[1,2] The study was approved by the ethical committee of *Aghia Sophia* Children's Hospital and the parents gave their written informed consent.

The promoter region [A(TA)nTAA] of UGT1A1 gene was analyzed using methods previously described.[6] Three different GS promoter genotypes were characterized: homozygous $(TA)_7/(TA)_7$, heterozygous $(TA)_6/(TA)_7$ and normal homozygous $(TA)_6/(TA)_6$.

χ^2 test or Fischer's exact test (SPSS program) was used for the comparison of the frequency of the three different UGT1A1 promoter group genotypes between the groups of children with symptomatic cholelithiasis and the healthy controls. All *p* values less than 0.05 were considered to indicate statistical significance. Both population samples (control group and children with cholelithiasis) were also tested for agreement with the assumption of the Hardy-Weinberg equilibrium. Although there seemed to be an excess of homozygotes and a corresponding deficiency of heterozygotes, these differences between observed and expected were not statistically significant ($p>0.05$), therefore the population is in Hardy-Weinberg equilibrium.

The frequency of the GS genotypes in the UGT1A1 gene promoter region in the group of children with cholelithiasis, as well as in the control group, is shown in Table 1. The $(TA)_7$ allele frequency was significantly higher in the group of children with cholelithiasis than in the healthy group of children ($p< 0.05$).

Gilbert's syndrome is a benign condition characterized by mild unconjugated hyperbilirubinemia in the absence of liver disease or overt hemolysis and its frequency varies in different populations.[6,7] Our previous report on GS in 105 healthy Greek children aged 2-10 years, showed that 18.6% were homozygous for the $(TA)_7$ allele. In the same group the total bilirubin levels and genotype frequencies for males and females showed no significant sex differences between the

three different genotypes.[6,7]

In the present study, eleven of the 30 children (36.7%) with gallstones and six of the 40 healthy children (15%) had the $(TA)_7/TA)_7$ genotype suggesting, for the first time, that GS promoter genotype might be a risk factor for the development of gallstones in children, in the absence of any other predisposing factors.

Previous studies have demonstrated an increased risk for developing gallstones in patients with co-inheritance of GS and hereditary spherocytosis (HS) or homozygous β-thalassemia.[5,8] More recently, Galanello *et al.* reported that the presence of GS genotype is associated with a statistically increased prevalence of cholelithiasis in both thalassemia major and intermedia patients.[8] Passon *et al.* also report that children with sickle cell anemia and cholelithiasis have a higher frequency of the abnormal $(TA)_7$ UGT1A promoter allele.[9]

All the above evidence and the results of the present study should alert pediatricians to investigate the GS promoter genotype in children with cholelithiasis even in the absence of other risk factor.

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Immunologic reconstitution in long-term survivors of thalassemia major after hematopoietic stem cell transplantation

We studied the immune function of 33 long-term survivors of thalassemia after hematopoietic stem cell transplantation. Lymphocyte subsets, lymphoproliferative response and immunoglobulin were normal but the level of natural killer cells was low. Five and seven patients had suboptimal antibody response at 4 week after pneumococcal and hepatitis B vaccine, respectively, but this response returned to normal by 6 months.

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Immune recovery after allogeneic haematopoietic stem cell transplantation (HSCT) is affected by many factors such as age and graft-versus-host disease (GVHD).[1] Transfusion-dependent thalassemia major (TM) patients have altered immune function,[2] and this study was aimed at evaluating the immune recovery of long-term survivors of TM after HSCT. Thirty-three TM patients who had survived more than 2 years after HSCT were studied. The donors were HLA-identical siblings and the conditioning was busulphan and cyclophosphamide. Antithymocyte globulin (ATG) was given at a daily dose of 10 mg/kg from day −5 to day +5 in 20 patients, 11 patients received 30 mg/kg/day for 3 days from day −4, and two did not receive ATG. GVHD prophylaxis was cyclosporin A and short course methotrexate.[3] The patients were re-immunized at one year with diphtheria, pertussis and tetanus vaccine. Three doses of hepatitis B vaccine were to be given if the antibody against hepatitis B (anti-HBs) was below the protective level (<10 IU/L).

At the study baseline, lymphocyte subsets were assessed by flow cytometry and were compared with those of an age- and sex-matched reference.[4] Lymphoproliferative response (LPR) was studied for mitogens and specific recall antigens (herpes simplex virus, cytomegalovirus, varicella-zoster virus). Serum immunoglobulins and anti-tetanus antibody were measured. Thirty patients were given a booster intramuscular injection of hepatitis B vaccine (Engerix-B), 33 patients received one dose of polyvalent pneumococcal vaccines (Pneumovax-23). Anti-HBs and anti-pneumococcal bodies were measured at baseline, and four weeks and six months following vaccination. An antibody titer more than double the baseline level was considered to represent an optimal response.

At the time of the study, the median age of the patients was 15.8 years (5.8 to 26.9 years) and their median follow-up from HSCT was 68 months (24 to 106 months). None of the patients was receiving desferrioxamine and their mean serum ferritin was 1383±1157 pmol/L. Acute GVHD grade I or II had occurred in 19 patients but all responded to a short course of steroids. None of them developed chronic GVHD and immunosuppressive treatment had been stopped more than one year prior to