our PCR primers. The comparison of CDRIII sequences with the germ line sequences did not suggest mutation. However, since we used PCR to detect the CDRIII region we could not further evaluate the sequence and mutational status of the VH genes.

Our first patient (brother I) presented with an indolent disease. The second patient (brother II) was diagnosed approximately 5 years later, but his disease was more aggressive and he died after developing Richter's syndrome. Richter's transformation was linked to a DNA mismatch-repair defect-initiated microsatellite instability.[4] This genetic alteration was initially identified in the hereditary non-polyposis colorectal cancer syndrome (HNPCC).[5] We tried to confirm the diagnosis of both mother and sister. Unfortunately, because of the long period between their death and diagnosis of the first brother, the old medical data were no longer available. Nevertheless our patients' history is suggestive of familiar CLL.

The mutational status of VH genes does not change during the course of the disease and has been documented to hold within a family with CLL.[6] In contrast, the behavior of CLL in both our patients was different (indolent versus aggressive) despite both having unmutated Ig status (poor prognosis). The second patient (brother II) was not tested for the presence of CLL-like cells prior to diagnosis, nor for VH gene mutations because he had been diagnosed and treated before studies of mutational status of VH genes[7] and data by Rawstron *et al.*[1] had been published. That is also why we had to use limited archive samples and PCR products for sequencing.

While recent data suggest that ZAP-70 expression should be included in the diagnostic work-up of patients with CLL as a more convenient prognostic marker than VH genes mutation,[10] the studies showing a high incidence of CLL-like cells in healthy first-degree relatives of CLL patients indicate that an early FACS analysis of these relatives should be considered as a screening for pre-CLL.[1] As we now have a variety of relatively non-toxic therapeutic modalities (e.g. monoclonal antibodies anti-CD20 or anti-CD52) available and early stages of CLL[8,9] respond better to such therapy, CLL with its later consequences, including Richter's transformation, could perhaps have been avoided and CLL, at least in its initial form, may become a preventable type of cancer.

Jan Cerny,# Alena Slavickova,* Anna Krepelova,° Marek Trneny,* Josef Karban,* Pavel Klener**

**First Department of Internal Medicine, °Institute of Biology, Charles University General Hospital, Prague, Czech Republic; #University of Massachusetts Memorial Medical Center, Department of Medicine, 55, Lake Avenue North, Worcester, MA, USA*

Key words: leukemia, familial, clonality, IgH rearrangement, polymerase chain reaction, clonal lymphocytosis of uncertain significance.

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Correspondence: Jan Cerny, University of Massachusetts Memorial Medical Center, Department of Medicine, 55 Lake Avenue North, Worcester, MA 01605, USA. E-mail: jcern@hotmail.com

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A rare β-thalassemia mutation (C-T) at position -90 of the β-globin gene discovered in a Chinese family

We provide the first description of a Chinese family with three heterozygotes for a rare β-thalassemia mutation previously observed in a Portuguese carrier. The mutation (-90 C-T) changes the conserved promoter sequence within the proximal CACCC box of the β-globin gene; this can reduce β-globin transcription significantly.

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β-thalassemia is a varied group of disorders of hemoglobin (Hb) synthesis, most of which result from point mutations within the β-globin gene or the immediate flanking sequence. Over 200 different β-thalassemia mutations have now been characterized worldwide.[1] Within each population at risk for β-thalassemia a small number of common mutations are found. For example, in the Chinese population, five mutations, of the 30 known, account for more than 90% of all cases.[2-4] Here we describe a rare β-thalassemia mutation previously unreported in the Chinese population, the C-T substitution at position -90 in the proximal CACCC box of the β-globin gene.

The proband was a 27-year old woman from a Chinese family originating from Sihui county of Guangdong Province, southern China. We studied this family because the proband was found to have a typical hypochromic microcytosis during routine genetic screening for β-thalassemia but no known mutations reported in the Chinese population could be identified. Standard hematologic techniques were used to measure RBC counts and Hb concentration. Reverse dot blots (RDB)

Table 1. Results of blood analysis, DNA diagnosis and mRNA detection in the family members.

Sex	*Proband female*	*Mother female*	*Father male*	*Brother male*	*Husband male*
RBC ($\times 10^{12}$/L)	5.7	4.8	4.7	5.8	6.4
Hb (g/L)	139	105	154	144	145
MCV (fL)	73.6	68.2	88.2	73.4	67.1
MCH (pg)	23.6	21.8	33.0	24.7	22.8
MCHC (g/L)	357	320	374	336	340
RDW (%)	0.149	0.152	0.132	0.144	0.159
HbA (%)	94.3	93.6	97.2	94.0	97.1
HbA2 (%)	5.7	6.4	2.8	6.0	2.9
Genotype	β^{-90}/β^{N}	β^{-90}/β^{N}	β^{N}/β^{N}	β^{-90}/β^{N}	β^{N}/β^{N}
β/α ratio	2.130	2.219	3.716	2.238	5.341

were employed to investigate the 18 known types of Chinese β-thalassemia mutations.[5] Gap-polymerase chain reaction (PCR) assays were performed to detect the three known deletional forms of α-thalassemia (−−^{SEA}/, −$\alpha^{3.7}$/ and −$\alpha^{4.2}$/) as previously reported by our laboratory.[6,7] The entire α-globin gene fragments of the proband, her mother and her brother were sequenced. Reverse-transcription(RT)-PCR was performed to determine the β-globin transcription using the One Step RNA PCR kit protocol (TaKaRa Biotechnology, Dalian Co. Ltd, China), with primers modified from Lin's method.[8] The hematologic data obtained from this family and the proband's husband, who was found to be a carrier of α-thalassemia with −−^{SEA} allele, are listed in Table 1. Hematologic findings in the proband, her mother and brother were increased HbA_2 (5.7-6.4%), and low values of MCV (68.2-73.6fL), indicating the possibility of the three subjects being carriers of the same type of β-thalassemia allele. We think that the proband's mother might be heterozygous with β-thalassemic trait since iron deficiency was excluded although she showed a mild anemia and greater reduction of MCV (see Table 1). No known β-thalassemia mutations were found by RDB assay in any of the family members. DNA analysis of the β-gene region of the three subjects revealed heterozygosity for the C-T substitution at position -90 within the proximal CACCC box of the β-globin gene. The representative sequence of the β-gene region from genomic DNA of the proband is illustrated in Figure 1 and the mutant sequence was registered with GeneBank (AY260740). The possible co-inheritence of β-thalassemia and one of the three common α-thalassemia determinants (−−^{SEA}/, −$\alpha^{3.7}$/ and −$\alpha^{4.2}$/) in the Chinese population were excluded in all three subjects of the family. To study the effects of this mutation on β-globin gene transcription further, we performed semi-quantitative RT-PCR analysis in the family members. The transcription level of this β-globin gene in heterozygosity (2.233±0.01, n=3) was comparable with that of a heterozygous IVS-2-654 (C-T) mutation (2.110±0.53; n=10 95%CI 1.732-2.488) but significantly lower than that of normal individuals (3.779±1.19; n=13 95%CI 3.060-4.499), confirming a reduction in β-globin gene transcription. However, our present result did not provide evidence on whether the

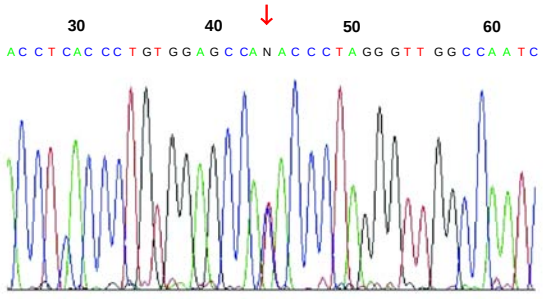

Figure 1. Sequence analysis of the β-globin gene amplified from the proband. The downward arrow indicates the heterozygosity for the C→T substitution at position –90 of the β-globin gene.

mutation results in β^{+}-thalassemia or β^{0}-thalassemia although such a promoter mutation generally causes a β-globin mRNA level compatible with that of the relatively mild phenotype of β^{+}-thalassemia. Further work is needed to elucidate the detailed information on the output of β-globin mRNA level affected by this transcriptional mutation.

Thus, the proband, her mother and brother were proven to be carriers of a transcriptional mutation of β-thalassemia, whereas her father was a normal individual. The genotype-phenotype relationship observed in all family members is presented in Table 1. This rare β-thalassemia mutation was previously described in a Portuguese carrier many years ago, accounting for 0.97% of all β-thalassemia alleles in the Portuguese population.[9] To the best of our knowledge, we provide the first description of Chinese heterozygotes for this mutation. Based on the data from the population of Guangdong Province, where thalassemias are very common, in our current study on molecular epidemiology of thalassemias, only one case was detected among 9,316 random samples screened for β -thalassemia, and this mutation accounted for 0.39% of all 256 β-thalassemia chromosomes identified.

Shiqi Jia, Xiongwu Lao,§ Wenjun Li,* Jianying Ma,§ Qiuhua Mo,* Xiangmin Xu**

**Department of Medical Genetics, First Military Medical University, Guangzhou, Guangdong, P.R. China; §Sihui Municipal Maternity and Child Healthcare Hospital, Sihui, Guangdong, P.R. China*

Key words: β-thalassemia, transcription mutation, β-globin gene.

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Correspondence: Xiang-Min Xu, MD, Department of Medical Genetics, First Military Medical University, Tonghe 510515, Guangzhou, Guangdong, P.R. China. Phone: international +86.020.61648293. Fax: international +86.020.87644466 . E-mail: gzxuxm@pub.guangzhou.gd.cn

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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