Familial chronic lymphocytic leukemia

Familial aggregation of chronic lymphocytic leukemia (CLL) has been observed more frequently than familial aggregation of any other type of oncohematologic disorder. The presence of cells with a CLL-like immunophenotype (CLL-like cells) was recently documented in 13.5% healthy first-degree relatives of CLL patients.[1] We present a family with CLL in which 2 brothers, a sister and their mother were affected.

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We used polymerase chain reaction (PCR) and flow activated cell sorting (FACS) to investigate bone marrow samples (BM) and peripheral blood samples (PB) obtained from patients as described elsewhere.[2] PCR products were extracted from 3% Metaphor agarose gel using the crush and soak technique[3] and purified with Microcon-100 (Millipore) columns, sequenced with a BigDye Terminator Cycle Sequencing kit (Applied Biosystems) and analyzed on an ABI Prism 310 Genetic Analyzer (PE BioSystems).

Two patients (brothers) who were diagnosed (brother I at age 52; brother II at 60) and treated for CLL carried a PCR-detectable IgH rearrangement. Their mother and sister died (both had enlarged lymph nodes; Figure 1). In both cases the disease became refractory to chemotherapy. Transformation to a high-grade malignancy was suspected in brother I, but not confirmed. He died approximately 9 years after the initial diagnosis. In the second brother, the transformation to Richter's syndrome was confirmed and he died approximately 3 years after the diagnosis.

Both cases had a typical immunophenotype characterized by monotypic surface expression of CD19, CD5, CD23, CD20, CD11c, HLA-DR, and immunoglobulin light chain κ. Clonality (as mentioned above) was verified by PCR. Nucleotide sequence data analysis revealed the presence of two different CDRIII rearrangements (109 bp in patient I and 110 bp in patient II, Figure 2). The first patient's CDRIII sequence consisted of V(?) – D1-7 – JH6c gene rearrangement, was in- frame (see Figure 2 A). The second patient's CDRIII consisted of V(?) – D6-19 – JH3b segments. When we looked for the position of the reading frame, we found a TGA stop codon (unproductive rearrangement; Figure 2B). That means we have detected the unproductive allele for IgH. Since FACS confirmed monoclonality, the other allele was rearranged and productive, but not detected by

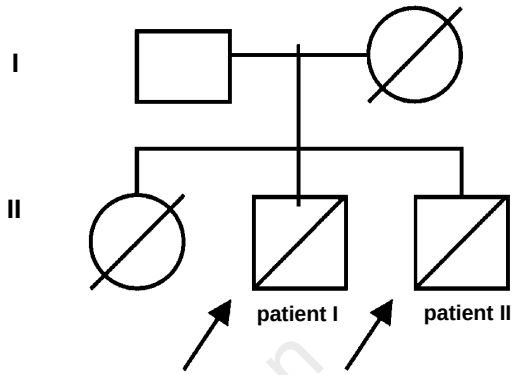

Figure 1. Pedigree of a family with CLL: Mother and daughter died from leukemia (both had enlarged lymph nodes). Two brothers (sons) were diagnosed as having CLL: one died 9 years after diagnosis, the other developed Richter's syndrome and died within 3 years of diagnosis.

Figure 2A. Sequence of CDRIII rearrangement obtained from PCR positive samples from patient I.

5'- AC-ACG-GCY-STG-TAT-TAC-TGT-GCG-AGA-
FR3A primer 3' end of VH gene

-GAG-G GA-
(N-nucleotides)

-ATA-ACT-GGA-ACT-ACG-TAC-
D 1-7 segment

-ATT-ACT-ACT-ACT-ACT-ACA-TGG-ACG-TCT-GGG-GGA-AAG-

-GGA-CCA-C-
JH6c sequence

-GTC-ACC-GTC-TCC-TCA-G- 3'
JH primer

FR3A: framework region 3; D: diversity segment; JH: joining gene; N: nucleotides (small letters); sequence between rearranged VH, D and JH genes (capital letters); primer and D sequences underlined.

Figure 2B. Sequence of CDRIII rearrangement obtained from PCR positive samples from patient II.

5'- AC-ACG-GCY-STG-TAT-TAC-TGTGCG-AGA-
FR3A primer 3' end of VH gene

-GGA-CTG-GAC-CCA-C-

-AT-AGC-AGT-GGC-TGG-CTG-GTT-GGG-
D 6-19 segment (N- nucleotides)

Stop codon
-TGA-TGC-TTT-TGA-TTC-TGG-GGC-CAA-GGG-ACA-ATG-
JH3b sequence

-TCA-CCG-TCT-CCT-CAG- 3'
JH primer

FR3A: framework region 3; D: diversity segment; JH: joining gene; N: nucleotides (small letters); sequence between rearranged VH, D and JH genes (capital letters); primer and D sequences underlined.

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*

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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)