



## Low number of DNA copy number changes in small lymphocytic lymphoma

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### Abstract

**Background and Objective.** Small lymphocytic lymphoma (SLL) is morphologically and immunologically similar to chronic lymphocytic leukemia (CLL), and the new REAL classification refers to them as a single disease termed SLL/CLL. Recently, frequent losses in 6q, 11q and/or 13q were observed in CLL using comparative genomic hybridization (CGH).<sup>1</sup>

We performed CGH analyses in order to find out whether these two entities contain the same DNA copy number changes.

**Design and Methods.** Seventeen patients with stage IV disease and one with stage III disease were studied by CGH. CGH is based on quantitation of the fluorescence intensity of differentially labeled DNAs. For this purpose tumor DNA labeled with FITC-12dUTP and normal DNA labeled with Texas red-5dUTP were hybridized to normal metaphase chromosomes. The ratio of fluorescence intensity of hybridized tumor and normal DNA was measured using computerized image microscopy to identify over- or under-represented regions in the tumor genome. All findings were confirmed using a confidence interval of 99% with a 1% error probability.

**Results.** The most consistent finding was a gain of the entire chromosome 12 observed in three patients and a loss in 14q24 in one patient. No other changes were detected. All abnormal cases presented with stage IV disease and had bone marrow infiltration. Two 12+ cases had a leukemic disease.

**Interpretation and Conclusions.** Our results indicate that trisomy 12 is one of the most frequent chromosomal aberrations in SLL. Losses regarded as typical of CLL were not present in SLL. This may indicate that the genetic pathways in the development of SLL/CLL in patients presenting with enlarged lymph nodes (SLL) with or without leukemia are different from those in patients presenting with leukemia (CLL) without enlarged lymph nodes.

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Key words: small lymphocytic lymphoma, chronic lymphocytic leukemia, comparative genomic hybridization, trisomy 12

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Small lymphocytic lymphoma (SLL) is a subtype of low grade non-Hodgkin's lymphoma. SLL in lymph nodes is histologically characterized by diffuse proliferation of small lymphocytes with regular round nuclei. Based on morphological and immunological features SLL is regarded as a tissue counterpart of B-cell chronic lymphocytic leukemia (CLL). In the new REAL-classification they are regarded as a single disease termed SLL/CLL.<sup>2</sup> Although most patients with SLL have bone marrow and peripheral blood involvement at diagnosis, it is possible that some may not develop leukemia.<sup>2</sup> CLL has been classified as typical when more than 85% of the circulating lymphocytes have clumped chromatin with no visible or small nucleoli, and a small amount of cytoplasm. In CLL/PL (prolymphocytic leukemia) 10-54% of the cells are prolymphocytes, and in atypical CLL more than 15% of the cells have a cleaved nucleus or lymphoplasmacytic features.<sup>3</sup> The prognosis of patients with SLL/CLL is highly variable. Some patients survive with an indolent disease for many years without any treatment and succumb to other diseases. Some patients develop a more aggressive lymphoma and die from it. The median survival is approximately ten years.

The molecular pathogenesis of SLL/CLL is largely unknown. Cytogenetic studies in SLL have revealed some chromosomal aberrations, such as trisomy 12 and deletions in 13q and 14q.<sup>4</sup> However, no specific gene of pathogenetic importance in SLL/CLL has yet been identified. Comparative genomic hybridization (CGH) is a molecular cytogenetic method that enables indirect screening for gains and losses of DNA copy numbers throughout the genome in a single hybridization. So far, no data are available from studies of SLL using CGH. We used CGH to study 18 patients with SLL to identify genomic areas which may harbor oncogenes of SLL.

### Materials and Methods

Lymph node specimens were collected during diagnostic procedures carried out between 1989 and 1996 from 18 SLL patients referred to the Helsinki University Central Hospital. The median age of the patients (2 females and 16 males) was 64 years (range 44-84 years). Seventeen patients presented with stage IV disease and one with stage III disease. Bone marrow involvement was seen in all but one

**Table 1. Clinical and hematologic characteristics and CGH findings of patients with small lymphocytic lymphoma.**

Case	Age /Sex at diagnosis	Stage*	BM infiltration	B-lymphocytes $\times 10^9/L$	Copy number changes in lymph node
1	61/M	IVB	+	6.1	+12
2	57/M	IVA	+	4.4	+12
3	83/M	IVA	+	15.8	+12
4	92/M	IVA	+	1.4	-14q24-qter
5	44/M	IVA	+	23.6	None
6	82/M	IVA	+	2.7	None
7	52/M	IVA	+	5.2	None
8	65/F	IVA	+	3.7	None
9	70/F	IVA	+	72.3	None
10	54/M	IVB	+	75.0	None
11	70/M	IVB	+	17.9	None
12	63/M	IIIA	-	3.4	None
13	67/M	IVA	+	6.8	None
14	67/M	IVA	+	9.9	None
15	53/M	IVA	+	10.2	None
16	60/M	IVA	+	17.6	None
17	84/M	IVA	+	75.0	None
18	50/M	IVA	+	9.1	None

\*TNM classification according to the International Union Against Cancer.

patient. A leukemic disease was detected in eight patients according to the IWCLL criteria (*International Workshop on Chronic Lymphocytic Leukemia*)<sup>5</sup> (lymphocyte count  $> 10 \times 10^9/L$ ) and in 13 patients according to NCI-WG criteria (lymphocyte count  $> 5 \times 10^9/L$ ).<sup>6</sup> Five patients had a normal lymphocyte count. Lactic acid dehydrogenase level was normal in all except three patients. The median follow-up of the patients was 36 months (range 1- 85 months). Two patients have died of unrelated diseases (one of cardiac arrhythmia and one of pre-existing renal disease), the other 16 patients are alive with lymphoma. In all cases the histologic features were typical of SLL/CLL.

There was diffuse proliferation of small lymphocytes with round nuclei and clumped chromatin. There were also proliferation centers composed of slightly larger lymphoid cells (mainly prolymphocytes, occasional paraimmunoblasts) giving a pseudofollicular pattern. The immunohistochemical features were also typical of SLL/CLL.<sup>2</sup> In each case the lymphoma cells were positive for the following antigens (frozen section immunohistochemistry): IgM, IgD, Ig- $\kappa$ , or Ig- $\lambda$ , CD5, CD20, and CD23. In all cases the CD3-positive T-cells accounted for less than 20% of all cells, and in 16 of the cases for less than 10%. In all specimens the proportion of tumor cells was  $\geq 50\%$ .

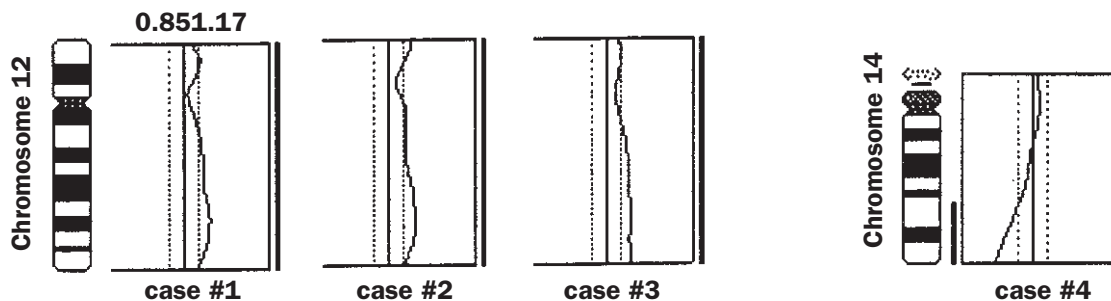
CGH was performed using direct fluorochrome-conjugated DNAs for all samples as described previously.<sup>7,8</sup> Hybridizations were analyzed using an Olympus fluorescence microscope and the ISIS digital image analysis system (Metasystems Hard & Software, Altlusheim, Germany). Chromosomal regions were interpreted as over-represented when the green-to-red ratio was higher than 1.17 (gains), and as under-represented when the ratio was lower than 0.85 (losses).<sup>7</sup>

## Results

The clinical and hematologic data and CGH findings of patients with SLL are summarized in Table 1. CGH analysis revealed that only four of the 18 patients (14%) showed DNA copy number changes (Table 1). The most consistent finding was a gain of the whole chromosome 12 seen in three patients and a loss in 14q24 in one patient (Figure 1). No other changes were found. All abnormal cases presented with stage IV disease and had bone marrow infiltration. Two 12+ cases had a leukemic disease (lymphocyte counts:  $6.1 \times 10^9/L$  and  $15.8 \times 10^9/L$ ).

## Discussion

In our study a gain of the whole chromosome 12 was observed in three specimens. Cytogenetic studies have shown that this gain is one of the most fre-



**Figure 1. DNA copy number changes detected by CGH in four cases of SLL. Gain of the whole chromosome 12 in cases #1, 2 and 3, and loss at 14q24 in case #4 were revealed by CGH. To the left and the right of the base line (1.0), cut-off values of 0.85 (losses) and 1.17 (gains), respectively.**

quent chromosomal aberrations in SLL/CLL<sup>1,9-11</sup> and that it occurs in 30-40% of all cytogenetically abnormal cases. Recent cytogenetic studies have, however, demonstrated trisomy 12 to be more frequent in atypical CLL.<sup>3,12</sup> Trisomy 12 seems to indicate poorer survival than does normal karyotype or a single aberration of chromosome 13q.<sup>9,12</sup>

Loss in 14q24-qter was another change observed in this study. This aberration has also been reported by chromosome banding analysis.<sup>4</sup>

Frequent loss of 11q14-q24 has recently been reported in typical CLL (48%) and a gain of the entire chromosome 12 has also been observed but only in 16% of the CLL cases studied, suggesting that loss at 11q is one of the most common abnormalities in patients with typical CLL.<sup>1</sup> Losses in 6q and 13q have also been frequently found in typical CLL.

Morphologically and immunologically SLL and CLL are similar. In our series of 18 typical cases of SLL, of which 13 were leukemic (according to the NCI-WG criteria), we could not, however, find any losses in 11q nor any other losses observed by CGH in typical CLL.<sup>1</sup> This may indicate that genetic pathways in the development of the SLL/CLL in patients presenting with enlarged lymph nodes (SLL) with or without leukemia are different from those in patients presenting with leukemia (CLL) without enlarged lymph nodes.

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*All the authors were equally responsible for the design of the study. All the authors also contributed to the analysis and writing of this paper. YA, KA and SK were responsible for the CGH analysis.*

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#### **Disclosures**

*Conflict of interest: none.*

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#### **Manuscript processing**

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