Array-based karyotyping in chronic lymphocytic leukemia (CLL) detects new unbalanced abnormalities that escape conventional cytogenetics and CLL FISH panel

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Aims. Chronic lymphocytic leukemia (CLL) is the most common adult leukemia with a very heterogeneous course. Progress in molecular genetic characterization of CLL has confirmed the prognostic role of unbalanced chromosomal abnormalities currently defined by molecular cytogenetic methods: conventional karyotyping and FISH. However, a significant percentage of genomic abnormalities escapes routine investigation due to the limitations of these methods. It is presently clear that some of these aberrations have impact on prognosis and disease progression.
Methods. We examined copy number changes in the tumor genomes of 50 CLL patients using bacterial artificial chromosome (BAC) and/or oligonucleotide array platforms.
We compared the results of arrayCGH with those obtained by FISH and conventional cytogenetics and evaluated their clinical importance.
Results. A total of 111 copy number changes were detected in 43 patients (86%) with clonal abnormalities present in at least 23% of the cells. Moreover, 14 patients (28%) were found to have 39 genomic changes that had not been detected by standard cytogenetic and/or FISH analyses. These included possibly prognostically important recurrent 2p and 8q24 gains. The most frequent unbalanced changes involved chromosomes 18, 7, 3, 9 and 17. We also determined the minimal deleted region on chromosome 6q in 7 cases by chromosome 6/7 specific array.
Conclusions. The results showed that a subset of potentially significant genomic aberrations in CLL is being missed by the current routine techniques. Further, we clearly demonstrated the robustness, high sensitivity and specificity of the arrayCGH analysis as well as its potential for use in routine screening of CLL.

Key words: chronic lymphocytic leukemia, cancer, arrayCGH, copy number changes, chromosomal abnormalities, del(6)(q)

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INTRODUCTION

B-cell chronic lymphocytic leukemia is the most common adult leukemia in the Western world. The clinical course of CLL is heterogeneous. Some patients with CLL survive for many years without therapy, showing minimal signs and symptoms throughout the course of the disease. They have a survival time similar to age-matched controls. Other patients, however, have rapidly deteriorating blood counts and organomegaly, leading to death. The Rai and Binet clinical staging systems[1,2] were developed to estimate the prognosis of CLL patients but are unable to predict a poor prognosis at early stages of the disease. Recently, there has been an active interest in identifying molecular and cytogenetic markers useful for predicting the clinical course in these patients. Among others, the prognostic role of *IgVH* mutational status and expression of CD38 and ZAP-70 was established. A subset of genomic aberrations was identified as important independent predictors of disease progression and survival[3]. Deletions of 11q and 17p, affecting the ataxia telangiectasia mutated (*ATM*) gene and p53 tumor suppressor gene (*TP53*) respectively, have been associated with disease progression and reduced survival[4-7]. Early identification of these deletions could allow clinicians to choose appropriate therapy for these patients[8,9].

Other recurrent aberrations are detected in CLL. Trisomy 12 was described as the first recurrent chromosome aberration in CLL by Gahrton et al. in 1980 (ref.[10]). This aberration initially correlated with an advanced stage of the disease and shorter survival time[11]. Deletion of 13q14, likely to target mir-15 and mir-16 (ref.[12]), is the most common cytogenetic abnormality in CLL correlated with a favorable prognosis in the absence of other abnormalities[13]. Additionally, deletion of 6q is observed in approximately 6% of CLL patients. While Oscier et al.[14] reported shorter treatment-free intervals for CLL patients with a 6q deletion, no adverse prog-

nostic effect was found in other large CLL studies[15,16]. Currently, no gene contributing to CLL pathogenesis has been identified at 6q even though some candidate genes located in the deleted region have been identified, such as *PIC3A* and *MYB* (ref.[16]). Translocations and deletions of 14q32 involving the *IGH* gene were found in up to 5% of CLL patients[17-19]. Other chromosomal abnormalities involving regions of 3q, 8q, 9p and other regions are less frequent in CLL. Therefore, routine diagnostic investigation of CLL patients includes conventional cytogenetics and interphase FISH analysis; a panel of 4-6 probes was designed to analyze the 11q22, 12, 13q14/13q34, 17p13, 6q21 and 14q32 regions as these regions are recurrently targeted by numerical and structural aberrations in CLL. While cytogenetics is informative in up to 50% of CLLs, FISH detects chromosomal aberrations of the above loci in more than 80% of patients. However, the latter technique provides a very limited view of the genomic aberrations because only a number of loci are analyzed. It is presently unclear whether the CLL-associated aberrations that are not included in the standard FISH panel have any impact on prognosis and disease progression. With the advent and availability of arrayCGH technology, high-resolution, locus-specific analysis and genome-wide evaluation can be combined into a single test[20]. To utilize the ability of arrayCGH, we studied 50 CLL patients recently diagnosed and treated at our institution. This study aimed at comparing arrayCGH results with conventional cytogenetics and FISH and identifying possible unbalanced chromosomal aberrations that escaped banding and FISH analysis. Moreover, using the tiling path chromosome 6 arrayCGH platform, we mapped 6q deletions in selected CLL patients and defined the smallest commonly deleted region likely to harbor the CLL-related tumor suppressor gene. Another aim of this pilot study was to demonstrate the potential clinical utility of arrayCGH method.

MATERIALS AND METHODS

Patients

A total of 34 male and 16 female patients with a median age of 61 years were diagnosed with CLL and treated at the Department of Hemato-oncology, University Hospital Olomouc, Czech Republic. Peripheral blood was examined at the time of diagnosis or during the course of the disease after obtaining informed consent. Of the patients, 30 were examined at the time of diagnosis while the remaining 20 were examined during the course of the disease. All patients of the latter group were treated with different chemotherapy regimens, such as CHOP (cyclophosphamide, doxorubicin, vincristine and prednisone), R-CHOP (rituximab-CHOP), and FLUCY (fludarabine and cyclophosphamide).

Overall, 18 patients were Binet A, 3 patients were Binet A/B, 16 were Binet B and 13 were Binet C (Table 1). The median overall survival (OS) was 37.3 months (range 12.5-215.9). A total of 43 patients are alive with a median OS of 37.9 months.

Conventional cytogenetics and FISH

Cytogenetic examinations were performed on metaphases obtained by parallel short-term culture in the presence of 12-O-tetradecanoylphorbol-13-acetate (TPA) (Sigma-Aldrich, Munich, Germany), the immunostimulatory CpG-oligonucleotide DSP30 (TIBMolbiol, Berlin, Germany) and interleukin-2 (IL-2) (Sigma-Aldrich). G-banding analysis as well as fluorescence *in situ* hybridization (FISH) were performed as described in standard procedures.

The probes used for FISH analysis included LSI D13S319(13q14)/13q34/CEP12, LSI p53, LSI ATM, LSI IGH, LSI ALK, LSI NMYC, LSI MYC, CEP 2, 11, 17, 18 (Abbott Molecular Inc, Des Plaines, USA), ON MM 15q22/6q21, GLI(12q13) (Kreatech Diagnostics, Amsterdam, Netherlands), XCAP 2 (MetaSystems, Altlussheim, Germany) and BAC derived probes RP11-373L24 and RP11-498O5 covering the *REL* locus (BlueGnome, Cambridge, UK). At least 200 nuclei were analyzed, and the number of abnormal nuclei was expressed as a percentage of the scored nuclei. The cut-off level for reliable determination of abnormality was established as the presence of at least 2.5% of abnormal nuclei. In patients with a complex karyotype, WCP probes (Cambio, Cambridge, UK; MetaSystems) and MFISH (MetaSystems) were used.

ArrayCGH analysis

DNA for arrayCGH examination was isolated by the standard phenol-chloroform method from the samples taken at the same time as samples for cytogenetic and FISH examination.

Three different BAC arrayCGH platforms with a resolution of approximately 1 Mb were used. The most frequently used arrayCGH chip (20 patients) was manufactured by LUMC, Leiden, Netherlands. A 1Mb BAC array platform (VIB MicroArray Facility, Catholic University Leuven, Leuven, Belgium) was used for 12 patients. For other 12 patients, arrayCGH analysis was performed using CytoChip_Haem_v0.1 (BlueGnome). Finally, the commercially available oligonucleotide 4x44K array (Agilent Technologies, Santa Clara, USA) was utilized for 6 patients. These 4 platforms were compared to each other using hybridisation with control male and female sample. The bias detected across platforms was in all cases under the detection limits defined for this study.

The tiling path chromosome 6 array was constructed by VIB MicroArray Facility, Leuven, Belgium and was used for 7 patients with detected del(6)(q). The arrayCGH experiments followed the standard protocols and manufacturers' recommendations[21-24]. Hybridized microarrays were scanned with the Axon 2400A microarray scanner, and the results were evaluated according to microarray types. The microarrays manufactured in Leuven and Leiden were evaluated using a homemade spreadsheet application; microarrays manufactured by BlueGnome were analyzed with the BlueFuse software (Bluegnome) and the Agilent oligo 4x44K array was analyzed with the Agilent Feature Extraction Software (Agilent Technologies).

Table 1. Clinical, cytogenetic and FISH findings in 50 CLL patients.

No.	Sex/ Age	Binet stage	Mutation status *IgVH*	ISCN Cytogenetics	FISH					
					TP53	ATM	c12	13q14	6q21	IGH
1	M/62	B	UM	UNS	N	N	N	N	ND	N
2	M/50	B	M	48~49,XY,?+19,?+20,1-2 mar[cp4]/46,XY[2]	N	N	N	D81%	ND	ND
3	F/52	C	M	46,XY,t(3;14)(q?;q?)[9]/46,XY[1]	N	N	N	N	N	R64%
4	M70	C	UM	UNS	N	D22%	N	D96%	ND	D5'95%
5	M/60	C	UM	46,XY,del(6)(q?),der(7)t(7;12)(q3?6;q?12q?ter), ?del(13)(q)[12]/46,XY[1]	N	N	N	D84%	ND	D5'80%
6	M/57	B	M	46,XY[5]	N	N	N	D48%	ND	ND
7	M/74	A	M	45~46,X,-Y,?-9,?-19,+3mar[cp3]/46,XY[9]	N	N	N	D88%	ND	N
8	M46	B	UM	46,XY,der(11),der(13)[10].ish t(11;13)(q?21;q?) ins(13;14)(q?;q?)(wcp11+,wcp13+,wcp14+)/46,XY[4]	N	N	N	D81%	ND	N
9	M/61	C	UM	45,XY,-15,-17,+mar[9]	N	D81%	N	N	ND	N
10	F/71	B	UM	46,XX[5]	N	N	N	N	ND	N
11	M/42	B	UM	UNS	N	N	N	D39%	ND	N
12	M/56	C	UM	UNS	N	N	N	N	ND	N
13	M/72	B	M	46,XY[21]	N	N	N	D79%	ND	N
14	F/42	A	M	46,XX[30]	N	N	N	D94%	ND	D5'87%
15	M/51	A	UM	45,XY,?der(1),del(6)(q),-13[16]	N	N	N	D75%	D78%	D5'85%
16	M/66	C	UM	UNS	N	N	N	N	D83%	N
17	M/63	A	UM	47~47,XY,der(3),?der(4),?-5,-7,der(11),+12,?der(14), +2-3mar[cp11]	N	D77%	+74%	N	N	N
18	F/63†	B	UM	ND	D97%	N	N	D87%	N	N
19	M/58	B	UM	46,XY[13]	N	N	N	D88%	D89%	N
20	M/70	A	M	46,XY[15]	D84%	N	N	D82%	N	ND
21	F/71	C	UM	46,XX,der(1),?der(2),der(8),der(11)t(11;?),?der(14) [cp9]	N	D83%	N	N	N	N
22	M/78	A	M	44~45,XY,r(12)(p13q24.3),inc[cp3]/46,XY[4]	D43%	N	N	D78%	N	D5'47%
23	M/58†	A	UM	46,XY,del(11)(q?)[6]/46,XY[5]	N	D91%	N	D76%	N	N
24	F/60	A	M	UNS	N	N	N	D94%	N	N
25	M/46†	B	UM	46,XY[4]	N	D88%	N	D86%	N	N
26	M/73	A	M	46,XY[13]	N	N	+43%	D43%	N	N
27	M/70	C	UM	UNS	N	D94%	N	D91%	N	D5'93%
28	F/70	A/B	UM	UNS	N	N	+46%	N	N	D3'53%
29	M/68†	C	UM	46,XY[5]	D93%	N	N	D93%	ND	D3'97%
30	M/83	A/B	UM	46,XY,del(14q)[8]	N	N	N	N	N	D60%
31	M/60	C	UM	46,XY,del(11)(q?)[5]/46,XY,?der(1),del(11)(q?), ?der(17)[4]/46,XY[2]	N	D53%	N	D93%	N	N
32	F/50	B	UM	46,XX,der(13),der(16)[3]/45~46,XX,-19,+mar[cp5]	N	N	N	N	D60%	UNS
33	F/52	A	UM	46,XY,del(11q)[10]	N	D76%	N	N	N	N
34	F/56	A	UM	46,XX[14]	N	N	N	D92%	N	UNS
35	M/47	B	UM	46,XY[4]	N	N	N	D77%	N	N
36	M/60	A	UM	UNS	N	D48%	N	D45%	D50%	UNS
37	F/52	A/B	M	46,XX,del(13q)[5]/46,XX[10]	N	N	N	D92%	N	N
38	M/65	B	UM	47,XY,+12[29]/46,XY[1]	N	N	+80%	N	N	N
39	M/59	C	UM	45,XY,der(2)dup(2)(p13)ins(2;13)(p?;?q),der(11) t(11;17)(q?24;?p),der(13)t(2;13)(p?;q?13),?dic(17;22) (p11;p11)[5]/45,X,der(Y)t(Y;15)(q?11;q?21),idem[11]	D85%	N	N	N	N	N
40	M/68†	B	UM	UNS	D55%	N	N	D97%	N	N
41	F/65	A	boundary value	46,XX,der(7)t(2;7)(p?;q?36),del(13q)[cp9]	N	N	N	D95%	N	N
42	F/71	A	UM	46,XX,der(11)del(11)(q?)t(11;20)(q?;?),der(16) t(16;19)(?;p?),der(20)t(17;20)(?q;?)[13]	N	D90%	N	N	N	N
43	M/61	A	UM	46,XY[30]	N	N	N	D82%	N	N
44	M/54	B	UM	46,XY[3]/46,XY,del(11q)[3]	N	D47%	N	D94%	N	D5'90%
45	F/77†	C	M	47,XX,+12[3]/46,XX[2]	N	N	+79%	N	N	D5'85%
46	F/83	A	M	UNS	N	N	N	D74%	N	N
47	M/65	A	UM	46,XY[10]	N	N	N	D66%	N	D5'80%
48	M/50	A	UM	UNS	N	D92%	N	D53%	N	N
49	F/62	C	UM	46,XX[23]	N	N	N	D69%	N	D5'63%
50	M/52†	B	UM	44~46,XY,-6,-14,-17,+1-3mar[cp10]	N	N	N	N	D67%	N

Abbreviations: N – normal findings; D – deletion; + – three copies of CEP 12; ND – not done; R – the rearrangement of gene; UNS – unsuccessful examination; † patient died

Table 2. ArrayCGH results in 50 CLL patients.

No.	gains	losses
1	-	-
2	-	13q13.1q14.2(33,894,709-48,999,920)
3	-	-
4	-	-
5	12q13.13q24.33(52,742,592-133,525,406)	6q16.3q22.31(103,336,301-120,550,918), 13q13.3q21.33(38,155,980-71,519,829)
6	-	13q13.1q14.3(33,894,709-52,514,204)
7	-	13q14.2q21.2(47,651,918-59,886,576)
8	-	13q14.2q14.3(48,862,679-51,573,841), 13q21.31(63,104,009-64,200,914)
9	-	15q11.2q15.1(25,185,941-41,157,098)
10	-	-
11	-	-
12	-	-
13	-	13q14.2q14.3(48,999,921-52,514,204)
14	-	13q14.2q14.3(50,062,881-51,573,841)
15	-	6q12q27(65,101,827-170,581,850), 18p11.32p11.21(179,428-14,989,963)
16	-	6q16.1(97,717,588-97,817,598), 6q16.3(103,336,301-103,386,329) 6q21(108,047,534-108,108,291)
17	12(133BAC), 13q21.2q21.32(61,823,181-66,517,269), 18q23(73,359,922-77,875,127), 19q13.1 2q13.2(38,161,572-42,940,785)	11q22.3(103,461,090-108,886,109)
18	-	1p35.1p12(331,03,405-118,884,319), 3p26.3p26.1(968,957-4,677,964), 8p23.3p23.1(81,046-11,941,743), 13q14.2q21.32(50,368-66,517), 13q31. 3q34(93,702,860-114,120,603), 17p13.3p11.2(2,990,938-19,808,088), 20q12q13(38,672,967-41,981,799)
19	-	6q21(106,778,974-109,197,165), 13q14.2(50,368,875-50,546,925), 22q11.21(23,071,750-23,221,729)
20	18q21.31q23(54,518,395-77,875,127)	7q31.1q34(107,642,872-141,079,377), 13q14.2q14.3(48,589,512-51,573,841), 17p13.3p11.2(622,586-20,289,789)
21	8q24.21(128,508,282-129,064,398), 8q24.2 2q24.3(134,803,426-146,227,426)	11q22.3(108,721,042-108,886,109), 14q32.33(106,196,305-107,146,073)
22	-	13q14.2(50,368,875-50,952,073), 14q32.33(106,475,175-107,146,073), 17p13.3p11.2(2,990,938-17,304,683)
23	-	11q14.1q22.3(85,061,154-108,886,109), 13q14.2q14.3(50,368,875-53,457,910)
24	-	13q14.2(50,368,875-50,952,073)
25	-	11q22.3q23.2(106,579,547-114,372,284), 13q13.1(32,807,050-33,944,539)
26	12(172 BAC), 18(103 BAC), 19(75 BAC)	13q14.3(50,062,881-51,573,841)
27	2p25.3p12(235,263-77,339,292)	11q14.1q23.2(78,965,050-115,755,274), 13q13.1(32,806,050-33,944,539)
28	12(172 BAC), 18q22.1q22.3(66,676,923-68,777,622)	7p22p15.1(94,136-2,863,6921), 14q24.1q32.3(69,340,601-107,206,128), 18q21.2(52,781,830-53,160,754)
29	18q21.32q22.3(53,348,598-69,890,646)	3p26.3p21.31(212,817-50,018,329), 7p22.3p15.1(94,136-28,636,921), 9q22.33q34.3(101,849,491-140,997,194), 14q24.1q32.33(69,340,601-104,313,141), 17p13.1(7,495,272-7,741,642), 18q21.2q21.32(53,348,598-56,877,019)
30	-	14q24.1q32.33(69,340,601-104,313,141)
31	-	11q22.2q24.1(102,281,372-122,793,220), 13q13.3q21.1(39,679,272-57,752,460)
32	-	6q15q25.3(88,252,488-158,934,425)
33	-	11q14.2q23.2(87,242,809-114,372,284)
34	-	13q12.3q22.1(31,951,813-74,404,613)
35	5q34q35.3(164,820,779-180,124,695), 7q11.2 2q36.3(68,613,015-159,128,663), 8q23.1q24.23(108,911,914-139,384,083)	13q14.2q22.2(47,651,918-76,542,003), 17q25.3(76,125,623-80,781,537)
36	8q21.3q24.3(87,570,656-146,162,080)	6q21(108,047,534-108,540,335), 11q11q13.1(55,421,065-64,250,509), 11q22.3q25(106,579,547-132,886,220), 13q12.11q12.12(20,936,836-24,158,187), 13q14.2q14.3(48,999,921-52,514,204)
37	-	13q14.3(51,430,618-51,573,841)
38	12(143 BAC)	-
39	2p25.3p16.3(1,633,836-49,658,363), 2p16. 1p14(59,105,825-64,822,652), 2p14(66,540,604-69,168,739), 2p12(76,319,546-80,955,167), 4q28.2q35.2(130,877,638-190,782,221), 13q14. 3q21.32(53,285,718-65,890,658), 13q22.2q31.1(75,560,502-81,845,753), 13q31. 1q34(82,012,303-114,912,704), 17q21.31q25.3(41,576,520-80,781,537)	17p13.3p11.2(1,618,017-18,169,260)
40	-	9q21.13q34.12(76,396,515-133,480,644), 13q14.2(50,546,926-50,952,073), 14q32.33(106,406,318-107,267,432), 17p13.3p11.2(622,586-17,304,683)
41	2p25.3p12(2,271,126-68,307,621)	13q12.3q22.1(31,150,628-31,789,825)
42	-	11q14.1q25(79,727,361-133,610,439)
43	-	13q14.2q14.3(4,707,348-50,925,202)
44	-	11q22.3(102,611,654-107,782,670), 13q14.2(50,368,875-50,952,073)
45	8q21.3q24.23(90,050,966-137,910,471), 12(139 BAC)	8p23.2-p12(2,317,216-31,967,062)
46	-	13q13.3q32.3(35,997,178-100,551,259)
47	-	13q14.2q14.3(48,894,899-50,454,033), 14q23q24.3(68,877,801-74,421,176)
48	-	11q22.1q23.3(10,172,554-116,704,710), 13q14.2q14.3(48,894,899-49,645,837)
49	-	13q14.2q14.3(48,467,994-52,285,963), 14q32.33(105,354,886-106,072,530)
50	2p25.2(300,000-89,163,193), 8q22. 3q24.3(103,802,271-103,959,652)	6q16.3q25.3(103,441,042-159,904,765)

RESULTS

The relevant clinical, cytogenetic, FISH and mutational status data of 50 CLL patients are summarized in Table 1. Cytogenetic results were obtained for 37 patients, and an abnormal karyotype was detected in 23 (46%) patients. Translocations were detected and confirmed by FISH in 11 patients. A commercially available FISH panel revealed recurrent chromosomal changes in 47 (94%) patients. In addition to already well-known changes (such as losses at 6q (7 patients/14%), 11q22 (12 patients/2%), 13q14 (33 patients/66%), 17p13 (6 patients/12%) and trisomy 12 (5 patients/10%)), additional abnormalities were detected. Deletions or partial deletions and/or rearrangement involving 14q32 were found in 14 patients using FISH with a locus-specific probe for the *IgH* gene, which covers both the constant and variable gene region. We confirmed deletion of the 5' *IgVH* flanking sequences in 10 cases, while 3' *IgH* deletion was detected in only 2 cases. Deletion of the whole *IgH* gene was found in only 1 case, as well as rearrangement of the *IGH* gene (Table 1). FISH targeted to non-routinely analyzed CLL regions was performed in selected cases to confirm and complete cytogenetic and arrayCGH findings (data not shown).

Copy number aberrations revealed by arrayCGH

ArrayCGH revealed copy number changes in 44 (88%) out of 50 patients. We detected a total of 84 regions of loss and 34 regions of gain (Table 2). Using arrayCGH, we detected well-known aberrations as well as various novel imbalances. These new abnormalities were found in 14 patients and included losses of chromosomes 1p (no. 18), 3p (nos. 18 and 29), 7p (nos. 28 and 29), 7q (no. 20), 18 (no. 15), 8p (nos.18 and 45), 9q (nos. 29 and 40), 15q (no. 9), 17q (no. 35), 18p (no. 15), 18q (nos. 28 and 29), 20q (no. 18) and 22q (no. 19). Novel chromosomal gains included gains of chromosomes 5q (no. 35), 7q (no. 35), 17q (no. 39), 18q/18 (nos. 17, 20, 26, 28 and 29) and 19q/19 (nos. 17 and 27). In four cases (nos. 27, 39, 41 and 50), gains of chromosome 2p were detected. In one (no. 39), the gained region was heterogeneous, as shown in Figure 1. The result of patient no. 50 was included in the data published previously[24]. The most frequent gains were detected on chromosomes 8q and 18q, each in 5 cases (10%). These gains could be considered recurrent abnormalities. The most frequent losses were recognized on chromosome 6q (7 cases, 14%).

Loss of 6q and determination of the smallest commonly deleted region

Seven patients (nos. 5, 15, 16, 19, 32, 36 and 50) with 6q loss detected by FISH or arrayCGH were successfully subjected to 32K tiling path chromosome 6 arrayCGH to more precisely define the smallest commonly deleted region (SCDR). We confirmed that the SCDR included 6q21q32 comprising a 1.4 Mb region (Figure 2). The deleted region involved band q21 where a number of genes are located, among them the *FOXO3A* gene.

Comparison of FISH and arrayCGH results

All 50 CLL samples had been previously analyzed using commercially available FISH probes mapping to routinely analyzed loci; 11q22 (*ATM*), centromeric region of chromosome 12, 13q14 and 17p13 (*TP53*). These results were compared to assess array performance versus interphase FISH. A total of 95% of the FISH findings were concordant with the arrayCGH results. Thirteen findings in 10 patients (nos. 4, 5, 9, 11, 14, 15, 27, 29, 44 and 45; Table 1, 2) were discordant for various reasons. This discordance involved deletion of 11q22 containing the *ATM* gene, which was detected in 12 cases by FISH but was not confirmed in 2 cases by arrayCGH. Trisomy of chromosome 12 was approved in all 5 FISH positive cases. Moreover in 1 patient (no. 5) partial trisomy of 12q13q24 was detected only by arrayCGH and later confirmed by FISH with GLI (12q13) probe. Deletion of 13q14 was confirmed by arrayCGH in only 29 out of the 33 cases detected by FISH with LSI D13S319 probe. On the other hand, arrayCGH revealed high heterogeneity of the deleted 13q14 region in other patients, which was displayed mainly in the results of oligonucleotide arrays (Table 2). Deletion of chromosome 17p13 detected by arrayCGH was concordant with FISH results in all examined cases.

DISCUSSION

We used 4 different arrayCGH platforms available in our laboratory during the study. This could cause inhomogeneous results due to platform-specific copy number variations. Gunnarsson et al.[25] showed that all arrayCGH platforms concordantly detect large and known copy number alterations but diverge in detection of small ones. Therefore we took into account in this study, only abnormalities represented by at least three subsequent clones or copy number changes confirmed by FISH.

The arrayCGH revealed a total of 84 regions of loss and 34 regions of gain. We were able to detect copy number changes in regions with already well-known impact on CLL prognosis, such as deletions of the *ATM* gene at 11q22 (ref.[26, 27]), *miR-15a/16-1* and *RB1* genes at the 13q14 region[28-30], *TP53* gene at 17p13 (ref.[31,32]) and trisomy 12 / gain of 12q (ref.[33]). We also identified other recurrent imbalances such as gains of 2p and 8q24 and loss of 14q32. In addition, we noticed a large number of non-recurrent imbalances, most of them as new unbalanced changes (Table 2). Our results indicate that the genomic instability of CLL is higher than previously thought. We suspect that regions of recurrent chromosomal imbalances could result in altered expression of genes contributing to the pathogenesis of CLL but this was not aim of our study.

The frequent gain of the 2p14-p25 sequences detected by arrayCGH in 4 patients is of particular interest. These findings were confirmed by FISH with the LSI ALK, LSI NMYC, CEP 2 and REL-specific BAC probes. Our research team published finding 2p gains in another cohort of CLL cases[24], and its prognostic significance was re-

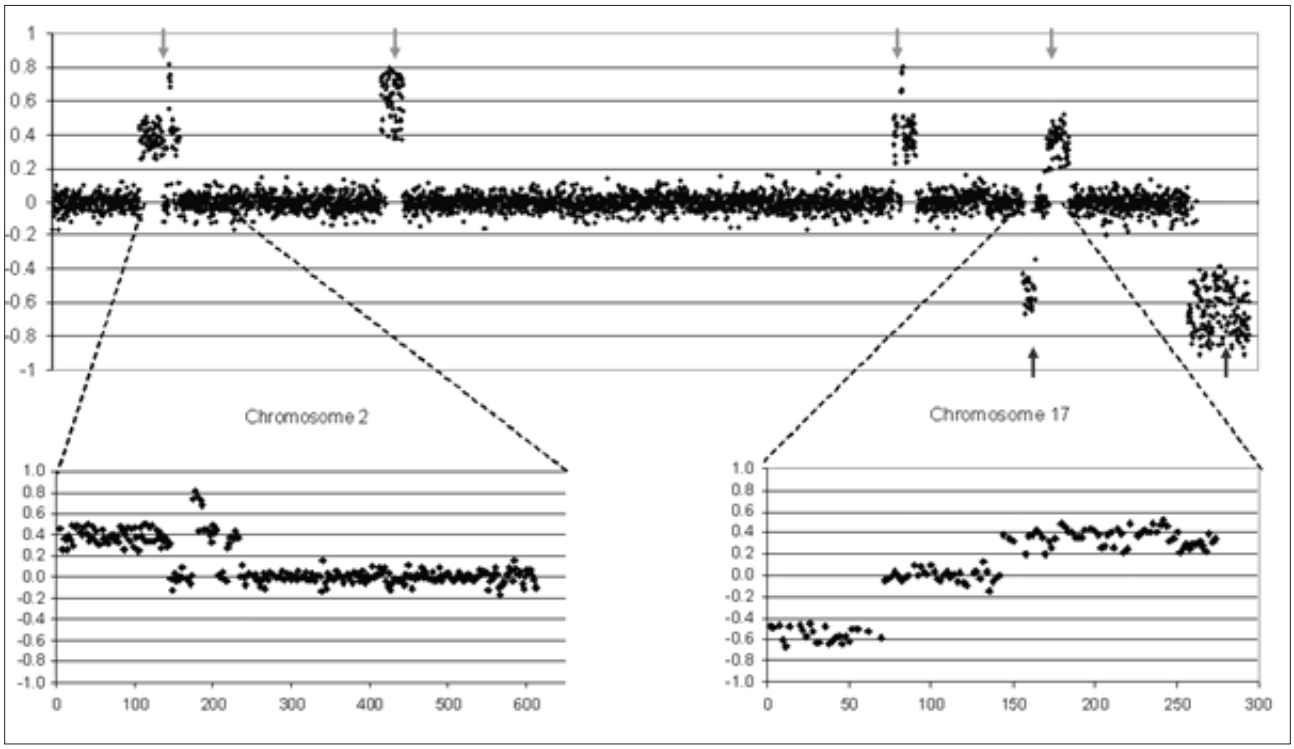

Fig. 1. Example of arrayCGH result in patient no. 39.
ArrayCGH profile is showing log2 ratios of Cy5/Cy3 signal intensities along all chromosomes, from chromosome 1 to chromosome X, from the left to the right side, respectively. ArrayCGH revealed gains of regions 2p25.3p16.3, 2p16.1p14, 2p14, 2p12, 4q28.2q35.2, 13q21.1q21.32, 13q22.2q31.1, 13q31.1q34, 17q21.31q25.3 (upper grey arrows) and losses of 17p13.3p11.2 region and chromosome X (due to sex-mismatch control) (lower black arrows). Lower part of figure is displaying detailed profiles of chromosome 2, note the 4 different regions of 2p gain, and chromosome 17 lost and gained regions.

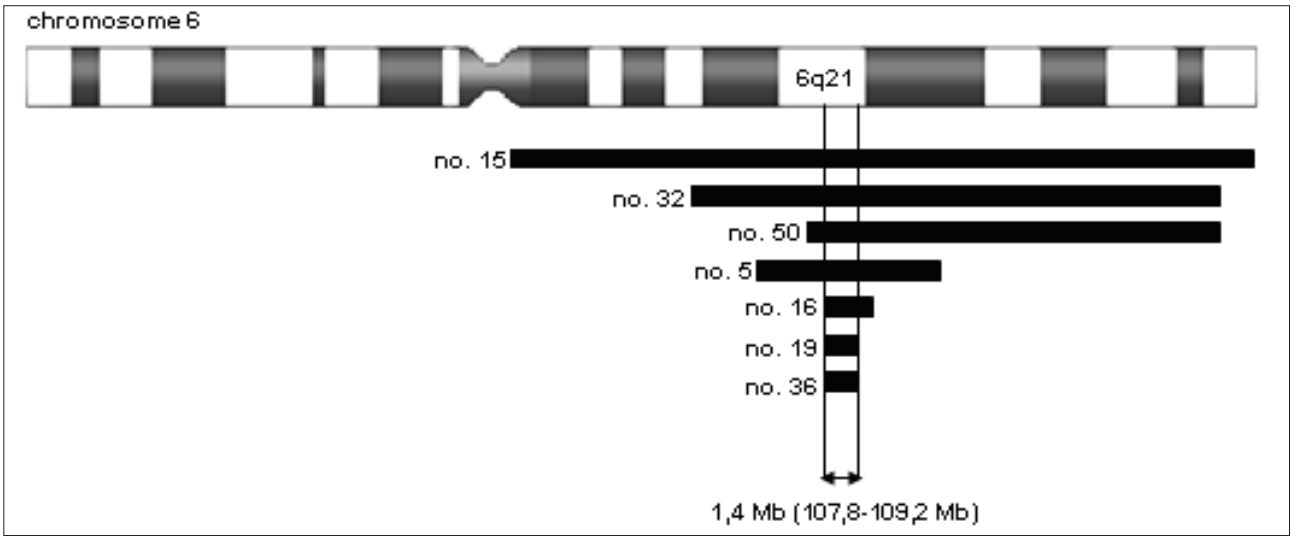

Fig. 2. Schematic summary of 32K tiling path chromosome 6 arrayCGH results.
Black bars are representing deleted regions along chromosome 6q in 7 patients. The established SCDR covered 1,4 Mb at 6q21 (107,8-109,2 Mb).

cently confirmed by Chapiro et al.[34]. In 3 patients, the 2p gain was accompanied by other cytogenetic abnormalities (nos. 27, 39 and 50) while another only had a 13q deletion (no. 41). The heterogeneity of the gained region is shown in Fig. 1, case no. 39. According to the literature[3,35,36], a gain of 2p sequences occurs in 7-17% of CLL. This aberration is associated with a poor prognosis and could play an important role in the pathogenesis of the disease. For this reason, we again suggest that 2p probes (e.g., NMYC or ALK) are included in the routine FISH investigation of CLL (ref.[24]).

Gains of the 8q region detected in 5 patients, 4 with an unmutated *IgVH* pattern, involved region q24 with the location of the *CMYC* gene. This finding was confirmed

by FISH with the LSI MYC probe. The 8q gain would have remained undetected in 4 out of 5 patients if the conventional cytogenetics and routine FISH panel had alone been used.

The loss of 14q32 sequences was detected by FISH in 13 cases, whereas arrayCGH recognized this aberration in only 6 of these. This was probably caused by the already well-known presence of polymorphic sequences in this region. Losses in the *IGH* region presumed to represent deletions occurring during the physiological *IgH* gene rearrangement within B-cell development and is not associated with the pathogenesis of CLL (ref.[37]). On the other hand, the detected deletions tend to associate with additional chromosomal changes, such as trisomy 12 and *IgVH* unmutated status[38]. Both cases with 3´*IgH* deletion were unmutated, as well as 7 out of 10 cases with 5´*IgVH* deletion, and in all cases additional changes were observed. A constant loss of 6q sequences, confirmed by FISH with the ON MM 6q21/15q22 probe, was detected by arrayCGH in 7 CLL patients. All these patients showed an unmutated *IgVH* pattern related to a poor prognosis. In two patients, del(6)(q) was detected as the sole aberration; in the remaining five patients, del(6)(q) was accompanied by other molecular cytogenetic abnormalities, such as deletions of 11q22, 13q14, 17p13 and other regions. These findings are in agreement with published data that classify del(6)(q) as an adverse prognostic factor in lymphoid malignancies[39,40]. The smallest commonly deleted region at 6q established in 7 patients was defined by 32K tiling path chromosome 6 arrayCGH and covered 1.4 Mb at 6q21 (107.8-109.2 Mb). This region includes only one *FOXO3A* gene previously reported as a gene that plays a role in B-cell activation, differentiation and pathogenesis[40]. The other genes with the same role in CLL, such as *CCNC*, *CD24*, *PRDM1* located at 6q21 (106.5 Mb)(ref.[41-44]), were excluded from consideration in our patients because they are located outside of our established SCDR.

We also suggest that an unknown tumor suppressor gene could be present within this region. Expression profiling[45] may shed light on the events connected with 6q21 deletions as well as on other copy number changes in patients with B-CLL and their role in the malignancy.

Six out of 50 analyzed patients showed a balanced karyotype in arrayCGH. In 3 patients, this finding correlated with normal cytogenetic and FISH results. In 2 patients, only FISH detected deletions of 13q14, 11q22 and 5'*IGH*. We presume that these findings are either below the detection level of the arrayCGH technique (22%) or that the 2 patients who escaped arrayCGH analysis have a deletion of 13q14 of a very small size, which was not depicted by arrayCGH platform (Leiden, Netherlands) due to absence of BAC referring to this approximately 100 kb large region. A balanced chromosomal translocation t(3;14) with rearrangement of the *IgH* gene was found in 1 patient with an arrayCGH-balanced profile.

Our findings are in concordance with findings of other groups[36,46-49].

CONCLUSIONS

In summary, the CGH array proved to be a reliable tool for detecting quantitative genomic aberrations that escape cytogenetic and routine FISH analyses in CLL. It also helped to precisely map the breakpoints of unbalanced regions but failed to identify aberrations in samples with small deletion in region not presented on the platform and samples with low involvement of pathological cells. The detection limit of our arrayCGH platforms was 22% of abnormal cells in analyzed sample.

Cytogenetic examination of the studied CLL patients revealed chromosomal abnormalities in 23 (46%) patients, whereas FISH analysis of six CLL monitored loci depicted 77 aberrations in 47 (94%) patients. ArrayCGH, however, identified 118 genomic imbalances in 44 (88%) patients, including new aberrations in 14 patients which had not been detected by FISH or cytogenetics. These results suggest that a subset of potentially significant genomic alterations in CLL is being missed by using only cytogenetics and FISH. Without conventional cytogenetics and FISH, however, it would not be possible to resolve complex karyotypes, balanced translocations and approximately 5% of abnormalities would be missed due to limits of arrayCGH.

In conclusion, we propose a combination of conventional cytogenetics, FISH and arrayCGH as a necessary tool to unravel the molecular karyotype of CLL as it is an important indicator of disease prognosis. Further detailed analysis of aberrant regions detected by arrayCGH, and complemented by cytogenetic and molecular findings as well as clinical parameters, could be of help in identification of new biomarkers in CLL with a potential prognostic value.

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