Array Comparative Genomic Hybridization Analysis Identifies Recurrent Gain of Chromosome 2p25.3 Involving the *ACP1* and *MYCN* Genes in Chronic Lymphocytic Leukemia

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Abstract

Chromosomal aberrations are independent prognostic markers in chronic lymphocytic leukemia (CLL). Recent studies using genomic arrays have shown recurrent gains of the short arm of chromosome 2 (2p) in a subset of CLL. We evaluated 178 CLL cases for 2p gains using custom-designed oligonucleotide array-based comparative genomic hybridization (aCGH). A high frequency of 2p gains was observed in 53 of 178 (30%) cases, which ranged from a small 29-kb region to large segments involving the entire short arm. Besides several common chromosomal aberrations associated with 2p gain, we demonstrated a novel observation that gain of the telomeric region 2p25.3 harboring the *ACP1* gene is common in CLL (25%, 44 of 178 cases). The *ACP1* gene has been previously shown to regulate T-cell receptor signaling through *ZAP-70*, and both genes are unfavorable clinical markers for CLL. Quantitative polymerase chain reaction (qPCR) confirmed the presence of 3-6 copies of *ACP1* in 35 of 40 (88%) of these cases. Interestingly, none of the aCGH diploid CLL cases showed gain of *ACP1*. Assessment of 73 healthy individuals by qPCR revealed *ACP1* copy number gain in only two cases (2.7%). Gain of 2p25.3 was associated with ZAP-70 expression ($P < .002$) and unmutated immunoglobulin heavy chain variable (*IGHV*) gene mutation ($P < .0001$). A high frequency of *MYCN* co-amplification with *ACP1* was observed (14 of 40 cases, 35%). The frequent 2p25.3 gain involving the *ACP1* and *MYCN* genes may help define the critical region of 2p that contributes to pathogenesis of CLL together with other chromosomal abnormalities.

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Introduction

Chronic lymphocytic leukemia (CLL) is characterized by a highly variable clinical course and outcome. Specific recurrent chromosomal aberrations, traditionally assessed in clinical laboratories by fluorescence in situ hybridization (FISH) or less-sensitive conventional cytogenetics, are important independent biomarkers for disease progression and survival.[1] Deletions (del) of *ATM* at 11q22-23 and *TP53* at 17p13.1 are associated with a worse clinical course in CLL whereas del13q14.3 and trisomy 12 are associated with a better overall outcome.[1-3]

Array-based comparative genomic hybridization (aCGH) is a powerful high-resolution tool for detection of chromosomal aberrations, and currently this platform is gaining acceptance as a clinical tool for the analysis of CLL genome.[4,5] Recently, two independent studies using bacterial artificial chromosome (BAC) CGH showed gains of the short arm of chromosome 2 (2p) in a subset of CLL cases.[6,7] This region of 2p harbors several oncogenes, including

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MYCN, *ALK,* and *REL*, suggesting a possible pathogenic role for these genes in CLL.

Compared with BAC arrays, high-density oligonucleotide arrays delineate the boundaries of chromosomal alterations more precisely and allow the detection of genomic aberrations at a single gene level.[8-10] In this study, we used a customized oligonucleotide aCGH platform validated in our laboratory to assess for 2p genomic alterations in a large number of CLL patients.[5] We demonstrate that gain of the telomeric region 2p25.3 harboring the acid phosphatase 1 (*ACP1*) gene is a common aberration in CLL. *ACP1* is a low molecular weight phosphotyrosine phosphatase (*LMWPTP*) family member that plays a key role in cell proliferation by dephosphorylating/activating both tyrosine kinase receptors and docking proteins.[11-13] Overexpression of *LMWPTP*s has been demonstrated to be sufficient to induce neoplastic transformation.[14,15] Earlier studies have shown that *ACP1* regulates T-cell receptor signaling through activation of zeta-chain associated protein kinase 70kDa (ZAP-70).[11] Because *ZAP-70* expression is known to correlate with unmutated immunoglobulin heavy chain variable (*IGHV*) gene and poorer prognosis, we determined whether there is correlation between 2p25.3 gain with *ZAP-70* expression and *IGHV* mutation status. In addition, using quantitative polymerase chain reaction (qPCR), we assessed copy number gains of *ACP1* and two genes, *SH3YL1* and *FAM150B*, immediately flanking *ACP1*. We also assessed copy number changes of *MYCN*, *ALK,* and *REL*, three genes downstream of *ACP1* that have been previously reported to be amplified in CLL associated with 2p gains.[6,7] The copy numbers of *ACP1* were also determined in healthy individuals by qPCR to rule out the possibility that the gain of *ACP1* was due to copy number variation (CNV).

Materials and Methods

Case Selection and Review

Two hundred patient samples, diagnosed as CLL according to the World Health Organization criteria at The University of Texas M. D. Anderson Cancer Center (Houston, TX), were initially screened according to the CD19+/CD5+ cell count determined by flow cytometry.[3] One hundred seventy-eight cases (mixed treated and untreated patients) with previously established criteria of greater than 25% CD19+/CD5+ cells in the samples were included in the study.

DNA Purification and Labeling

Isolation and labeling of genomic DNA was performed as described previously.[5] Briefly, genomic DNA (gDNA) from peripheral blood (PB) or bone marrow (BM) aspirates was isolated using the Autopure extractor (Qiagen/Gentra, Valenica, CA). Five hundred nanograms of gDNA was digested with Alu and RsaI restriction enzymes for 2 hours at 37 °C. Digested gDNAs from patients and the reference DNA (human female DNA, Promega Corporation, Madison, WI) were labeled with Cy5-dUTP and Cy3-dUTP, respectively, using Agilent Genomic DNA labeling kit plus (Agilent Technologies, Polo Alto, CA). The labeled DNA was purified using Micron YM-30 columns (Millipore, Billerica, MA) and the volume was adjusted by 1 x Tris-EDTA buffer (pH 8.0) to 20 to 25 μL.

Genomic Array Design and Hybridization

A custom-designed, 4 x 44K, 60-mer oligonucleotide genomic array, with gene-centric full genome coverage augmented with high-density probe tiling in the 15 common aberrant loci in CLL was used.[5] For hybridization, labeled patients' gDNA and reference DNA were mixed and co-precipitated with 5 μg of human Cot-1 DNA (Invitrogen, Carlsbad, CA) using 11 μL of 10X blocking reagent and 55 μL of 2X hybridization buffer (Agilent Technologies) in a total volume of 110 μL. After denaturing at 93°C for 3 minutes, the mixture was incubated at 30°C for 30 minutes. Hybridization was performed at 65°C for 40 hours in a rotating oven (Robbins Scientific, Mountain View, CA) at 10 rpm. The slides were washed in oligo-aCGH wash Buffer 1 at room temperature, followed by washes for 1 minute at 37°C in oligo-aCGH wash Buffer 2 (Agilent Technologies), for 1 minute at room temperature in acetonitrile (Sigma-Aldtrich, St Louis, MO), and a final 30 seconds wash in stabilization and drying solution (Agilent Technologies). Slides were scanned using an Agilent 2565BA DNA microarray scanner.

aCGH Data Analysis

Data were normalized using the Feature Extraction Software version 9.5.3.1 (Agilent Technologies), and analyzed by CGH analytics software version 3.5.14 (Agilent Technologies) and Nexus CGH 4.1 (Biodiscovery Inc, EL Segundo, CA). Previously developed analysis protocols were applied.[5] For analysis using CGH analytics, low and high stringency settings with the Aberration Detection Method 2 (ADM-2) statistical algorithm and the default analytics settings for the centralization and fuzzy zero correction were used. The "low stringency setting" included an ADM-2 threshold of 4.0 and an aberration level filter with a minimum number of probes in an aberrant region of 2 and a minimum absolute average Log2 ratio of 0.05. The "high stringency" setting included an ADM-2 threshold of 6.0 and an aberration level filter with a minimum number of probes in an aberrant region of 25 and a minimum absolute average Log2 ratio of 0.2. For the Nexus CGH analysis, the Rank Segmentation algorithm with significance threshold of 1.0×10^{-5} was used. The settings for aberration calls in Nexus CGH were: 0.15 for gain, 0.4 for high gain, -0.1 for loss and -0.4 for high loss. Human genome assembly hg18 (NCBI Build 36) was applied for the analysis.

Quality Control Measures and Assay Performance Parameters

Only samples with a post-labeling yield of 5 to 7 μg DNA and a specific activity of 25 to 40 pmol/μg for Cy3 and 20 to 35 pmol/μg for Cy5 were used for array hybridization. Following array hybridization the signal intensity, signal to noise ratio, background noise, the derivative log ratio spread, and the reproducibility were evaluated using the Feature Extraction software version 9.5.3.1 (Agilent Technologies), with cutoffs for sample rejection used according to the manufacturer's recommendations.

Cytogenetics and FISH

Conventional cytogenetics and FISH analyses were performed on cultures of whole BM or PB samples as described earlier.[5] Briefly, 20 metaphase cells were used for cytogenetics analysis using standard Giemsa-banding techniques. A multiprobe FISH panel included locus specific probes to *ATM* (11q23), the centromeric region of chromosome 12 (12p11.1-q11), D13S319 (13q14.3), *LAMP1* (13q34), and *TP53* (17p13.1). Two hundred interphase nuclei were examined and counted for each probe.

Copy Number Analysis By Real Time qPCR

To validate aCGH findings of 2p gain, qPCR was performed using TaqMan Copy Number Assays (Applied Biosystems, Carlsbad, CA) for *SH3YL1*, *ACP1*, *FAM150B*, *TPO*, *MYCN*, *ALK*, and *REL* according to manufacturer's instructions. The *RNase P* gene, which is known to exist in two copies in a diploid genome, was used as the endogenous copy number reference in multiplex reactions. Healthy female genomic DNA from Promega was applied as diploid control. The PCR reactions were performed in triplicates using 50 ng of genomic DNA, 1× TaqMan Universal PCR master mix, 1x TaqMan Copy Number Assay mix in a total volume of 20 μL per each reaction. PCR was performed on a 7900HT Sequence Detection System (Applied Biosystems) in a 96-well format, and the amplification was achieved using the standard amplification protocol as follows: 50°C for 2 minutes, 95°C for 10 minutes, followed by 40 cycles at 95°C for 15 seconds and 60°C for 1 minute. Post-PCR copy number analysis was performed by Applied Biosystems CopyCaller Software v1.0 (Applied Biosystems), which performs a comparative C_T ($\Delta\Delta C_T$) relative quantitation analysis of the real-time data using RNase P and Promega Female DNA as controls. The software uses the statistical model $\Delta CT = K - \log_{1+E} CN$, where K is a constant, E is the PCR efficiency of the assay of interest, and CN is copy number with the range [1, ∞).

Statistical Analysis

The two-sided chi-square tests were applied to test against the null hypothesis of no association between chromosome 2p25.3 gain or *IGHV* mutation status and ZAP-70 expression, using P = .05 as the threshold of significance.

Results

Chromosomal Alterations Detected By aCGH

For aCGH analysis, the "low stringency setting" with an ADM-2 threshold of 4.0 and an aberration level filter with a minimum number of probes in an aberrant region of 2 and a minimum absolute average Log2 ratio of 0.05 was used first followed by a "high stringency" setting with ADM-2 threshold of 6.0 and an aberration level filter with a minimum number of probes in an aberrant region of 25 and a minimum absolute average Log2 ratio of 0.2. The chromosomal aberrations seen in the study group are summarized in Table 1. The well- documented CLL-associated chromosomal deletions involving 13q12-13q14, 11q14.3-q23.3, and 17p13.1 were observed in 60%, 46%, and 28% of the cases, respectively. Trisomy 12 was observed in 23% of the cases. Using Nexus CGH software, which allows analysis of large datasets simultaneously, we observed gains of 2p region of various lengths in 53 of 178 cases (30%). Forty-four of 53 cases (83%) showed a gain in the telomeric region (2p25.3) involving known oncogenes including *ACP1, TPO, MYCN,* etc (Figure 1A). Because there is still no report about the copy gain of *ACP1* in CLL, the rest of this study concentrates on the *ACP1* gene. Figure 1B shows a closer view of the copy gain of the *ACP1* gene observed by Nexus software.

Except in one case, none of the 2p25.3 gains detected by aCGH were identified by conventional cytogenetics (Supplemental Table 1). Chr 2p25.3 gain was always associated with concurrent abnormalities in other chromosomes including del13q14 (60.0%), del11q23 involving the *ATM* gene (42.9%), del14q (35.6%), del6q (33.3%), del17p13.1 involving *TP53* (31.4%), and trisomy 12 (11.4%; Table 2).

Table 1 Distribution of Recurrent aCGH-Detected Aberrations in CLL[a]

Chromosome	Cytoband	Event	Frequency (%)
Chr1	q23.2-q23.3	CN Loss	15.7
Chr2	p25.3	CN Gain	25.1
Chr2	p12-25.3	CN Gain	32.4
Chr3	p24.3	CN Loss	16.9
Chr3	q26.1	CN Gain	15.7
Chr4	q28.1-q28.3	CN Gain	18.5
Chr6	q21-q23	CN Loss	11.0
Chr6	q25-q27	CN Loss	8.0
Chr8	q24.3	CN Gain	17.4
Chr9	q33.3-q34.3	CN Loss	15.7
Chr11	q14.3-q23.3	CN Loss	45.9
Chr12	q13-q15	CN Gain	23.0
Chr13	q12-q14.3	CN Loss	59.9
Chr14	q24.2-q24.3	CN Loss	15.5
Chr16	p13.3	CN Loss	15.2
Chr17	p13.1	CN Loss	28.0

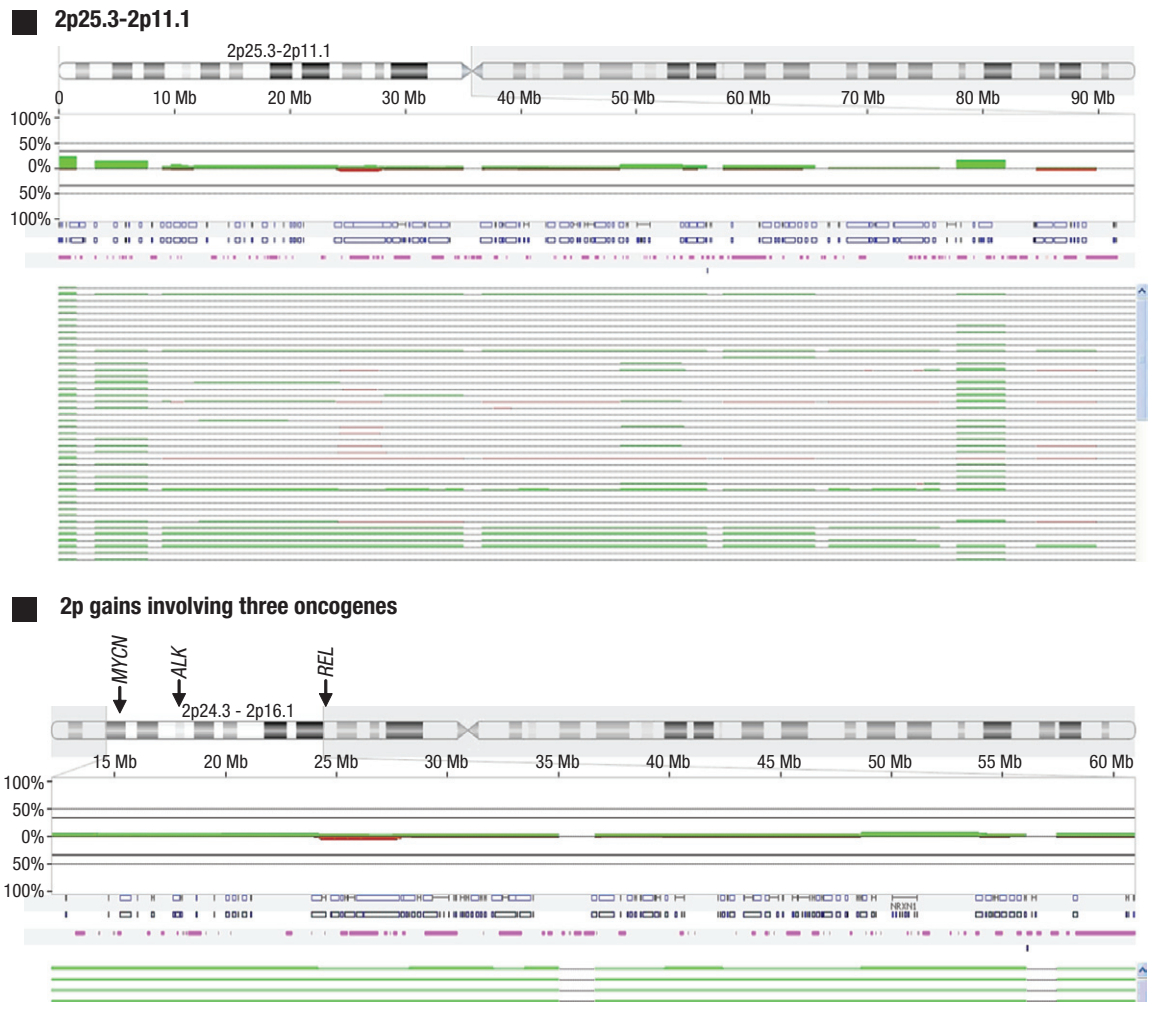
Abbreviations: aCGH = array-based comparative genomic hybridization; CLL = chronc lymphocytic leukemia; CN = copy number.
[a] The percentage of aberrations was calculated based on CN gains or losses detected by aCGH. Only changes present in more than 15% of the cases (n=178) are listed.

The 2p region is known to harbor three important oncogenes, *MYCN* (2p24.3), *ALK* (2p23.2), and *REL* (2p13-p12). Gains in 2p involving these three loci have been reported in CLL previously.[6,7,16] In the report by Jarosova et al, 2p gain was identified in 16 of 200 (8%) CLL cases, all involving *MYCN*, *ALK,* and *REL.*[7] The minimally duplicated region in their study involved *MYCN* at 2p24.3 and *REL* at 2p13-p12. Using aCGH, we identified 2p gains involving the *MYCN* and *ALK* loci in 13 of 178 (7.3%) cases and gains of the *REL* locus in 11 of 178 (6.2%) cases, respectively. 2p gains involving all three oncogenes was observed in only 8 of 173 (5%) cases.

Copy Number and Gene Expression Analysis By qPCR

To confirm 2p gains identified by aCGH, we performed copy number analysis by qPCR in 40 positive cases with available DNA (Supplemental Table 2 and Fig 2). More than two copies of *ACP1* were detected in 32 of 40 (80%) cases using the criteria/cutoffs defined by Applied Biosystems' CopyCaller software. Four to six copies of *ACP1* were detected in 11 (31%) of these cases. In contrast, copy gain of the most telomeric gene on chromosome 2p, *SH3YL1*, which is 0.8 kb upstream of *ACP1*, was observed in only 4 of 40 (10%) cases. Similarly, gain in *FAM150B*, a gene 1.3 kb downstream of *ACP1*, was observed in only 9 of 40 (23%) cases. Thus, the low frequency copy gains of genes immediately flanking *ACP1* indicates that the gain of *ACP1* was not associated with telomeric alterations commonly seen in cancer. Furthermore, gains of *TPO* (2p25.3), *MYCN* (2p24.1), *ALK* (2p23), and *REL* (2p13-p12) were observed

Figure 1 Screen Shots of Nexus Analysis Results of 178 CLL Cases. For the Nexus CGH Analysis, the Rank Segmentation Algorithm With Significance Threshold of 1.0 x 10^{-5} Was Used. The Settings for Aberration Calls in Nexus CGH Were: 0.15 for Gain, 0.4 for High Gain, -0.1 for Loss and -0.4 for High Loss. Human Genome Assembly hg18 (NCBI Build 36) Was Applied for the Analysis. (A) The Copy Gains in the 2p. (B) A Zoomed Region of 2p in View of the Three Oncogenes. Green Color: Copy Gain. Grey Color: Diploid



in 20 (50%), 17 (42.5%), 4 (10%), and 2 (5%) of 40 cases, respectively. Concurrent gain of *ACP1* was seen in all except three cases in which gain of *MYCN* was the sole aberration (Table 3 and Fig 3A). Three cases (#1, 29 and 36) showed gains of all three oncogens by qPCR. Gains involving these loci were also observed by aCGH.

Two CNVs for *ACP1* gene have been reported based on the first-generation CNV map of the human genome in a study with 270 individuals.[17] To test the possibility that *ACP1* gain observed in CLL is due to CNV, 73 healthy controls were tested by qPCR for *ACP1* copy number. Three copies of *ACP1* were observed in 2 (2.7%) healthy individuals (Fig 3B). One copy was detected in only one individual (1.3%). Furthermore, *ACP1* copy number gain was not observed in 29 diploid CLL cases called by aCGH and only one case was found to be one copy (6.9%). Gain of *ACP1* correlated with a 1.5-fold increase in RNA level in comparison with the diploid *ACP1* CLL cases. However, the differences were not statistically significant, possibly due to the small number of cases (data not shown).

Correlation of Copy Number Alterations With ZAP70 Expression and IGHV Mutation Status

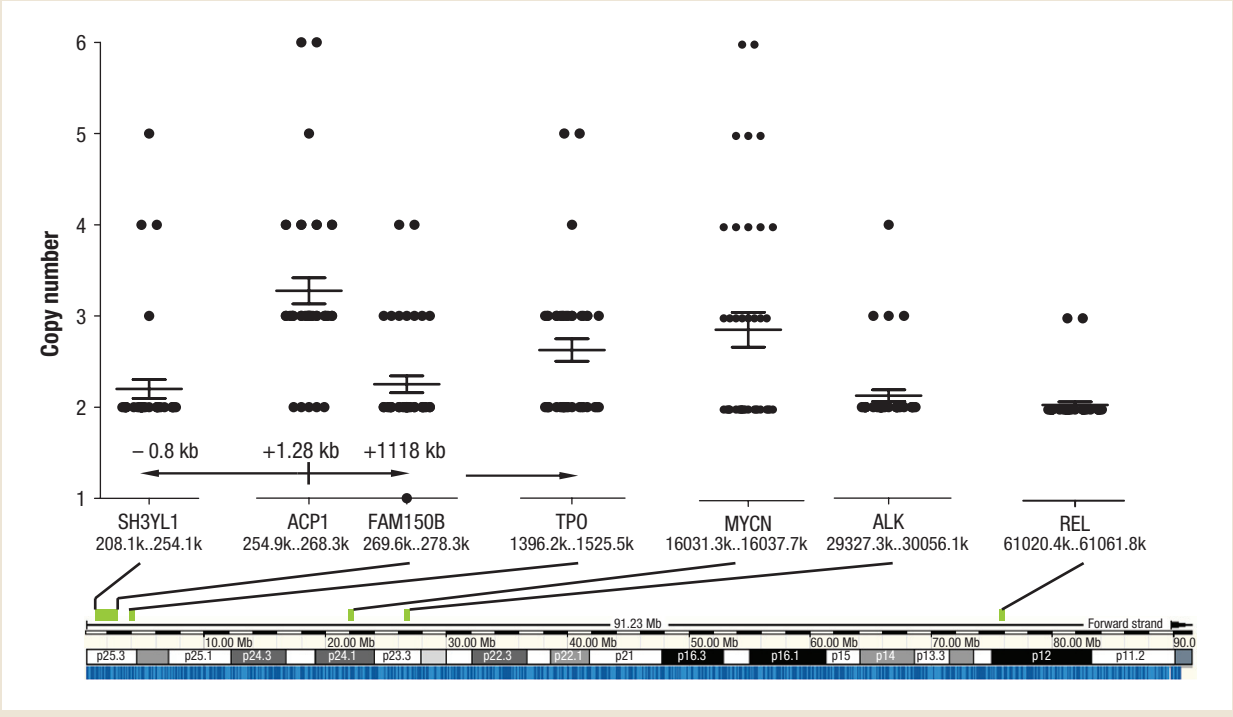
Similar to *CD45*, *ACP1* plays a crucial role in T-cell signaling by specifically dephosphorylating Tyr-292, a negative regulator of *ZAP-70*, thereby counteracting inactivation of ZAP-70 at the post-transcriptional level.[11, 18] Expression of ZAP-70 is associated with enhanced B-cell signaling by allowing more effective IgM signaling in CLL B cells.[19,20] In CLL, higher expression of ZAP-70 is associated with unmutated *IGHV* status and poor prognosis. We tested CLL

Table 2 Comparison of Chromosomal Aberrations in CLL With and Without 2p25.3 Gain Detected by aCGH[a]

Chromosome	With 2p25.3 Gain	Without 2p25.3 Gain	*P* Value
del 17p13.1(FISH)	11/35 (31.4%)	28/133 (21.1%)	.285
del 11q23 (FISH)	15/35 (42.9 %)	22/133 (16.5%)	.002
del 13q14 (FISH)	21/35 (60%)	26/133 (19.5%)	< .0001
Trisomy 12 (FISH)	4/35 (11.4%)	23/133 (17.3%)	.561
del 6q (aCGH)	15/45 (33.3%)	14/133 (13.5%)	.001
del 14q (aCGH)	16/45 (35.6%)	17/133 (12.8%)	.001
Unmutated *IGHV*	26/34 (76.5%)	52/133 (39.1%)	< .0001
ZAP-70 expression	19/27 (70.4%)	19/56 (33.9%)	.002

Abbreviations: aCGH = array-based comparative genomic hybridization; CLL = chronic lymphocytic leukemia; FISH = fluorescent in situ hybridization.
[a] Chromosomal aberrations were detected by aCGH. *IGHV* somatic hypermutation status was evaluated by Sanger sequencing. Mutated *IGHV* is defined as ≥ 2% mutated bases in the sequenced region. *ZAP-70* expression was detected by flow cytometry. *P* values were calculated using chi-square test.

Figure 2 Location and Copy Numbers of Selected Genes Involved in Chromosome 2p Gain. Real Time qPCR for Copy Number Analysis Was Performed as Described in Materials and Methods and the Results Were Analyzed by CopyCaller Software (Applied Biosystems). The Bar Represents Means and S.D



cases with available RNA for ZAP-70 expression, and observed that amplification of *ACP1* only led to a 1.4-fold increase in ZAP-70 mRNA level (data not shown). ZAP-70 protein data assessed by flow cytometry was available for 83 cases and we observed that ZAP-70 was expressed in 38 (45.8%) cases, including 19 of 27 (70.4%) cases with 2p25.3 gain and 19 of 56 (33.9%) cases without 2p25.3 gain, ($P = .002$; Table 2). Thus, *ACP1* copy gain was associated significantly with the expression of ZAP-70 protein.

IGHV mutational analysis was assessed in 167 CLL cases. *IGHV* was unmutated in 78 (43%) cases, including 26 of 34 (76.5%) showing 2p25.3 gain and 52 of 133 (39.1%) without 2p25.3 gain ($P < .0001$; Table 2).

Discussion

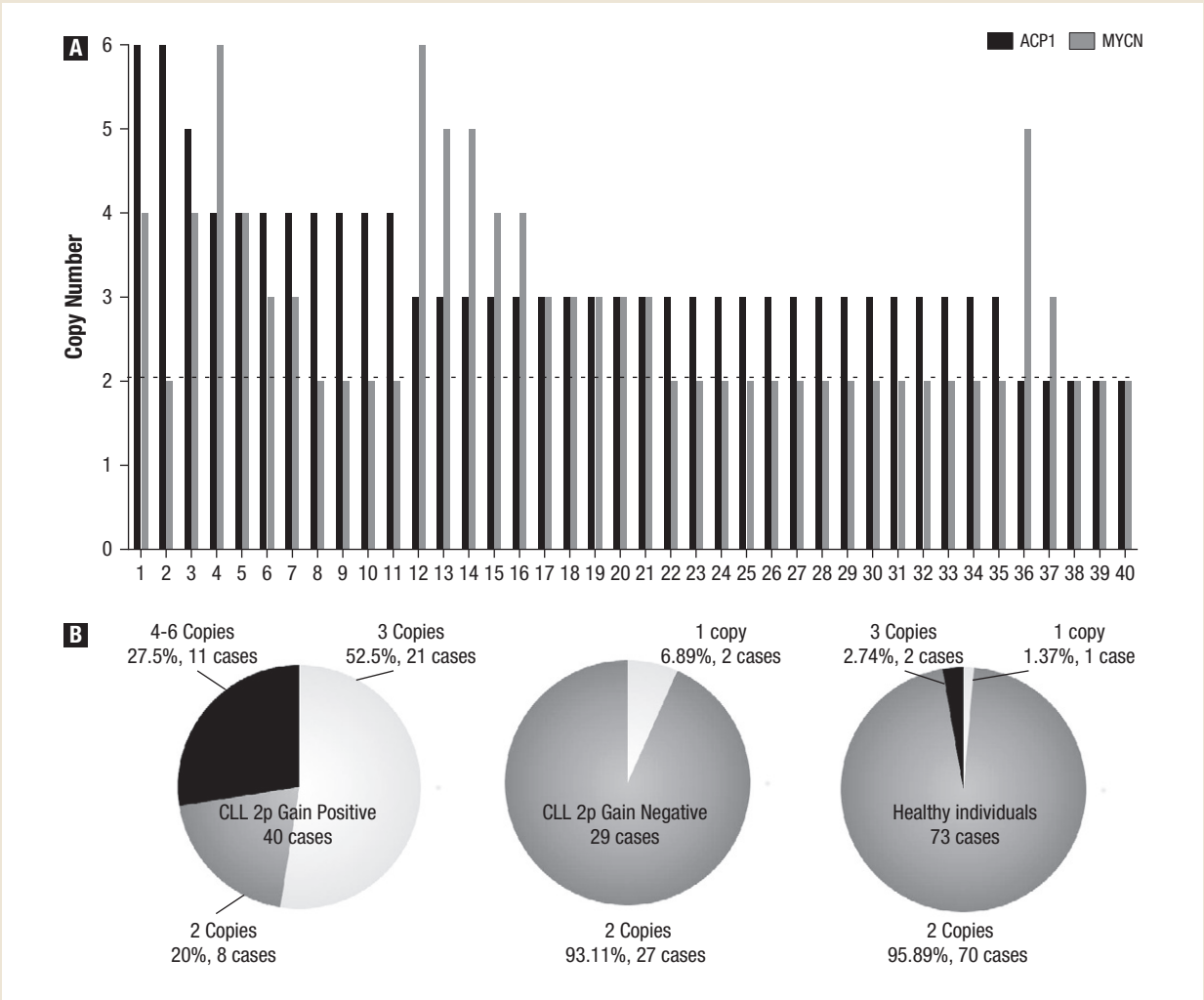
Chromosomal aberrations are detected in 50% to 80% of CLL cases and have prognostic and diagnostic importance.[18] The most commonly recognized chromosomal abnormalities, typically detected by FISH in clinical laboratories, are del13q, del11q, del17p13, and trisomy 12. Whole or partial gain of chromosome 2p is an attractive addition to this list because of the presence of the three

Table 3 Copy Number Determination *ACP1* and Neighboring Genes by qPCR

Cases	SH3YL1 2p25.3	ACP1 2p25.3	FAM150B 2p25.3	TPO 2p25.3	MYCN 2p24.1	ALK 2p23	REL 2p13-p12
1	4	6	3	5	4	4	2
2	2	3	1	3	2	2	2
3	5	6	4	5	2	2	2
4	2	3	2	3	2	2	2
5	2	4	2	2	2	2	2
6	2	3	2	3	2	2	2
7	2	3	2	2	2	2	2
8	2	4	2	3	2	2	2
9	2	3	2	2	2	2	2
10	2	3	2	2	5	2	2
11	2	4	3	3	4	3	2
12	2	3	2	3	3	2	2
13	2	4	2	3	3	2	2
14	2	2	2	2	2	2	2
15	2	3	2	2	2	2	2
16	2	4	2	2	2	2	2
17	2	3	3	2	2	2	2
18	2	4	2	3	2	2	2
19	2	3	2	3	2	2	2
20	2	3	3	2	5	2	2
21	2	4	3	3	3	2	2
22	2	3	3	2	3	2	2
23	2	2	2	2	2	2	2
24	2	2	2	2	3	2	2
25	2	3	2	3	6	2	2
26	2	3	2	3	2	2	2
27	2	3	2	3	2	2	2
28	2	3	2	2	3	2	2
29	3	4	4	3	6	3	2
30	2	3	2	3	2	2	2
31	2	2	2	2	5	2	2
32	2	2	2	2	3	2	2
33	2	3	2	2	4	2	2
34	2	3	2	3	3	2	2
35	2	3	2	3	2	2	2
36	4	5	3	4	4	3	2
37	2	2	2	2	2	2	2
38	2	2	2	2	2	2	2
39	2	3	2	2	2	2	3
40	2	2	2	2	4	2	3

Copy numbers of *SH3YL1*, *ACP1*, *FAM150B*, *MYCN*, *ALK*, and *REL* were assessed by qPCR using TaqMan Copy Number Assays according to manufacturer's instruction.
Abbreviation: qPCR = quantitative polymerase chain reaction.

Figure 3 (A) qPCR Analysis of *ACP1* and *MYCN*. qPCR Analyses Were Performed With gDNA Using TaqMan Copy Number Assays and the Copy Numbers were Determined by CopyCaller Software (Applied Biosystems). X Axis, Patient Samples; Y Axis: Copy Numbers. (B) qPCR-based Copy Number Nnalysis of *ACP1* in 2p Gain Positive/Negative CLL Samples and Healthy Individuals. TaqMan Copy Number Assays Were Performed on 40 Cases of CLL 2p Gain Positive and 30 Cases of 2p Gain Negative Samples Defined by aCGH. The *ACP1* Copy Numbers Were Also Tested in 73 Cases of Healthy Individuals. The Copy Numbers Were Determined by CopyCaller Software. The Percentage of 1 Copy, 2 Copies, 3 Copies or 4-6 Copies Samples Were Illustrated in the Figure



known oncogenes in this region, *MYCN*, *ALK,* and *REL*. Until now, the few studies that have reported recurrent 2p gains in CLL were performed using either BAC or single-nucleotide polymorphism (SNP) arrays, and focused on these oncogenes.[6,7,16] Here, we evaluated 2p gains using an oligonucleotide array that offers the ability to interrogate the copy number state of defined genomic regions or entire genomes at a higher resolution than BAC arrays. Validated by qPCR, aCGH in our laboratory achieves 100% accuracy when calling the negative cases and 77% when calling the positive. We report for the first time a recurrent gain of 2p25.3 harboring the *ACP1* gene at a frequency of approximately 25% in a large cohort of CLL patients. qPCR-based copy number analysis confirmed the aCGH findings and revealed the presence of more than two copies of the *ACP1* gene in 32 of 40 (80%) cases with aCGH detected 2p25.3 gain. The gain of *ACP1* was observed in only 2.7% healthy individuals, which confirmed the presence of CNVs of *ACP1*.[17] Though the possibility that *ACP1* CNV is associated with CLL cannot be ruled out, the fact that *ACP1* copy number gain in CLL cases was significantly higher, ie, 50% (35/70) compared to 2.7% (2/73) in healthy individuals indicates that the *ACP1* gene is amplified in CLL.

Gains of 2p in CLL have been reported in several studies. Pfeifer et al identified 2p gain in four cases using a SNP array and one case showed a whole-arm 2p gain as the sole aberration.[16] Unlike their observations, isolated 2p gain was not seen in our study. In our cohort, all cases with 2p gain were associated with other aberrations, including common chromosomal abnormalities in CLL such as del13q14, del11q22, del17p13.1, and trisomy 12. Chapiro et al observed a high frequency of del6q and chr 1 gain in 32% and 19% of

cases, respectively, in association with 2p gain in untreated CLL patients in Binet stages B or C.[6] In our cohort consisting of mixed treated and untreated patients, del6q (15 of 44; 34%) was also present at a high frequency in association with 2p gain. However, no increase in chromosome 1 aberrations was observed.

The correlation of 2p gain with *MYCN* transcript levels was recently confirmed in CLL.[6, 21] In the current study, using qPCR, we found a high frequency of *MYCN* co-amplification with *ACP1* in 14 of 40 (35%) cases. The concurrent amplification of *ACP1* and *MYCN* may help define the critical region of 2p gain and also suggests a possible link between *ACP1* and *MYCN* amplification. In malignant neoplasms, *MYCN* is abnormal almost exclusively in neuroblastoma. *MYCN* amplification is seen in up to 40% of neuroblastoma cases and correlates with poor prognosis. A few downstream targets of *MYCN* have been identified. Hatzi et al showed that MYCN overexpression resulted in down-regulation of leukemia inhibitory factor (*LIF*).[21] *LIF* was initially identified by its ability to induce macrophage differentiation of M1 murine cells and suppressed their proliferation in vitro.[22] However, the link between *LIF* and hematopoietic malignancies remains to be identified. *MYCN* is a homologue of *c-MYC*. A 1.3- to 2.9-fold increase in *MYCN* copy number was detected in B-cell chronic CLL patients compared with the healthy controls.[23] More interestingly, gain of *MYCN* was recently reported to be a recurrent lesion in Richter's syndrome[24] which represents the transformation of CLL to a more aggressive lymphoma, usually diffuse large B-cell lymphoma. It is possible that *MYCN* not only plays a pivotal role in CLL pathogenesis but also may serve as a potential prognostic marker in CLL patients.

In summary, using oligonucleotide aCGH platform and Nexus CGH analysis software, we were able to identify recurrent gains in the telomeric region of 2p in CLL, and particularly 2p25.3 involving the *ACP1* gene. Gains of 2p25.3 are associated with unmutated *IGHV* status, ZAP-70 expression, and a high frequency of concurrent *MYCN* gain. These results suggest that *ACP1* gain may be involved in CLL pathogenesis and/or prognosis, which warrant future clinical correlative and mechanistic investigations using homogeneous subsets of CLL patients.

Disclosures

William Wierda, MD, PhD, *Grant or research support*: GlaxoSmithKline. Michael Keating, MB, BS, *Paid consultant*: Genzyme, Roche, Celgene; *Speaker's Bureau*: Xcenda. *Authors contributed equally.

Supplementary Materials

Note: to access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at doi:10.1016/j.clml.2011.03.031.

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