Comparative genome profiling across subtypes of low-grade B-cell lymphoma identifies type-specific and common aberrations that target genes with a role in B-cell neoplasia

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ABSTRACT

Background

Low-grade B-cell lymphomas are a very heterogeneous group of tumors, whose differential diagnosis is frequently compromised by the lack of specific cytogenetic or molecular features. Our objective was to search for genomic features that allow a better molecular identification of the different types of lymphoma studied.

Design and Methods

We selected a panel of 87 low-grade B-cell lymphoma tumor samples that were unambiguously diagnosed (clinically and cytogenetically) as: follicular, splenic marginal zone, nodal marginal zone, lymphoplasmacytic, mantle cell, extranodal marginal zone MALT-type lymphoma or B-cell chronic lymphocytic leukemia. All samples were subjected to the same high-resolution genomic DNA analysis (array-based comparative genomic hybridization): a whole genome platform that contained 44000 probes distributed across the genome. Genomic imbalances were recorded, compiled and analyzed.

Results

Eighty percent of analyzed cases showed genomic imbalances (deletions and gain/amplifications) but the frequency of these imbalances ranged from 100% in mantle cell lymphomas to 33% in MALT lymphomas. A total of 95 new genomic imbalances affecting all lymphoma subtypes, were defined. We evaluated the extension of the genomic instability, detecting distinct patterns of genomic instability within subtypes. Specific pathways, such as nuclear factor kB (gains of *REL* and *BCL11A*, and losses of *COMMD3, BIRC1, IKK1* and *NFKB2*), Polycomb group proteins (gain of *BMI1* and deletion of *PCGF6*), DNA repair checkpoint pathways (deletion of 16q24 involving *CDT1*), or miRNA with a role in B-cell lymphoma pathogenesis (*MIRN15A*, *MIRN16-1*), were targeted by this genomic instability.

Conclusions

Although all subtypes of lymphomas showed gains and losses of DNA, the analysis of their genomic profiles indicated that there are specific aberrations in almost every subtype as well as frequent aberrations that are common to a large number of lymphoma types. These common aberrations target genes that are important in B-cell lymphomagenesis.

Key words: lymphoma, genomic profile, arrayCGH, genomic instability

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Introduction

Low-grade B-cell lymphomas account for approximately 50% of all lymphomas. They are distinguished by a relatively low proliferative index, small cell size, relatively large tumor masses and a paradoxical combination of advanced clinical stages associated with low clinical aggressiveness.[1] The World Health Organization (WHO) classification includes at least seven major subtypes of low-grade B-cell lymphomas: follicular, splenic marginal zone, nodal marginal zone, lymphoplasmacytic, mantle cell, extranodal marginal zone MALT type and B-cell chronic lymphocytic leukemia.[2,3] Each of these subtypes is considered a distinct disease, with specific molecular and morphological features, susceptible in many cases to lymphoma-type adjusted therapeutic protocols.[4] Although some of the most common conditions are relatively well defined, this is not the case for the most infrequent, such as lymphoplasmacytic and nodal marginal zone lymphomas. Equally, the differential diagnosis between these low-grade B-cell types of lymphoma is frequently a hard task, compromised by the lack of specific cytogenetic or molecular features. As a consequence of the difficulties in the differential diagnosis, therapies for many lymphoma types are still frequently based on common protocols with variable success. The knowledge so far accumulated on molecular diagnosis has not been translated into the development of new, targeted therapies. Within this context, the development of new high-throughput techniques provides an opportunity for genome-wide analysis at different levels. In this study we made use of array-based comparative genomic hybridization (array-CGH) to scan genomic imbalances across a panel of *gold-standard* cases of different subtypes of low-grade lymphoma. Although conventional CGH (based on chromosome analysis) had been extensively used for the search of chromosome changes in lymphomas,[5,6] only certain subtypes of low-grade lymphomas such as mantle cell,[7-11] nodal marginal zone[12-15] and chronic lymphocytic leukemia[16] have been studied with array-CGH technology. These analyses have been done with different genomic platforms at very variable levels of resolution, preventing an exhaustive comparative study. We systematically applied the same high resolution assay to the seven subtypes of lymphoma to avoid misinterpretation of the data, to define specific genomic imbalances (deletions and gain/amplifications), to compare their distribution among subtypes and to produce, for the first time, a genomic instability profile of these tumors.

Design and Methods

Tumor samples and clinical data

Genetic and clinical diagnostic data were obtained for a series of 87 indolent non-Hodgkin's lymphomas (NHL) provided by participating Institutions through the CNIO Tumour Bank. The series included 15 cases of follicular lymphoma, 15 of B-cell chronic lymphocytic leukemia, 14 of mantle cell lymphoma, 9 of nodal marginal zone lymphoma, 10 of lymphoplasmacytic lymphoma, 15 of splenic marginal zone lymphoma, and 9 cases of extranodal marginal MALT-type lymphoma. The histological diagnosis of each case was centrally reviewed, following the established WHO criteria.[2] All cases were CD20-positive and Ki67-negative. Clinical and genetic data are summarized in *Online Supplementary Table S1*. All cases presented the standard morphology, clinical picture, analytical features, and peripheral blood morphology and phenotype described for each entity.

Array-CGH assays, fluorescence in situ hybridization (FISH) validation and data analysis

All samples were hybridized against Human Genome CGH 44K microarrays (Agilent Technologies, Palo Alto, CA, USA), spanning the entire human genome at a median resolution of ~75Kb. Genomic DNA was obtained from peripheral blood of ten healthy female donors. DNA was extracted with DNeasy tissue kits (Qiagen, Germantown, MD, USA). The same amount of each donor DNA was mixed in a female DNA pool to be used as reference DNA in all the hybridizations. Hybridizations were done according to the manufacturer's protocols and all data analyzed as previously described.[17,18] Copy number karyotypes of each tumor are recorded in *Online Supplementary Table S1*.

Recurrent regions affected by genomic imbalances were defined as a sequence of at least five consecutive altered probes common to a set of array-CGH profiles and smallest overlapping regions of imbalance (SORI) as a recurrent region that contains no smaller recurrent region.[19] FISH assays were performed with commercial probes (Vysis Inc., Downers Grove, IL, USA) for the aberrations listed in *Online Supplementary Table S1*. The UCSC genome browser (*http://genome.ucsc.edu*) was used to select the bacterial artificial chromosome (BAC) clones covering the 7q31 region in splenic lymphoma: RP11-154N21, RP11-597L6, RP11-166D01, RP11-706J21, RP11-140I14, RP11-10I12 and RP11-138A9. The BAC were obtained from BACPAC Resource Centre (BPRC) at the Children's Hospital Oakland Research Institute (Oakland, CA, USA). A commercial centromeric probe for chromosome 7 (Vysis Inc., Downers Grove, IL, USA) was used as a control. FISH assays were carried out according to the manufacturer's instructions. FISH scoring of the 7q31 region fluorescence signal was carried out in each sample by counting the number of single copy gene and control probe signals in an average of 100 nuclei. A sample was considered deletion-positive when the ratio between the test region and control signal was near 0.5 in more than 50% of the tumor cells. To avoid overestimation of the number of genomic imbalances, due to such polymorphisms being detected by the array-CGH platform, we eliminated all DNA copy changes (genomic imbalances) observed in our samples that had at least 80% of their sequence overlapping with known polymorphisms included in the Database of Genomic Variants (version: 20th December 2006). GEPAS Suite software (*http://gepas.bioinfo.cipf.es/*) was used for unsupervised

Table 1. Description of the recurrent genomic aberrations (SORI) according to the lymphoma subtype.

Type of change	*Cytoband*	*First gene*	*START (Kbp)*	*Size (Mbp)*	*Freq. (%)*	SORI analysis (*)
Follicular Lymphoma (FL - 15 samples)						
Loss	1p36.33p36.22	CCNL2	371	9.6	13	Redefined from: 22; 23; 25; 26
Loss	1p36.12	ALPL	21589	0.1	13	Redefined from: 22; 23; 25; 26
Loss	1p36.11	PIGV	26807	0.4	13	Redefined from: 22; 23; 25; 26
Loss	2q31.1	HOXD1	176791	0.01	13	New data. Only involves HOXD1 gene
Loss	6q13	COL9A1	71006	1.1	20	Redefined from: 21; 22; 23; 24; 25; 26
Loss	6q16.2q16.3	POU3F2	99391	3.2	20	Redefined from: 21; 22; 23; 24; 25; 26
Loss	6q21q22.31	chr6:105278175-105278234	105278	14.6	20	Redefined from: 21; 22; 23; 24; 25; 26
Loss	6q23.3q24.1	chr6:138136891-138136950	138137	1.2	20	Redefined from: 22; 23; 24; 25; 26
Loss	10q23.1q25.1	ENST00000332738	83653	24.5	13	Redefined from: 25
Loss	17q21.33	PPP1R9B	45568	0.1	13	Redefined from: 21; 25
Loss	21q22.3	PKNOX1	43283	0.1	13	Redefined from: 21; 25
Gain	1q23.2	FCGR2A	158292	0.1	13	Redefined from: 22; 23; 25; 26
Gain	chr 2	AL137761	29	242.4	13	Redefined from: 21; 24; 25; 26
Gain	5ptelomerq12.3	BG325888	148	68.6	13	Redefined from: 21; 22; 23; 24
Gain	5q14.3qtelomer	TNPO1	72151	108.5	13	Redefined from: 21; 22; 24
Gain	7p	CENTA1	753	56.7	26	Redefined from: 21; 22; 24; 25; 26
Gain	8p	chr8:062197601-062197660	62198	84.1	20	Redefined from: 21; 23; 25; 26
Gain	12q12q21.1	PRICKLE1	41140	34.6	13	Redefined from: 21; 22; 23; 24; 25; 26
Gain	16p13.11	THC2207062	14956	0.9	13	Redefined from: 21; 23; 24; 26
Gain	chr 18	USP14	170	58.2	16	Redefined from: 21; 22; 23; 24; 25; 26
Chronic Lymphocytic Leukemia (CLL - 15 samples)						
Loss	11q22.1q23.2	BC033698	96058	18.8	46	Redefined from: 29
Loss	13q14.2	AY211923	48868	0.1	13	Redefined from: 29
Loss	13q14.2q14.3	DLEU2	49542	0.3	20	Redefined from: 29. Includes *MIRN15A/MIRN16-1*
Loss	22q11.22	IGLC2	21241	0.3	13	*Indicates Ig light chain gene rearrangement
Gain	2p16.1	FLJ31438	55314	0.1	20	Redefined from: 29. Includes REL
Gain	12p13.33p13.31	IQSEC3	50	6.4	13	New data
Mantle Cell Lymphoma (MCL - 14 samples)						
Loss	1p22.1p21.1	LOC388650	93012	10.3	28	Redefined from: 7; 8; 9
Loss	1p13.3	ENST00000294656	108858	2.6	28	Redefined from: 7; 9
Loss	1q21.3	AK127884	151803	0.1	28	Redefined from: 9
Loss	1q22q23.1	BC054045	153212	2.8	28	Redefined from: 9
Loss	5q13.2	SLC30A5	68435	2.5	14	New data. Also in LPL. Includes *BIRC1* gene
Loss	6q13.2	chr6:091152410-091152469	91152	5.8	36	Redefined from: 9
Loss	6q16.3q24.3	chr6:097479298-097479344	97479	53.7	36	Redefined from: 7; 8; 9
Loss	6q25.2q27	C6orf97	151931	18.9	36	Redefined from: 7; 9
Loss	7q31.1q34	chr7:108034769-108034828	108035	31.1	21	New data. Also found in SMZL
Loss	8ptelomp12	ZNF596	182	35.8	14	Redefined from: 7; 8; 9
Loss	centromer	FLJ32731	43156	3.9	14	Redefined from: 7; 8; 9
Loss	8q11.21q12.1	chr8:047655222-047655281	47655	10.4	14	Redefined from: 9
Loss	8q12.1q13.1	chr8:060942453-060942512	60942	6.9	21	Redefined from: 9
Loss	9p23	chr9:010676662-010676721	10677	2.1	36	Redefined from: 9
Loss	9p21.3	chr9:021733410-021733469	21733	2.1	36	Redefined from: 7; 8; 9
Loss	9p21.3p21.2	chr9:025357325-025357384	25357	2.5	36	Redefined from: 7; 8; 9
Loss	9q33.3	C9orf74	128222	0.1	14	Redefined from: 9
Loss	10p15.3p15.2	chr10:000138206-000138265	138	3.5	29	Redefined from: 7; 9
Loss	10p15.1	CR749391	4657	1.3	29	Redefined from: 7; 9
Loss	10p14p13	ITIH5	7643	7.5	29	Redefined from: 7; 8; 9
Loss	10q24.33	INA	105040	0.1	21	New data. Overlaps with FL. Includes *PCGF6, PDCD11* and *TAF5*
Loss	11q13.3	FGF4	69297	0.04	14	New data
Loss	11q23.1q23.2	MGC14839	111299	2.7	29	Redefined from: 7; 8; 9; includes *ATM* gene
Loss	12q13.12	WNT1	47660	0.1	14	New data
Loss	12q24.13q24.32	MAPKAPK5	110743	16.8	14	Redefined from: 9
Loss	13q13.1q14.11	APRIN	32074	12.9	29	Redefined from: 7; 8; 9
Loss	13q14.2q21.2	chr13:047066073-047066132	47066	13.8	29	Redefined from: 7; 8; 9
Loss	13q32.1q34	DCT	93889	20.2	29	Redefined from: 7; 8; 9
Loss	14q24.2q32.12	ZFP36L1	68324	24.0	14	New data
Loss	15q11.2q22.2	chr15:019109124-019109183	19109	44.0	14	Redefined from: 7; 9
Loss	17ptelomerp11.2	chr17:000048539-000048598	49	18.8	36	Redefined from: 7; 8; 9
Loss	17q11.2	DKFZp667M2411	25965	1.4	21	New data. Includes *MIRN365-2/MIRN193A*
Loss	17q32.2	LOC339210	52248	1.7	14	New data
Loss	18p11.31p11.23	LAMA1	6932	0.5	14	Redefined from: 9
Loss	19q13.32	MGC15476	51843	0.9	14	New data: includes *PUMA*
Loss	22q11.22	SUHW2	21175	0.1	14	Indicates Ig light chain gene rearrangement
Gain	3q26.1q29	chr3:163571061-163571120	163571	35.7	36	Redefined from: 7; 8; 9
Gain	6p25.3p24.3	OFCC1	9839	9.3	14	Redefined from: 7; 9
Gain	7p22.3p15.1	chr7:000149268-000149327	149	23.1	14	Redefined from: 7; 8; 9
Gain	8q24.21q24.3	chr8:126466498-126466557	126466	19.8	14	Redefined from: 7; 8; 9 *(continued →)*

Table 1 (continued). Description of the recurrent genomic aberrations (SORI) according to the lymphoma subtype.

Gain	10p12.2	COMMD3	22646	1.1	21	Redefined from: 7; 8; 9; includes *BMI1*
Gain	11q21.3	chr11:069333898-069333957	69334	0.2	21	New data. Includes *FGF3* gene
Gain	12p12.1	ABCC9	21860	2.8	14	Redefined from: 9
Gain	12q13.3q14.1	LOC144501	50855	9.5	14	Redefined from: 8; 9
Gain	14q32.33	MTA1	104961	0.1	21	Redefined from: 9; indicates Ig Heavy chain gene rearrangement
Gain	15q22.31q25.3	NOPE	63472	24.1	36	Redefined from: 8; 9
Gain	18q21.31q22.1	chr18:053859886-053859945	53860	1.0	21	Redefined from: 8; 9
Gain	18q22.3q23	CR749350	70139	5.9	21	Redefined from: 9
Lymphoplasmacytic Lymphoma (LPL - 10 samples)						
Loss	5q13.2	SLC30A5	68435	2.3	30	New data. Also found in MCL. Includes *BIRC1* gene
Loss	6q13	DDX43	74175	0.1	44	New data
Loss	6q23.3q24.1	chr6:136184224-136184283	136184	6.6	30	New data
Loss	6q25.1	KATNA1	149988	0.2	30	New data
Loss	15q15.1q21.1	AK098781	38611	5.2	30	New data. Also found in MCL
Loss	chr 19	PPAP2C	232	63.6	44	New data
Splenic Marginal Zone Lymphoma (SMZL - 15 samples)						
Loss	6p25.3	chr6:000204528-000204587	205	0.1	13	New data
Loss	7q31.32q34	FAM3C	120592	22.5	40	Redefined from: 36; 38
Loss	13q14.2q14.3	DLEU2	49542	0.3	13	New data. Also found in CLL. Includes *MIRN15A/MIRN16-1*
Loss	16p13.3	NAGPA	5020	0.2	13	New data
Loss	16p12.2p12.1	IMAA	21383	0.4	13	New data
Loss	22q11.22	SUHW2	21175	0.1	13	Indicates Ig light chain gene rearrangement
Gain	3q26.33q29	ZNF639	180535	18.8	20	Redefined from: 37
Gain	12q13.11q15	SFRS2IP	44608	25.4	13	Redefined from: 37
Nodal Marginal Zone Lymphoma (NMZL - 9 samples)						
Loss	1p36.11	CNR2	23979	4.2	22	Redefined from: 11
Loss	19q13.2	DEDD2	47395	0.05	22	New data
Gain	3q11.2q29	chr3:082994708-082994767	82995	116.3	22	Redefined from: 41
Gain	6p	chr6:000204528-000204587	205	62.9	22	New data
Gain	6q14.1	ELOVL4	80693	1.8	22	New data
Gain	12q14.1	IQSEC3	50	132.3	22	New data
Gain	18q12.3qtrlom	CD691586	34427	41.7	22	New data

**SORI analysis: Comparative study of the literature regarding the description of the identified aberrant region.*

clustering, supervised analysis and gene annotation. Additional unsupervised clustering was performed with CLUSTER.[20]

Results

Genomic imbalances in the different subtypes of lymphoma

We analyzed 87 samples of unambiguously classified into the more frequent subtypes of low-grade B cell lymphoma. All samples were assayed by high resolution array-CGH in the same way and those regions affected by genomic imbalances were annotated for each case (*Online Supplementary Table S1*). The complete data set has been deposited in the NCBI Gene Expression Omnibus (*GEO, http://www.ncbi.nlm.nih.gov/projects/geo/*) and is accessible through GEO series accession number GSE8918. Overall, 80% of the lymphomas showed an altered genome with this frequency ranging from 100% in mantle cell lymphoma to 33% in MALT lymphomas. *Online Supplementary Figure S1* represents the frequency and localization of the imbalances found in each subtype of lymphoma. All genomic imbalances were compiled to define the SORI, both in the complete set of samples and separately according to subtype. The complete list of the 95 SORI (corresponding to 65 deleted and 30 gained regions) found is provided in Table 1, together with their size, frequency and other features. Novel findings were observed in all subtypes of lymphoma.

We found that 87% of the low-grade follicular lymphomas showed genomic imbalances (represented by 21 SORI; 12 deletions and 9 gains). The most frequent gains were those of the short arms of chromosomes 7 and 8 (Table 1). 6q deletions (the most frequent imbalance) appeared as four independent SORI scattered through 6q13 to 6q24.1. A new aberrant small deletion on chromosome 2q31.1, which only affected the *HOXD1* gene, was observed.

Eighty percent of B-cell chronic lymphocytic leukemias showed genomic imbalances that were used to identify six SORI (4 deletions and 2 gains). We were able to define the extent of some previously known imbalances more precisely. For example, deletion of 11q was narrowed down to a region of 18.8 Mb (which included the *ATM* gene). 13q deletions were observed as two recurrent deletions of 100 Kb and 300 Kb (Table 1), including the miRNA genes *MIRN15A/MIRN16-1*. Finally, we identify a discrete gain on 2p16 (100 Kb) that included the *REL* and *BCL11A* genes.

Mantle cell lymphoma was the most complex entity,

with 48 SORI (12 gains and 36 deletions) being detected. As in follicular lymphoma, the deletion of 6q appeared as three independent SORI. We identified a deletion of 7q31.34 in three out of 14 samples – a region similar to the one that appears in splenic marginal zone lymphomas. This deletion was confirmed by FISH. As new findings we noted the small deletions on 10q24.3 (involving *PCGF6*, *PDCD11* and *TAF5* genes, which overlaps with the deletion observed in 13% of the follicular lymphoma cases that included the *IKK1* and *NFKB2* genes), 12q13.12 (*WNT1*), 19q13.32 (*PUMA*), 17q11.2 (*MIRN365-2/MIRN193A*) and 5q13 (also seen in lymphoplasmacytic lymphomas).

Lymphoplasmacytic lymphomas have never been studied by array-CGH. We observed aberrations in nine out of ten cases and defined six SORI, all of them corresponding to deletions. The most frequent event (44%) was monosomy of chromosome 19 and 6q deletions. A deletion on 5q13, similar to the one identified in mantle cell lymphoma, was observed. Splenic marginal zone lymphomas revealed genomic imbalances in 11 out of 15 samples. The specific 7q deletion was precisely identified from 7q31.32 to 7q34, spanning 22.5 Mb (Table 1) and was recurrently seen in six samples (40%). New regions of imbalance are deletions on 13q14.2 (overlapping with those seen in chronic lymphocytic leukemia and mantle cell lymphoma) and on 16p12. The gain of chromosome 3 was narrowed down to 3q26.33q29. Finally, seven out of nine nodal marginal zone lymphomas showed genomic imbalances. Trisomy of chromosome 3 was recurrently seen and it could be re-defined as a region located at 3q11q29. Newly defined genomic imbalances in nodal marginal zone lymphomas were gain of 6p and deletions on 1p36 and 19q13.2. Three out of nine MALT type lymphomas showed genomic imbalances. However, no recurrent SORI were found. Therefore, the MALT subgroup of samples was not considered for subsequent analyses.

Genomic analysis of the whole series

The analysis of the complete series revealed that some SORI were present in several different subtypes of lymphoma. Overall, we found that 35 genomic regions were altered in more than four different subtypes of low-grade lymphoma (Table 2). The deletion in 6q that appears in almost all subtypes was delineated by ten independent SORI extending from 6q12 to 6q25. The gained region on the short arm of chromosome 2 was delineated by three SORI and included the *REL* gene. Gain of 3q26q29 and 12q13q14 affected five and six out of the six investigated subtypes of lymphoma, respectively. We also found deletions in 11q23, 13q14, 17p13 and 19q13.12 and gains of 8q24, 18q21.23 that were present in most subtypes. Taking advantage of the predefined SORI, we conducted a supervised clustering analysis to define the most representative genomic markers from each subtype (Figure 1). The 50 most significant SORI were then tested in an unsupervised clustering analysis (Figure 2). The different subtypes of low-grade lymphoma were segregated with different efficiencies, probably due to the sample size. While cases of mantle cell lymphoma and chronic lymphocytic leukemia clustered approximately all together, cases of follicular lymphoma and splenic marginal zone lymphoma segregated into at least two branches, possibly as a reflection of two distinct patterns of genomic instability as we describe below. The lymphoplasmacytic and nodal marginal zone lymphomas failed to cluster properly.

Apart from the global profiles based on regions of genomic imbalances that were provided by array-CGH for each tumor, we conducted a genomic instability quantitative study of all subtypes of low-grade lymphoma assessed by different parameters, which gives a unique view of the genomic instability that a tumor has undergone at diagnosis.[21] This novel approach enables greater understanding of the behavior of each lymphoma subtype. We took into account the number of whole chromosome aberrations, number of DNA breaks (referred to as copy number transitions) within a chromosome, high-level amplifications or homozygous deletions, and the number of chromosomes containing such transitions[21] (*Online Supplementary Table S2*). We could infer different patterns of genomic instability among the subtypes of lymphoma. Mantle cell lymphoma and chronic lymphocytic leukemia showed a homogeneous pattern of genomic instability characterized mainly by the occurrence of copy number transitions and structural aberrations (Figure 3). Mantle cell lymphoma was the most genomically unstable subtype of lymphoma with a median of 14 copy number transitions per case (range, 2 to 38) and four high level amplifications. Chronic lymphocytic leukemia presented the same type of homogenous instability profile as mantle cell lymphoma, although the median value of copy number transitions was lower (median 4; range, 0 to 17). In contrast, splenic marginal zone, follicular and lymphoplasmacytic lymphomas were heterogeneous in terms of genomic instability. Each subtype seemed to contain at least two distinct patterns. While some samples behaved similarly to those of mantle cell lymphoma and chronic lymphocytic leukemia, others either failed to show any type of aberration or only had numerical changes.

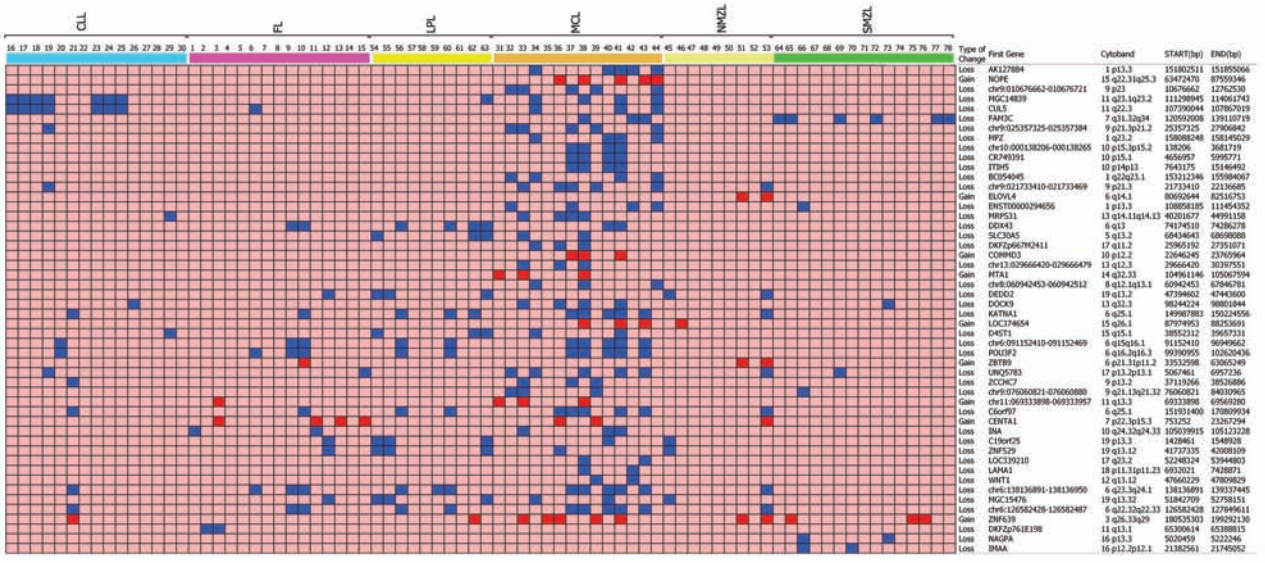
Discussion

We present here a detailed comparative analysis of the DNA genomic imbalances found in a representative set of the most relevant subtypes of low-grade B-cell lymphoma. Although several works have been published describing genomic imbalances detected by array-CGH in B-cell lymphomas, each one of them was focused on a single subtype such as mantle cell lymphoma,[7-11] nodal marginal zone lymphoma[12-15] or chronic lymphocytic leukemia.[16] In addition, almost every one of the studies were done using different genomic platforms with different types of clones (BAC, oligos, etc), densities, resolution and data analysis approaches. This tremendous variability has hampered the comparison of the incidence and nature of genomic imbalances among subtypes of low-grade lymphomas. Our

Table 2. Distribution of recurrent genomic aberrations that are found in more than four subtypes of low-grade B-cell lymphoma.

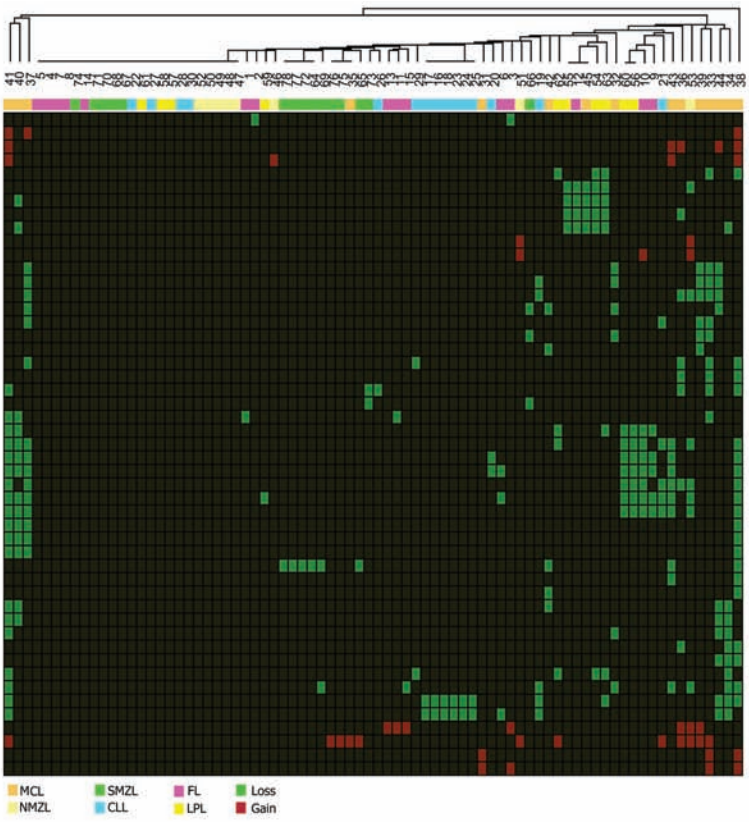
Type of change	*Cytoband*	*First gene*	*START (bp)*	*Size (Mbp)*	*% FL*	*% CLL*	*% MCL*	*% LPL*	*% SMZL*	*% NMZL*
loss	1p36.13	*MGC12760*	16693433	0.8	7	0	7	0	7	11
loss	1p36.11	*PIGV*	26807319	0.4	13	0	0	10	7	22
gain	2p24.2p24.1	*FAM49A*	16655528	3.9	13	13	0	10	0	11
gain	2p23.1	*chr2:030171297-030171356*	30171297	1.4	13	13	0	10	0	11
gain	2p16.1p15	*BCL11A* (includes *REL*)	60515739	3.1	13	13	0	10	0	11
gain	3p22.1p21.31	*BU431659*	42048704	4.9	0	7	7	0	13	11
gain	3q26.33q29	*ZNF639*	180535303	18.8	0	7	36	10	20	22
gain	6p24.3p22.3	*OFCC1*	9839390	9.3	7	0	14	10	0	22
loss	6q12	*chr6:067633130-067633189*	67633130	1.7	13	7	14	20	0	0
loss	6q13	*COL9A1*	71005657	0.6	20	7	14	20	0	0
loss	6q14.1	*THC2097824*	82699543	1.0	13	7	21	20	0	11
loss	6q15q16.1	*chr6:091152410-091152469*	91152410	5.8	13	7	36	20	0	0
loss	6q16.2q16.3	*POU3F2*	99390955	3.2	20	7	36	20	0	0
loss	6q21	*chr6:105759408-105759467*	105759408	3.4	20	7	36	20	0	11
loss	6q22.32q22.33	*chr6:126582428-126582487*	126582428	1.3	13	7	36	20	0	11
loss	6q23.3q24.1	*chr6:138136891-138136950*	138136891	1.2	20	7	36	30	0	11
loss	6q25.1	*KATNA1*	149987883	0.2	7	7	36	30	0	11
loss	6q25.1	*C6orf97*	151931400	18.9	7	7	36	20	0	11
gain	8q24.21q24.3	*chr8:127458201-127458260*	127458201	18.8	20	0	14	0	7	11
loss	11q23.1q23.2	*MGC14839*	111298945	2.8	0	47	29	10	0	0
gain	12p13.33p13.31	*IQSEC3*	49967	6.4	7	13	7	10	7	22
gain	12p12.1	*ABCC9*	21859982	2.8	7	7	14	10	7	22
gain	12q13.13q14.1	*LOC144501*	50854508	9.5	13	7	14	10	13	22
loss	13q14.2	*SETDB2*	48923674	0.0	0	13	29	0	7	11
loss	13q14.2q14.3	*DLEU2* (includes *MIRN365-2/MIRN193A*)	49542355	0.3	0	20	29	0	13	11
loss	16q24.2q24.3	*ZNF469*	87033189	0.7	7	7	7	0	7	0
loss	17p13.2p13.1	*UNQ5783*	5067461	1.9	7	7	36	0	7	11
gain	18q21.31q21.33	*chr18:053859886-053859945*	53859886	4.5	27	0	21	10	7	22
loss	19p13.3	*C19orf25*	1428461	0.1	7	0	7	30	0	11
loss	19q13.12	*ZNF529*	41737335	0.3	7	0	7	30	0	11
gain	19q13.31q13.32	*ZNF45* (includes *BCL3*)	49079469	2.1	0	0	7	10	7	11
loss	19q13.32	*MGC15476* (includes *PUMA*)	51842709	0.9	7	0	14	30	0	11
gain	19q13.33q13.43	*FLJ32658*	54593738	9.2	7	0	0	10	7	11
loss	22q11.22	*SUHW2*	21174548	0.1	7	20	14	0	13	0
gain	22q13.1q13.33	*C1QTNF6*	35907828	13.6	7	0	7	0	7	11

FL, follicular lymphoma; CLL, chronic lymphocytic leukemia; MCL mantle cell lymphoma; LPL, lymphoplasmacytic lymphoma; SMZL, splenic marginal zone lymphoma; NMZL, nodal marginal zone lymphoma.



FL, follicular lymphoma; CLL, chronic lymphocytic leukemia; MCL mantle cell lymphoma; LPL, lymphoplasmacytic lymphoma; SMZL, splenic marginal zone lymphoma; NMZL, nodal marginal zone lymphoma.

Figure 1. Supervised clustering of the genomic imbalances detected by array-CGH. This analysis was conducted considering the six predefined subtypes of low-grade lymphoma.



Type of Change	First Gene	Cytoband	START (bp)	END (bp)	Size (bp)	N° Probes
Loss	DKFZp761E198	11 q13.1	65300614	65388815	0.0882	6
Gain	COMMD3	10 p12.2	22646245	23765964	1.11972	24
Gain	NOPE	15 q22.31q25.3	63472470	87559346	24.0869	446
Gain	LOC374654	15 q26.1	87974953	88253691	0.27874	10
Loss	SLC30A5	5 q13.2	68434643	68698088	0.26345	14
Loss	DEDD2	19 q13.2	47394602	47443600	0.049	5
Loss	C19orf25	19 p13.3	1428461	1548928	0.12047	5
Loss	ZNF529	19 q13.12	41737335	42008109	0.27077	10
Loss	MGC15476	19 q13.32	51842709	52758151	0.91544	46
Gain	ELOVL4	6 q14.1	80692644	82516753	1.82411	11
Gain	ZBTB9	6 p21.31p11.2	33532598	63065249	29.5327	457
Loss	chr9:010676662-010676721	9 p23	10676662	12762530	2.08587	16
Loss	chr9:025357325-025357384	9 p21.3p21.2	25357325	27906842	2.54952	26
Loss	chr9:021733410-021733469	9 p21.3	21733410	22136685	0.40328	13
Loss	ENST00000294656	1 p13.3	108858185	111454352	2.59617	80
Loss	ZCCHC7	9 p13.2	37119266	38526886	1.40762	26
Loss	chr9:076060821-076060880	9 q21.13q21.32	76060821	84030965	7.97014	90
Loss	WNT1	12 q13.12	47660229	47809829	0.1496	10
Loss	MRPS31	13 q14.11q14.13	40201677	44991158	4.78948	75
Loss	chr13:029666420-029666479	13 q12.3	29666420	30397551	0.73113	14
Loss	DOCK9	13 q32.3	98244224	98801844	0.55762	13
Loss	NAGPA	16 p13.3	5020459	5222246	0.20179	5
Loss	INA	10 q24.32q24.33	105039915	105123228	0.08331	5
Loss	DDX43	6 q13	74174510	74286278	0.11177	6
Loss	KATNA1	6 q25.1	149987883	150224556	0.23667	8
Loss	chr6:091152410-091152469	6 q15q16.1	91152410	96949662	5.79725	39
Loss	POU3F2	6 q16.2q16.3	99390955	102620436	3.22948	46
Loss	C6orf97	6 q25.1	151931400	170809934	18.8785	244
Loss	chr6:138136891-138136950	6 q23.3q24.1	138136891	139337445	1.20055	20
Loss	chr6:126582428-126582487	6 q22.32q22.33	126582428	127849611	1.26718	15
Loss	chr10:000138206-000138265	10 p15.3p15.2	138206	3681719	3.54351	37
Loss	CR749391	10 p15.1	4656957	5995771	1.33881	26
Loss	ITIH5	10 p14p13	7643175	15146492	7.50332	90
Loss	FAM3C	7 q31.32q34	120592008	139110719	18.5187	262
Loss	LOC339210	17 q23.2	52248324	53944803	1.69648	44
Loss	LAMA1	18 p11.31p11.23	6932021	7428871	0.49685	5
Loss	AK127884	1 p13.3	151802511	151855066	0.05256	2_5
Loss	MPZ	1 q23.2	158088248	158145029	0.05678	5
Loss	BC054045	1 q22q23.1	153212346	155984067	2.77172	76
Loss	DKFZp667M2411	17 q11.2	25965192	27351071	1.38588	38
Loss	chr8:060942453-060942512	8 q12.1q13.1	60942453	67846781	6.90433	77
Loss	D4ST1	15 q15.1	38552312	39657331	1.10502	31
Loss	UNQ5783	17 p13.2p13.1	5067461	6957236	1.88978	58
Loss	MGC14839	11 q23.1q23.2	111298945	114061743	2.7628	63
Loss	CUL5	11 q22.3	107390044	107867019	0.47698	18
Gain	CENTA1	7 p22.3p15.3	753252	23267294	22.514	256
Gain	ZNF639	3 q26.33q29	180535303	199292130	18.7568	308
Gain	MTA1	14 q32.33	104961146	105067594	0.10645	11
Gain	chr11:069333898-069333957	11 q13.3	69333898	69569280	0.23538	6

FL, follicular lymphoma; CLL, chronic lymphocytic leukemia; MCL mantle cell lymphoma; LPL, lymphoplasmacytic lymphoma; SMZL, splenic marginal zone lymphoma; NMZL, nodal marginal zone lymphoma.

Figure 2. Hierarchical unsupervised clustering of the genomic imbalances detected by array-CGH. This analysis was done using SORI as variables. SORI are defined in detailed in the table at the right of the dendogram. A color (shown at the bottom of the figure) was attributed to each subtype for better visualization.

approach was intended to minimize these sources of variability and to facilitate comparisons. To this aim, all samples were assayed with the same high resolution genomic platform, including about 44,000 probes covering the whole genome at an average resolution of 75Kb, with a redundant coverage of known cancer genes. This platform has been used for other types of tumors[17,18] but never for the analysis of low-grade lymphomas. The second major point was the careful choice of samples, which were *gold-standard* representative cases of each subtype of lymphoma. For this purpose, all pathological diagnoses were performed in a single institution. All samples were carefully chosen to fulfil the standard clinical and pathological criteria of each of the subtypes.

Excluding MALT lymphomas, which in our series were not found to show recurrent changes, 85% of the samples displayed some degree of genomic instability. This percentage ranged between 100% for mantle cell lymphoma and 73% for the splenic marginal zone lymphomas. Novel findings were observed in all studied groups and are summarized in Table 1. Recurrent SORI were compared only with recurrent aberrations that have been reported based on array-CGH, whenever possible, or on conventional CGH approaches. With regards to follicular lymphomas,[22-27] we confirmed some data and we redefined the gain on chromosome 7, which was reduced to 7p, and the nature of the 6q deletions,[4] which appeared as four independent SORI scattered through 6q13 to 6q24.1. Additionally, a deletion of the *HOXD1* gene, likely affecting of the NOTCH pathway via *RBPJk*,[28] was observed. As reported previously,[5,16,29,30] 80% of chronic lymphocytic leukemia samples show genomic imbalances. Comparing our data with those of a recent study that used 10 K and 50 K single nucleotide polymorphism arrays to scan for genomic aberrations in peripheral blood or bone marrow from 70 patients with chronic lymphocytic leukemia,[30] we better defined two small recurrent deletions on 13q to 100 Kb and 300 Kb. The latter deletion included the miRNA genes *MIRN15A/MIRN16-1*, which have been found to be deleted or down-regulated in the majority of patients with chronic lympocytic leukemia.[31] Of interest, we redefined the gain of the 2p16 region as a frequent finding in chronic lympocytic leukemia. This common region includes the *REL* and *BCL11A* genes. All mantle cell lymphoma samples showed a highly aberrant genome. We were able to confirm the majority of published data,[8-10] but also discovered some new altered regions. For the first time, we found, in 21% of the mantle cell lymphoma samples, the same 7q deletion

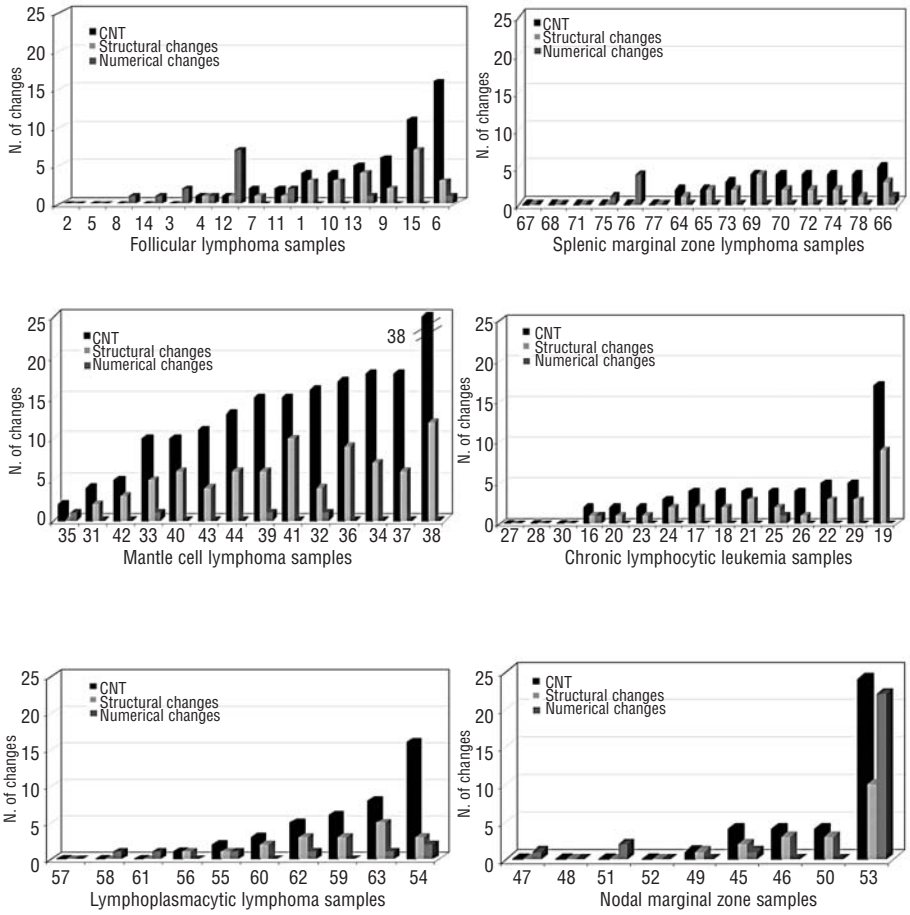


Figure 3. Genomic instability profile of the six subtypes of low-grade lymphoma. Each tumor was analyzed by the same 44K array-CGH. All DNA changes were categorized in eight different parameters (*Online Supplementary Table S2*) but only three of them are shown in this figure: number of chromosome transitions (CNT) (black bars), number of chromosomes with structural changes (light gray bars) and the number of aneuploid chromosomes (dark gray bars). Different patterns of genomic instability may be observed: while all mantle cell lymphomas showed a regular profile of structural and numerical aberrations, splenic marginal zone lymphomas seemed to configure two classes (no changes or a few recurrent changes).

that has been seen in splenic marginal zone lymphoma (*discussed below*). A new set of deletions, involving several genes of interest, was also identified. The deletion on 10q24 affects *PCGF6* and *PDCD11*. *PCGF6* codes for a protein that contains a RING finger motif (closely related to those of the polycomb group (PcG) proteins, RNF110/MEL-18 and BMI1) which has been shown to interact with some polycomb group proteins and act as a transcription repressor. *PDCD11*, which shares 82% identity with *NFBP*, codes for a protein that induces activation of NFκB and the FasL promoter, leading to apoptosis in Jurkat cells.[32,33] On the other hand, the *COMMD3* genomic sequence is disrupted by the aberration found on 10p12 that also resulted in the gain of BMI1. *COMMD3* in a suppressor of NFκB activation[34] and *BMI1* is a well known oncogene from the polycomb group that represses the *INK4A* locus.[35] A deletion on 17q11.2 included the loss of the miRNA *MIRN365-2/MIRN193A* genes, which have been described to be altered in human cervical cancer.[36]

Analysis of lymphoplasmacytic lymphoma samples helped us to re-define the reported deletion of 6q[4] as two SORI within 6q22.3-q25.1. A deletion on 5q13, similar to the one detected in mantle cell lymphomas, was recurrently observed. This deletion involved *BIRC1*, a gene that contains BIR domains that have been shown to be essential to prevent apoptosis in PC12 cells after stimulation of the tumor necrosis-α receptor (a key component of the NFκB pathway).[37]

Splenic marginal zone lymphomas have never been analyzed by a genomic approach. Comparing our results with those obtained using chromosome-based CGH,[38-40] we defined several deletions in chromosomes 6p, 13q 16p, 16q, and 22q. The well-known deletion on 7q was precisely identified from 7q31.32 to 7q34, spanning 22.5 Mb, confirming recent data obtained from other molecular cytogenetic analyses.[41] We found that this deletion occurs almost exclusively in 45% of splenic marginal zone lymphomas with a likely effect on the expression of microRNA genes that cluster within this region.[42] Surprisingly, we detected the same lesion in three out of 14 cases of mantle cell lymphoma that were clearly diagnosed as such (CD20$^+$, CCND1$^+$ and FISH$^+$ for the *CCND1* rearrangement).

With regards to nodal marginal zone lymphomas, data from a chromosome-based study[43] and from a recent paper that included another seven cases[12] showed that genetic aberrations resulting in the gain of several regions of chromosome 3 constitute a common marker for this subtype of lymphoma. We found this change in two of nine samples in our series and, more importantly, we have narrowed down the gain to a smaller region located at 3q11q29. Newly defined genomic imbalances in nodal marginal zone lymphomas were the gain of 6p and deletions on 1p36 and 19q13.2, also partially described in other marginal zone

lymphomas.[12]

There were several interesting findings from the genomic analysis conducted on all the samples pooled together (Table 2). Apart from the complete description of the deletions on 6q, several markers may be considered as the general profile of low-grade lymphoma: gains of 2p16, 3q26q29, 12q13 8q24 and 18q21 as well as losses of 11q23, 13q14, 17p13 and 19q13.12 have emerged as significant genomic regions of interest. All of them harbor genes that play a role in B-cell proliferation. As an example, the gained region of the short arm of chromosome 2 is delineated by three SORI and includes the *REL* gene.[44] Amplification of this gene has been previously described in diffuse large B-cell lymphoma[45] and we also demonstrated its overexpression in splenic marginal zone lymphoma.[42] Generalizing, our results identify a set of commonly deleted or gained regions, independently of the diagnosis, which point to specific pathways, such as NF-κB (gains that involved *REL* and *BCL11A*, and losses that involved *COMMD3*, *BIRC1*, *IKK1* and *NFKB2*), polycomb group proteins (gain of *BMI1* and deletion of *PCGF6*), DNA repair checkpoint pathways (deletion of 16q24 involving *CDT1*), or miRNA with a role in B-cell lymphoma pathogenesis (*MIRN15A*, *MIRN16-1*).

Reports of genomic instability (Figure 3) have been published for bladder, breast, and neuroblastoma tumors.[21,46,47] An emerging feature of this type of analysis is that genomic variables such as the number of transitions and the fraction of the genome altered in a single tumor may correlate with clinical evolution. Further follow-up and recruitment of more samples for our series will facilitate, in due time, a similar analysis for low-grade lymphomas. It is notable that the differences in genomic instability reports from each subtype of lymphoma reflect the same heterogeneity that is observed in the clustering analysis (Figure 1). Additionally, the majority of the genomic imbalances found in lymphoplasmacytic and nodal marginal zone lymphomas were also observed in other subtypes (mostly mantle cell and follicular lymphomas) and practically no specific aberrations were identified. These findings raise doubts about the diagnostic criteria for lymphoplasmacytic and nodal marginal zone lymphomas, and suggest that these tumors may not correspond to lymphoproliferative diseases with a distinctive molecular pathogenesis.

Authorship and Disclosures

BIF and JS performed the experiments and analyzed the data. JFG, MM, FC, and SM provided, diagnosed and selected the cases. AC helped with the management of the bioinformatics of data. MAP and JCC designed the experiments. BIF, MAP and JCC analyzed the data and wrote the paper. The authors reported no potential conflicts of interest.

References

1. Sanchez-Beato M, Sanchez-Aguilera A, Piris MA. Cell cycle deregulation in B-cell lymphomas. Blood 2003; 101: 1220-35.
2. Jaffe ES, Harris NL, Diebold J, Muller-Hermelink HK. World Health Organization classification of neoplastic diseases of the hematopoietic and lymphoid tissues. A progress report. Am J Clin Pathol 1999;111[Suppl 1]:S8-12.
3. Dogan A. Modern histological classification of low grade B-cell lymphomas. Best Pract Res Clin Haematol 2005;18:11-26.
4. Gascoyne RD. Hematopathology approaches to diagnosis and prognosis of indolent B-cell lymphomas. Hematology Am Soc Hematol Educ Program 2005:299-306.
5. Lichter P, Joos S, Bentz M, Lampel S. Comparative genomic hybridization: uses and limitations. Semin Hematol 2000;37:348-57.
6. Monni O, Joensuu H, Franssila K, Knuutila S. DNA copy number changes in diffuse large B-cell lymphoma-comparative genomic hybridization study. Blood 1996; 87: 5269-78.
7. de Leeuw RJ, Davies JJ, Rosenwald A, Bebb G, Gascoyne RD, Dyer MJ, et al. Comprehensive whole genome array CGH profiling of mantle cell lymphoma model genomes. Hum Mol Genet 2004;13: 1827-37.
8. Tagawa H, Karnan S, Suzuki R, Matsuo K, Zhang X, Ota A, et al. Genome-wide array-based CGH for mantle cell lymphoma: identification of homozygous deletions of the proapoptotic gene BIM. Oncogene 2005; 24:1348-58.
9. Rubio-Moscardo F, Climent J, Siebert R, Piris MA, Martin-Subero JI, Nielander I, et al. Mantle-cell lymphoma genotypes identified with CGH to BAC microarrays define a leukemic subgroup of disease and predict patient outcome. Blood 2005; 105:4445-54.
10. Kohlhammer H, Schwaenen C, Wessendorf S, Holzmann K, Kestler HA, Kienle D, et al. Genomic DNA-chip hybridization in t(11;14)-positive mantle cell lymphomas shows a high frequency of aberrations and allows a refined characterization of consensus regions. Blood 2004; 104: 795-801.
11. Jarosova M, Papajik T, Holzerova M, Dusek L, Pikalova Z, Lakoma I, et al. High incidence of unbalanced chromosomal changes in mantle cell lymphoma detected by comparative genomic hybridization. Leuk Lymphoma 2004;45:1835-46.
12. Kim WS, Honma K, Karnan S, Tagawa H, Kim YD, Oh YL, et al. Genome-wide array-based comparative genomic hybridization of ocular marginal zone B cell lymphoma: comparison with pulmonary and nodal marginal zone B cell lymphoma. Genes Chromosomes Cancer 2007; 46:776-83.
13. Fukuhara N, Nakamura T, Nakagawa M, Tagawa H, Takeuchi I, Yatabe Y, et al. Chromosomal imbalances are associated with outcome of Helicobacter pylori eradication in t(11;18) (q21;q21) negative gastric mucosa-associated lymphoid tissue lymphomas. Genes Chromosomes Cancer 2007;46:784-90.
14. Chuang SS, Liu H, Ye H, Martin-Subero JI, Siebert R, Huang WT. Pulmonary mucosa-associated lymphoid tissue lymphoma with strong nuclear B-cell CLL/lymphoma 10 (BCL10) expression and novel translocationt(1;2)(p22;p12)/immunoglobulin kappa chain-BCL10. J Clin Pathol 2007;60:727-8.
15. Zhou Y, Ye H, Martin-Subero JI, Hamoudi R, Lu YJ, Wang R, et al. Distinct comparative genomic hybridisation profiles in gastric mucosa-associated lymphoid tissue lymphomas with and without t(11;18) (q21;q21). Br J Haematol 2006;133:35-42.
16. Stilgenbauer S, Lichter P, Dohner H. Genetic features of B-cell chronic lymphocytic leukemia. Rev Clin Exp Hematol 2000;4:48-72.
17. Suela J, Alvarez S, Cifuentes F, Largo C, Ferreira BI, Blesa D, et al. DNA profiling analysis of 100 consecutive de novo acute myeloid leukemia cases reveals patterns of genomic instability that affect all cytogenetic risk groups. Leukemia 2007;21: 1224-31.
18. Largo C, Saez B, Alvarez S, Suela J, Ferreira B, Blesa D, et al. Multiple

myeloma primary cells show a highly rearranged unbalanced genome with amplifications and homozygous deletions irrespective of the presence of immunoglobulin-related chromosome translocations. Haematologica 2007;92:795-802.
19. Rouveirol C, Stransky N, Hupe P, Rosa PL, Viara E, Barillot E, et al. Computation of recurrent minimal genomic alterations from array-CGH data. Bioinformatics 2006; 22: 849-56.
20. Eisen MB, Spellman PT, Brown PO, Botstein D. Cluster analysis and display of genome-wide expression patterns. Proc Natl Acad Sci USA 1998; 95:14863-8.
21. Blaveri E, Brewer JL, Roydasgupta R, Fridlyand J, DeVries S, Koppie T, et al. Bladder cancer stage and outcome by array-based comparative genomic hybridization. Clin Cancer Res 2005; 11:7012-22.
22. D'Haese JG, Tsukasaki K, Cremer FW, Fischer C, Bartram CR, Jauch A. Chromosomal aberrations in follicular non-Hodgkin lymphomas of Japanese patients, detected with comparative genomic hybridization and polymerase chain reaction analysis. Cancer Genet Cytogenet 2005;162: 107-14.
23. Zhang X, Karnan S, Tagawa H, Suzuki R, Tsuzuki S, Hosokawa Y, et al. Comparison of genetic aberrations in CD10+ diffused large B-cell lymphoma and follicular lymphoma by comparative genomic hybridization and tissue-fluorescence in situ hybridization. Cancer Sci 2004;95: 809-14.
24. Martinez-Climent JA, Alizadeh AA, Segraves R, Blesa D, Rubio-Moscardo F, Albertson DG, et al. Transformation of follicular lymphoma to diffuse large cell lymphoma is associated with a heterogeneous set of DNA copy number and gene expression alterations. Blood 2003;101:3109-17.
25. Viardot A, Martin-Subero JI, Siebert R, Harder S, Gesk S, Bentz M, et al. Detection of secondary genetic aberrations in follicle center cell derived lymphomas: assessment of the reliability of comparative genomic hybridization and standard chromosome analysis. Leukemia 2001; 15:177-83.
26. Avet-Loiseau H, Vigier M, Moreau A, Mellerin MP, Gaillard F, Harousseau JL, et al. Comparative genomic hybridization detects genomic abnormalities in 80% of follicular lymphomas. Br J Haematol 1997;97:119-22.
27. Bentz M, Werner CA, Dohner H, Joos S, Barth TF, Siebert R, et al. High incidence of chromosomal imbalances and gene amplifications in the classical follicular variant of follicle center lymphoma. Blood 1996;88:1437-44.
28. Zakany J, Kmita M, Alarcon P, de la Pompa JL, Duboule D. Localized and transient transcription of Hox genes suggests a link between patterning and the segmentation clock. Cell 2001;106:207-17.
29. Schwaenen C, Nessling M, Wessendorf S, Salvi T, Wrobel G, Radlwimmer B, et al. Automated array-based genomic profiling in chronic lymphocytic leukemia: development of a clinical tool and discovery of recurrent genomic alterations. Proc Natl Acad Sci USA 2004;101: 1039-44.
30. Pfeifer D, Pantic M, Skatulla I, Rawluk J, Kreutz C, Martens UM, et al. Genome-wide analysis of DNA copy number changes and LOH in CLL using high-density SNP arrays. Blood 2007;109:1202-10.
31. Calin GA, Dumitru CD, Shimizu M, Bichi R, Zupo S, Noch E, et al. Frequent deletions and down-regulation of micro- RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. Proc Natl Acad Sci USA 2002;99:15524-9.
32. Sweet T, Khalili K, Sawaya BE, Amini S. Identification of a novel protein from glial cells based on its ability to interact with NF-kappaB subunits. J Cell Biochem 2003; 90:884-91.
33. Lacana E, D'Adamio L. Regulation of Fas ligand expression and cell death by apoptosis-linked gene 4. Nat Med 19995:542-7.
34. Burstein E, Hoberg JE, Wilkinson AS, Rumble JM, Csomos RA, Komarck CM, et al. COMMD proteins, a novel family of structural and functional homologs of MURR1. J Biol Chem 2005;280: 22222-32.
35. Itahana K, Zou Y, Itahana Y, Martinez JL, Beausejour C, Jacobs JJ, et al. Control of the replicative life span of human fibroblasts by p16 and the polycomb protein Bmi-1. Mol Cell Biol 2003;23:389-401.
36. Lui WO, Pourmand N, Patterson BK, Fire A. Patterns of known and novel small RNAs in human cervical cancer. Cancer Res 2007;67:6031-43.
37. Gotz R, Karch C, Digby MR, Troppmair J, Rapp UR, Sendtner M. The neuronal apoptosis inhibitory protein suppresses neuronal differentiation and apoptosis in PC12 cells. Hum Mol Genet 2000;9:2479-89.
38. Andersen CL, Gruszka-Westwood A, Atkinson S, Matutes E, Catovsky D, Pedersen RK, et al. Recurrent genomic imbalances in B-cell splenic marginal-zone lymphoma revealed by comparative genomic hybridization. Cancer Genet Cytogenet 2005; 156:122-8.
39. Boonstra R, Bosga-Bouwer A, van Imhoff GW, Krause V, Palmer M, Coupland RW, et al. Splenic marginal zone lymphomas presenting with splenomegaly and typical immunophenotype are characterized by allelic loss in 7q31-32. Mod Pathol 2003; 16:1210-7.
40. Hernandez JM, Garcia JL, Gutierrez NC, Mollejo M, Martinez-Climent JA, Flores T, et al. Novel genomic imbalances in B-cell splenic marginal zone lymphomas revealed by comparative genomic hybridization and cytogenetics. Am J Pathol 2001; 158:1843-50.
41. Gazzo S, Chudoba I, Traverse-Glehen A, Baseggio L, Felman P, Berger F, et al. Detailed characterization of 7q deletions by multicolor banding (mBAND) in marginal zone cell lymphoma. Cancer Genet Cytogenet 2007;175:159-65.
42. Ruiz-Ballesteros E, Mollejo M, Mateo M, Algara P, Martinez P, Piris MA. MicroRNA losses in the frequently deleted region of 7q in SMZL. Leukemia 2007;21:2547-9.
43. Aamot HV, Micci F, Holte H, Delabie J, Heim S. G-banding and molecular cytogenetic analyses of marginal zone lymphoma. Br J Haematol 2005;130: 890-901.
44. Rao PH, Houldsworth J, Dyomina K, Parsa NZ, Cigudosa JC, Louie DC, et al. Chromosomal and gene amplification in diffuse large B-cell lymphoma. Blood 1998;92:234-40.
45. Gilmore TD, Kalaitzidis D, Liang MC, Starczynowski DT. The c-Rel transcription factor and B-cell proliferation: a deal with the devil. Oncogene 2004;23:2275-86.
46. Tomioka N, Oba S, Ohira M, Misra A, Fridlyand J, Ishii S, et al. Novel risk stratification of patients with neuroblastoma by genomic signature, which is independent of molecular signature. Oncogene 2007; 27:441-9.
47. Fridlyand J, Snijders AM, Ylstra B, Li H, Olshen A, Segraves R, et al. Breast tumor copy number aberration phenotypes and genomic instability. BMC Cancer 2006;6:96.