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Genome-wide DNA profiling of marginal zone lymphomas identifies subtype-specific lesions with an impact on the clinical outcome

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Marginal zone B-cell lymphomas (MZLs) have been divided into 3 distinct subtypes (extranodal MZLs of mucosa-associated lymphoid tissue [MALT] type, nodal MZLs, and splenic MZLs). Nevertheless, the relationship between the subtypes is still unclear. We performed a comprehensive analysis of genomic DNA copy number changes in a very large series of MZL cases with the aim of addressing this question. Samples from 218 MZL patients (25 nodal, 57 MALT, 134 splenic, and 2 not better specified MZLs) were analyzed with the Affymetrix Human Mapping 250K SNP arrays, and the data combined with matched gene expression in 33 of 218 cases. MALT lymphoma presented significantly more frequently gains at 3p, 6p, 18p, and del(6q23) (*TNFAIP3*/*A20*), whereas splenic MZLs was associated with del(7q31), del(8p). Nodal MZLs did not show statistically significant differences compared with MALT lymphoma while lacking the splenic MZLs-related 7q losses. Gains of 3q and 18q were common to all 3 subtypes. del(8p) was often present together with del(17p) (*TP53*). Although del(17p) did not determine a worse outcome and del(8p) was only of borderline significance, the presence of both deletions had a highly significant negative impact on the outcome of splenic MZLs. (*Blood*. 2011;117(5):1595-1604)

Introduction

In the early 1990s, the term marginal zone B-cell lymphoma (MZL) was proposed in the Revised European-American Classification for Lymphoid Neoplasms[1] to encompass 2 apparently closely related lymphoma subtypes, namely, the "low grade B-cell lymphoma of the mucosa-associated lymphoid tissue (MALT) type," currently named MALT lymphoma, and the "nodal marginal zone B-cell lymphoma," also known as "monocytoid lymphoma." A third MZL subtype, with similar immunophenotype but distinct clinical features was also provisionally included (ie, the "primary splenic marginal zone lymphoma with or without villous lymphocytes"). Currently, each MZL category is now considered a unique lymphoma subtype in the World Health Organization classification: extranodal MZLs of MALT (MALT lymphoma), nodal MZLs, and splenic MZLs.[2] Although the general clinical presentations vary and unique recurrent translocations have been described in MALT lymphoma, the genetic relationship between the 3 MZL categories is still unresolved. Similarities in histologic features, such as neoplastic cell morphology, follicular colonization, presence of a plasmacell differentiation, immunophenotype, as well as genetic lesions (eg, gains of chromosomes 3 and 18 occurring at a frequency higher than other types of lymphomas) suggest that the 3 MZL subtypes differ from other lymphomas, but there are not widely accepted criteria able to differentiate among them. Indeed, in the presence of clinically disseminated disease, the differential diagnosis among different MZL subtypes is challenging. Hence, a better understanding of the underlying genetic aberrations is warranted to clarify these issues.

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To elucidate the molecular pathogenesis of MZLs, assess the relationship among the subtypes, and evaluate the impact of genomic aberrations on the clinical outcome, we performed genome-wide DNA profiling (array-CGH) on a large series of MZLs.

Methods

Tumor panel

All clinical specimens were derived from involved sites and obtained in the course of routine diagnostic procedures before therapy initiation. Cases were selected based on the availability of frozen material with a fraction of neoplastic cells in the specimen representing more than 70% of overall cellularity as determined by morphologic and/or immunophenotypic studies. Informed consent was obtained in accordance with the Declaration of Helsinki following the procedures approved by the local ethical committees and institutional review boards of each participating institution.

Array-CGH

DNA samples were analyzed using the GeneChip Human Mapping 250K NspI (Affymetrix), as previously described.[3] Data acquisition was performed using the GeneChip Operating Software, Version 1.4 and Genotyping Analysis Software, Version 4.1. Genotype calls were calculated using the BRLMM algorithm (Bayesian Robust Linear Model with Mahalanobis distance classifier). Mapping data for probes were derived from the National Center for Biotechnology Information Human Genome Build 36, as provided by Affymetrix, which was used for all subsequent analyses. The modified Bayesian Piecewise Regression method[4] was used to estimate the copy number (CN) starting from raw CN values obtained with Affymetrix CNAT 4.01 using a Gaussian bandwidth of 0 kb and 46 Caucasian normal female samples of the HapMap Project as reference samples. After normalization of each profile to a median log2-ratio of zero, thresholds for loss and gain were defined as 6 times the median absolute deviation symmetrically around zero with an associated P value less than .001 after Bonferroni multiple test correction. Loss of heterozygosity (LOH) profiles were obtained applying the method with haplotype correction for tumor-only LOH inference available in the dChip software[5] using as reference the 60 Centre d'Etude du Polymorphisme Humain parents of the HapMap Project and computing the allele A frequency from the data. The recurrent minimal common regions (MCRs) were defined using the algorithm by Lenz et al[6]: abnormal chromosome arm, abnormal whole chromosome, short recurrent abnormality, and long recurrent abnormality. MCR were identified for gains, losses, LOH, homozygous deletions, and amplifications. To be identified as an MCR, a minimal type-specific frequency of 5% was set for gains, losses, and LOH MCR, whereas homozygous deletions and amplifications had to occur in at least 2 cases. Reported MCR frequencies aggregate across all patients who carry a lesion minimally containing the MCR region irrespective of its type. For MCR occurring in at least 15% of cases, differences in MCR frequencies between subgroups were evaluated using a Fisher exact test followed by multiple test correction (false discovery rate, q value): results were considered statistically significant with a q value less than or equal to 0.10. The commonly affected regions were compared with the Database of Genomic Variants (http://projects.tcag.ca/variation): regions showing an overlap more than 80% between probes and known copy number variations (CNV) were considered bona fide CNV and discarded from further analyses. MCR containing the genes coding the immunoglobulin heavy chain genes and the κ and λ light chains were also similarly discarded because CN changes in these regions probably represent the physiologic rearrangements occurring in B cells. Frequency plots for DNA gains, DNA losses, and LOH were calculated from modified Bayesian Piecewise Regression segmented CN data, thresholded for gain or loss or for LOH, without removing the known CNV and immunoglobulin gene loci.

The University of California, Santa Cruz Genome Browser (http://genome.ucsc.edu)[7] was used to retrieve additional information. Unsupervised clustering was performed using the non-negative matrix factorization (NMF) algorithm.[8] Raw data are available at the National Center for Biotechnology Information Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo) database under accession number GSE24881.

Combination of array-CGH and gene expression profiling

Matched gene expression profile (GEP) was obtained in 18 cases with the Affymetrix GeneChip HGU95av2 arrays (9 MALT lymphomas, 2 splenic MZLs, and 7 nodal MZLs) and in 15 additional cases with the Affymetrix GeneChip U133 plus 2.0 arrays (9 MALT lymphomas, 2 splenic MZLs, and 4 nodal MZLs). Samples were processed and hybridized as previously described.[9,10] Expression values were calculated from CEL files using the Robust Multiarray Average (RMA) algorithm.[11] To each expression probe set on the array, we associated the mean CN of SNP probes that were flanking or inside the associated gene region. Probe sets with an SD less than the median SD, for either expression or CN, were regarded as nonchanging or nondiffering and were filtered out for further analysis. Furthermore, for each gene expression probe set, we required at least 20% of the samples to be expressed (ie, RMA $>$ 5.7). After filtering we retained 1819 probe sets for the U95A array and 4268 probe sets for the U133A array. To identify the genes most correlated with CN, we used the pseudo-inverse projection as described by Alter and Golub.[12] We filtered the GEP onto the CN profile basis before the correlation analysis. We use the complete, nonfiltered GEP and CN profile data (ie, before applying the SD filter). The projection filter was constructed by computing the pseudo-inverse using singular value decomposition of the CN profile data. By projecting the GEP onto the singular vectors of the CN profiles, we only retained those in gene expression that "fit" the characteristic genome-wide patterns of CN profiles. Any GEP changes that could not be explained by CN profiles patterns, for example, because of indirect mechanisms or epigenetic causes, were filtered out. After performing pseudo-inverse filtering, we calculated the Pearson correlation coefficient. For genes that corresponded to multiple probe sets, we considered their best P value. For each MCR, we reported the genes that were significantly correlated at false discovery rate less than 0.05. The Database for Annotation, Visualization and Integrated Discovery, Version 6.7 Web-accessible program[13] was used to map the genes to KEGG, Biocarta, and Panther Pathways and UniProt keywords and statistically examine the enrichment of gene members for each pathway, using a P value $<$ .001.

Analysis of clinical data

To evaluate the impact of the genetic aberrations on overall survival (OS), calculated from diagnosis to the last follow-up or death, univariate analysis was performed only for MCR with at least 5 patients with data necessary to calculate OS. Such data were available for 169 of 218 (77.5%) cases. Internal validation was quantified by calculating the Harrell concordance index (C-index).[3,14] The bootstrap procedure as implemented in the "validate" function of the R-package design[15] was used, allowing for computation of an unbiased estimate of the C-index. We used 500 bootstrap samples. The actuarial durations of OS were plotted as curves according to the Kaplan-Meier method. Statistical analyses were performed with R package[15] and with SPSS Version 17.0.2.

Results

MALT lymphoma, splenic MZLs, and nodal MZLs: subtype-specific genomic alterations

A total of 218 MZL samples were analyzed by high-resolution genome-wide DNA profiling: 57 MALT lymphoma, 134 splenic MZLs, 25 nodal MZLs, and 2 MZLs where allocation to a specific category was not possible. Supplemental Table 1 (available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article) summarizes the main clinical and pathologic features.

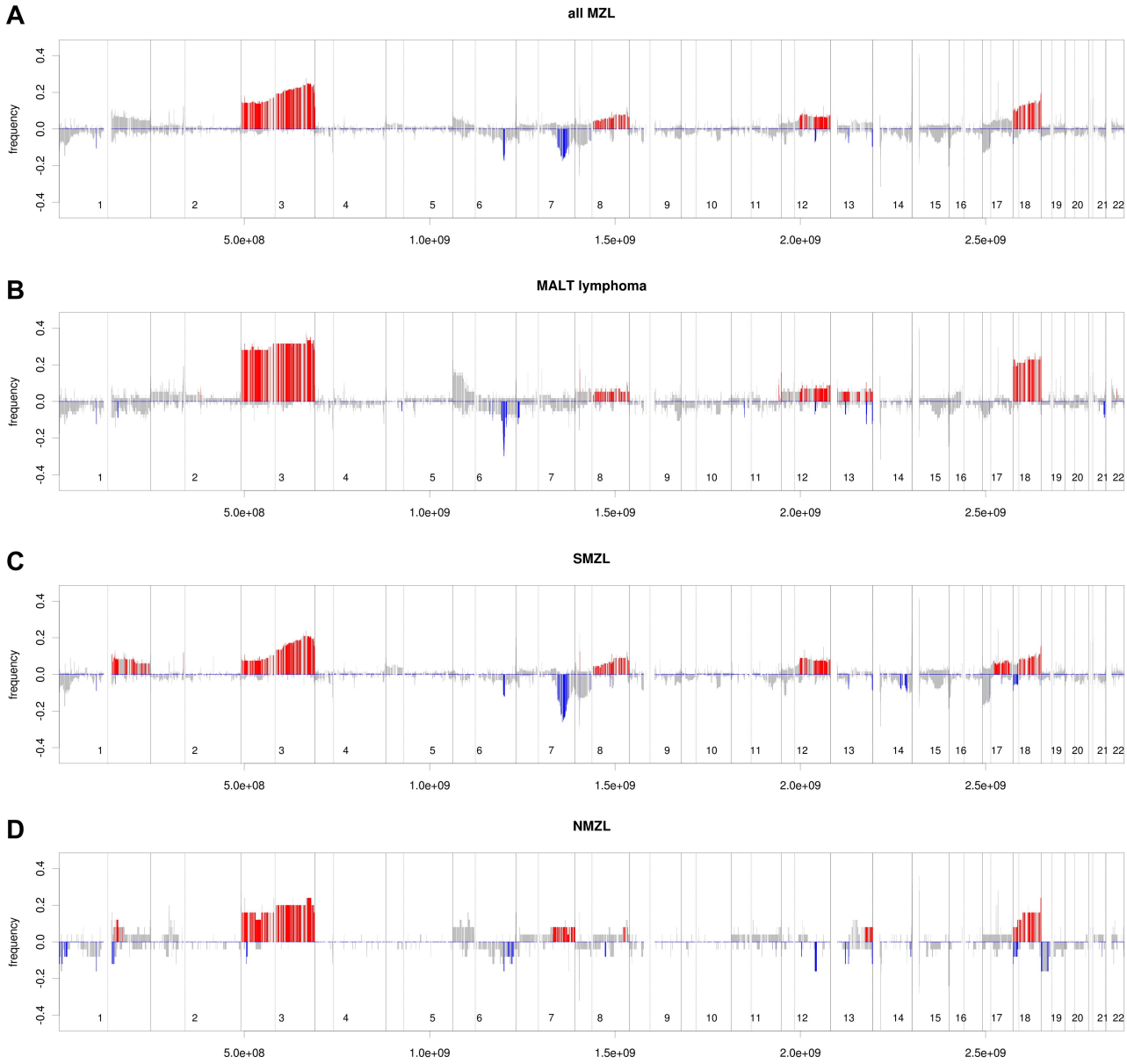


Figure 1. Frequency of DNA gains (up) and losses (down) observed in MZL. (A) 218 MZL, (B) 57 MALT lymphoma, (C) 134 splenic MZL, and (D) 25 nodal MZL samples. Red represents gains with associated up-regulated transcripts; and blue, losses with associated down-regulated transcripts. x-axis represents chromosome localization and physical mapping; and y-axis, proportion of cases showing the aberrations.

To have a precise representation of the lesions characterizing these disorders and the genes more likely to be affected, we determined the minimal recurrent alterations that contain loci possibly relevant for the tumor (MCR), in the whole group and in each subgroup of MZL cases, and we combined them with gene expression data (supplemental Tables 2-3). The frequencies of DNA gains and losses, with the regions correlated with a significant effect on gene expression highlighted, are represented in Figure 1, whereas supplemental Figure 1 shows the frequency of LOH.

Among all MZLs, 59 MCRs were identified composing 32 gains, 25 losses, one LOH, and one homozygous deletion. Homozygous deletion was detected at 6q23.3 in 1.8% (4 of 218) of MZL cases, containing 2 genes, *TNFAIP3* and *PERP*, which indeed were down-regulated. Three cases were MALT lymphoma, and one was splenic MZL. Table 1 shows the most common MCRs, containing genes affected by CN changes, such as *NFKBIZ*, *BCL6, NFATC1,* and *TNFAIP3*. The LOH region overlapped with a recurrent region of loss at 7q31.33. No amplifications were observed.

Transcripts mapped within MCR and affected by CN changes were enriched of genes belonging to relevant pathways (cell cycle, $P < .0001$; colorectal cancer, $P < .0001$; ubiquitin proteasome, $P < .0001$) or involved in apoptosis ($P < .0001$). Similar enrichment was observed in both MALT lymphomas and splenic MZLs.

A series of MCRs were significantly different between MALT lymphoma and splenic MZLs (Figure 2; supplemental Table 2). MALT lymphoma was characterized by gains at 6p25, gains affecting chromosomes 3 (affecting *FOXP1*, *NFKBIZ*, and *BCL6*) and 18 (affecting *NFATC1* and *BCL2*), and losses at 6q (affecting *TNFAIP3*) and 1p. Splenic MZL was mainly associated with losses at 7q (affecting *POT1*, containing *MIR29A* and *MIR29B*), 17p (containing *TP53*), and 8p. The latter deletion was validated by analyzing 2 samples with the loss using a different higher-density array

Table 1. Most common MCR observed in the 218 MZL and in the individual subgroups

Histotype	Lesion	Cytoband	Frequency, %	Start	Size	Correlation with gene expression
MZL	Gains	3q11.2-q29 (*NFKBIZ*, *BCL6*)	24	9.50×10^7	1.04×10^8	Yes
		6q27	16	1.70×10^8	1.01×10^6	
		13q11-q12.11	25	1.80×10^7	5.33×10^5	
		18q23 (*NFATC1)*	18	7.40×10^7	2.12×10^6	Yes
		21p	15			
	Losses	1p36.21-p36.13	12	1.51×10^7	1.62×10^6	Yes
		4p12-p11	13	4.82×10^7	5.72×10^5	
		6q23.2-q24.1 (*TNFAIP3*)	16	1.35×10^8	4.78×10^6	Yes
		7q31.31-q32.3	17	1.20×10^8	1.15×10^7	Yes
		17p13.3-p13.1 (*TP53*)	13	1.89×10^4	9.71×10^6	Yes
MALT lymphoma	Gains	3q (*NFKBIZ*, *BCL6*)	30			Yes
		3p (*FOXP1*)	26			Yes
		6p25.1-p21.32	14	4.35×10^6	2.85×10^7	
		7p15.1	14	3.15×10^7	1.78×10^4	
		7q11.22	18	6.88×10^7	5.68×10^5	
		8p21.2	14	2.72×10^7	1.36×10^5	
		18p	21			Yes
		18q (*BCL2, NFATC1)*	21			Yes
	Losses	1p36.21-p36.13	12	1.56×10^7	1.12×10^6	Yes
		4p11–12	14	4.81×10^7	6.81×10^5	
		6q23.3-q24.1 (*TNFAIP3*)	30	1.37×10^8	2.35×10^6	Yes
		13q32.1-q32.2	16	9.69×10^7	8.28×10^5	Yes
Splenic MZL	Gains	3q13.13-q29 (NFKBIZ, BCL6)	20	1.10×10^8	8.89×10^7	Yes
		6q27	19	1.70×10^8	4.83×10^5	
		8p23.1-p22	15	1.23×10^7	5.08×10^5	Yes
		9q31.1	13	1.05×10^8	3.20×10^4	
		12q21.31	13	8.07×10^7	4.06×10^4	
		13q11-q12.11	28	1.80×10^7	5.33×10^5	
		18q23 (*NFATC1)*	15	7.35×10^7	2.57×10^6	Yes
		21p	17			
	Losses	1p36.21-p36.13	13	1.51×10^7	1.62×10^6	
		4p12-p11	13	4.82×10^7	5.72×10^5	
		7q31.31-q32.3	26	1.20×10^8	1.15×10^7	Yes
		8p	13			
		17p13.3-p13.1 (*TP53*)	17	1.89×10^4	9.71×10^6	Yes
Nodal MZL	Gains	2p16.3	20	4.95×10^7	2.11×10^6	
		3p25.3	24	1.04×10^7	6.06×10^5	
		3p14.3-q29 (*FOXP1*, *NFKBIZ*, *BCL6*)	24	5.46×10^7	1.45×10^8	Yes
		6p21.2-p21.1	16	4.01×10^7	1.46×10^6	
		6q27	16	1.70×10^8	1.04×10^6	
		18q23 (*NFATC1)*	24	7.42×10^7	1.95×10^6	Yes
	Losses	1p36.33-p36.22	16	7.76×10^5	9.76×10^6	Yes
		1p21.2	16	9.98×10^7	1.86×10^6	Yes
		6q23.3 (*TNFAIP3*)	16	1.37×10^8	1.44×10^6	Yes
		12q21.33-q23.1	16	9.07×10^7	4.81×10^6	Yes
		19p13.3-p12	16	2.12×10^5	1.98×10^7	Yes

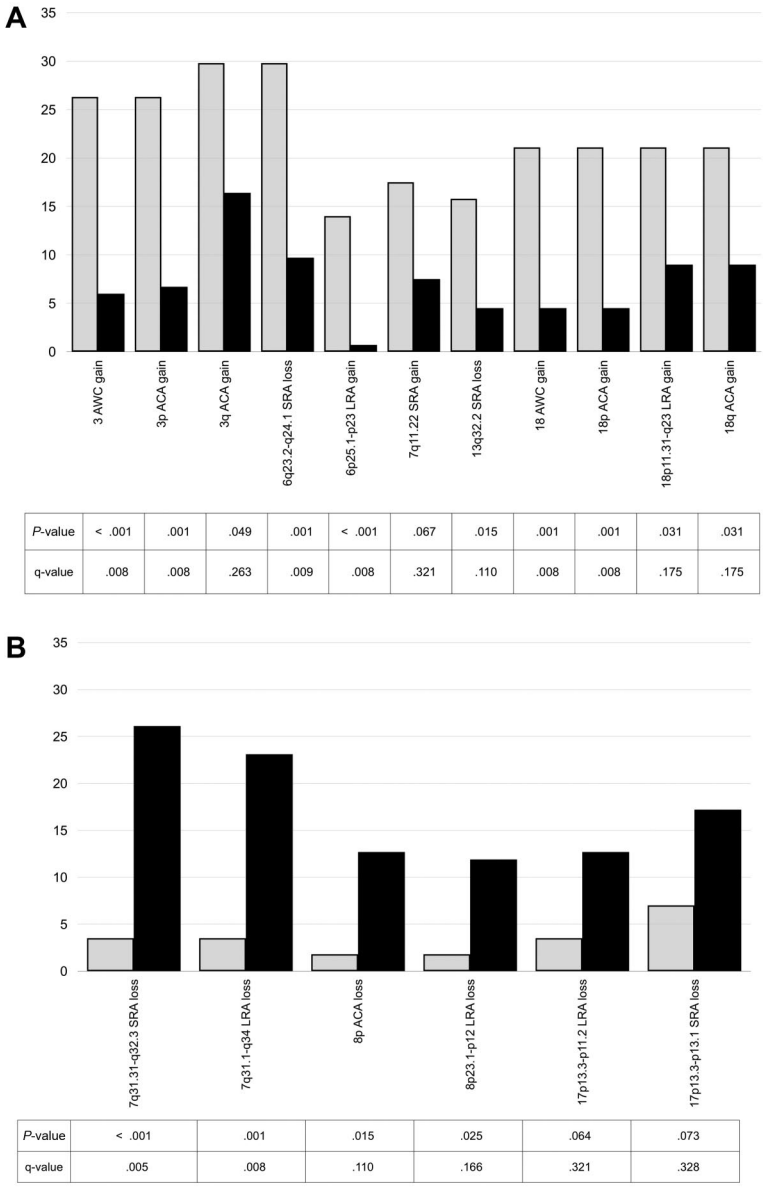
(Affymetrix Genome-Wide Human SNP Array, Version 6.0, following the manufacturer's instructions). No statistically significant differences were observed on comparing MALT lymphoma with nodal MZLs, but the latter completely lacked the splenic MZL-specific 7q deletions: del(7q31.31-q32.3) (affecting *POT1*, containing *MIR29A*, *MIR29B*) occurred in 35 of 134 (26%) splenic MZLs, and in none (0%) of the 25 nodal MZLs ($P = .0014$; $q = 0.04$).

To further genetically characterize the individual subtypes, we applied the MCR algorithm to each MZL category separately (Table 1; supplemental Table 3).

A total of 127 MCRs were identified in the 57 MALT lymphoma: 66 gains, 43 losses, 17 LOH, and one homozygous deletion. The most frequent lesions were gains of chromosomes 3 (different MCRs, affecting *FOXP1*, *NFKBIZ*, and *BCL6*) and 18 (affecting *BCL2*, *MALT1*, *NFATC1*) and del(6q23) (affecting *TNFAIP3*). Recurrent homozygous deletions were identified at 6q23 (affecting *TNFAIP3* and *PERP*) in 5.4% (3 of 57) of cases. An analysis considering specific anatomic sites of involvement was not performed because of the relatively small number of available MALT lymphoma cases.

In splenic MZLs, 72 MCR were identified: 35 gains, 34 losses, and 3 LOH. No recurrent homozygous deletion was observed. The most frequent lesions were del(7q31-q32) (affecting *POT1*, containing *MIR29A* and *MIR29B*), gain at 13q11 (60% overlap with known bona fide CNV), and gain at 3q (affecting *NFKBIZ* and *BCL6*).

In nodal MZLs, 78 MCR were identified: 29 gains, 36 losses, and 13 LOH. The most frequent lesions, all occurring in 24% (6 of 25) of cases, were gains of chromosome 3 (affecting *FOXP1*, *NFKBIZ*, and *BCL6*) and 18q23 (affecting *NFATC1*).



P-value	< .001	.001	.049	.001	< .001	.067	.015	.001	.001	.031	.031
q-value	.008	.008	.263	.009	.008	.321	.110	.008	.008	.175	.175

P-value	< .001	.001	.015	.025	.064	.073
q-value	.005	.008	.110	.166	.321	.328

Figure 2. The most significantly different MCRs between extranodal MZLs and splenic MZLs. (A) Regions more common in MALT lymphomas MZLs. (B) Regions more common in splenic MZLs.

Patterns of concomitant genomic aberrations

To identify patterns of unbalanced genomic aberrations, which might suggest the existence of genes with a cooperative pathogenetic role, we performed both unsupervised and supervised analyses.

The application of the unsupervised NMF clustering algorithm to the whole series of MZL cases first identified 2 clusters, characterized by the presence or absence of gains of the long arm of chromosome 3. Then, when applied on these 2 clusters separately, NMF identified 4 robust groups (Figure 3). Cluster 1 (37 of 218 patients, 17%) was mainly characterized by gains at 3q (*NFKBIZ*, *BCL6*), plus gains at 1q, 8q, 17q, 18q (*BCL2*, *NFATC1*), del(7q) (*POT1*, *MIR29A*, *MIR29B*), del(8p), del(17p) (*TP53*), and del(18p). The main lesions of cluster 2 (20 of 218 cases, 9%) were +3, +18, and del(6q). Cluster 3 (142 of 218 cases, 65%) had very few recurrent lesions, with only a low frequency of del(7q) (*POT1*, *MIR29A*, and *MIR29B*) and del(17p) (*TP53*) observed. Cluster 4 (19 of 218, 8%) mainly displayed del(7q) (*POT1*, *MIR29A*, *MIR29B*), del(8p), del(17p) and gain at 12q. Half (11 of 20, 55%) of cluster 2 members were MALT lymphoma, despite the latter representing only 26% (57 of 218) of the whole MZL series analyzed. In contrast, 17 of 19 (89%) of cluster 4 cases were splenic MZLs, and only 1 of 19 (5%) MALT lymphoma. The 4 clusters had no differences in terms of OS and clinical characteristics.

We then studied the co-occurrence of MCRs in individual patients by applying a Fisher exact test (*P* value) followed by multiple test correction (Table 2). Gains of 3q (*NFKBIZ*, *BCL6*) were associated with different genomic lesions in MALT lymphoma and splenic MZLs. An association with gains affecting chromosome 18 (*BCL2*, *NFATC1*) was the only lesion shared between MALT lymphoma and splenic MZLs. MALT lymphoma cases bearing gains at 3q also presented more frequent gains at 13q, 5p15.2, del(17p13.1-p13.3) (*TP53*), and LOH at 2p16.3, compared with cases without 3q gain. On the converse, in splenic MZLs, lesions associated with 3q gain included gain at 17q22-q25.3, 1q, del(6q23.2-q24.1) (*TNFAIP3*), and del(6q25). Similar patterns were observed for cases with and without gain at 18q because almost all cases with this lesion had a concomitant gain at 3q (data not shown).

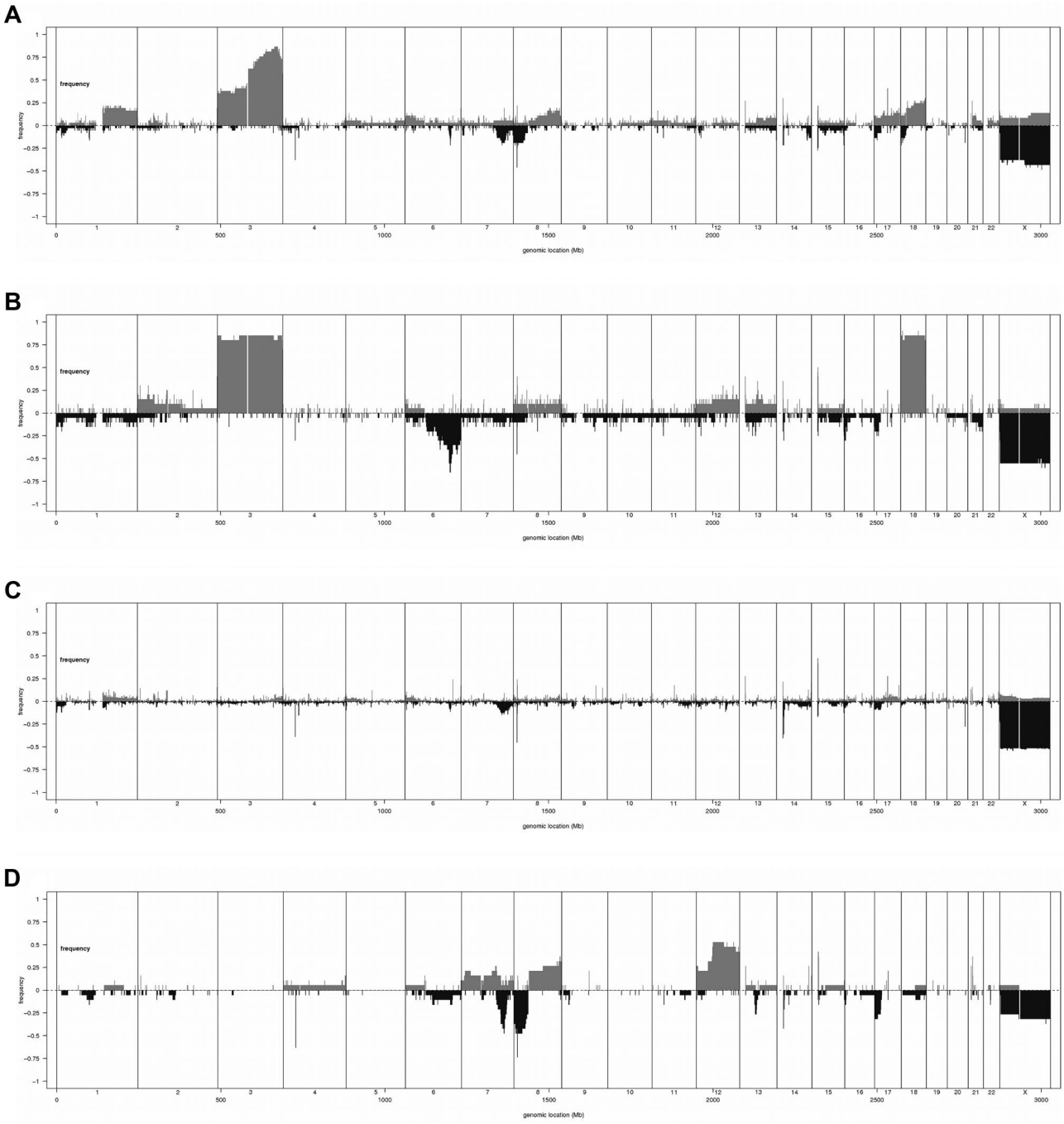


Figure 3. Frequency of DNA gains (up) and losses (down) in 4 MZLs clusters identified using the unsupervised NMF algorithm. (A-D) NMF clusters 1-4. x-axis represents chromosome localization and physical mapping; and y-axis, proportion of cases showing the aberrations.

Splenic MZLs with del(7q31-q32) (*POT1*, *MIR29A*, and *MIR29B*) showed a trend for higher occurrence of concomitant del(8p12-p23). Cases with del(17p) (*TP53*) had concomitant deletions of del(8p), del(13q14) (*MIR15/MIR16*), and gains at 17q22-q25.3. The del(8p) was associated with del(17p13.1-p13.3) (*TP53*), del(13q14.2-q14.3) (*MIR15/MIR16*), and gain at 8q. Similar results could be obtained comparing cases with and without del(8p23) (data not shown). Cases with del(13q14.3) (*MIR15/MIR16*), a lesion common in chronic lymphocytic leukemia (CLL) and targeting *MIR15/MIR16*, had concomitant del(8p), del(6q25.2), del(17p) (*TP53*), and gain at 18q11-q23 (*BCL2*, *NFATC1*). No differences were observed regarding the occurrence of del(7q31-q32).

Genomic lesions correlate with worse outcome

A clinical score has been proposed for splenic MZLs,[16] in which low hemoglobin and albumin levels and increased serum lactate dehydrogenase level divide patients into 3 prognostic categories: low risk (with no adverse factors), intermediate risk (with one adverse factor), and high risk (with 2 or 3 adverse factors). Because in our series, the OS curve for intermediate risk and high risk were comparable (supplemental Figure 2), we compared the genomic profiles of these 2 categories with the low-risk patients. Intermediate- or high-risk patients displayed more del(1p36.21-p36.13) (7 of 43, 16% vs 0 of 29, 0%; $P = .037$; $q = 0.28$), del(8p12-p23) (17 of

Table 2. Patterns of association among MCR as evaluated by applying Fisher exact test (*P*) followed by multiple test correction (q)

	Associated regions	No. with lesion	% with lesion	No. without lesion	% without lesion	*P*	q
MALT lymphoma, 3q gain	18q gain (*BCL2*, *NFATC1*)	9/17	53	3/40	8	.00036	0.0018
	del(17p13.1-p13.3) (*TP53*)	5/17	29	0/40	0	.0015	0.0067
	2p16.3 LOH	3/17	18	0/40	0	.023	0.082
	13q gain	3/17	18	0/40	0	.023	0.082
	5p15.2 gain	4/17	23	1/40	3	.024	0.082
Splenic MZL, 3q gain	18q gain (*BCL2*, *NFATC1*)	8/22	36	4/112	4	< .00001	0.0003
	17q22-q25.3 gain	6/22	27	4/112	4	.0014	0.0061
	1q gain	5/22	23	3/112	3	.0031	0.012
	del(6q23.2-q24.1) (*TNFAIP3*)	6/22	27	7/112	6	.0079	0.027
	del(6q25)	5/22	23	3/112	3	.011	0.035
Splenic MZL, del(7q31-q32)	del(8p12-p23)	8/35	23	8/99	8	.032	0.19
Splenic MZL, del(17p) (*TP53*)	del(8p)	8/23	35	9/111	8	.002	0.012
	del(13q14.2-q14.3) (*MIR15/MIR16*)	5/23	22	6/111	5	.022	0.078
	17q22-q25.3 gain	5/23	22	5/111	4	.014	0.06
Splenic MZL, del(8p)	del(17p13.1-p13.3) (*TP53*)	8/17	47	15/117	13	.002	0.019
	del(13q14.2-q14.3) (*MIR15*/*MIR16*)	5/17	29	6/117	5	.005	0.034
	8q gain	4/17	24	3/117	3	.005	0.034
Splenic MZL, del(13q14.2-q14.3) (*MIR15*/*MIR16*)	del(6q25.2)	4/11	36	6/123	5	.004	0.061
	del(8p)	5/11	45	11/123	9	.006	0.062
	18q gain (*BCL2*, *NFATC1*)	4/11	36	7/123	6	.006	0.062
	del(17p) (*TP53*)	5/11	45	13/23	10	.007	0.061

43, 39% vs 5 of 29, 17%; $P = .067$; q = 0.28), del(7q31.31-q32.3) (12 of 43, 28% vs 3 of 29, 10%; $P = .08$), and del(17p) (10 of 43, 23% vs 2 of 29, 7%; $P = .1$). The observation of a higher number of recurrent genomic lesions in splenic MZL patients with an intermediate to high Intergruppo Italiano Linfomi score strongly underlines the rationale of such a prognostic score.

We then analyzed the MCRs for their direct impact on survival. del(8p) was associated with worse OS ($P = .048$; q = 1) among all the MZL cases. The lesion showed a similar effect also when analyzed separately in MALT lymphoma and splenic MZLs, although not reaching statistical significance. Based on the association of del(8p) with del(17p), also reported in other lymphoid tumors,[3,17] we compared the outcome in patients with splenic MZLs bearing these 2 lesions. As shown in Figure 4, the presence of both lesions had a significant impact on the outcome (Gehan-Breslow-Wilcoxon test: $P = .004$, q = 0.33, C-index = 0.58), whereas del(8p) alone was of borderline statistical significance ($P = .067$, q = 0.9, C-index = 0.57) and isolated del(17p) had no significant impact. Importantly, only the presence of both del(8p) and del(17p) was also able to better define the outcome of patients with an intermediate- or high-risk clinical score ($P = .003$; C-index = 0.655). In MALT lymphoma, gain at 3q11.2-q29 (*NFKBIZ*, *BCL6*) was associated with worse OS ($P = .048$; q = 0.75). As already mentioned in "Patterns of concomitant genomic aberrations," the 4 NMF clusters that we identified were not associated with differences in outcome.

Discussion

We analyzed a large series of MZLs using a high-density genome-wide DNA microarray to better define differences among MALT lymphoma, splenic MZLs, and nodal MZLs and to identify regions with prognostic significance. The integration with gene expression profile data was used to identify genes probably targeted by genomic events. In support of the current World Health Organization classification,[2] differences were detected among the 3 subtypes, especially between MALT lymphoma and splenic MZLs by both supervised and unsupervised analyses. The splenic MZLs presented more frequently the well-known del(7q31-q32), but also deletions at 8p and 17p, whereas MALT lymphomas more commonly displayed deletions at 1p and 6q, as well gains on chromosomes 3 and 18, and on the short arm of chromosome 6.

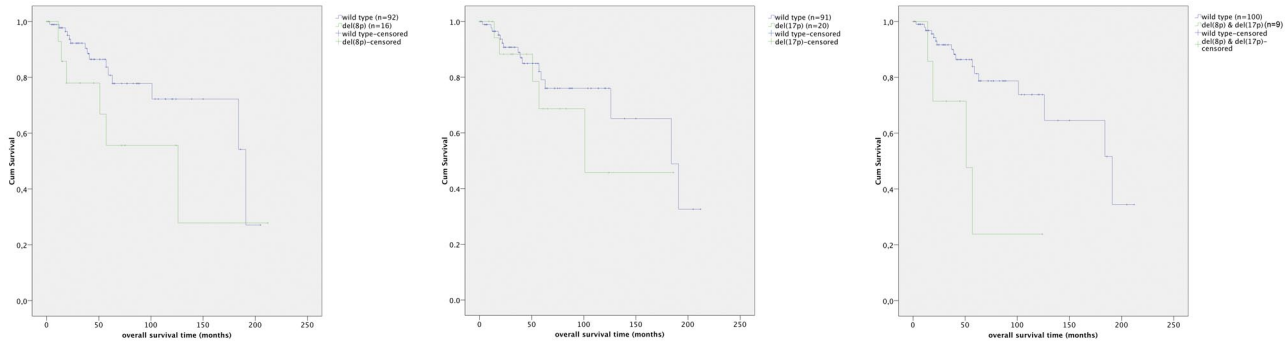


Figure 4. Kaplan-Meier graph showing OS in splenic MZLs according to the presence of del(8p) only ($P = .068$), del(17p) only ($P = .256$), or del(8p) and del(17p) together ($P = .004$). Green represents patients bearing the lesion(s); and blue, patients without the lesion(s). x-axis represents time (months); and y-axis, percentage of patients alive.

Deletion of the long arm of chromosome 7, which was present in more than one-fourth of splenic MZLs but in less than 5% of the MALT lymphoma cases, is the best known lesion in splenic MZLs.[18-25] The presence of del(7q31-q32) was the most common lesion in splenic MZLs; but despite the large number of samples analyzed, the minimal common deleted region was still very large, composing more than 4 Mb. *POT1*, a gene reported as possibly affected by the deletion,[23] was down-regulated in patients carrying del(7q31-q32), but it was not the only affected gene. The MCRs contained *MIR29A* and *MIR29B*, suggested as altered in splenic MZLs with del(7q),[22] and were also mapped within the MCR. Our analysis, performed with a high-resolution array, strongly suggests that the deletion could target not single, but multiple, transcripts.

Aberrations affecting chromosome 6, losses of its long arm and gains of the short arm, were more common in MALT lymphoma and in nodal MZLs than in splenic MZLs. The 6q MCR contained the gene *TNFAIP3*, coding for a negative NFKB regulator, which has been recently identified as frequently inactivated by somatic mutations and deletions in different lymphoma subtypes, including MALT lymphoma and diffuse large B-cell lymphoma (DLBCL).[26-28] Our data, showing also the down-regulation of the transcripts in patients with del(6q23), strongly underline the importance of the gene, particularly among MALT lymphomas. Deletions of *TNFAIP3* have been reported as being associated with gains at 6p, involving the TNFA/B/C locus, in MALT lymphoma[29] and in Waldenstrom macroglobulinemia (WM).[30] In our series, losses of the *TNFAIP3* locus were not associated with 6p gains affecting the TNFA/B/C locus. These differences could be the result of different primary sites of MALT lymphoma analyzed, but also of different technical approaches because we used an array covering the entire 6p arm, whereas some of the published data[29] were obtained with fluorescence in situ hybridization using probes targeting the TNFA/B/C region only, thus unable to detect more telomeric regions, but with a higher sensitivity for perhaps subclonal lesions of the TNFA/B/C locus. Indeed, the scenario might be complex, involving the genetic background of the host (single nucleotide polymorphisms and CNV).

Similar to follicular lymphomas and DLBCL,[3,31,32] 6p was the site of copy-neutral LOH, suggesting the presence of advantageous mutations or polymorphisms in this region. Polymorphisms of genes mapped in 6p, such as *TNFA* itself (6p21.33) and *IRF4* (6p25), have been associated with risk of lymphoma, particularly of MZLs.[33,34] The 6p region also contains the genes coding for the human leukocyte antigens, which can be down-regulated in lymphoma cells via copy-neutral LOH.[35] Further studies are needed to understand the potential role of the specific patterns of 6q deletion and 6p gain and their relation to the microenvironment involved in the pathogenesis of MZLs, especially of MALT lymphoma. It is worth mentioning that, different from other lymphoma subtypes,[3,31,32] copy-neutral LOH did not appear to be a common event in MZLs.

Although also present in other lymphomas, such as DLBCL,[6] recurrent gains affecting chromosomes 3 and 18 are considered hallmarks of MZLs. Interestingly, the short arms of both chromosomes were more commonly affected in MALT lymphoma or nodal MZLs than in splenic MZLs. Differences in the prevalence of whole chromosome gains or of preferential gains of long arms could reflect different underlying mechanisms of lymphomagenesis, some of which could be the result of exogenous or host factors, such as DNA polymorphisms of the pericentromeric region.[36,37] The 2 aberrations determined an overexpression of different genes, including *FOXP1*, *NFKBIZ*, and *BCL6* (on chromosome 3) and *BCL2*, *MALT1*, and *NFATC1* (on chromosome 18). Whereas genes mapped on the long arms might provide a survival advantage to neoplastic cells in all MZLs, genes mapped to the short arms might only be useful for MZLs developing in an extranodal or nodal environment. Here, gains affecting 3q and 18 seemed to affect B-cell receptor signaling pathways ($P = .04$), Wnt signaling ($P = .014$), cell cycle ($P = .02$), and apoptosis ($P = .04$), whereas genes associated with 3p and 18p gains composed transcripts involved in chemokine and cytokine signaling pathway ($P = .01$), ubiquitin proteasome pathway ($P = .01$), Ras signaling ($P = .04$), and in tight junction regulation ($P = .05$). *FOXP1* (3p14.1) is overexpressed in a small fraction of MALT lymphomas because of its juxtaposition to the immunoglobulin heavy chain gene promoter after chromosomal translocation.[38] The 3p21.31 region contains a cluster of genes coding for chemokine receptors: *CCR9*, *CXCR6*, *XCR1*, *CCR3*, *CCR1*, *CCR3*, *CCR5*, and *CCRL2*. CXCR6 and the ligand of CCR5, CCL5, have recently been reported as part of the gene expression signature of pulmonary MALT lymphoma,[39] and CCR5 has been shown to be overexpressed in MALT lymphoma cells compared with normal MZL cells.[40] *CCR4* (3p22.3) has been previously reported as overexpressed in MALT lymphoma bearing trisomy 3,[40] and it could be involved in the differential homing of the MZL subtypes. In our series, 3q gains were associated with a worse outcome in MALT lymphoma, but not in the remaining MZL subtypes. Similar results have been reported in mantle cell lymphoma[41] and DLBCL.[6] Besides the different frequency of gains, the pattern of concomitant lesions associated with gains at 3q and 18q were also different in the 2 main MZLs subgroups. Although MALT lymphoma showed an association with gain at 13q (containing also *MIRHG1/MIR-17–92*) and del(17p) (*TP53*), in splenic MZLs the lesions were associated with gains at 1q, and 17q22-q25.3, del(6q23.2-q24.1) (*TNFAIP3*), and del(6q25), suggesting different pathogenetic mechanisms in the 2 MZL types.

Both del(8p) and del(17p) (*TP53*) were more common in splenic MZLs than in MALT lymphoma. In our series, the loss of the *TP53* locus did not affect the prognosis of splenic MZLs. *TP53* somatic mutations, and not the simple allelic loss, might confer a poor outcome in splenic MZLs,[42-44] similar to DLBCL.[45] On the converse, del(8p) was associated with a poorer outcome among all MZL cases. In splenic MZLs, similar to recent observations in CLL and DLBCL,[3,17] del(8p) was often present together with del(17p13.1-p13.3). Importantly, although del(17p) did not determine a worse outcome and del(8p) was only of borderline significance, the presence of both deletions had a highly significant impact on outcome of patients with splenic MZLs. The demonstration of both losses also better defined the prognosis of patients with an intermediate- or high-risk clinical score. *TNFRSF10A* (*TRAILR1*) and *TNFRSF10B* (*TRAILR2*), coding for the transmembrane pro-apoptotic death receptors, and *MCPH1*, coding for microcephalin 1 involved in DNA damage repair, have been proposed as targets of deletions in aggressive B-cell lymphomas.[46,47] Genes mapped to 8p have also been linked to a higher risk of autoimmune disorders.[48] Here, we did not observe down-regulation of any of these genes in association with del(8p).

Chromosomal translocations identified in MALT lymphoma are usually absent in nodal MZLs.[2] Similar to splenic MZLs, nodal MZL cases with both mutated and unmutated IgH genes exist.[19,49] In our series, nodal MZLs appeared extremely similar to MALT lymphoma and different from splenic MZLs. In particular, nodal MZLs lacked the main recurrent lesions specific for splenic MZLs, such as 7q loss, and presented gains of both short and long arms of

chromosomes 3 (*FOXP1*, *NFKBIZ*, and *BCL6*) and 18 (*BCL2* and *NFATC1*), as well of 6p, all lesions associated with MALT lymphoma. No unbalanced genomic lesions specific for nodal MZLs were identified. Nodal MZLs might represent a distinct entity derived from a heterogeneous B-cell counterpart similar to the progenitors of splenic MZLs but that have undergone a selection process more similar to MALT lymphoma cells. Alternatively, nodal MZLs could represent MALT lymphomas clinically presenting as nodal MZLs.

Differential diagnosis of small B-cell lymphomas is often difficult, and this could be partially the result of the existence of a large overlapping of common genomic aberrations across the different lymphoma subtypes.[50] Of particular interest is the comparison of MZLs with lymphoplasmacytic lymphoma, whether accompanied by an IgM component (WM) or not, and CLL. The genomic profile, which has been reported for WM,[30] partially overlaps with what we see in MZLs: 3q and 18q gains, common to all MZLs; 6p gains and del(6q23) (*TNFAIP3*), common in MALT lymphoma; and del(8p) and 8q gains, common in splenic MZLs. On the converse, del(6q21) (*PRDM1*), gains of chromosome 4, and del(14q32.32) (*TRAF3*) appear specific for WM. Genome-wide data are not available for lymphoplasmacytic lymphoma without an IgM component. The del(13q14.3) (*MIR15*/*MIR16*), observed in less than 10% of MZL cases, is considered to be the typical lesion of CLL, in which it is present in at least half of the cases, but it has also been reported in other types of low-grade lymphomas.[50] Importantly, cases of splenic MZLs with del(13q14.3) showed a genetic profile, which was consistent with the diagnosis of splenic MZLs and not of CLL, with the frequency of 7q loss being similar to the remaining splenic MZL cases.

In conclusion, we have identified the differences in terms of unbalanced genomic lesions between the 2 main MZL subtypes and suggested that the rare subtype of nodal MZLs is intermediate between them. We also showed that gain of 3q and del(8p) possibly determine a poor OS in MZL patients and that the presence of the latter lesions concomitant with del(17p) determines a very poor outcome in splenic MZL patients. The implication of these last observations for the clinical practice will require validation on large independent prospective series.

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Authorship

Contribution: A.R. designed research and performed the SNP-array analysis; M. Mian performed statistical analysis and interpreted data; E.C. interpreted data; G.B., M.P., G.G., M.A.P., and R.D.F. provided advice and collected and characterized MZL samples; L.A., U.N., S.G., A.J.M.F., L.B., R.D.G., J.S., C.T., V.C., V.G., R.M., S.F., M. Mollejo, F. Facchetti, A.T., S.U., M.G.T., S.D., C.T., F. Forconi, and C.D. collected and characterized MZL samples; P.M.V.R. and C.P.D.C. performed statistical analysis; F.C. and E.Z. designed research and provided advice; I.K. designed research and performed statistical analysis; and F.B. designed research, analyzed and interpreted data, and wrote the manuscript.

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