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Recurrent Mutations of *MYD88* and *TBL1XR1* in Primary Central Nervous System Lymphomas

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Abstract

Purpose: Our objective was to identify the genetic changes involved in primary central nervous system lymphoma (PCNSL) oncogenesis and evaluate their clinical relevance.

Experimental Design: We investigated a series of 29 newly diagnosed, HIV-negative, PCNSL patients using high-resolution single-nucleotide polymorphism (SNP) arrays ($n = 29$) and whole-exome sequencing ($n = 4$) approaches. Recurrent homozygous deletions and somatic gene mutations found were validated by quantitative real-time PCR and Sanger sequencing, respectively. Molecular results were correlated with prognosis.

Results: All PCNSLs were diffuse large B-cell lymphomas, and the patients received chemotherapy without radiotherapy as initial treatment. The SNP analysis revealed recurrent large and focal chromosome imbalances that target candidate genes in PCNSL oncogenesis. The most frequent genomic abnormalities were (i) 6p21.32 loss (*HLA* locus), (ii) 6q loss, (iii) *CDKN2A* homozygous deletions, (iv) 12q12-q22, and (v) chromosome 7q21 and 7q31 gains. Homozygous deletions of *PRMD1*, *TOX*, and *DOCK5* and the amplification of *HDAC9* were also detected. Sequencing of matched tumor and blood DNA samples identified novel somatic mutations in *MYD88* and *TBL1XR1* in 38% and 14% of the cases, respectively. The correlation of genetic abnormalities with clinical outcomes using multivariate analysis showed that 6q22 loss ($P = 0.006$ and $P = 0.01$) and *CDKN2A* homozygous deletion ($P = 0.02$ and $P = 0.01$) were significantly associated with shorter progression-free survival and overall survival.

Conclusions: Our study provides new insights into the molecular tumorigenesis of PCNSL and identifies novel genetic alterations in this disease, especially *MYD88* and *TBL1XR1* mutations activating the NF-κB signaling pathway, which may be promising targets for future therapeutic strategies. *Clin Cancer Res; 18(19); 5203–11. ©2012 AACR.*

Introduction

Primary central nervous system lymphomas (PCNSL) are extranodal non-Hodgkin lymphomas that arise within the brain or the eyes and account for approximately 3% of all primary brain tumors. In immunocompetent patients, the vast majority of PCNSLs (90%) are classified as diffuse large B-cell lymphomas (DLBCL) according to the World Health Organization (WHO) criteria (1). In addition, they are

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

Alberto Gonzalez-Aguilar, Ahmed Idbaih, and Blandine Boisselier have contributed equally to this work.

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Translational Relevance

Biology of primary central nervous system lymphoma (PCNSL) is poorly understood because of the rarity of the disease and the scare availability of biopsy-based tumor tissue. Better knowledge of PCNSL genomic landscape would contribute to improve the management of this tumor. To this end, we conducted a comprehensive genome wide DNA profiling on the basis of single-nucleotide polymorphism–based microarray and used for the first time in PCNSL a whole-exome sequencing approach. Recurrent alterations were identified, including *MYD88* and *TBL1XR1* mutations, which represent novel potential promising targets in PCNSL for future treatments, as they both might activate the NF-κB signaling pathway. Interestingly, other alterations, that is, *CDKN2A* homozygous deletion and chromosome 6q22 loss, were correlated through multivariate analysis with poor outcome and warrant to be evaluated as prognostic biomarkers in prospective trials. Our study provides new insights into PCNSL pathogenesis and support further investigations with important clinical relevance.

Epstein–Barr virus negative, in contrast with the PCNSLs observed in immunocompromised patients. The standard initial treatment relies on high-dose methotrexate (MTX)–based polychemotherapy with or without whole-brain radiotherapy (2). Little is known about the molecular pathogenesis of PCNSL. This is probably related to the lack of biologic material to study; indeed, most PCNSL samples are obtained from stereotactic biopsies. The PCNSL patients have a median overall survival (OS) ranging from 2 to 5 years, and it is still unclear whether their less favorable outcomes compared with those of patients with systemic DLBCL are linked to the organ-specific microenvironment of the lymphoma or whether they reflect a specific, aggressive, intrinsic biologic behavior. Systemic DLBCLs have been classified into 2 main prognostic subgroups according to their gene expression profile: "germinal center B-cell-like type" (GC) and the "activated B-cell-like type" (ABC; ref. 3). We and others have previously shown that PCNSL can be distinguished from systemic DLBCL in that it expresses both GC-like and ABC-like features, suggesting that it represents an overlapping histogenetic time point of B-cell differentiation that corresponds to an early post-GC stage (4, 5). Several genes and mRNAs have been reported to be highly differentially expressed between PCNSL and systemic DLBCL (6–8). However, it remains unknown whether these expression signatures represent molecular pathways that are specific to PCNSL (9).

Studies searching for genomic alterations in PCNSL are scarce. Recently, high-resolution genomic arrays and whole-genome sequencing have been shown to be efficient approaches to achieve comprehensive analyses of chromosome imbalances and gene mutations in solid cancers and hematologic malignancies, including systemic DLBCLs (10–12). These techniques were used to investigate a series of 29 PCNSL samples in this study. Several chromosome regions and genes were identified as being frequently abnormal, suggesting that they may play important roles in PCNSL tumorigenesis. The predictive and prognostic values of these variants were analyzed.

Materials and Methods

Patients and tumor samples

A total of 29 paired PCNSL frozen tumor and blood samples were obtained from 10 institutions. All the tumors were obtained in HIV-negative patients at diagnosis and classified as CD20+DLBCLs on the basis of morphology and immunohistochemistry according to the WHO classification (1). Clinical records were reviewed retrospectively; all the patients were newly diagnosed and systemic lymphoma was excluded by extensive work-up including at the minimum a body–computer tomography (CT) scan or a positron emission tomography (PET)–scan and a bone marrow biopsy. All the patients received chemotherapy without whole brain radiotherapy (WBRT) as initial treatment according to Anocef (Association des Neuroncologues d'Expression Française) protocols. Clinical data and molecular genetic results were analyzed retrospectively. Written consent of patients was obtained for sample collection and genetic analysis in the setting of research work.

DNA isolation and SNP array

Tumor DNA from cryopreserved samples was extracted using the DNA Midi Kit (QIAGEN) according to the manufacturer's instructions. The DNA was extracted from blood sample by conventional saline method. The DNA was quantified using the NanoVue spectrophotometer and qualified on gel agarosis. Tumor DNA was run upon Infinium Illumina Human 610-Quad SNP array (Illumina). Array processing, using 250 ng of tumor DNA, was outsourced to Integragen. Extracted data using Feature Extraction software were imported and analyzed using Nexus 5.1 (Biodiscovery).

Quantitative real-time PCR

Homozygous deletions were validated using TaqMan Copy Number Assay. Primers and a FAM-labeled minor groove binder TaqMan probe testing *CDKN2A* (Hs02738179_cn), *PRDM1* (Hs02133894_cn), *DOCK5* (Hs01042688_cn), *TOX* (Hs01944817_cn), and primers and VIC-labeled minor groove binder TaqMan probe testing reference gene RNASE P (ref.: 4403326, Applied Biosystems) were used to assess copy number status. All assays were carried in duplicate on LightCycler480 Multiwell Plate 96. Gene copy number status was determined using the method of $2^{-\Delta\Delta Ct}$.

Targeted-exome sequencing

Genomic DNA was captured using Agilent in-solution enrichment methodology (SureSelect Human All Exon Kits Version 2, Agilent) with their biotinylated oligonucleotides

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probes library (Human All Exon v2-46 Mb, Agilent), followed by paired-end 75b massively parallel sequencing on Illumina GAIIX (13). Sequence capture, enrichment, and elution were conducted according to manufacturer's instructions and protocols (SureSelect, Agilent). Each eluted-enriched DNA sample was sequenced on an Illumina GAIIX as paired-end 75b reads. Image analysis and base calling was conducted using Illumina Real Time Analysis Pipeline version 1.8 with default parameters. The bioinformatics analysis of sequencing data was on the basis of the Illumina pipeline (CASAVA1.7). CASAVA conducts alignment of a sequencing run to a reference genome (hg19), calls the SNPs on the basis of the allele calls and read depth, and detects variants (SNPs and Indels). The alignment algorithm used is ELANDv2 (conducts multiseed and gapped alignments). Only the positions included in the bait coordinates are conserved. Genetics variation annotation was conducted on the basis of in-house pipeline, consisting on genes annotation (RefSeq), known polymorphisms (dbSNP 131, 1,000 Genome) followed by a mutation characterization. For each position, the exomic frequencies were determined from all the exomes already sequenced at Integragen, and the exome results provided by HapMap. We also realize a coverage/depth statistical analysis for each bait.

Sanger sequencing

Primers and conditions for PCR used for *MYD88*, *PIM1*, and *TBL1XR1* sequencing are available upon request. The PCR products were purified using NucleoFast PCR (Macherey Nagel). The Big-Dye Terminator Cycle sequencing Ready Reaction (PerkinElmer) was used for the sequencing reactions. Both forward and reverse chains were analyzed on ABI Prism 3730 Genetic Analyzer (Perkin Elmer).

Statistical analysis

Progression-free survival (PFS) and OS were calculated from the first day of treatment. The survival distributions were estimated by the method of Kaplan–Meier. The Log-Rank test was used in univariate analysis to compare PFS and OS across groups. The Cox proportional hazards model was used to estimate univariate HRs and 95% CIs presented in Table 4. Variables that were found to be significant in univariate analysis with the Log-Rank test were included in a multivariate stepwise Cox regression model. Because age and Karnofsky performance status are known to be important prognostic factors, they were also introduced in the model, although they were not significant in univariate analysis. All tests were 2-sided. *P* values less than 0.05 were considered statistically significant. All analyses were conducted with the SAS software version 9.2 (SAS Institute).

Results

Chromosome imbalances

Nine chromosome regions were recurrently imbalanced in at least 20% of the cases (Fig. 1 and Table 1). The most frequent deletion was 6p21.32 (79%) corresponding to the *HLA* locus. Three minimal common regions of deletion were identified on chromosome arm 6q: (i) 6q14.1-q16.3 (27%), (ii) 6q16.3 (37%), and (iii) 6q21.1-q25 (34%). Chromosome 12q12-q22 (27%), 7q21.11-q21.12 (20%), and 7q31.1-q31.2 (20%) were the most frequent material gained.

Homozygous gene deletion and gene amplification

CDKN2A was by far the most frequently homozygously deleted gene (13 cases, 45%). Other genes were homozygously deleted at a lower rate (Table 2): *DOCK5* (3 cases), *PRDM1* (2 cases), *TOX* (1 case). High-level amplifications involved the 7p21.1 region targeting *HDAC9* (1 case). *CDKN2A*, *TOX*, *PRMD1*, and *DOCK5* homozygous deletion were validated using qPCR.

Somatic gene mutations

High quality and large amount (3 μg) of DNA were requested for the whole-exome sequencing experiments. From the cohort of 29 samples, 4 tumor DNA and 4 matched blood DNA fulfilling these criteria were selected for targeted-exome capture. Whole-exome sequencing

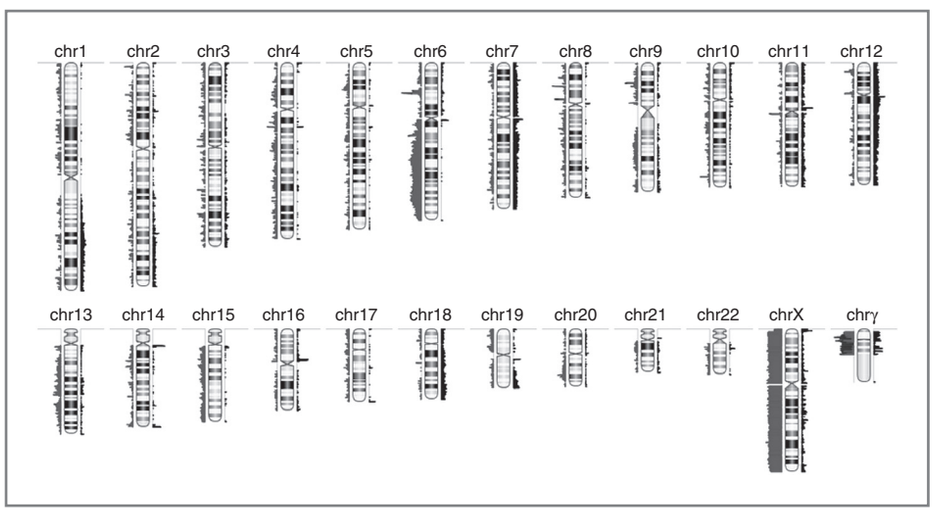

Figure 1. Overview of the genomic aberrations found in 29 PCNSL using SNP. Bars at the right of the chromosomes, gains and bars at the left, losses.

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Table 1. Most frequent chromosome imbalances in PCNSL (candidate genes are listed in all regions smaller than 5 Mb)

Chromosome region	Cytoband	Size (Mb)	Event	Cases, *n* (%)	Genes, *n*	Gene symbols
chr6:32,561,968-32,616,499	p21.32	0.1	Loss	23 (79)	1	*HLA-DRB5*
chr6:31,281,893-32,550,756	p21.33-p21.32	1.3	Loss	9 (31)	80	
chr6:32,611,800-33,081,054	p21.32	0.5	Loss	10 (34)	16	
chr6:102,757,131-104,010,647	q16.3	1.3	Loss	11 (37)	0	
chr6:77,085,561-102,662,047	q14.1-q16.3	25.6	Loss	8 (27)	84	
chr6:104,992,890-151,197,385	q21-q25.1	46.2	Loss	10 (34)	243	
chr7:83,113,802-86,268,286	q21.11-q21.12	3.2	Gain	6 (20)	4	*SEMA3E*; *SEMA3A*, *SEMA3D*, *GRM3*
chr7:113,788,480-115,513,297	q31.1-q31.2	1.7	Gain	6 (20)	3	*FOXP2*, *MDFIC*, *TFEC*
chr12:37,332,160-91,334,471	q12-q22	54.0	Gain	8 (27)	452	

revealed 33,628 base substitutions involving 8,991 genes and 3,467 insertions and deletions involving 2,683 genes in tumor DNA. SNP reported in the Hapmap, 1,000 genomes and ncbi refseq databases were removed. Comparison of tumor and matched normal lymphocyte genome showed 1,678 somatic mutations involving 1,424 genes and 71 small insertions and deletions involving 65 genes. We focused on somatic mutations changing protein-coding sequences and found in at least 2 of 4 tumors investigated. We identified 12 recurrent nonsynonymous mutations and 2 insertions involving 7 and 2 genes, respectively. Finally after Sanger sequencing, 5 somatic mutations in 3 genes (*MYD88*, *TBL1XR1*, and *PIM1*) were validated. To specify the incidence of these gene mutations, we screened the 29 frozen sample DNAs of the series by standard Sanger sequencing. Two additional mutations in *TBL1XR1* were identified in 2 cases. Altogether, *MYD88*, *TBL1XR1*, and *PIM1* showed nonsynonymous mutations in 38%, 14%, and 3% of cases, respectively (Table 3).

Correlation between molecular genetic alterations and patient outcome

The cohort comprised 15 men and 14 women, median age was 65 years (range: 23 to 81) and the median Karnofsky performance status (KPS) was 70 (range: 40 to 100). Seventeen patients had multiple enhancing lesions on MRI. All but 1 received high dose MTX–based polychemotherapy without WBRT as initial treatment: MTX 3 g/m^2–lomustine–procarbazine ± prophylactic intrathecal chemotherapy (n = 11); MTX 3.5 g/m^2-procarbazine–vincristine–cytarabine (n = 8); MTX 3.5 g/m^2–temozolomide (n = 9), temozolomide (n = 1). At the time of analysis, the median follow-up was 79 months. The 3-year PFS and OS were 40% (95% CI, 22 to 58) and 61% (95% CI, 41 to 76), respectively. Salvage treatments in the 22 relapsing or refractory patients were: chemotherapy alone (n = 12), WBRT with or without chemotherapy (n = 5), palliative care (n = 5). Age (<60 vs. ≥60 years), KPS (<70 vs. ≥70), number of enhancing lesions on MRI (single vs. multiple), initial chemotherapy regimen (with or without cytarabine), and the most frequent or relevant genomic alterations were investigated for prognostic significance: 6p21.32 loss (*HLA-DRB5* locus), 6q22 loss; 12q12-22 gain, *CDKN2A* homozygous deletions, *MYD88* and *TBL1XR1* mutations. Chromosome 6q loss is complex in our series with 3 minimal common regions of deletions. To assess its previously reported adverse prognostic significance on survival (14), we have tested the chr6:128,591,541-128,845,913 region [including

Table 2. Homozygous deletions (HD) and high copy gains (HCG) found in 29 PCNSLs

Cytoband	Genes (*n*)	Candidate gene	Gene product	Biologic function	Event	Cases (*n*)
6q21	1	*PRDM1*	PR domain zinc finger protein 1 Transcriptional repressor	B-cell differentiation into plasma cell	HD	2
8p21.2	1	*DOCK5*	Dedicator of cytokinesis 5	Guanine nucleotide exchange factor for GTPases	HD	3
8q12.1	1	*TOX*	Thymocyte selection-associated high mobility group box	B-cell differentiation	HD	1
9p21	1	*CDKN2A*	Cyclin-dependent kinase inhibitor 2A	Cell-cycle G_1 control	HD	13
7p21.1	1	*HDAC9*	Histone deacetylase 9	Chromatine condensation	HCG	1

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Table 3. Genes recurrently mutated in PCNSL

Gene	Gene product	Function/pathway	Wt codon	Mutant codon	Amino acid change	Mutation type	Exome capture ($n = 4$)	Validation exome ($n = 4$)	Whole population ($n = 29$)
MYD88	Signaling adaptor protein	NF-κB and JAK/STAT3 activation	CTG	CCG	L265P	Nonsynonymous	3	3	11
PIM1	Proviral integration site for MuLV[a]	Ser/Thr kinase	CTC	TTC	L177F	Nonsynonymous	2	1	1
TBL1XR1	Transducin β-like 1 X-linked receptor 1	Transcriptional regulation of NF-κB and Wnt	GAT	CAT	D421H	Nonsynonymous	1	1	1
			TCC	TTC	S419F	Nonsynonymous	1	1	1
			TCC	ACC	S419T	Nonsynonymous	1	1	1
			TTT	TCT	F541S	Nonsynonymous	—	—	1
			GTG	GTT	V445V	Synomynous	—	—	1

[a]Molony murine leukemia virus.

the RP11-151E20 and CTD-2378A7 BACs (14)]. In univariate analysis, only 6q22 loss ($P = 0.006$; $P = 0.006$) and *CDKN2A* homozygous deletion ($P = 0.01$; $P = 0.004$) were associated with significantly shorter PFS and OS. In multivariate analysis, age > 60 years ($P = 0.009$ and $P = 0.03$), 6q22 loss ($P = 0.006$ and $P = 0.01$), and *CDKN2A* deletion ($P = 0.02$ and $P = 0.01$) were significantly associated with poor PFS and OS, respectively (Table 4 and Fig. 2A–D).

Discussion

To our knowledge, the present study is the most comprehensive analysis of genetic alterations in PCNSL to date, using a whole-exome sequencing approach and SNP. We reported novel recurrent somatic gene mutations of *MYD88* and *TBL1XR1* in PCNSL. We identified focal genetic abnormalities with new putative candidate genes in PCNSL oncogenesis. We also confirmed and extended reports from previous studies that chromosome 6p21.32 deletion, chromosome 6q loss, and *CDKN2A* homozygous deletion are frequent genetic changes in PCNSL. Finally, the correlation of genetic profiles with clinical outcomes through multivariate analysis showed that 6q22 and *CDKN2A* deletions were associated with a significant unfavorable impact on prognostic both in term of PFS and OS.

Whole-genome sequencing strategies have successfully identified critical genes in the pathogenesis of several

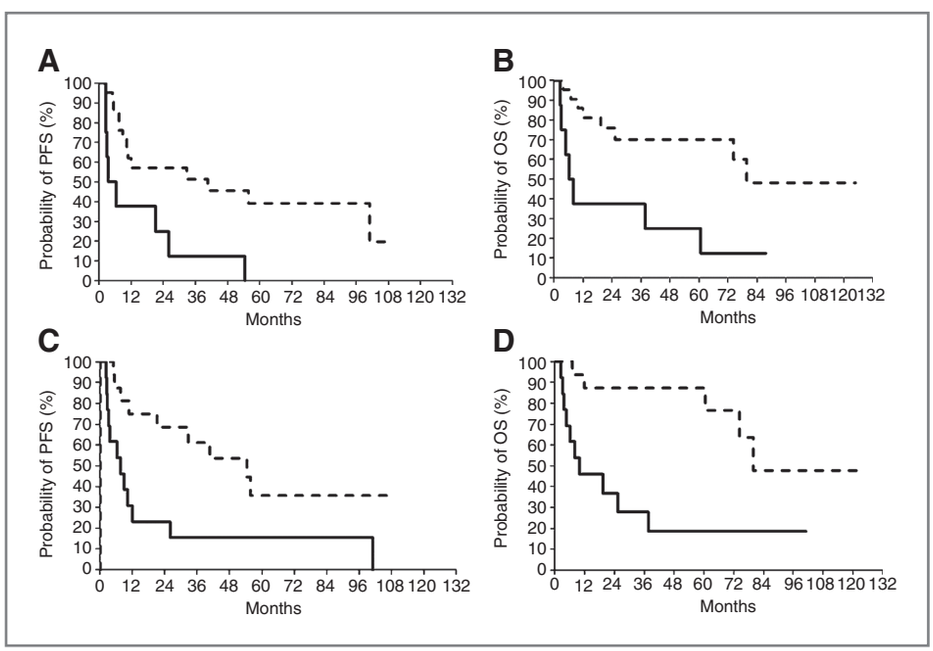

Figure 2. Kaplan–Meier curves according with: chromosome 6q22 status (loss: solid line; retained: dotted line) for (A) PFS ($P = 0.006$). B, OS ($P = 0.006$), and *CDKN2A* status (homozygous deletion: solid line; no homozygous deletion: dotted line) for (C) PFS ($P = 0.01$) and (D) OS ($P = 0.004$).

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Table 4. Univariate and multivariate analysis for PFS and OS

	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P* value (Log-Rank test)	HR (95% CI)	*P* value
PFS				
Age[a]	2.25 (0.88–5.75)	0.08	4.21 (1.42–12.50)	0.009
KPS[b]	1.22 (0.48–3.08)	0.67	—	n.s.
Chemotherapy[c]	1.07 (0.39–2.98)	0.89	—	—
6q22 loss	3.42 (1.36–8.62)	0.006	4.62 (1.52–14.01)	0.006
6p21.32 loss	2.1 (0.79–5.42)	0.13	—	—
12q12-22 gain	1.01 (0.39–2.63)	0.98	—	—
CDKN2A hd	2.99 (1.24–7.20)	0.01	3.08 (1.13–8.39)	0.02
MYD88 mut	1.44 (0.61–3.40)	0.41	—	—
*TBL1XR1*mut	2.55 (0.82–7.90)	0.09	—	—
OS				
Age[a]	2.33 (0.77–7.01)	0.13	4.26 (1.13–16.10)	0.03
KPS[b]	1.15 (0.39–3.38)	0.8	—	n.s.
Chemotherapy[c]	1.15 (0.32–4?13)	0.83	—	—
6q22 loss	3.78 (1.36–10.53)	0.006	4.45 (1.38–14.35)	0.01
6p21.32 loss	1.43 (0.40–5.26)	0.58	—	—
12q12-22 gain	1.48 (0.50–4.35)	0.47	—	—
CDKN2A hd	4.37 (1.47–12.95)	0.004	4.56 (1.35–15.30)	0.01
MYD88 mut	1.59 (0.56–4.43)	0.38	—	—
TBL1XR1 mut	2.60 (0.68–9.90)	0.16	—	—

Variables included in multivariate analysis were: age, KPS, 6q22 loss, and CDKN2A hd.

Mut, mutation; Hd, homozygous deletion; n.s., nonsignificant.

[a]Age (<60 vs. > or = 60 years).

[b]KPS (<70 vs. > or = 70 years).

[c]Chemotherapy (with vs. without high intravenous dose cytarabine).

cancers, including hematologic malignancies (12, 15). However, this approach has yet not been used to screen PCNSL. We detected *MYD88* mutations in 38% of PCNSL samples, and *MYD88* represents the most frequently mutated gene in PCNSL that is currently known. *MYD88* encodes a signaling adaptor protein that activates the NF-κB pathway after stimulation of the Toll-like receptor and the IL1 and IL18 receptors. Notably, all PCNSLs harbored the same mutation, L265P. Recently, Ngo and colleagues (16) reported the same L265P mutation in *MYD88* as a hot spot in 29% of systemic DLBCLs, although it occurred exclusively in the "activated B-cell" type (ABC). *MYD88* mutations have also been observed at lower rates in other hematologic malignancies, such as mucosa-associated lymphoid tissue lymphomas and chronic lymphocytic leukemia (15, 16). The L265P mutation resides in the Toll/IL-1 receptor (TIR) domain and would induce a gain of function by increasing NF-κB activity and enhancing JAK-STAT3 (Janus kinase-signal transducer and activator of transcription 3) signaling and interferon-β production. We also found recurrent, potentially functionally relevant, nonsynonymous somatic mutations in *TBL1XR1* in 14% of cases. *TBL1XR1* encodes transducin-β–like 1 X-linked receptor 1, which is a transcriptional regulator that interacts with nuclear hormone receptor corepressors (17) and may play a regulatory role in the NF-κB pathway and *Wnt*-mediated transcription. Braggio and colleagues (18) recently described a focal deletion on the 3q26.32 region in 3 of 7 PCNSL samples investigated. Interestingly, this region encompasses the *TBL1XR1* locus. However, to our knowledge, no sequence mutations in this gene have yet to be described in PCNSL or in systemic DLBCLs (12). Moreover, 1 tumor of our series displayed biallelic inactivation of *TBL1XR1* with a somatic mutation of 1 allele and a deletion of the other allele suggesting that *TBL1XR1* may be inactivated as a tumor suppressor gene. Recurrent deletions and mutations of *TBL1XR1* have also been reported in acute lymphoblastic leukemias (19, 20). However, the precise mechanisms by which *TBL1XR1* mutations contribute to tumorigenesis remain unclear. Indeed, *TBL1XR1* amplification has been shown in breast cancer, and its inactivation reduced tumor cell invasion in *in vitro* and *in vivo* knockdown experiments in malignant mammary cell lines (21). These observations might suggest contradictory tumor-specific roles for *TBL1XR1* mutations in oncogenesis. *PIM-1*, which is located on 6p21.2, encodes a serine/threonine kinase that may contribute to tumor progression in many cancers by promoting early transformation, cell

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proliferation and potentially angiogenesis (22). *PIM1* is a target proto-oncogene that is reported in DLBCLs with an aberrant somatic hypermutation and has been proposed as an important component of the transformation process (12). A previous study reported mutations in *PIM1* in 5 of 10 cases of PCNSL (23). In contrast, we found somatic mutations in only 2% of cases in our series. Our identification of recurrent somatic mutations in *MYD88* and *TBL1XR1* have extended the list of genes, such as *PRDM1* (24) and *CARD11* (25), that when mutated contribute to the constitutive activation of NF-κB, emphasizing the importance of this key signaling pathway in PCNSL. Hence, patients with PCNSL may be likely to benefit from treatments that inhibit the *MYD88* signaling pathway, especially those with tumors bearing the L265P mutation.

High-resolution genomic arrays (aCGH and SNP) are powerful tools for the comprehensive analysis of chromosome imbalances. To date, only 3 genomic array studies of PCNSL have been published and were limited to 7, 12, and 19 cases (refs. 18, 26, 27; Supplementary Table S1). Our study confirmed the main imbalanced region previously reported and identified novel altered region and candidate targeted genes. The most frequent changes were losses at 6p21.3 and 6q and gains at 12q12.22. The recurrent loss of chromosome 6p21.32 involving the *HLA* locus was observed in 79% of cases. This feature seems specific to DLBCLs of immune-privileged sites, including PCNSL and testicular DLBCLs, and might represent a mechanism of immune escape from CD8+ and CD4+ cytotoxic T cells via the downregulation of HLA class II expression (28, 29). However, few studies have addressed its clinical significance. In systemic DLBCLs, 6p21.32 deletion has been reported in chemoresponsive tumors (30); on the other hand, the loss of MHC class II gene and protein expression have been correlated with poor prognosis (31). In this study, we found no significant correlation between the loss of 6p21.32 (*HLA* locus) and outcome. Another recurrent aberration in PCNSL was the loss of chromosome 6q (27% to 37%), which seems to occur at a higher rate than in systemic DLBCLs, where a lower frequency has been reported (32, 33). We found 3 distinct minimal common deletion regions (MCRs), 6q14.1-16.3, 6q16.3, and 6q21-25.1. Several MCRs have been reported in previous studies (18, 34, 35), suggesting a highly complex rearrangement of this chromosome, which likely involves more than one targeted gene. Altogether, up to 4 nonoverlapping MCRs have been reported to date. Several candidate genes, such as *PRDM1* and *PTPRK*, have been identified in this region. *PRDM1*, located on 6q21, encodes a positive regulatory domain I protein with a Zinc Finger domain (BLIMP1 protein), which acts as a tumor suppressor (36). *PRDM1* inactivation would contribute to lymphomagenesis by blocking B-cell differentiation into antibody-secreting plasma cells. *PRMD1* mutations have been reported in 24% of systemic DLBCLs (37, 38) and 10% of PCNSLs (4 *PRDM1* mutations out of a total of 40 PCNSL samples screened in 2 studies; refs. 24, 26). We observed recurrent homozygous deletions of *PRDM1*, which have not been reported elsewhere to date, in 2 cases. *PTPRK*, located on 6q22-23 within a MCR refined by LOH mapping, encodes a tyrosine phosphatase protein that is involved in the regulation of cell contact and adhesion (39). A loss of *PTPRK* protein expression was observed in most of the PCNSLs tested. Further studies to identify gene mutations and/or rearrangements are needed to ascertain the involvement of *PTPRK* in PCNSL tumorigenesis. Interestingly, the loss of the *PTPRK* locus on 6q22 has been associated with a poor outcome in PCNSL patients in 2 previous studies. However, patient clinical characteristics were not available (39) or treatments not detailed (14, 39). In the present study, we observed a significantly shorter PFS and OS in PCNSL patients with 6q22 loss in multivariate analysis. The most frequent chromosome gains in our series were 12q12-22, 7q21.11-q21.12, and 7q31.1-q31.2. The minimal regions of interest on both 12q and 7q should be refined further; they overlap with loci previously reported to be gained in PCNSL and encompass many potential candidate genes (26, 29, 34).

Genomic array techniques detecting also small genomic alterations, such as gene amplifications and homozygous deletions, may identify target genes more accurately. In accordance with previous studies, *CDKN2A* was the most frequently deleted gene in PCNSL (45% of cases; refs. 18, 26, 27, 40). Epigenetic silencing has also been reported (41). *CDKN2A* is a well-known tumor suppressor gene, located on 9p21.3, which encodes a cyclin-dependent kinase inhibitor (p16) that regulates cell-cycle progression. Inactivation of *CDKN2A* has been reported in many malignancies. To our knowledge, the correlation between *CDKN2A* inactivation and outcome has been has been suggested in only 1 small study that included 14 patients (42). Our multivariate analyses confirm the negative prognostic impact of the *CDKN2A* genotype on both PFS and OS in PCNSL. Several other genes are of interest because they were found to be targeted by homozygous deletions (*TOX*, *PRDM1*, and *DOCK5*) or by amplification (*HDAC9*) in PCNSL. *TOX* is located on 8q12.1 and encodes a DNA-binding factor that is required for the development of many cell lineages in the immune system, such as CD4 (+) T cells, natural killer T cells, and regulatory T cells (43, 44) and may contribute to the observed arrest of B-cell differentiation in PCNSL. Deletions of *TOX* in one previous case of PCNSL (18) and in 4% of childhood acute lymphatic leukemias have been reported (45). *HDAC9*, which is a unique gene located in the 7p21.1 amplified region, encodes a member of the class IIa histone deacetylase (HDAC) enzyme family, which is involved in chromatin condensation and transcriptional repression (46). *HDAC9* gain has been described in squamous cell cervical carcinomas (47), and higher *HDAC9* expression has been associated with poor prognosis in childhood acute lymphatic leukemias (48). *DOCK5*, which maps to 8p21.2-p21.3, encodes the dedicator of cytokinesis 5 protein and is recurrently deleted and underexpressed in osteosarcoma (49, 50). All of these candidate genes should be further analyzed to explore their potential and specific contributions to PCNSL pathogenesis.

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In conclusion, although our study is retrospective and includes a limited number of patients, it expands our knowledge of the molecular genetic heterogeneity of PCNSL. It highlights novel genetic events with potential clinical and biologic relevance, identifies prognostic biomarkers, and supports further investigations in larger prospective studies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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