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

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 PRDM1, 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 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.
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 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-kB 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 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 Anocaf (Association des Neuronoctologistes d’Expression Francaise) 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−ΔΔCt.

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
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 \(\mu\)g) 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
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/m²–lomustine–procarbazine ± prophylactic intrathecal chemotherapy (n = 11); MTX 3.5 g/m²–procarbazine–vincristine–cytarabine (n = 8); MTX 3.5 g/m²–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

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

<table>
<thead>
<tr>
<th>Chromosome region</th>
<th>Cytoband</th>
<th>Size (Mb)</th>
<th>Event</th>
<th>Cases, n (%)</th>
<th>Genes, n</th>
<th>Gene symbols</th>
</tr>
</thead>
<tbody>
<tr>
<td>chr6:32,561,968-32,616,499</td>
<td>p21.32</td>
<td>0.1</td>
<td>Loss</td>
<td>23 (79)</td>
<td>1</td>
<td>HLA-DRB5</td>
</tr>
<tr>
<td>chr6:61,800-33,081,054</td>
<td>p21.32</td>
<td>0.5</td>
<td>Loss</td>
<td>10 (34)</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>chr6:102,757,131-104,010,647</td>
<td>q16.3</td>
<td>1.3</td>
<td>Loss</td>
<td>11 (37)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>chr6:77,085,561-102,662,047</td>
<td>q14.1-q16.3</td>
<td>25.6</td>
<td>Loss</td>
<td>8 (27)</td>
<td>84</td>
<td></td>
</tr>
<tr>
<td>chr6:104,992,890-151,197,385</td>
<td>q21-q25.1</td>
<td>46.2</td>
<td>Loss</td>
<td>10 (34)</td>
<td>243</td>
<td></td>
</tr>
<tr>
<td>chr7:83,113,802-86,268,286</td>
<td>q21.11-q21.12</td>
<td>3.2</td>
<td>Gain</td>
<td>6 (20)</td>
<td>4</td>
<td>SEMA3E; SEMA3A; SEMA3D, GRM3</td>
</tr>
<tr>
<td>chr7:113,788,480-115,513,297</td>
<td>q31.1-q31.2</td>
<td>1.7</td>
<td>Gain</td>
<td>6 (20)</td>
<td>3</td>
<td>FOXP2, MDFIC, TFEC</td>
</tr>
<tr>
<td>chr12:37,332,160-91,334,471</td>
<td>q12-q22</td>
<td>54.0</td>
<td>Gain</td>
<td>8 (27)</td>
<td>452</td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Cytoband</th>
<th>Genes (n)</th>
<th>Candidate gene</th>
<th>Gene product</th>
<th>Biologic function</th>
<th>Event</th>
<th>Cases (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6q21</td>
<td>1</td>
<td>PRDM1</td>
<td>PR domain zinc finger protein</td>
<td>B-cell differentiation</td>
<td>HD</td>
<td>2</td>
</tr>
<tr>
<td>7p21.1</td>
<td>1</td>
<td>HDAC9</td>
<td>Histone deacetylase 9</td>
<td>Chromatin condensation</td>
<td>HCG</td>
<td>1</td>
</tr>
<tr>
<td>8p21.2</td>
<td>1</td>
<td>DOCK5</td>
<td>Dedicator of cytokinesis 5</td>
<td>Guanine nucleotide exchange factor for GTPases</td>
<td>HD</td>
<td>3</td>
</tr>
<tr>
<td>8q12.1</td>
<td>1</td>
<td>TOX</td>
<td>Thymocyte selection-associated high mobility group box</td>
<td>B-cell differentiation</td>
<td>HD</td>
<td>1</td>
</tr>
<tr>
<td>9p21</td>
<td>1</td>
<td>CDKN2A</td>
<td>Cyclin-dependent kinase inhibitor 2A</td>
<td>Cell-cycle G1 control</td>
<td>HD</td>
<td>13</td>
</tr>
</tbody>
</table>

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 malignancies.
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-kB 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-kB 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-kB 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 vitro and in vivo knock-down 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

| 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             |                 |                 |                 |                 |
| Agea           | 2.25 (0.88–5.75) | 0.08             | 4.21 (1.42–12.50) | 0.009 |
| KPSb           | 1.22 (0.48–3.08) | 0.67             | –               | n.s.          |
| Chemotherapyc  | 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             | –               | –             |
| 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             | –               | –             |
| TBL1XR1mut     | 2.55 (0.82–7.90) | 0.09             | –               | –             |
| OS              |                 |                 |                 |                 |
| Agea           | 2.33 (0.77–7.01) | 0.13             | 4.26 (1.13–16.10) | 0.03 |
| KPSb           | 1.15 (0.39–3.38) | 0.8              | –               | n.s.          |
| Chemotherapyc  | 1.15 (0.32–4.71) | 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             | –               | –             |
| TBL1XR1mut     | 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.

aAge (<60 vs. > or = 60 years).
bKPS (<70 vs. > or = 70 years).
cChemotherapy (with vs. without high intravenous dose cytarabine).
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 6p21.3.2 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).

Several other genes are of interest because they represent 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 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 Ila 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.
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.

Authors' Contributions
Conception and design: A. Gonzalez-Aguilar, A. Idbaïb, A. Laurence, K. Hoang-Xuan
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Recurrent Mutations of MYD88 and TBL1XR1 in Primary Central Nervous System Lymphomas

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