The most frequent tumors in the ocular adnexa are lymphoproliferative disorders (LPDs), including malignant lymphoma and orbital inflammation with lymphoid hyperplasia or infiltration. The rate of LPDs with orbital tumors and simulating lesions is 24% to 49%. Orbital lymphoma accounts for 53% to 55% of malignant orbital tumors in adults. Most orbital lymphomas are primary low-grade B-cell non-Hodgkin lymphomas, and the most common subtype is extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT). Other ocular adnexal LPDs include several benign, noninfectious, chronic inflammatory diseases, including IgG4-related ophthalmic disease, reactive lymphoid hyperplasia (RLH), and idiopathic orbital inflammation. Immunoglobulin G4-ROD, in particular, is becoming increasingly recognized and, based on a recent study, accounts for 61% of benign ocular adnexal LPDs. Discrimination of orbital lymphoma from benign ocular adnexal LPDs is important due to the different therapeutic implications. Orbital lymphoma is amenable to low-dose radiation therapy, whereas benign ocular adnexal LPDs are expected to respond well to corticosteroid therapy.

Ocular adnexal MALT lymphomas are associated with chromosomal abnormalities, such as t(11;18)(q21;q21), t(14;18)(q32;q21), t(3;14)(p14.1;q32), trisomy 3, 16,15 18,14,16-18,19 gain of 6p, 16,17,20 21q, 16,15 loss of 6q23, 16,17 and 6q23 uniparental disomy (UPD). 16 as revealed by FISH, 14,15,18-20 comparative genomic hybridization (CGH), 17 or single-nucleotide polymorphism array (SNPA, Table 1). Differences in the chromosomal abnormalities in ocular adnexal LPDs, and the correlation between these chromosomal abnormalities and the clinical features, however, remain unknown. Govi et al. 21 reported that MALT lymphomas of conjunctival origin have a lower risk for systemic involvement than those arising in the orbit or eyelid, but previous studies did not discriminate between conjunctival and orbital MALT lymphomas. The majority of patients with ocular adnexal MALT lymphoma present with localized disease, although among those with complete staging information, < 10% cases are stage IV at the time of diagnosis due to bone marrow involvement. For management of ocular adnexal MALT lymphomas, radiotherapy provides excellent local control with a prolonged clinical course. 22-25 The disease can recur, however,
at local, initial, and distant sites. Therefore, the clinical characteristics may correlate with chromosomal abnormalities in ocular adnexal MALT lymphoma.

Single nucleotide polymorphism-A karyotyping, which is an exhaustive and high-resolution technique, allows for the detection of minute genomic abnormalities. By taking advantage of very large numbers of allele-specific probes synthesized on microarrays at high density, copy number (CN) alterations as well as allelic imbalances can be detected with high sensitivity. Most importantly, SNP-A karyotyping represents the only platform currently available for genome-wide detection of copy neutral loss of heterozygosity (CN-LOH) or UPD, which is observed commonly in cancer genomes. In the present study, we used high-resolution SNP-A to detect comprehensive genomic alterations of ocular adnexal LPDs for the discrimination of malignant lymphomas from benign ocular adnexal LPDs, including copy number variations (CNVs) and UPD, which may be associated with the clinical features and outcome.

**Materials and Methods**

**Patient and Sample Collection**

We studied 52 cases (25 men and 27 women; mean age, 59 ± 18 years; range, 22–88 years) with ocular adnexal LPDs (13 orbital MALT lymphomas [mean age, 71 ± 11 years; range, 45–85 years], 16 conjunctival MALT lymphomas [mean age, 50 ± 17 years; range, 25–79 years], 13 IgG4-ROD [mean age, 53 ± 16 years; range, 27–80 years], 4 RLH [mean age, 42 ± 20 years; range, 22–70 years], 6 diffuse large B-cell lymphomas [DLBCL; mean age, 70 ± 17 years; range, 39–88 years]) diagnosed between 2008 and 2012 at Tokyo Medical University Hospitals. Surgically resected or biopsied specimens of ocular adnexal LPDs were delivered immediately to the Cytology and Molecular Laboratory. Genomic DNA was extracted from the tumor tissue and stored immediately at −80°C until assayed.

**Diagnosis**

Ocular adnexal LPDs were diagnosed according to the pathologic features, based on immunohistochemical staining, flow cytometric analysis, and gene rearrangement analysis according to the latest World Health Organization criteria in 2008. Flow cytometric and gene rearrangement analysis were used to identify B-cell clonality by analyzing k and l ratios in a B-cell population and immunoglobulin heavy chain (IgH) gene monoclonal rearrangement, respectively. For diagnosis of IgG4-ROD, the following two main criteria were adopted: (1) serum IgG4 concentration > 135 mg/dL and (2) ratio of IgG4+ cells to IgG+ cells of 40% or above, or more than 50 IgG+ cells per high-power field (×400). Distributions of B- and T-cells in a...
lesion were evaluated by immunohistochemical staining, and a normal distribution of these cells was considered to indicate RLH. The clinical stage of the disease at diagnosis was determined by the Ann Arbor staging system and the tumor, node metastasis (TNM)–based clinical staging system based on gallium-67 (67Ga) scintigraphy, computed tomography (CT) and/or magnetic resonance imaging for all patients, complete blood count, serum lactate dehydrogenase levels, and, for the majority of patients, a CT scan of the chest and abdomen, and bone marrow biopsies.

SNP Microarray
Genomic DNA was subjected to SNP-A karyotyping using GeneChip Human Mapping 250 K SNP arrays (Affymetrix, Santa Clara, CA, USA) according to the manufacturer’s instructions, as described previously. The standard protocol included: NSP-I digestion, ligation of NSP-I adaptor oligonucleotides, polymerase chain reaction amplification using a single primer, fragmentation with DNase I, labeling with a biotinylated oligonucleotide and hybridization to the microarray, and scanning and analysis using the Copy Number Analyzer for Affymetrix GeneChip Mapping arrays (CNAG) and the hidden Markov model.

The study complied with the principles of the Declaration of Helsinki and was approved by the ethics committee of Tokyo Medical University. Informed consent was obtained from all subjects.

RESULTS

Patients
We analyzed 52 ocular adnexal LPDs, including 13 orbital MALT lymphomas, 16 conjunctival MALT lymphomas, 13 IgG4-RODs, 4 RLHs, and 6 DLBCLs. The clinical information is summarized in Tables 2 through 5.

Characteristics of CNVs in Ocular Adnexal LPDs
Copy number variations in all LPD samples are shown in Figure 1. As in our previous report, we defined CNVs as CN gains, losses, or UPD involving > 3 Mb segments (Fig. 2). In ocular adnexal MALT lymphomas, the most frequent CN gain region was trisomy 3, detected in 31% (9/29), followed by...
Comparison Between CNVs of Orbital and Conjunctival MALT Lymphomas

Next, we compared the CNVs in orbital and conjunctival MALT lymphomas. Copy number variations were detected in 77% (10/13) of orbital MALT lymphomas and in 67% (11/16) of conjunctival MALT lymphomas (Fig. 1). In addition, CN gain was more frequent in orbital MALT lymphomas 69% (9/13) than in conjunctival MALT lymphomas 50% (8/16; Fig. 1). Moreover, the number of regions containing CNVs was higher in orbital MALT lymphoma (31 regions) than in conjunctival MALT lymphoma (24 regions; Fig. 1).

The most frequent regions of CNVs differed between orbital and conjunctival MALT lymphomas (Fig. 4). Trisomy 3 was most frequent (>50%) in orbital MALT lymphomas. On the other hand, trisomy 18 was most frequent (>20%) in conjunctival MALT lymphomas (Fig. 4A). With regard to CN loss, the most frequent region of orbital and conjunctival MALT lymphomas was 9p (>15%) and 6q (>15%), respectively (Fig. 4B). In addition, the most frequent UPD region was 6q (>20%) in orbital MALT lymphomas, whereas 3q (>15%) was most frequent in conjunctival MALT lymphomas (Fig. 4C).

Discussion

Discrimination of malignant lymphoma from benign LPDs is crucial due to the different therapeutic implications. Making a differential diagnosis of malignant lymphoma from benign LPDs often is challenging. Moreover, several cases of ocular adnexal MALT lymphoma arising in the background of IgG4-ROD and IgG4-producing MALT lymphoma have been described.15–46 In this present study, we determined the CNVs of ocular adnexal LPDs using high-resolution SNP-A. Our results revealed CNVs in malignant lymphomas, including MALT lymphoma and DLBCL. In contrast, cases with benign LPDs, which include IgG4-ROD and RLH, exhibited no CNVs. Thus, SNP-A is useful for discriminating ocular adnexal MALT lymphoma from benign LPDs.

We detected CNVs in trisomy 3 (31%), 18 (17%), and gain of 6p (14%) in ocular adnexal MALT lymphomas using SNP-A. In previous studies, CNVs of ocular adnexal MALT lymphoma were detected in trisomy 3 (11%–68%), trisomy 18 (11%–56%), and gain of 6p (8%–20%) by FISH or CGH array.15–18 In addition to these CNVs, we detected 6q UPD and gain of 21q in 14% of cases of ocular adnexal MALT lymphoma. Kwee et al16 also detected trisomy 3, 18, gain of 6p and 21q, and 6q UPD in the ocular adnexal MALT lymphomas using SNP-A. Moreover, comparing orbital and conjunctival MALT lymphomas, CNVs in orbital MALT lymphomas were more frequent than those in conjunctival MALT lymphomas.

Table 3. Clinical Information and Laboratory Findings of IgG4-ROD Patients

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age, y/Sex</th>
<th>Site/Laterality</th>
<th>Clonality by FCM</th>
<th>Serum IgG4, 4.8–105 mg/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>75/F</td>
<td>LG/L</td>
<td>-</td>
<td>202</td>
</tr>
<tr>
<td>31</td>
<td>75/F</td>
<td>LG/L</td>
<td>-</td>
<td>202</td>
</tr>
<tr>
<td>32</td>
<td>67/F</td>
<td>LG/L</td>
<td>-</td>
<td>862</td>
</tr>
<tr>
<td>33</td>
<td>27/M</td>
<td>LG/R</td>
<td>-</td>
<td>1110</td>
</tr>
<tr>
<td>34</td>
<td>75/M</td>
<td>LG/L</td>
<td>-</td>
<td>229</td>
</tr>
<tr>
<td>35</td>
<td>52/F</td>
<td>LG/R</td>
<td>-</td>
<td>826</td>
</tr>
<tr>
<td>36</td>
<td>37/F</td>
<td>LG/R</td>
<td>-</td>
<td>295</td>
</tr>
<tr>
<td>37</td>
<td>56/M</td>
<td>LG/R</td>
<td>-</td>
<td>626</td>
</tr>
<tr>
<td>38</td>
<td>47/M</td>
<td>LG/R</td>
<td>-</td>
<td>262</td>
</tr>
<tr>
<td>39</td>
<td>56/M</td>
<td>LG/L</td>
<td>-</td>
<td>440</td>
</tr>
<tr>
<td>40</td>
<td>52/F</td>
<td>Orbit/R</td>
<td>-</td>
<td>348</td>
</tr>
<tr>
<td>41</td>
<td>44/F</td>
<td>LG/R</td>
<td>-</td>
<td>228</td>
</tr>
<tr>
<td>42</td>
<td>80/M</td>
<td>LG/L</td>
<td>-</td>
<td>1110</td>
</tr>
</tbody>
</table>

LG, lacrimal gland.

Table 4. Clinical Information and Laboratory Findings of RLH Patients

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age, y/Sex</th>
<th>Site/Laterality</th>
<th>Clonality by FCM</th>
<th>Serum LDH, 106–211 U/L</th>
<th>sIL-2R, 145-519 U/mL</th>
<th>Serum IgG4, 4.8–105 mg/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>43</td>
<td>42/F</td>
<td>Orbit/R</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>44</td>
<td>70/F</td>
<td>Orbit/L</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>45</td>
<td>22/M</td>
<td>Conjunctiva/R</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>46</td>
<td>35/M</td>
<td>Conjunctiva/R</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 5. Clinical Information and Laboratory Findings of DLBCL Patients

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age, y/Sex</th>
<th>Site/Laterality</th>
<th>Clonality by FCM</th>
<th>67Ga Uptake in Extraocular Organs</th>
<th>Ann Arbor Staging</th>
<th>TNM Staging</th>
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<tr>
<td>47</td>
<td>39/M</td>
<td>Conjunctiva/R</td>
<td>+</td>
<td>Maxillary sinus/L, parotid gland/R</td>
<td>II</td>
<td>T1aN2M0</td>
</tr>
<tr>
<td>48</td>
<td>80/M</td>
<td>Orbit/B</td>
<td>+</td>
<td>Submandibular lymph nodes/B</td>
<td>II</td>
<td>T2aN2M0</td>
</tr>
<tr>
<td>49</td>
<td>69/F</td>
<td>Orbit/L</td>
<td>+</td>
<td>Nasal cavity, forearm/L</td>
<td>IV</td>
<td>T3N4M1a</td>
</tr>
<tr>
<td>50</td>
<td>67/M</td>
<td>Orbit/L</td>
<td>+</td>
<td>Mediastinal, hilar lymph node/R, spleen, iliac bone/L</td>
<td>IV</td>
<td>T2aN4M1c</td>
</tr>
<tr>
<td>51</td>
<td>88/F</td>
<td>Orbit/L</td>
<td>+</td>
<td>None</td>
<td>I</td>
<td>T2bN0M0</td>
</tr>
<tr>
<td>52</td>
<td>75/M</td>
<td>Orbit/B</td>
<td>+</td>
<td>Cervical lymph node, supraclavicular lymph nodes/B</td>
<td>II</td>
<td>T2aN3M0</td>
</tr>
</tbody>
</table>
conjunctival MALT lymphomas. Ruiz et al. reported that gains in chromosome 3 have been observed most frequently in orbital rather than conjunctival ocular adnexal MALT lymphoma. Our results indicated that, in addition to gains of chromosome 3, loss of 9p and 6q UPD also are more frequent in orbital MALT lymphomas than in conjunctival MALT lymphoma. Previous studies reported that MALT lymphomas of conjunctival origin have a lower risk of systemic involvement than those arising in the orbit or eyelid. Our results are consistent with this report. Thus, the frequency of CNVs might be lower in conjunctival MALT lymphomas than in orbital MALT lymphoma.

The pathogenesis of MALT lymphoma is largely unknown. In gastric MALT lymphoma, *Helicobacter pylori* has been

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**Figure 1.** Distribution of CNVs in ocular adnexal LPDs. Genetic lesions are color-coded and plotted for each sample, as indicated. Regions with frequent CNVs are indicated by arrows (arrows, CN gains, losses, and UPDs ≥20% in MALT lymphomas, ≥50% in DLBCLs). Note that genetic changes involving small regions are lacking in this Figure due to limited resolution. Pink indicates CN gains, blue CN loss, and green UPD.
shown to be the causative agent in almost all cases. On the other hand, in ocular adnexal MALT lymphoma, *Chlamydo- phila psittaci* infection has been suggested. In this study, the association between *Chlamydo phila* infection and CNVs was not identified. In addition, Nakayama et al. reported that IgG4-ROD can predispose to the development of ocular adnexal MALT lymphoma. A difference in “gene expression” between IgG4+ and IgG4- orbit adipose tissues also was reported. However, there were no CNVs of IgG4-ROD patients in this study. Therefore, further studies are needed to reveal the association between IgG4-ROD and ocular adnexal MALT lymphoma.

![Figure 2](https://arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/934118/)
Figure 3. Representative cases (Patient No. 8) of orbital MALT lymphoma with CNVs. (A) Patient 8. Left orbital MALT lymphoma case. (B) Axial gadolinium-enhanced T1-weighted magnetic resonance image shows a mass lesion in the left orbit. (C) Scintigraphy with $^{67}$Ga showing uptake in the left orbit, mediastinum, bilateral thighs, retroperitoneal space, and right supraclavicular fossa. (D) Copy number variations of chromosomes 3, 6, and 18 generated by CNAG software. The CNAG software is freely available (available in the public domain at http://www.genome.umin.jp/). This patient showed CNVs, including trisomy 3, gain of 6p and 18q, and 6q UPD.
Patients with conjunctival and orbital MALT lymphomas can have poor or good clinical outcomes without any treatment after pathologic diagnosis by biopsy. Patients with a good clinical outcome without treatment might be those with fewer or no CNVs in our study. In addition, DLBCL of the ocular adnexal region is characterized by aggressive growth and poor outcome compared to ocular adnexal MALT lymphoma. The frequency of CNVs was lower in patients with ocular adnexal MALT lymphomas than in DLBCL patients. Therefore, the clinical outcome also might be better for patients with ocular adnexal MALT lymphomas than for patients with DLBCL.

There are several limitations to this study. First, our study included only a small sample size. A larger number of patients should be examined in multicenter studies. Second, we examined systemic involvement with $^{67}$Ga scintigraphy. Fluorodeoxyglucose positron emission tomography is reported to have significantly higher site and patient sensitivity than $^{67}$Ga scintigraphy. Thus, patients in whom systemic involvement was not detected by $^{67}$Ga scintigraphy did not necessarily lack systemic involvement.

In conclusion, SNP-A is a useful method for discriminating ocular adnexal lymphomas from benign LPDs based on a combined analysis of the pathology and analysis of the gene rearrangement. Even among cases of ocular adnexal lymphomas with the same histopathologic diagnosis, some exhibit different chromosomal abnormality patterns. Thus, differences in the chromosomal abnormality patterns may reflect the activity of ocular adnexal LPDs.
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References


