Clinicopathological features of 49 primary gastrointestinal diffuse large B-cell lymphoma cases; comparison with location, cell-of-origin, and frequency of MYD88 L265P

Authors

Keina Nagakita[1], Katsuyoshi Takata[1], Kohei Taniguchi[1], Tomoko Miyata-Takata[1], Yasuharu Sato[1], Akira Tari[2], Nobuhiko Ohnishi[1], Mai Noujima-Harada[1], Shizuma Omote[1], Naoya Nakamura[3], Masaya Iwamuro[4], Yoshinobu Maeda[5], Hiroyuki Okada[4], Mitsune Tanimoto[5], and Tadashi Yoshino[1]

Affiliations

[1] Department of Pathology, Okayama University, Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama, Japan

[2] Department of Internal Medicine, Hiroshima Red Cross Hospital and Atomic-Bomb Survivors Hospital, Hiroshima, Japan

[3] Department of Pathology, Tokai University School of Medicine, Isehara, Kanagawa, Japan

[4] Department of Gastroenterology and Hepatology, Okayama University Hospital, Okayama,

Japan

[5] Department of Hematology and Oncology, Okayama University Hospital, Okayama, Japan.

Short title: MYD88 L265P in GI DLBCL

Corresponding author:

Katsuyoshi Takata, M.D., Ph.D.

Department of Pathology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences

2-5-1 Shikata-cho, kita-ku, Okayama City, Okayama 700-8558, Japan

Tel: (+81)-86-235-7150

Fax: (+81)-86-235-7156

E-mail: katsuyoshi.t@h5.dion.ne.jp

Abstract

The gastrointestinal (GI) tract is the most common primary site of extranodal diffuse large B-cell lymphoma (DLBCL), approximately one-third of extranodal DLBCL occurred in the GI tract. We investigated the clinicopathological features and immunohistochemically-assessed cell-of-origin of 49 GI DLBCL (stomach: 24, small intestine: 10, colon: 15) and also examined the presence of MYD88 L265P as recently this mutation has been frequently identified in ABC-like DLBCL, particularly in extranodal sites. Small intestinal DLBCL was characterized by preponderance of women (P = 0.041) and elevated LDH (P = 0.002) and soluble interleukin-2 receptor (P = 0.033). Small intestinal DLBCL more frequently showed anemia (P = 0.031) and elevated CRP (P = 0.029) than gastric DLBCL. ABC-like phenotype was seen in 71.4% cases (stomach: 79%, small intestine: 70%, colon: 60%). MYD88 L265P was detected in 6.1% cases; all were primary gastric DLBCL with ABC-like phenotype but had no distinct clinicopathological features. In conclusion, GI DLBCL had different clinicopathological features according to the primary site especially in small intestine. And MYD88 L265P had little involvement in GI DLBCL compared with other extranodal DLBCLs, suggesting that its pathogenesis might be different from that of organs with a high frequency of MYD88 L265P.

Key words: diffuse large B-cell lymphoma, gastrointestinal tract, MYD88 L265P

INTRODUCTION

The gastrointestinal (GI) tract is the most common primary site of extranodal diffuse large B-cell lymphoma (DLBCL).[1] From our consultation files (1989–2014), approximately one-third of extranodal DLBCL occurred in the GI tract, with DLBCL being the most common subtype in the small intestine and large intestine and the second most common subtype in the stomach and duodenum, following mucosa-associated lymphoid tissue (MALT) lymphoma and follicular lymphoma, respectively.[2]

From comprehensive gene expression profiles, DLBCL was divided into activated B-cell (ABC)-like or germinal center B-cell (GCB)-like phenotype.[3,4] In addition, an immunohistochemical algorithm was generated to classify ABC/ non-GCB-like or GCB-like phenotype.[5,6] The ratio of ABC/ non-GCB-like and GCB-like phenotypes in GI DLBCL varied according to the sites of the GI tract. Specifically, the frequency of ABC/ non-GCB-like DLBCL was 42%–73% in the stomach, 40% in the duodenum, 6%–14% in the small intestine, and 57% in the large intestine.[7-11]

Recently, ABC/ non-GCB-like DLBCL has been reported to harbor several gene mutations related to the NFκB pathway, such as the *myeloid differentiation primary response protein 88* (*MYD88*), *TNFAIP3*, and *CARD11*.[12] MYD88 is an adaptor protein that binds to the

intracellular domain of toll-like/IL-1 receptors to form a dimer. This dimer phosphorylates IRAK1 and IRAK4 before activating the NFκB pathway via TRAF6 and TAK1. MYD88 L265P is the most frequent mutation and is known to be a gain of function mutation that contributes to tumor cell growth.[13]

MYD88 L265P was reported to be frequently identified in ABC/ non-GCB-like DLBCL (21.6%–31.2%) compared with GCB-like DLBCL (6.0%–9.7%).[13-17] Recent reports have revealed that MYD88 L265P was frequently detected in primary testicular DLBCL (68%–71%), primary central nervous system (CNS) DLBCL (75%–94%), and primary breast DLBCL (58.7%), where the ABC/ non-GCB-like phenotype dominated.[14, 18-20]

In primary GI DLBCL, the frequency of MYD88 L265P and its relationship with clinicopathological features have not been well studied to date. The GI tract, which has its own lymphoid tissues, has immunologically-specific environments where antigen stimuli are fulminant. In the present study, we aimed to investigate the clinicopathological features and immunohistochemically-assessed cell-of-origin and to examine the frequency and clinicopathological relationship of MYD88 L265P with primary GI DLBCL.

MATERIALS AND METHODS

Patient Selection

Cases were selected from the consultation files of our institution and our collaborating hospitals from 2001 to 2014. Overall, 49 cases were recruited; 24 in the stomach, 10 in the small intestine, and 15 in the colon (ileocecal: 3). Cases with Stage IV by Lugano classification[21] and those who had larger lesions in sites other than the GI tract were excluded from this study. Case 3 and 6 were clinically diagnosed and treated as primary GI DLBCL which was less than Stage 3. All cases were diagnosed by expert hematopathologists (KT, TM-T, and TY) according to the WHO classification 2008.[1] 40 biopsies and 9 resected materials were used in this study. No material after chemotherapy was included. The study protocol was approved by the Institutional Review Board of Okayama University, Okayama, Japan. All study procedures were conducted in accordance with the guidelines of the Declaration of Helsinki.

DNA Extraction, Polymerase Chain Reaction (PCR), and Sanger Sequencing

DNA was extracted from 10% formalin-fixed paraffin-embedded tissues (FFPET) using QIAGEN DNeasey kit (QIAGEN, Venlo, Netherland), according to the manufacturer's instructions. The extracted DNA was amplified by semi-nested PCR using AmpliTaq Gold

360 PCR Master MIX (Applied Biosystems, CA, USA). The primer sequences for this study were as follows: *MYD88*-1F (5′-GTTGAAGACTGGGCTTGTCC-3′), *MYD88*-6R (5′-GGTTGGTGTAGTCGCAGACA-3′), and *MYD88*-7R (5′-GTGCAGGGGTTGGTGTAGTC-3′). PCR and Sanger sequencing were performed as previously reported.[20]

Immunohistochemistry

Immunohistochemical analysis was performed on FFPET using Bond-Max Autostainer (Leica Biosystems, Melbourne, Australia), according to the manufacturer's instructions. The primary antibodies used are shown in Table S1. The cutoff values were set as follows: 80% for GCET1, FOXP1, and MUM1, and 70% for c-MYC, and 30% for BCL6 and CD10. We classified the cases into ABC-like or GCB-like DLBCL, and non-GCB-like or GCB-like DLBCL, according to Choi's and Hans' algorithms, respectively.[6] Ki-67 labeling index was evaluated by counting the average of 1220 (822–1642) cells in each case using Patholoscope (MITANI CORPORATION, Fukui, Japan).

Statistical analysis

Chi-square test and Fisher's exact test were performed using the Statcel3 (OMS publishing Inc., Saitama, Japan). Statistical significance was defined as $P < 0.05$.

RESULTS

Characteristics of Patients with GI DLBCL

The clinicopathological characteristics of patients with GI DLBCL are shown in Table 1 and Table S2; 24 patients were men and 25 patients were women; the age ranged from 47 to 92 (median, 74) years. B symptoms were observed in 10 of 35 cases (28.6%). LDH level was elevated in 20 of 49 patients (40.8%). An increase in soluble interleukin-2 receptor (sIL-2R) more than 1000 U/ml was seen in 19 of 45 patients (42.2%). Anemia (14/49, 28.6 %), thrombocytopenia (4/49, 8.2 %), hypoalbuminemia (13/47, 27.7%), and CRP elevation (21/45, 46.7%) were observed. Among 49 patients in this study, 17 were at Stage I, 21 were at Stage II1, 7 were at Stage II2, 2 were at Stage IIE; and 2 were unknown, according to Lugano classification. One case (Case 22) with gastric DLBCL Stage IIE had metastasis to a gastric lymph node and direct invasion to the spleen. Another case (Case 49) with rectal DLBCL had direct invasion to the urinary bladder. International Prognostic Index (IPI) information was 0–2 in 34 cases and 3–5 in 3 cases. *Helicobacter pylori* infection was

detected in 10 of 25 cases (40%). Eight out of 10 small intestinal cases had surgical resection at the time of diagnosis or in the clinical course, in contrast to the gastric cases, none of which had surgical resection. Among 8 surgical resected cases, 6 cases had emergency resection for obstruction or peritonitis. One case had prophylactic resection before chemotherapy and the other case had surgical resection for the obstruction during chemotherapy. In colonic DLBCL, 8 out of 15 cases had surgical resection. Three cases underwent operation at the time of diagnosis, 5 cases had resection due to perforation, obstruction or hemorrhage during chemotherapy.

Therapeutic information was obtained from 47 cases; 42 patients (89.4%) underwent chemotherapy (39 patients received rituximab combination therapy). Eleven patients (22.4%) underwent radiation therapy after chemotherapy. One patient (2.1%) underwent rituximab monotherapy. One patient (2.1%) was observed without any therapy. Surgical resection was performed on 12 patients (small intestine: 6, colon: 6). Three patients died from postoperative complications and did not receive any chemotherapy. Two patients (small intestine: 1, colon: 1) died of DLBCL, whereas nine patients (stomach: 5, small intestine: 3, colon: 1) died of causes other than lymphoma.

Comparison of the clinicopathological characteristics among gastric, small intestinal, and

colonic DLBCLs are shown in Table 1. Females sex ($P = 0.041$) and elevation in levels of LDH ($P = 0.002$) and sIL-2R ($P = 0.033$) were frequently seen in small intestinal DLBCL. Compared with gastric DLBCL, small intestinal DLBCL more frequently showed anemia ($P = 0.031$) and elevation of CRP level ($P = 0.029$), although there was no significant difference when compared with colonic DLBCL.

Histology and Immunophenotype of GI DLBCL

All cases demonstrated diffuse proliferation of large lymphoid cells with CD20+ and CD3−. The Ki-67 labeling index was 68.3% on average (range, 35%–90%) (Fig. 1 and Table 2). CD10 was positive in 22 of 49 cases (44.9%); nine in the stomach, four in the small intestine, and nine in the colon. CD5 was positive in 2 of 49 cases (4.1%); one was in the stomach and the other was in the small intestine. c-MYC was positive in 11 of 47 cases (23.4%); six in the stomach, three in the small intestine, and two in the colon. The results by Choi's algorithm[6] are shown in Table 2; 35 of 49 cases (71.4%) showed ABC-like phenotype, whereas 14 of 49 cases (28.6%) showed GCB-like phenotype. The ratio of ABC-like/ GCB-like phenotype was 19/5 for the stomach, 7/3 for the small intestine, and 9/6 for the colon.

Detection of MYD88 L265P

MYD88 L265P was detected in only 3 of 49 cases (6.1%) (Fig. 2); all of them were primary gastric DLBCL with ABC-like phenotype. Moreover, MYD88 L265P was not detected in primary small intestinal or colonic DLBCL. Comparison of the clinicopathological features between wild type and MYD88 L265P cases of primary gastric DLBCL showed that there was no distinct clinicopathological difference between the two (Table S3). In addition, there was no case in present series including 3 MYD88 L265P cases, which had MALT lymphoma or lymphoplasmacytic lymphoma in the background or other sites of DLBCL.

Comparison of MYD88 L265P frequency with Other Extranodal DLBCLs

MYD88 L265P was reported to be frequently detected in extranodal DLBCL where the ABC-like phenotype dominated, such as testis CNS breast and skin.[14,18-20,22] We re-classified GI DLBCL by Hans' algorithm and compared with other organs of the previous studies. As a result, non-GCB-like DLBCL in the GI DLBCL was 49%(24/49) [58%(14/24) in the gastric one, 40%(4/10) in the small intestinal one, and 40%(6/15) in the colonic one]. The frequency of MYD88 L265P in non-GCB like DLBCL was 8.3%, which was significantly rare compared with other extranodal DLBCLs (57.1-100%) ($P < 0.001$). (Table S4 and Fig. 3)

Clinicopathological Characteristics of the Cases with MYD88 L265P

The clinicopathological features of the patients with MYD88 L265P (Cases 1, 13, and 14) are shown in Table 3; the average age of the patients was 74 years; two cases were men and the remaining one was a woman. Two cases (Cases 13 and 14) were Stage I; whereas Case 1 was Stage II2. Involvement of gastric, iliac arterial, and para-aortic lymph nodes was observed in Case 1. All cases showed type 2-like gross appearance of the tumor. *H. pylori* infection was detected in Case 1.

Anemia, thrombocytopenia, hypoalbuminemia, and LDH elevation were not seen in any of the cases. In Cases 1 and 13, sIL-2R was less than 1000 U/ml. IPI was low to low-intermediate (0–2) in all cases. All patients underwent chemotherapy with rituximab. Case 1 underwent radiation therapy after chemotherapy. All patients achieved complete remission (CR) and were alive without recurrence during the follow-up period. In the immunostaining, CD5 was negative in all cases, CD10 was positive in Case 1, and c-MYC was positive in Case 13.

DISCUSSION

Recent reports showed that MYD88 L265P was detected in 59%–61% of primary testicular

DLBCL, in 75%–94% of primary CNS DLBCL, and in 68%–71% of primary cutaneous DLBCL leg type.[13,18,19,22,23] Recently, we reported that MYD88 L265P was detected in 58.7% of primary breast DLBCL.[20] One report mentioned an 11% (2/18) frequency of MYD88 L265P in GI DLBCL, but the percentage of mutation and clinicopathological correlations in each GI organ were unknown.[14]

In this study, the frequency of MYD88 L265P was 6.1%, and all mutated cases were gastric DLBCL. Previously, MYD88 L265P was frequently identified in the organs where ABC-like/ non-GCB-like phenotype dominated. On the other hand, GI DLBCL, as we mentioned in the Results, was on the line, half of which(49%) was non-GCB-like phenotype by Hans' algorithm and 71% of which was ABC-like phenotype by Choi's algorithm. At any rate, it is noted that when Hans was applied, the frequency of MYD88 L265P in non-GCB-like DLBCL was also significantly low ($P < 0.001$) compared with other extranodal DLBCLs. We think that this suggested the uniqueness of the GI DLBCL. (Table S4 and Fig. 3) These results suggested that GI DLBCL develops via different pathways of lymphoma genesis compared with DLBCL at other sites wherein MYD88 L265P is frequently detected. The GI tract is known to be always exposed to antigen stimuli; in fact, it is suggested to be an immunologically-specific environment. Although this could partly explain the low mutation

rate in this study, some other organ-specific factors might be contributory because MYD88 L265P has been frequently detected in primary cutaneous DLBCL leg type, [22,23] which has an antigen stimulation that is similar to that on the GI tract. This hypothesis remains to be discussed in the future.

In this study, only three cases had MYD88 L265P and had relatively low tumor burden and low IPI. All patients achieved CR after chemotherapy and remained alive without relapse; however, there was no distinct clinicopathological factor that indicated a worse prognosis between MYD88 L265P and wild-type cases. In a review of previous studies, there was no consensus about the correlation of MYD88 L265P with the prognosis of DLBCL. It was reported that MYD88 L265P was a prognostic factor for primary cutaneous DLBCL leg type[22,23] but not for primary CNS DLBCL and primary breast DLBCL.[19,20] Therefore, the prognostic value of MYD88 L265P may depend on the organ involved in DLBCL. Because L265P is the most frequent mutation site, we examined only L265P in this study. It is possible that mutations of MYD88 other than L265P was seen in GI DLBCL, but this issue needs further study. So far, several kinds of primers and detection methods were used in previous reports. Allele-specific PCR (AS-PCR) was adopted in some papers[14, 18, 21], on the other hand, Sanger sequencing was adopted in others[15, 16, 17, 19, 20, 22, 23] including present study although

the primers were different among them. We used the same primers and protocols of our previous study[20]. There is a possibility of difference in MYD88 L265P frequency that is caused by the sensitivity of methods. But as our group reported before[20], the sensitivity of the AS-PCR for the Sanger sequencing was 92.6%, the specificity was 100%, and then the concordance rate was high between AS-PCR and Sanger sequencing. Therefore, we think that the low prevalence of MYD88 L265P in GI DLBCL could be attributed to the feature of GI DLBCL rather than the detection protocol itself. However, it needs to be assessed by other methods in the future.

In primary GI tract DLBCL, the frequency of ABC-like/ non-GCB-like DLBCL was reported to be 42%–73% in the stomach, 6%–14% in the small intestine, and 57% in the colon.[7-9,11] In this study, the frequency of ABC-like DLBCL was 79% (19/24) and 58% (14/24) in the stomach, 70% (7/10) and 40% (4/10) in the small intestine, and 60% (9/15) and 40% (6/15) in the colon by Choi's and Hans' algorithm, respectively. In this study, the frequency of ABC-like/ non-GCB-like DLBCL in the small intestine was higher than that in previous reports. The proportion of non-GCB-like phenotype in small intestinal DLBCL was reported as 6% and 14%,[9,11] both of which were according to Hans' algorithm.[5] The discrepancy in the percentage of ABC-like phenotype between previous reports and our study could be caused

by differences in the positivity of CD10 among these studies. The positivity of CD10 was reported by previous studies to be 75%–86%;[9,11] however, in this study, only 40% was positive for CD10. This may be considered as the most significant factor for the higher ABC-like DLBCL cases in this study than in previous reports.

When focusing on the primary site, primary small intestinal DLBCL showed significant LDH elevation ($P = 0.002$), high sIL-2R level ($P = 0.033$) and high necessary rate of operation compared with the other sites. This result might be reflective of the size of the tumor at the time of diagnosis. In gastric DLBCL, 11 of 24 cases had localized lesion without lymph node metastasis. However, in small intestinal DLBCL, 8 of 10 cases had lymph node metastasis. Furthermore, patients with small intestinal DLBCL had more frequent presentations of anemia ($P = 0.031$) and high CRP level ($P = 0.029$) than patients with gastric DLBCL. We deduced that patients with small intestinal DLBCL were prone to present with obstructive symptoms, probably because of a narrow lumen size and larger tumor volume at diagnosis. It is pointed that small intestinal DLBCL tended to be diagnosed at the progressed state of tumor even in the localized stage since the endoscopic exam was difficult. Although no gastric case had surgical resection, 8 out of 10 small intestinal cases and 8 out of 15 colonic cases had surgical resection. The most common cause of resection was obstruction for small

intestinal cases (6 cases) and complication for chemotherapy for colonic ones (5 cases). These need to be kept in mind in the clinical practice.

In conclusion, GI DLBCL had different clinicopathological features according to the primary site especially in small intestine. And MYD88 L265P had little involvement in GI DLBCL compared with other extranodal DLBCLs, suggesting that its pathogenesis might be different from that of organs with a high frequency of MYD88 L265P. A large cohort study is needed to confirm our results, and further molecular analysis might clarify the specific nature of GI DLBCL.

Disclosure Statement: None Declared.

REFERENCES

1 Swerdlow SH, Campo E, Harris NL, *et al*. *WHO classification of tumours of haematopoietic and lymphoid tissues*, 4th edn. Lyon: International Agency for Research on Cancer, 2008.

2 Nakamura S, Matsumoto T, Iida M, Yao T, Tsuneyoshi M. Primary gastrointestinal lymphoma in Japan: a clinicopathologic analysis of 455 patients with special reference to its time trends. *Cancer*. 2003; **97**: 2462-73.

3 Alizadeh AA, Eisen MB, Davis RE, *et al*. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature*. 2000; **403**: 503-11.

4 Rosenwald A, Wright G, Chan WC, *et al*. The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma. *N Engl J Med*. 2002; **346**: 1937-47.

5 Hans CP, Weisenburger DD, Greiner TC, *et al*. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. *Blood*. 2004; **103**: 275-82.

6 Choi WW, Weisenburger DD, Greiner TC, *et al*. A new immunostain algorithm classifies

diffuse large B-cell lymphoma into molecular subtypes with high accuracy. *Clin Cancer Res*. 2009; **15**: 5494-502.

7 Zhang Z, Shen Y, Shen D, Ni X. Immunophenotype classification and therapeutic outcomes of Chinese primary gastrointestinal diffuse large B-cell lymphoma. *BMC Gastroenterol*. 2012; **12**: 77.

8 Zizhen Z, Hui C, Yanying S, *et al*. Correlation between immunophenotype classification and clinicopathological features in Chinese patients with primary gastric diffuse large B-cell lymphoma. *Pathol Oncol Res*. 2013; **19**: 317-22.

9 Mitchell KA, Finn WG, Owens SR. Differences in germinal centre and non-germinal center phenotype in gastric and intestinal diffuse large B-cell lymphomas. *Leuk Lymphoma*. 2008; **49**: 1717-23.

10 Tamura M, Takata K, Sato Y, *et al*. Germinal center B-cell-like diffuse large B-cell lymphoma of the duodenum is associated with t(14;18) translocation. *Pathol Int*. 2011; **61**: 742-8.

11 Connor J, Ashton-Key M. Gastric and intestinal diffuse large B-cell lymphomas are clinically and immunophenotypically different. An immunohistochemical and clinical study. *Histopathology*. 2007; **51**: 697-703.

12 Jardin F. Next generation sequencing and the management of diffuse large B-cell lymphoma: from whole exome analysis to targeted therapy. *Discov Med*. 2014; **18**: 51-65.

13 Ngo VN, Young RM, Schmitz R, *et al*. Oncogenically active MYD88 mutations in human lymphoma. *Nature*. 2011; **470**: 115-9.

14 Kraan W, Horlings HM, van Keimpema M, *et al*. High prevalence of oncogenic MYD88 and CD79B mutations in diffuse large B-cell lymphomas presenting at immune-privileged sites. *Blood Cancer J*. 2013; **3**: e139.

15 Choi JW, Kim Y, Lee JH, Kim YS. MYD88 expression and L265P mutation in diffuse large B-cell lymphoma. *Hum Pathol*. 2013; **44**: 1375-81.

16 Kim Y, Ju H, Kim DH, *et al*. CD79B and MYD88 mutations in diffuse large B-cell lymphoma. *Hum Pathol*. 2014; **45**: 556-64.

17 Bohers E, Mareschal S, Bouzelfen A, *et al*. Targetable activating mutations are very frequent in GCB and ABC diffuse large B-cell lymphoma. *Genes Chromosomes Cancer*. 2014; **53**: 144-53.

18 Kraan W, van Keimpema M, Horlings HM, *et al*. High prevalence of oncogenic MYD88 and CD79B mutations in primary testicular diffuse large B-cell lymphoma. *Leukemia*. 2014; **28**: 719-20.

19 Yamada S, Ishida Y, Matsuno A, Yamazaki K. Primary diffuse large B-cell lymphomas of central nervous system exhibit remarkably high prevalence of oncogenic MYD88 and CD79B mutations. *Leuk Lymphoma*. 2015; **49**: 2141-5.

20 Taniguchi K, Takata K, Chuang SS, *et al*. Frequent MYD88 L265P and CD79B Mutations in Primary Breast Diffuse Large B-Cell Lymphoma. *Am J Surg Pathol*. 2016; **40**: 324-34.

21 Zucca E, Roggero E, Bertoni F, Cavalli F. Primary extranodal non-Hodgkin's lymphomas. Part 1: Gastrointestinal, cutaneous and genitourinary lymphomas. *Ann Oncol*. 1997; **8**: 727-37.

22 Pham-Ledard A, Prochazkova-Carlotti M, Andrique L, *et al*. Multiple genetic alterations in primary cutaneous large B-cell lymphoma, leg type support a common lymphomagenesis with activated B-cell-like diffuse large B-cell lymphoma. *Mod Pathol.* 2014; **27**:402-11.

23 Pham-Ledard A, Beylot-Barry M, Barbe C, *et al*. High frequency and clinical prognostic value of MYD88 L265P mutation in primary cutaneous diffuse large B-cell lymphoma, leg-type. *JAMA Dermatol*. 2014; **150**: 1173-9.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1 Primary antibodies used in this study.

Table S2 The clinical information of gastrointestinal DLBCL cases.

Table S3 Relationship between presence of MYD88 L265P and clinicopathological factors in gastric DLBCL

Table S4 The percentage of non-GCB-like DLBCL and MYD88 L265P in extranodal DLBCLs

FIGURE LEGENDS

Figure 1. The hematoxylin–eosin (HE) staining and immunohistochemical panel

(a) On HE staining, diffuse infiltration of large lymphoid cells is shown (Case 5). Immunohistochemical panel is (b) CD5-positive (Case 3), (c) CD10-positive (Case 15), (d) GCET1-positive (Case 5), (e) MUM1-positive (Case 5), (f) BCL6-positive (Case 23), (g) FOXP1-positive (Case 23), (h) c-MYC-positive (Case 23), and (i) Ki-67 labeling index is 62% (Case 5)

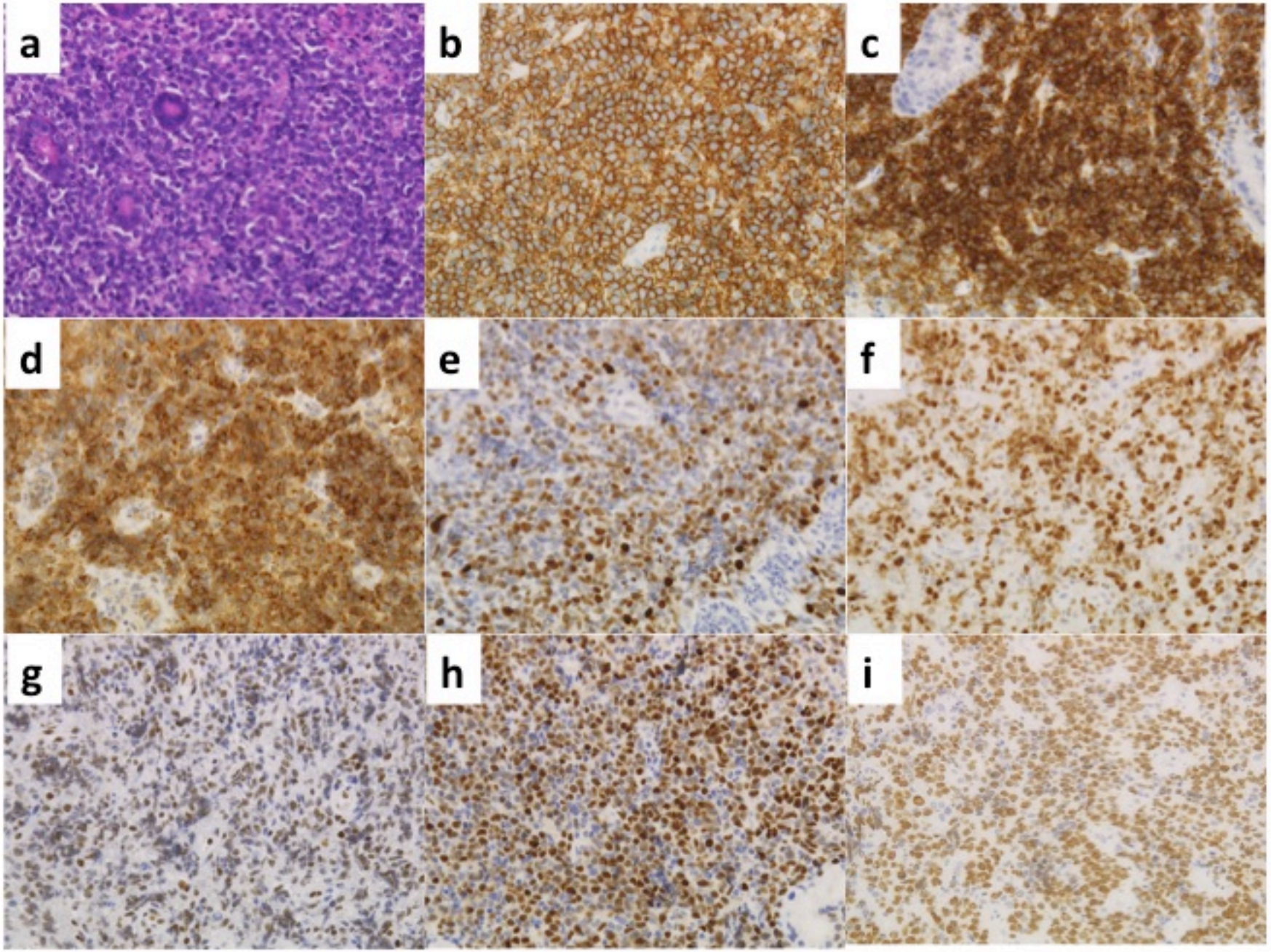
Figure 2. Sanger sequencing for MYD88 L265P

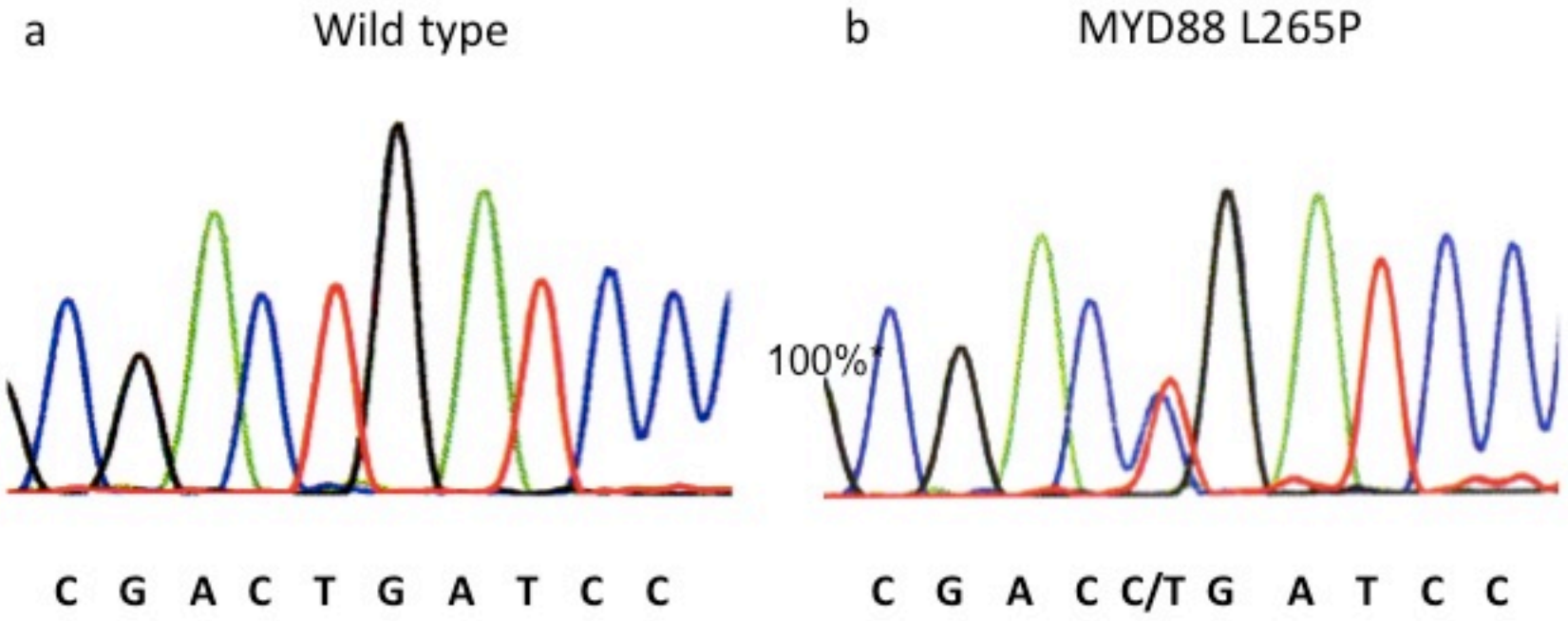
(a) MYD88 wild type (Case 23) and (b) MYD88 L265P (Case 13)

Figure 3. Presence of MYD88 L265P in extranodal diffuse large B-cell lymphomas (DLBCLs)

The percentage of non-GCB-like phenotype in extranodal DLBCLs was shown. White column shows wild type cases and black column shows MYD88 L265P cases. MYD88 L265P in non-GCB-like DLBCL was significantly rare in gastrointestinal DLBCL compared with other extranodal DLBCLs ($P < 0.001$). * the ratio of MYD88 L265P in non-GCB-like

DLBCL


a
b
c
d
e
f
g
h
i


a
Wild type
b
MYD88 L265P
100%
C G A C T G A T C C
C G A C C/T G A T C C

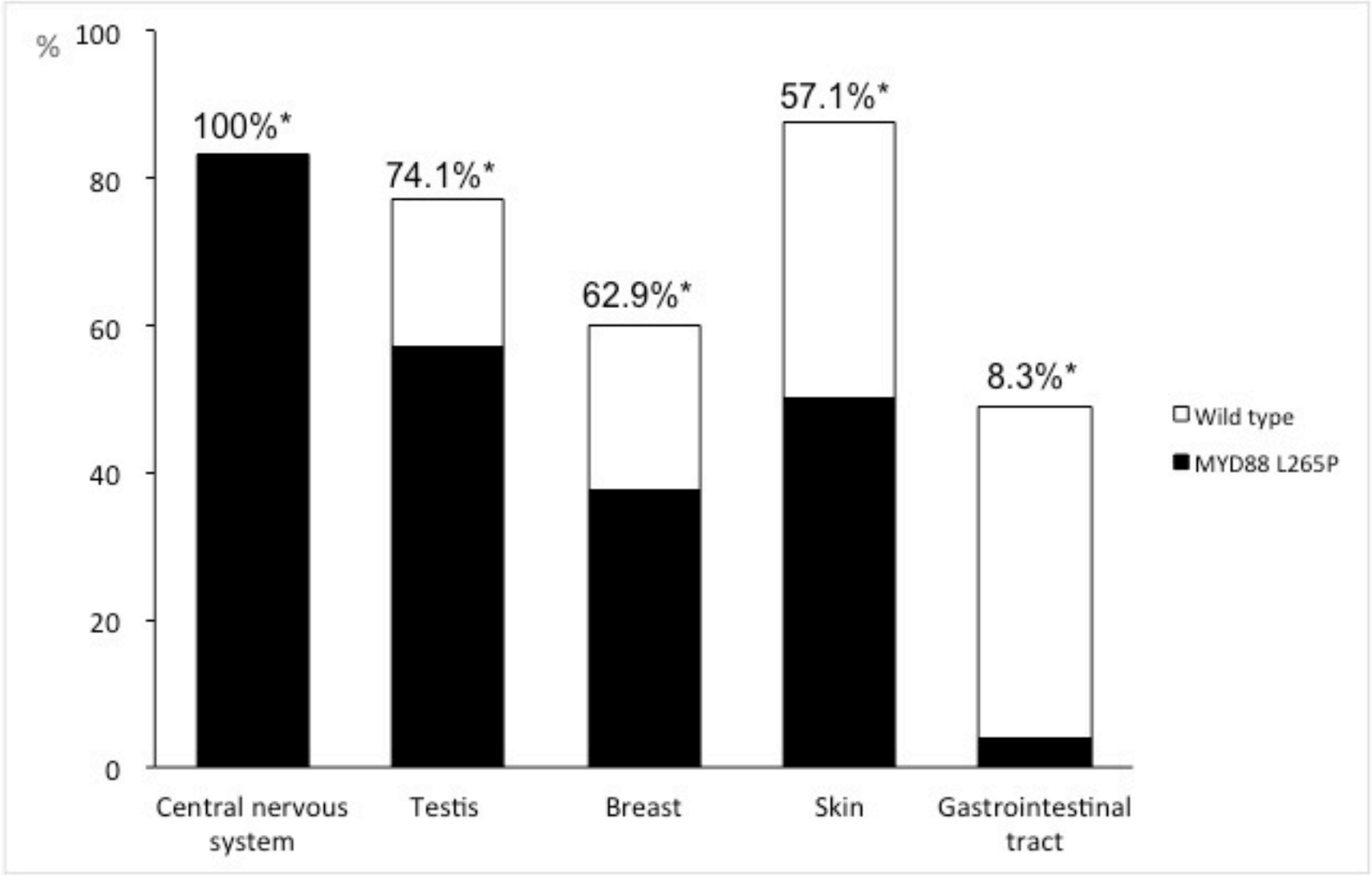

%
100
80
60
40
20
0
100%*
74.1%*
62.9%*
57.1%*
8.3%*
Central nervous system
Testis
Breast
Skin
Gastrointestinal tract
Wild type
MYD88 L265P

Table S4 The percentage of non-GCB-like DLBCL and MYD88 L265P in extranodal

Organ	Reference	non-GCB-like / Total	
Central nervous system	Yamada et al 19	15/18	(83.3%)
Testis	Kraan et al 18	27/35	(77.1%)
Breast	Taniguchi et al 20	27/45	(60.0%)
Skin	Pham-Ledard et al 22	14/16	(87.5%)
Gastrointestinal tract	Present study	24/49	(48.9%)

Abbreviations: DLBCL; diffuse large B-cell lymphoma.

* Compared with gastrointestinal DLBCL

DLBCLs.

MYD88 L265P / non-GCB-like DLBCL		*P**
15/15	(100.0%)	<0.001
20/27	(74.1%)	<0.001
17/27	(62.9%)	<0.001
8/14	(57.1%)	<0.001
2/24	(8.3%)	-