Gamma heavy chain disease lacks the *MYD88* L265p mutation associated with lymphoplasmacytic lymphoma

Gamma heavy chain disease (gHCD) is defined by an abnormally truncated IgG heavy chain monoclonal protein that lacks associated light chains and is secreted by a small B-cell neoplasm with plasmacytic differentiation.[1,2] Clinically, gHCD is associated with a female predominance and a high incidence of concurrent autoimmune conditions. As in the past, the 2008 WHO classification designates gHCD as a variant of lymphoplasmacytic lymphoma (LPL),[3] although it also states that gHCD is sufficiently distinctive to be considered a separate entity.[4]

In a recent study of the histopathological findings in 13 cases of gHCD,[5] we showed that gHCD is heterogeneous with some cases being morphologically typical of splenic marginal zone lymphoma (SMZL) or extranodal marginal zone (MALT) lymphoma, while other cases consist of a polymorphous B-cell neoplasm with predominantly small lymphocytes, plasmacytic differentiation, and variable numbers of admixed histiocytes and large transformed cells (Figure 1).[6] This latter group of cases technically meets the 2008 WHO definition of LPL, which is largely a diagnosis of exclusion, even though they are not typical of classic nodal LPL which is characterized by preservation of sinuses and a relatively monomorphic small lymphoplasmacytic infiltrate.[3,7,8] These histopathological differences suggested that gHCD and typical LPL may, in fact, represent unrelated disorders.

In recent landmark studies, the *MYD88* L265P mutation, which results in increased signaling through the NF-κB pathway, was identified in more than 90% of Waldenstrom's macroglobulinemia (WM), i.e. LPL with an IgM paraprotein.[9-14] This mutation has also been reported in diffuse large B-cell lymphoma, at least some cases of IgG or IgA positive LPL, and approximately 50% of IgM MGUS, but is present in only a small minority of other small B-cell neoplasms. The *MYD88* L265P mutation, therefore, represents a diagnostically useful marker to assist in distinguishing LPL from other small B-cell neoplasms that may show plasmacytic differentiation.[9-14] To further assess the potential relationship between gHCD and LPL, we analyzed a series of 11 gHCD cases involving lymph nodes, bone marrow or extramedullary tissues, and 10 cases of IgM positive, bone marrow-based LPL/WM for the *MYD88* L265P mutation.

PCR was performed using 20 ng DNA extracted from bone marrow aspirate, peripheral blood, or formalin-fixed, paraffin embedded tissues using the qBiomarker Somatic Mutation Assay for MYD88_85940 (Qiagen, Valencia, CA, USA) on the LightCycler 480 (Roche, Indianapolis, IN, USA). This assay employs two separate real-time reactions: one specific for the *MYD88* L265P mutation, and one that targets a reference, non-mutated portion of the *MYD88* gene. A crossing point (C_T) value is calculated for the mutant and reference *MYD88* reactions, and a ΔC_T is computed ($\Delta C_T = C_T\text{mutant} - C_T\text{reference}$). Analysis of 120 normal peripheral blood samples identified a cut off of ΔC_T less than 10 for interpretation as positive for the *MYD88* L265P mutation. Serial dilutions of the *MYD88* L265P mutant cell line OCI-LY-10 demonstrated a limit of detection of 0.5% mutant alleles (1% heterozygous tumor cells).

The clinical and pathological findings of the 11 cases of gHCD are shown in Table 1. Ten of these patients have been previously reported.[5,15] Remarkably, each of the 11 cases of gHCD was negative for the *MYD88* L265P mutation. In contrast, 9 of the 10 cases of LPL/WM were positive for the mutation. The single LPL/WM case negative for the mutation was a peripheral blood sample. Because PCR in this study was performed without prior B-cell purification, the neoplastic cells in this peripheral blood sample may have been below the limit of detection of this assay.

These findings show that gHCD lacks the *MYD88* L265P mutation that is characteristic of the vast majority of cases of LPL. Because this study utilized an allele-specific assay that targeted only the L265P mutation, the possibility of other *MYD88* mutations cannot be ruled out. Additional studies will be required to determine whether other recurrent abnormalities may be found in gHCD that may contribute to lymphomagenesis. In conjunction with

Table 1. Clinical and pathological features of gHCD cases studied including results of *MYD88* L265P mutation analysis.

Age	Sex	Autoimmune history	Bone marrow involved	Site	Histology	B-cell phenotype	*MYD88* L265P
39	F	None	Yes	Spleen	Splenic lymphoma, unclassifiable	CD5 Neg CD10 Neg	Neg
41	F	Hashimoto's	N/A	Thyroid	Extranodal MALT lymphoma	CD5 Neg CD10 Neg	Neg
45	F	AIHA	Yes	BM	MGUS	CD5 Neg CD10 Neg	Neg
46	M	RA	Yes	Lymph node	SBN-pc, NOS	CD5 Neg CD10 Neg	Neg
46	F	SLE	No	Lymph node	SBN-pc, NOS	CD5 Neg CD10 N/A	Neg
48	F	SLE	Yes	Lymph node	SBN-pc, NOS	CD5 Neg CD10 N/A	Neg
54	F	SLE, Sjögren's, Raynaud's	No	Lymph node	SBN-pc, NOS	CD5 Neg CD10 Neg	Neg
56	F	None	No	Spleen	SMZL	CD5 Neg CD10 Neg	Neg
64	F	SLE	No	Lymph node	SBN-pc, NOS	CD5 N/A CD10 Neg	Neg
69	F	None	Yes	Spleen	SBN-pc, NOS	CD5 Neg CD10 Neg	Neg
77	F	Hashimoto's	Yes	Salivary gland	SBN-pc, NOS	CD5 Neg CD10 Neg	Neg

AIHA: autoimmune hemolytic anemia; RA: rheumatoid arthritis; SLE: systemic lupus erythematosus; SBN-pc, NOS: small B-cell neoplasm with plasmacytic differentiation, not otherwise specified; SMZL: splenic marginal zone lymphoma; Neg: Negative; N/A: not available.

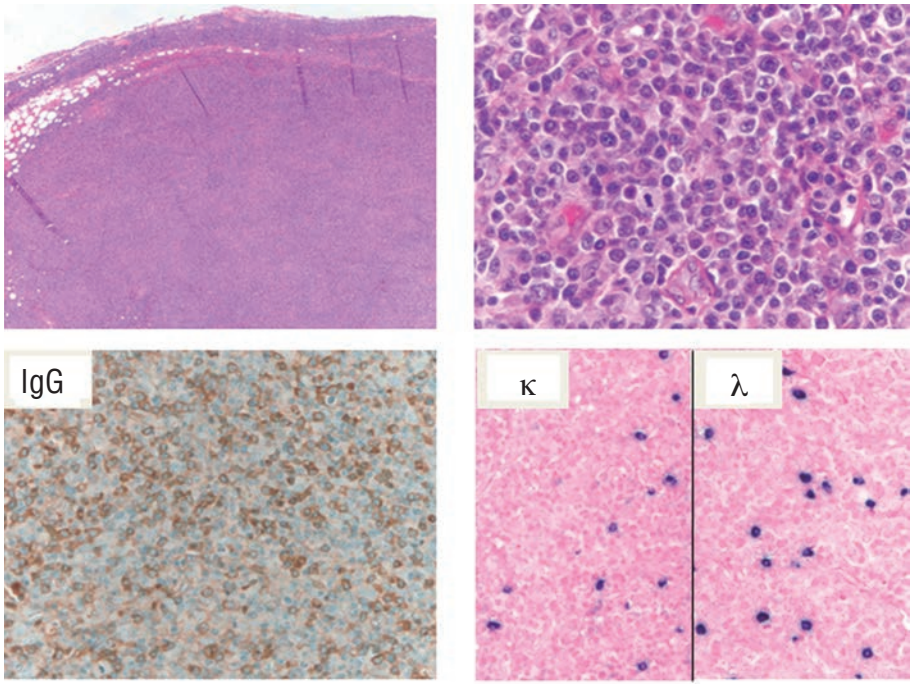

Figure 1. Seven cases (histologically classified as SBC-pc,NOS) showed similar morphological features with a diffuse infiltrate of small lymphocytes, occasional large cells, plasma cells and histiocytes. The plasma cells are positive for IgG and are negative for kappa and lambda light chains by *in situ* hybridization. [Images from Munshi *et al.*[15] *Copyright ©2008 Massachusetts Medical Society. Reprinted with permission from Massachusetts Medical Society*].

our prior observation that gHCD is histologically diverse and rarely, if ever, resembles typical nodal cases of LPL, these results argue strongly that gHCD should no longer be considered a variant of LPL. Similarly, Randen *et al.* have recently shown that primary cold agglutinin-associated lymphoproliferative disease lacks the *MYD88* L265P mutation and shows other features distinct from LPL.[6] Together, these reports help further refine LPL as a diagnostic category.

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