1

High prevalence of the *MYD88 L265P* mutation in IgM anti-MAG paraprotein-associated peripheral neuropathy

Authors:

Josephine M. Vos (1*), Nicolette C. Notermans (2), Shirley D'Sa (3), Michael P. Lunn (3), W. Ludo van der Pol (2), Willem Kraan (4, 5), Mary M. Reilly (6), Jane Chalker (7), Rajeev Gupta (3), Marie José Kersten (5,8), Steven T. Pals (4,5) and Monique C. Minnema (9).

Affiliations:

1) Department of Hematology, St. Antonius Ziekenhuis, Nieuwegein, The Netherlands

2) Brain Center Rudolf Magnus, UMC Utrecht, The Netherlands

3) University College London Hospitals NHS Foundation Trust, London, United Kingdom

4) Department of Pathology, Academic Medical Center, Amsterdam, The Netherlands

5) Lymphoma and Myeloma Center (Lymmcare), Amsterdam , the Netherlands

6) MRC Centre for Neuromuscular Diseases, UCL Institute of Neurology. London, United Kingdom

7) Great Ormond Street Hospital, London, United Kingdom

8) Department of Hematology, Academic Medical Center, Amsterdam, The Netherlands

9) Department of Hematology, UMC Utrecht Cancer Center, The Netherlands

* *Corresponding author:* email: jm.vos@antoniusziekenhuis.nl

Running head: MYD88 L265P in anti-MAG peripheral neuropathy

Word count: 1034

Tables: 1

Figures: 0

References: 15

Peripheral neuropathy (PN) is present in 30-50% of individuals with Immunoglobulin M Monoclonal gammopathy of unknown significance (IgM MGUS) and Waldenstrom Macroglobulinemia (WM). In approximately 50% of these cases, this is due to IgM anti-myelin-associated glycoprotein (MAG) paraprotein-associated peripheral neuropathy (anti-MAG PN)[1,2]. Anti-MAG PN typically presents as a chronic demyelinating disorder with progressive ataxia, tremor and sensory disturbance. The typical electrophysiological features associated with anti-MAG PN consist of slowed motor conduction velocities with a disproportionate prolongation of the distal motor latency, and significant involvement of sensory nerves.[1,3] By definition, a serum IgM paraprotein and anti-MAG antibodies are present. Up to 50% of patients develop significant disability after 10-15 years. Progressive disease-related disability is an indication to start treatment. However, while there is some evidence supporting clinical benefit of rituximab,[1,4] there is currently no consensus on the optimal treatment approach for anti-MAG PN and there is a high clinical need for effective therapies[1].

IgM paraproteinemia is a hallmark of WM and IgM MGUS. WM is an indolent B-cell malignancy with lymphoplasmacytic differentiation typically localized in the bone marrow (BM), while IgM MGUS is defined as asymptomatic IgM paraproteinemia with < 10% BM infiltration by lymphoplasmacytic cells.[5] The term "IgM related disease" is reserved for IgM MGUS with symptoms that are attributable to the paraprotein, such as cryoglobulinaemia, neuropathy and cold agglutinin disease[5]. Individuals with IgM MGUS are at increased risk for developing B-cell malignancies, mainly WM[6,7].

Recently, a recurrent somatic point mutation of the myeloid differentiation factor 88 (*MYD88*) gene, leading to an amino-acid change from leucine to proline (L265P), has been reported in the vast majority (> 90%) of WM patients and approximately 50% of IgM MGUS patients. The mutation is absent in healthy donors and multiple myeloma [8,9]. *MYD88* mutations, in particular the *MYD88 L265P* hot spot mutation, are found in a small subset of the aggressive large-cell B-cell lymphomas, including activated B-cell type diffuse large B-cell lymphoma and primary central nervous system lymphoma. [10,11]. Among indolent small-cell B-cell lymphomas, the *MYD88 L265P* mutation is largely confined to WM.[8] MYD88 is an adaptor protein of the interleukin-1R and toll-like receptor signalling pathways that ultimately lead to activation of nuclear factor-κB (NF-κB) and Janus kinase (JAK)/signal transducer and activator of transcription 3 (STAT3). The *MYD88 L265P* mutation results in aberrant

activation of these pathways and is considered the central driver mutation of WM and a diagnostic signature of the disease. In individuals with IgM MGUS, the presence of *MYD88 L265P* is associated with a higher risk of progression to WM[7]. In WM patients, treatment with ibrutinib, an oral Bruton's Tyrosine Kinase (BTK) inhibitor is less effective in WM patients with wild-type *MYD88* than in those with *MYD88 L265P*[12]. The incidence of *MYD88 L265P* is unknown in most IgM related disorders, with the exception of cold agglutinin disease (CAD), a chronic hemolytic disorder associated with IgM paraproteinemia. In CAD, *MYD88 L265P* was absent in all tested patients, which confirmed it as a distinct entity from WM.[13]

Thus far, the mutational status of *MYD88* has not been studied in IgM related PN. In the current study, we have found a high prevalence of *MYD88 L265P* in the BM of anti-MAG PN-patients.

Our study comprises 22 patients with anti-MAG PN. Inclusion criteria were: 1) presence of IgM paraprotein in the serum; 2) a positive serum IgM MAG-antibody test; 3) a confirmed clinical diagnosis of anti-MAG neuropathy by a neurologist specialized in peripheral nerve disorders, including electromyography (EMG); 4) availability of a BM sample for mutational analysis. Eligible patients were included at four centres: three in the Netherlands (AZN, AMC, and UMCU), and one in the UK (UCLH). Anti-MAG-antibody testing was performed per standard clinical care at each individual centre, using a commercially available ELISA (Bühlmann-Laboratories, Switzerland) with a cut-off point of at least 1500 Bühlmann titer units. Flow cytometry of the bone marrow aspirate for the detection of clonal B-cells was performed per standard clinical care in the subset of Dutch patients. *MYD88 L265P* mutation analysis was performed at two centres (UCLH and AMC) on DNA extracted from either stored BM aspirates or trephine biopsy specimens, using an allele specific PCR as described previously [10]. In samples that tested negative for the mutation, the presence of a B-cell clone was assessed using multiplex PCRs for the detection of B-cell receptor rearrangements [14].

The clinical characteristics of the patients, including neurological findings and relevant biochemical parameters, as well as the results of the mutation analysis are summarized in table 1. The *MYD88 L265P* mutation was detected in 13/22 patients (59%). Of the 9 patients (41%) that tested negative for the mutation, the presence of a B-cell clone was confirmed by rearrangement testing in 2 patients. In the other 7 patients that tested negative for the mutation, a clonal B cell population could not be detected.

Our study demonstrates that the *MYD88 L265P* mutation is highly prevalent in a cohort of well-characterized anti-MAG PN patients. The detected mutational rate of 59% most likely represents an underestimate since the tumour load was generally very low, as shown by the low quantity of clonal B-cells as detected by flow cytometry and negative B-cell receptor rearrangement-based clonality studies in 7/9 mutation negative patients. This indicates a very low frequency of neoplastic B cells in these samples, which may have precluded *MYD88 L265P* detection. Indeed, the reported prevalence of *MYD88 L265P* in IgM MGUS is highly variable (10-87%) which most likely relates to sensitivity issues in the setting of a low clonal B-cell burden.[9]

Our current results establish that the majority of anti-MAG PN cases contain the WM signature *MYD88 L265P* mutation. Furthermore, like B cells in WM, anti-MAG PN B cells have been shown to represent post-germinal center memory B cells that have undergone somatic mutation of their immunoglobulin-variable genes[15]. Low-titer anti-MAG antibodies have been found in up to 40% of WM/IgM MGUS patients[2]. Taken together, these data strongly suggest a pathogenetic link of IgM anti-MAG paraprotein-associated PN with WM. This supports the initiation of clinical trials for anti-MAG PN using agents that have proven efficacy in WM. Specifically, novel targeted agents with little (neuro-) toxicity such as the novel oral proteasome inhibitors or BTK inhibitors[1,12] could be of great therapeutic potential.

1. D'Sa S, Kersten MJ, Castillo JJ, Dimopoulos M, Kastritis E, Laane E, et al. Investigation and management of IgM and Waldenström-associated peripheral neuropathies: recommendations from the IWWM-8 consensus panel. Br J Haematol. 2017 Mar;176(5):728–42.

2. Baldini L, Nobile-Orazio E, Guffanti A, Barbieri S, Carpo M, Cro L, et al. Peripheral neuropathy in IgM monoclonal gammopathy and Wäldenstrom's macroglobulinemia: a frequent complication in elderly males with low MAG-reactive serum monoclonal component. Am J Hematol. 1994 Jan;45(1):25–31.

3. Joint Task Force of the EFNS and the PNS. European Federation of Neurological Societies/Peripheral Nerve Society Guideline on management of paraproteinemic demyelinating neuropathies. Report of a Joint Task Force of the European Federation of Neurological Societies and the Peripheral Nerve Societ. J Peripher Nerv Syst. 2010 Sep;15(3):185–95.

4. Hospital M-A, Viala K, Dragomir S, Levy V, Cohen-Aubart F, Neil J, et al. Immunotherapy-based regimen in anti-MAG neuropathy: results in 45 patients. Haematologica. 2013 Dec;98(12):e155-7.

5. Owen RG, Treon SP, Al-Katib A, Fonseca R, Greipp PR, McMaster ML, et al. Clinicopathological definition of Waldenstrom's macroglobulinemia: Consensus Panel Recommendations from the Second International Workshop on Waldenstrom's Macroglobulinemia. Semin Oncol. 2003 Apr;30(2):110–5.

6. Kyle RA, Therneau TM, Rajkumar SV, Remstein ED, Offord JR, Larson DR, et al. Long-term follow-up of IgM monoclonal gammopathy of undetermined significance. Blood. 2003 Nov 15;102(10):3759–64.

7. Varettoni M, Zibellini S, Arcaini L, Boveri E, Rattotti S, Pascutto C, et al. MYD88 (L265P) mutation is an independent risk factor for progression in patients with IgM monoclonal gammopathy of undetermined significance. Blood. 2013 Sep 26;122(13):2284–5.

8. Treon SP, Xu L, Yang G, Zhou Y, Liu X, Cao Y, et al. MYD88 L265P Somatic Mutation in Waldenström's Macroglobulinemia. N Engl J Med. 2012 Aug 30;367(9):826–33.

9. Paiva B, Corchete LA, Vidriales M-B, Garcia-Sanz R, Perez JJ, Aires-Mejia I, et al. The cellular origin and malignant transformation of Waldenstrom macroglobulinemia. Blood. 2015 Apr 9;125(15):2370–80.

10. Kraan W, Horlings HM, van Keimpema M, Schilder-Tol EJM, Oud MECM, Scheepstra C, 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 Sep;3(9):e139.

11. Ngo VN, Young RM, Schmitz R, Jhavar S, Xiao W, Lim K-H, et al. Oncogenically active MYD88 mutations in human lymphoma. Nature. 2011 Feb 3;470(7332):115–9.

12. Treon SP. How I treat Waldenstrom macroglobulinemia. Blood. 2015;126(6):721–32.

13. Randen U, Trøen G, Tierens A, Steen C, Warsame A, Beiske K, et al. Primary cold agglutinin-associated lymphoproliferative disease: a B-cell lymphoma of the bone marrow distinct from lymphoplasmacytic lymphoma. Haematologica. 2014 Mar 1;99(3):497–504.

14. van Dongen JJM, Langerak AW, Brüggemann M, Evans PAS, Hummel M, Lavender FL, et al. Design and standardization of PCR primers and protocols for detection of clonal immunoglobulin and T-cell receptor gene recombinations in suspect lymphoproliferations: report of the BIOMED-2 Concerted Action BMH4-CT98-3936. Leukemia. 2003 Dec;17(12):2257–317.

15. Mariette X, Tsapis A, Brouet JC. Nucleotidic sequence analysis of the variable domains of four human monoclonal IgM with an antibody activity to myelin-associated glycoprotein. Eur J Immunol. 1993 Apr;23(4):846–51.

Table 1:

Clinical characteristics and MYD88 L265P status of 22 patients with anti-MAG PN

Sex (M/F)	Age (years)	Bone marrow: Histological diagnosis	Bone marrow flow cytometry [A]; percentage of monoclonal B-cells	Serum IgM paraprotein level (g/L)	Clinical Pheno-type [B]	EMG Result [C]	*MYD88 L265P* mutation present	B-cell clone detected
M	72	MGUS	n.t.	< 3	1	1	+	
F	43	MGUS	n.t.	7	2	1	+	
M	70	MGUS	n.t.	<3	1	1	+	
F	74	MGUS	n.t.	<3	1	1	+	
F	72	MGUS	n.t.	9	1	1	-	+
M	62	MGUS	n.t.	10	1	1	+	
M	62	MGUS	n.t.	4	1	1	-	-
M	71	WM	n.t.	3	1	1	+	
M	66	MGUS	2%	5	1	1	+	
M	73	MGUS	Negative	7,6	2	2	+	
M	71	MGUS	1%	5,7	1	1	-	+
F	77	MGUS	Negative	8,7	2	2	-	-
M	64	MGUS	3%	16	1	1	+	
M	64	MGUS	Negative	1,7	1	1	-	-
F	79	MGUS	4%	5,7	1	1	+	
M	67	MGUS	Negative	2,3	1	1	-	-
F	59	MGUS	n.t.	4,7	1	1	+	
M	73	MGUS	Negative	2,3	1	1	-	-
M	76	MGUS	Negative	8,1	1	1	-	-
F	66	MGUS	<1%	5,4	1	1	-	-
F	75	MGUS	5%	3.16	1	1	+	
M	70	MGUS	Negative	0.6	1	1	+	

n.t: Not tested

[A] While bone marrow histology was performed in all cases, additional flow -cytometry of the bone marrow was only available in a subset.

[B] Clinical phenotype
1: Sensorimotor polyneuropathy, characterized by sensory disturbances, ataxia and ankle dorsiflexor weakness
2: Pure sensory polyneuropathy characterized by sensory disturbance and/or ataxia

[C] EMG result
1: Nerve conduction velocities consistent with a demyelinating polyneuropathy with a prolonged distal motor latency
2: Axonal polyneuropathy