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

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*Running head:* MYD88 L265P in anti-MAG peripheral neuropathy

*Word count:* 1034

*Tables:* 1

*Figures*: 0

*References*: 15

*Acknowledgement:* This research was supported by a Grant from Fonds Stimulans

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.

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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