Received: 4 December 2023 | Accepted: 23 February 2024

DOI: 10.1111/bjh.19383

BJHaem
BRITISH JOURNAL OF HAEMATOLOGY

LETTER TO THE EDITOR

Waldenstrom macroglobulinaemia with AA amyloidosis reveals a B-cell-restricted *NLRP2* variant

Dear Sir,

Waldenström's macroglobulinaemia (WM) is a rare haematological malignancy corresponding to an IgM-secreting lymphoplasmocytic lymphoma (LPL). The oncogenic driver of WM is a gain of function of MYD88.[1] The mutation overactivates the MYD88 signalling pathway, resulting in transcriptional activation of nuclear factor kappa B (NF-κB).[1,2] Some patients with WM have chronic inflammation, as evidenced by elevated C-reactive protein (CRP), and have been termed inflammatory WM (IWM).[3] The chronic inflammation in these patients may lead to the development of amyloid A (AA) amyloidosis.[4] AA amyloidosis is a rare disease resulting from the deposition of insoluble fibrils derived from the acute-phase reactant serum amyloid A (SAA) protein.[5] The mechanism leading to inflammation in IWM remains to be determined. Virtually all WM patients with *MYD88* mutations have activation of the transcription factor NF-κB and expression of pro-inflammatory cytokines.[6,7] Therefore, additional genetic events may be responsible for the increased inflammation.

We describe the case of a patient with AA amyloidosis complicating WM. Whole exome sequencing (WES) revealed a somatic mutation of *NLRP2* restricted to the B cells. We explored the consequences of this mutation. Detailed Material and Method are provided in the Supporting Information. Briefly, the patient gave informed consent for inclusion in

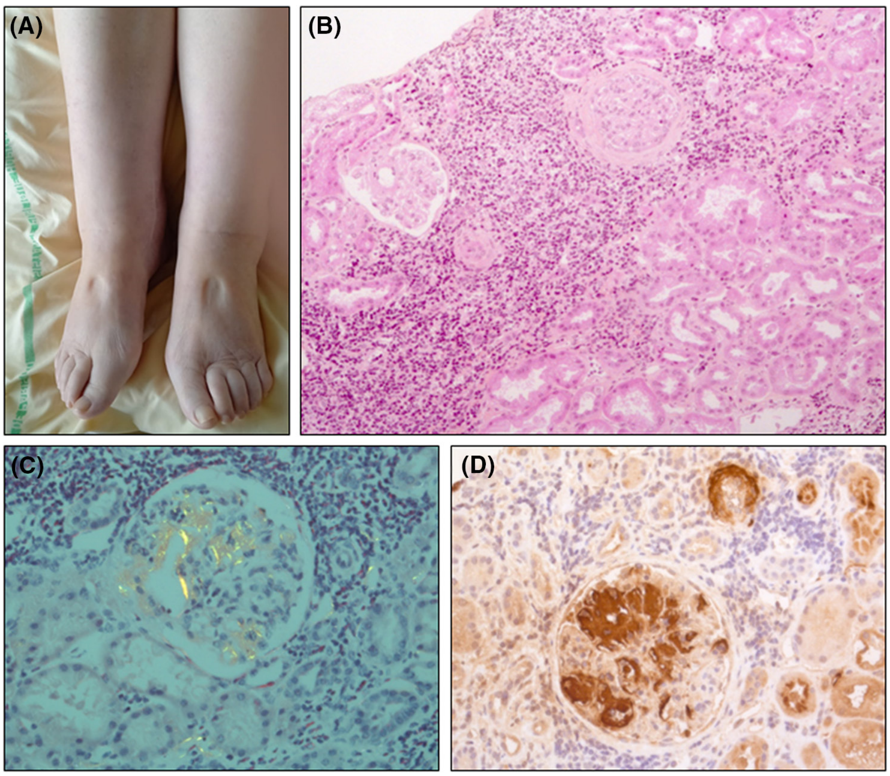

FIGURE 1 Clinical and pathological presentation of the patient. (A) Pitting oedema revealing nephrotic syndrome. (B) Haematoxylin and eosin stain of the kidney biopsy showing infiltrate with small lymphocytes. (C) Typical yellow-green bi-refringence of amyloid deposits under polarized light showing amyloid deposits in the glomeruli. (D) Immunostaining with antibodies directed against serum amyloid A protein, showing an intense marking of amyloid deposits.

the GEN-HEMO-INF study (NCT04702932, ethics review board approval 2020-A01928-31). ASC aggregates reporter cell lines were generated using HEK 293T. ASC protein fused to mCherry was expressed with the different NLRP2 variants. Flow cytometry analysis was performed according to the method described by Sester et al.[8] A representative gating of ASC aggregates is shown in Figure 2B. The BCWM-1 cell line[9] was used for NLRP2 knockdown experiments using short hairpin RNA.

The patient was an 86-year-old woman with a history of hypothyroidism and streptococcal bloodstream infection. She was diagnosed with IgM MGUS at 51 years of age. The MGUS progressed to overt WM with hyperlymphocytosis of 8.8 G/L and an IgM pic of 7.10 g/L. The bone marrow biopsy showed an infiltration of small lymphocytes and plasma cells, with a staining CD20+, IgM+, CD5− and IgG− accounting for 50% of the nucleated population. The disease was atypical due to the presence of a biclonal B-cell proliferation consisting of a CD20+, IgM+, CD5−, kappa subtype representing 50% of the circulating lymphocytes and a CD20+, IgM+, CD5−, lambda subtype representing 14% of the circulating lymphocytes. Consistently, she had two different *MYD88* mutations (the most common p.Leu265Pro and the atypical p.Ser219Cys).[10] She had no tumoral signs and no cytopenia. Observation was recommended. At the age of 86, she developed pitting oedema of the lower limbs (Figure 1A), revealing a nephrotic syndrome. A renal biopsy was performed and showed infiltration of the kidney by clonal B lymphocytes (CD20+/CD5−/CD3−/CD23−), consistent with lymphomatous infiltration (Figure 1B). The biopsy showed extra-cellular deposits with a positive Congo-red staining. The deposits were immunohistochemically stained with anti-SAA antibody, without significant staining by anti-kappa and anti-lambda antibodies (Figure 1C,D). Mass spectrometry deposits confirmed those findings. The diagnosis of AA amyloidosis was retained. A review of her past tests revealed that she had a sustained elevation of the CRP (>20 mg/L). She had no sign for inflammatory rheumatism, infection or inflammatory bowel disease. There were no detectable autoantibodies. A thoraco-abdomino-pelvian CT and a PET-CT were performed and showed other disease than WM. The diagnosis of AA amyloidosis caused by an IWM was retained. She was then treated with rituximab. At the age of 86, she developed a fatal SARS-Cov2 disease.

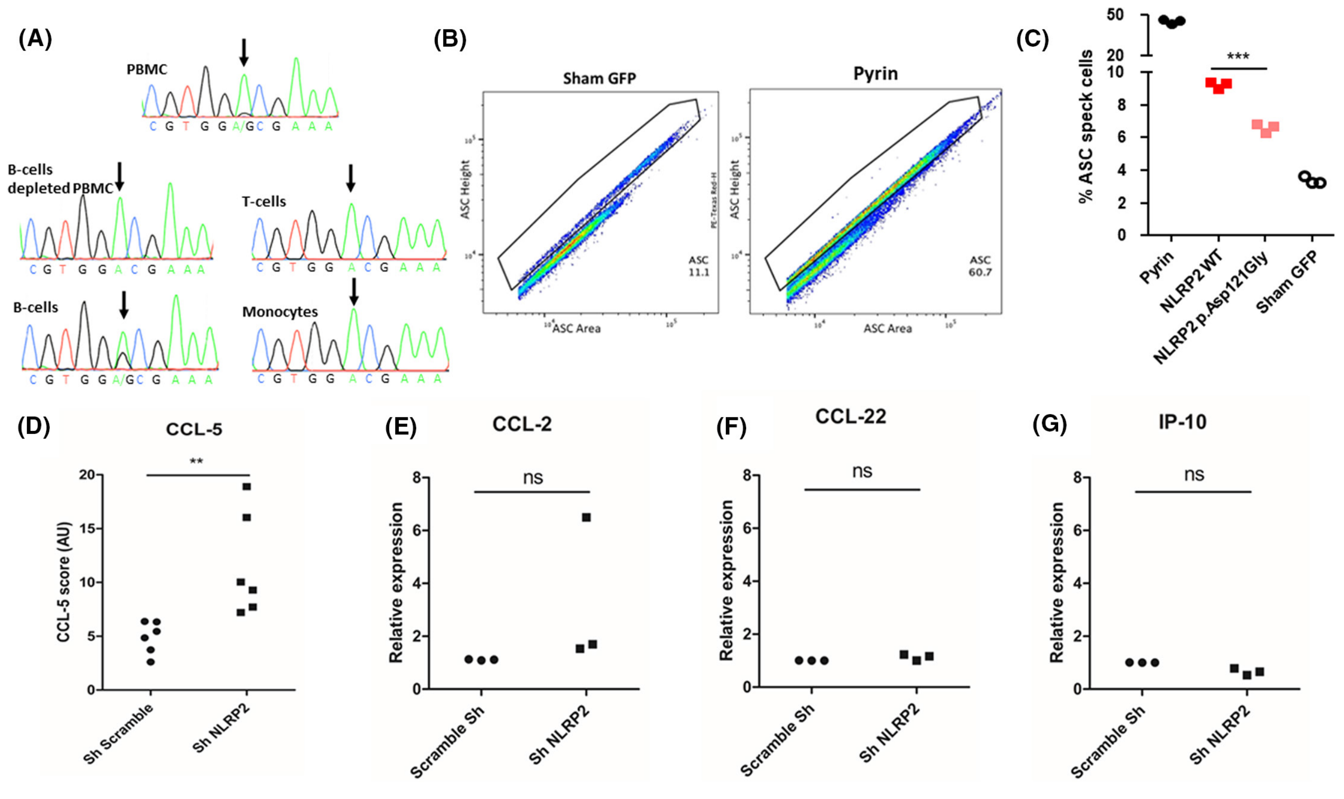

FIGURE 2 Genomic findings and functional exploration of NLRP2. (A) Sanger sequencing in different cell population of the patient revealing the somatic A>G substitution restricted to the B cell line (black arrows). (B) Typical gating for percentage of ASC speck cells measurement in the control positive (Pyrin) and negative (Sham GFP) conditions. (C) Percentage of ASC speck cells in the different inflammasome reporter cell lines. Three independent cultures for each condition were measured the same day. The experiment was triplicated with comparable results. (D) CCL-5 dosage by ELISA in the different Waldenstrom macroglobulinaemia cell lines, normalized by the number of cells in each experiment. Supernatant from six independent cell cultures was used. The results are expressed in arbitrary units. (E–G) Quantitative PCR experiments for CCL-2, CCL-22 and IP-10 in the different BCWM-1 cell lines. Three independent RNA extraction and cDNA generation were performed. Each measure was triplicated. The results are expressed relatively to the Scramble Sh BCWM-1 cell line. Each measure was triplicated. $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$.

Whole exome sequencing was performed on unsorted peripheral blood mononucleated cells. Apart from the *MYD88* mutations, two somatic mutations were isolated in immune regulatory genes: *NLRP2* (NM_001174082, c.362A>G, p.Asp121Gly) and *SDC4* (NM_002999, c.160G>A, p.Glu54Lys). Both were absent from GnomAD and our in-house exome library. We decided to investigate the pathogenicity of the *NLRP2* variant, as it is expressed in B cells, in contrast to *SDC4*. Sanger sequencing revealed that the variant was restricted to the B-cell population (Figure 2A) and most likely to the LPL population. The somatic *NLRP2* and *MYD88* variants were also found in WES on DNA extracted from the kidney biopsy displayed in Figure 1B.

NLRP2 has been shown to form an inflammasome.[11] To test the impact of the variant on inflammasome formation, WT and mutant NLRP2 were expressed in a reporter cell line (Figure S1A–C), and ASC specks were measured. Our experiments showed that NLRP2 p.Asp121Gly was associated with a decreased ASC aggregation compared to the NLRP2 WT (Figure 2C). This finding was consistent across cell with different expression of NLRP2 (Figure S1E).

To study the impact of NLRP2 loss of function in a WM model, we used ShRNA to knockdown *NLRP2* in the BCWM-1 cell line carrying the p.Leu265Pro *MYD88* mutation.[9] As the BCWM-1 cell line do not produce IL-1β or IL-6,[6] we measured CCL-5 production. Elsawa et al. demonstrated that CCL-5 is elevated in the bone marrow of WM patients and promotes Il-6 production by stromal cells.[6] We found an increased secretion of CCL-5 in the Sh NLRP2 cell line (Figure 2D). We measured CCL-2, CCL-22 and IP-10 expression by qPCR in the model, but we did not find any difference (Figure 2E–G).

Inflammatory diseases secondary to somatic mutations are now recognized due to advances in genome sequencing.[12–14] Genomic and functional investigations of the patient revealed a B-cell-restricted somatic mutation of *NLRP2* that may have contributed to the patient's inflammation. We demonstrated that the variant was associated with a decreased ability to form ASC aggregate. We also demonstrated in a WM model that NLRP2 is a regulator of CCL-5 production. Since CCL-5 produced by WM cells has been shown to enhance IL-6 production by stromal cells, and since IL-6 is a key cytokine for the induction of SAA production, our results support the hypothesis that the patient's *NLRP2* mutation might have contributed to the development of AA amyloidosis.

Extrapolation of *NLRP2* mutations consequence in a germline context should be done with caution. Further studies are required to confirm the role of NLRP2 in the physiopathology of IWM.

FUNDING INFORMATION

Reactives for the study were funded by grants from the Société Nationale Française de Médecine Interne (SNFMI, 20 000 euros), the Fondation Française pour la Recherche Contre le Myélome et les Gammapathies (46 000 euros) and AT was funded by a MD/PhD grant from Institut Imagine (Paris, France).

AUTHOR CONTRIBUTIONS

AT performed the experiments. SG-L, MPR and AT designed the research study. DB performed the pathological examination. AC and SG-L followed the patient. AT, MPR and SG-L wrote the initial draft. All authors participated in the redaction of the final manuscript.

ACKNOWLEDGEMENTS

We thank the patient for her participation in the study. We thank Quentin Riller (University of Paris, Laboratory of Immunogenetics of Pediatric Autoimmune Diseases, Imagine Institute, INSERM UMR 1163, Paris, France), Eric Allemand and Olivier Hermine for their help in the study design (Institut Imagine, INSERM U1163). We thank Cécile Masson (Bioinformatics Platform, Institut Imagine-Structure Fédérative de Recherche Necker, INSERM U1163 et INSERM US24/CNRS UAR3633, Paris Cité University, Paris, France) and Mélanie Parisot (Genomics Core Facility, Institut Imagine-Structure Fédérative de Recherche Necker, INSERM U1163 et INSERM US24/CNRS UAR3633, Paris Cite University, Paris, France) for their help for the whole exome sequencing. We thank Véronique Della Valle (INSERM U1170 Dynamique Moléculaire de la Transformation Hématopoïétique, Gustave Roussy, Villejuif, France) for the BCWM-1 cell line.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

All data are available upon request to the corresponding author.

ETHICS STATEMENT

The patient was recruited as part of the Gen-Hemo-Inf study (NCT04702932). The Gen-Hemo-Inf study is a physiopathological study recruiting patients with a haematological clonal disease complicated with inflammatory symptoms. The study was approved by a French national ethic committee (ID RCB: 2020-A01928-31). The study was conduct in accordance with the Declaration of Helsinki. The patient gave her informed consent for inclusion in the study and further publication of the data.

PATIENT CONSENT STATEMENT

The patient gave her informed consent for inclusion in the study and further publication of the data.

CLINICAL TRIAL REGISTRATION (INCLUDING TRIAL NUMBER)

NCT04702932.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.