Mutations in MYD88 are highly recurring in Waldenstrom’s Macroglobulinemia (WM) patients and are important for establishing the diagnosis of WM. The most common mutation in MYD88 is c.978T>C resulting in a proline substitution for leucine at amino acid position 265 (p.Leu265Pro). Both allele-specific PCR (AS-PCR) and clinical diagnostic next generation sequencing (NGS) panels are used to detect mutated MYD88, though they differ in sensitivity and scope. In this study we screened 734 patients with WM by AS-PCR for MYD88 c.978T>C MYD88 followed by Sanger sequencing to clarify negative results for non-MYD88 p.Leu265Pro mutations and compared the findings to clinical NGS panel data from the same biopsy when available. We also investigated MYD88 isoform dysregulation and isoform-specific effects of the observed mutations that may impact mutated MYD88 regulation which has not been previously studied in WM.

Methods

DNA from CD19-selected bone marrow mononuclear cells (BMMC) of 734 WM patients were used for the MYD88 c.978T>C AS-PCR assay previously described by us (Xu et al, Blood 2013). For patients wild-type for MYD88 c.978T>C by AS-PCR, Sanger sequencing of the open reading frame of MYD88 was performed for both DNA and RNA simultaneously isolated from CD19-selected BMMC. DNA was also used to validate the presence of c.978T>C by Sanger. Findings were compared to 319/734 (43.5%) patients who also underwent illumina miSeq targeted next generation sequencing on a clinical diagnostic platform using unselected BMMC. Next generation RNAseq isoform specific expression estimates were calculated using Salmon for 77 WM patients and 34 healthy donors (Hunter et al, Blood 2016).

Results

For 319 unique biopsies collected from 295 WM patients, both NGS panel data and AS-PCR were available. The finding between the NGS and AS-PCR studies were largely concordant. Discrepancies were observed in 86 (27%) cases where targeted NGS gave false negative results for c.978T>C but was detected by AS-PCR. Median sequencing read depth at p.Leu265Pro was 229 (range 120-922). Modeling these false negative results by age, previous treatment and bone marrow involvement revealed only bone involvement to be a significant predictor (p < 0.0001).