Detection of MYD88 L265P in Peripheral Blood of Patients with Waldenström's Macroglobulinemia and IgM Monoclonal Gammopathy of Undetermined Significance

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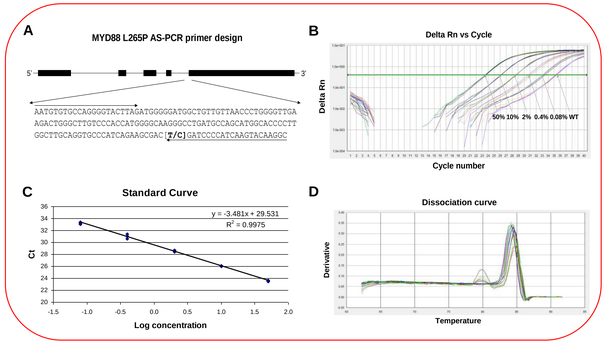
Key point

- MYD88 L265P is detected in the peripheral blood of WM and IgM MGUS patients, and correlates with bone marrow disease burden
- In the appropriate clinical setting, AS-PCR defined MYD88 L265P levels can support the diagnosis of WM, and discriminate WM from IgM MGUS

Abstract

MYD88 L265P is a highly prevalent somatic mutation in Waldenstrom's Macroglobulinemia (WM) and IgM MGUS. We investigated if MYD88 L265P status could be determined by peripheral blood (PB) AS-PCR examination. MYD88 L265P was detected in untreated WM (114/118; 96.6%); previously treated WM (63/102; 61.8%); IgG LPL (1/1; 100%); and IgM MGUS (5/12; 41.6%) patients, but none of 3 hyper-IgM or 40 healthy individuals. Median PB MYD88 L265P Δ*Ct* was 3.77, 7.24, 2.47, 10.89, 12.33, and 14.07 in patients with untreated WM, previously treated WM, IgG LPL, IgM MGUS, hyper-IgM syndrome, and healthy donors, respectively (p<0.0001). PB MYD88 L265P Δ*Ct* correlated to BM disease burden in untreated (r=-0.400; p=0.001) and previously treated (r=-0.442; p=0.0004) patients. In positive patients, MYD88 L265P Δ*Ct* was <6.5 in 40/49 (82%) smoldering, and 43/46 (94%) symptomatic WM patients, whereas all 5 IgM MGUS patients had a Δ*Ct* >6.5 (p<0.0001). Negative PB mutation status associated with lower BM disease (p=0.001) and serum IgM (p=0.019), and higher hemoglobin (p=0.004) levels in treated WM patients. These studies demonstrate the feasibility for determining MYD88 L265P status by PB examination, and use of MYD88 L265P Δ*Ct* to support the diagnosis of WM, and assessment of underlying BM disease burden.

Allele-specific PCR assay



The quantitative AS-PCR assay was developed as described previously (A) (Xu et al, Blood, 2013). DNA was extracted from CD19-selected cells with Dynabead selection kit. This quantitative AS-PCR assay can detect the MYD88 L265P mutation at a dilution of 0.08% with more than 2 cycle differences from the wild-type DNA background (B). Correlation coefficient of the standard curve was 0.998 with a slope value of -3.5 (C). The melting curve analysis revealed that the MYD88 L265P mutant-specific amplicon melted at 840C (D). MYD88 L265P Δ*Ct* of 9.6 was used as cutoff.

Baseline characteristics

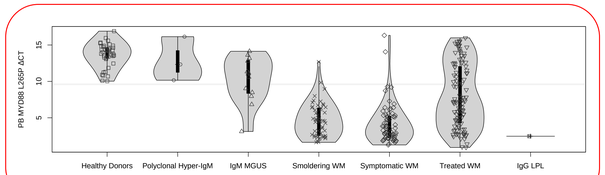
All patients

	N	Gender	Age	BM	WBC	HGB	HCT	PLT	ANEUT	ALYMP	IgG	IgA	IgM
IgM MGUS	12	6M / 6F	62.5	0	5.45	13.35	38.35	248.5	3.78	1.25	937	139.5	437
Smoldering WM	51	31M / 20F	61	20	7.2	13	38	247	4.43	1.78	658.5	72	1900
Symptomatic WM	67	33M / 34F	60	50	6	11.4	33	247	3.53	1.7	639.5	52	3320
Treated WM	102	72M / 30F	57.5	40	5.3	11.8	34.8	205	3.175	1.04	402	27	1700
ANOVA p-value			0.611	<0.0001	0.0006	<0.0001	<0.0001	0.0282	0.0016	0.225	<0.0001	<0.0001	<0.0001

Paired patients

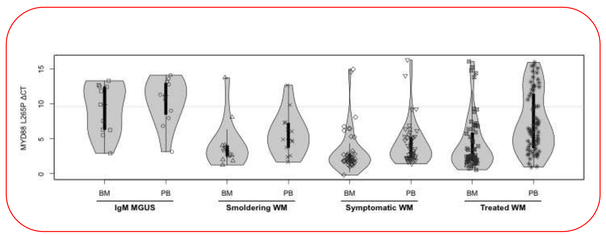
	N	Gender	Age	BM	WBC	HGB	HCT	PLT	ANEUT	ALYMP	IgG	IgA	IgM
IgM MGUS	12	6M / 6F	62.5	0	5.45	13.35	38.35	248.5	3.78	1.25	937	139.5	437
Smoldering WM	13	9M / 4F	63	15	6.9	12.4	37.1	208	3.655	1.89	651	81	1240
Symptomatic WM	48	24M / 24F	58.5	50	6.3	11.45	33	253	3.71	1.73	610	53	3285
Treated WM	66	49M / 17F	56	32.5	5.35	11.95	35	216	2.97	1.035	353.5	26	1540
ANOVA p-value			0.213	<0.0001	0.0513	0.0006	0.0015	0.0456	0.126	<0.0001	0.0062	<0.0001	0.0007

AS-PCR results - all patients



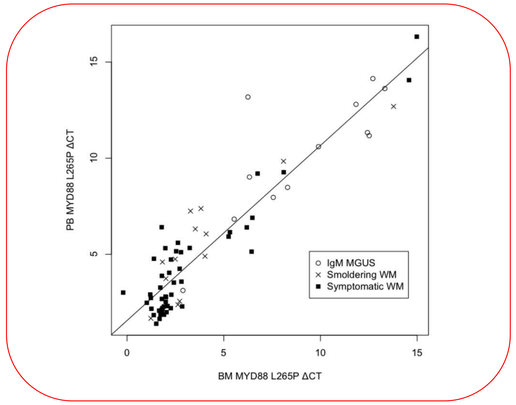
114/118 (96.6%) untreated WM patients, including 49/51 (96.1%) smoldering and 65/67 (97.0%) symptomatic patients were positive for MYD88 L265P. Lower frequency of MYD88 L265P was detected in previously treated WM patients (63/102, 61.8%) and IgM MGUS patients (5/12, 41.2%). All 3 hyper-IgM syndrome patients and 40 healthy donors were negative for MYD88 L265P.

AS-PCR results - paired patients



MYD88 L265P was detected in 12 (92.3%) and 46 (95.7%) CD19-selected BM samples from smoldering and symptomatic untreated WM patients, respectively. Among the positive patients, MYD88 L265P was detected in paired PB in 11/12 (92%) and 46/46 (100%) of smoldering and symptomatic patients, respectively. For IgM MGUS, MYD88 L265P was present in 6 (50%) and 5 (41.7%) of the BM and PB CD19-selected samples, respectively.

Correlation analysis



Among all untreated WM and IgM MGUS patients, PB MYD88 L265P Δ*Ct* strongly correlated to BM MYD88 L265P Δ*Ct* (r=0.835; p<0.00001). Negative PB mutation status associated with lower BM disease (p=0.001) and serum IgM (p=0.019), and higher hemoglobin (p=0.004) levels in treated WM patients. In positive patients, MYD88 L265P Δ*Ct* was <6.5 in 40/49 (82%) smoldering, and 43/46 (94%) symptomatic WM patients, whereas all 5 IgM MGUS patients had a Δ*Ct* >6.5 (p<0.0001).

Conclusion

We demonstrate the feasibility of detecting MYD88 L265P by use of PB AS-PCR testing, with high rates of sensitivity and specificity particularly for untreated WM and IgM MGUS patients. In the appropriate clinical context, the use of PB MYD88 L265P testing may provide a convenient, non-invasive, and inexpensive method to establish the diagnosis of WM, and follow changes in underlying disease burden .