Detection of the MYD88 L265P Mutation in Waldenström's Macroglobulinemia Using a Highly Sensitive Allele-Specific PCR Assay

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Abstract

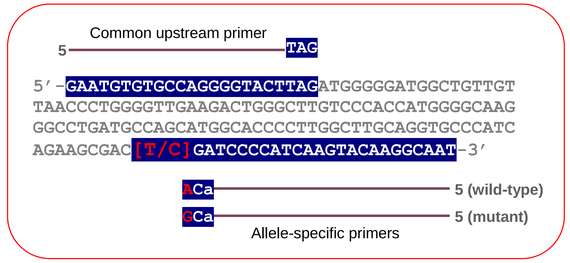
Introduction: Waldenström's macroglobulinemia (WM) is a B-cell malignancy characterized by bone marrow (BM) infiltration with lymphoplasmacytic cells and production of an IgM paraprotein. By whole genome sequencing, we recently identified a somatic mutation (L265P) in the MYD88 gene in 27/30 (90%) WM patients (Treon et al, ASH 2011). To expand this finding for possible diagnostic testing, we developed an allele-specific PCR assay for MYD88-L265P and evaluated this assay in a large cohort of WM patients.

Materials and methods: An allele-specific PCR assay was developed with a threshold of detection of 0.1% for detection of the MYD88-L265P mutation. DNA from bone marrow aspirates from 96 patients with the clinicopathological diagnosis of WM and 9 healthy controls was used for assessment of MYD88-L265P expression by both allele-specific PCR and Sanger sequencing. Findings were correlated with clinical parameters.

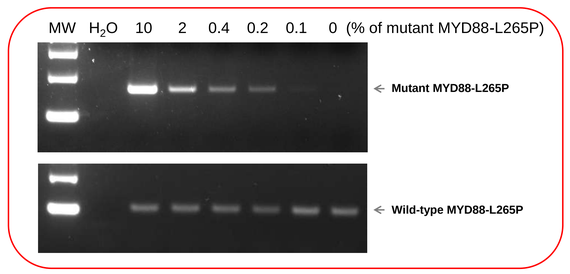
Results: We observed that 85/96 (89%) WM patients were positive for MYD88-L265P using the allele-specific PCR assay. Of the 85 allele-specific PCR positive patients, 80 demonstrated a detectable mutation peak by Sanger sequencing. All 11 allele-specific PCR negative patients remained negative by Sanger sequencing. By the allele-specific PCR assay, MYD88-L265P positive patients showed greater bone marrow involvement ($p<0.001$) and higher serum IgM ($p<0.001$) versus MYD88-L265P negative patients.

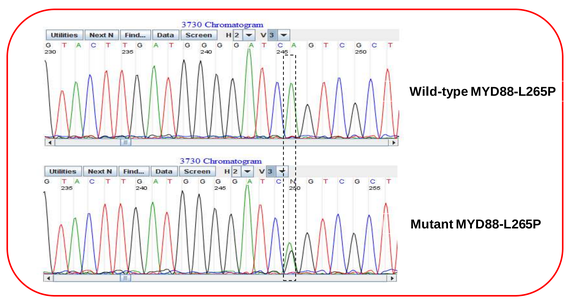
Conclusion: MYD88-L265P is highly expressed in BM samples of WM patients using an allele-specific PCR assay, and is associated with greater bone marrow disease burden and serum IgM levels. Use of allele-specific PCR provides a simple and sensitive diagnostic tool for detection of the MYD88-L265P mutation.

Allele-specific PCR design

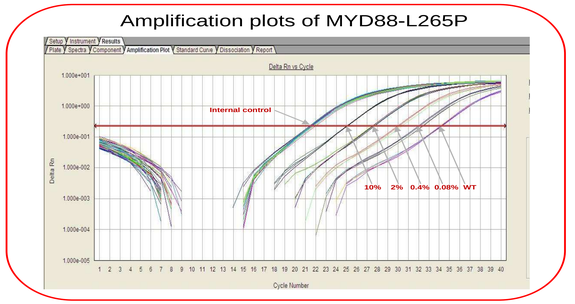


PCR reaction was performed in volume of 25 ul with 50 nM of forward primers and reverse primers and 100 ng DNA. Thermal cycling conditions were 2 min at 94^0C, followed by 40 cycles of 94^0C for 30s, 57^0C for 30s, and 68^0C for 30s, with a final extension at 68^0C for 5 min. Sensitivity was assessed by a serial dilution of the WM cell line DNA. PCR products were separated on 2% agarose gel.





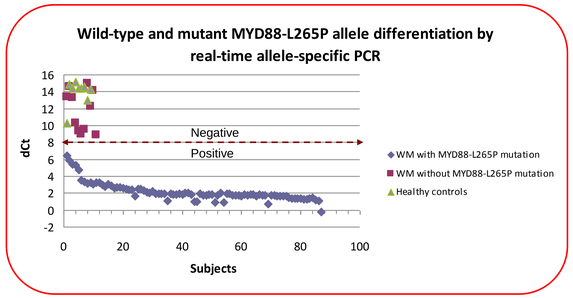
Real-time PCR implementation



Real-time quantitative PCR was performed on an ABI 7500 real-time PCR system using Power SYBR green PCR master mix according to manufacture's instruction.

Results

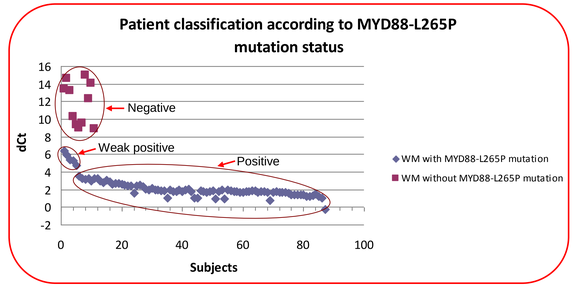
A total of 96 untreated WM patients and 9 healthy controls were analyzed by real-time allele-specific PCR and Sanger sequencing.



	MYD88-L265P mutation status					
Clinical parameter	Positive		Negative		p-value*	p-value**
BM involvement, %	n=85	54.13 (29.65)	n=11	17.73 (18.35)	0.0003	0.0002
Serum IgM (mg/dL)	n=84	3276.23 (1966.39)	n=11	973.00 (1576.88)	<0.0001	0.0003

Values are mean (SD).
*Non-parametric ANOVA
**ANCOVA. Age at diagnosis and gender were adjusted.

Five WM patients showed negative MYD88-L265P by Sanger sequencing but weak positive by allele-specific real-time PCR. Correlation with clinical parameters was further analyzed.



	MYD88-L265P mutation status					
Clinical parameter	Positive		Weak positive		Negative		p-value*	p-value**
BM involvement, %	n=80	55.14 (29.42)%	n=5	38.00 (31.94)%	n=11	17.73 (18.35)	0.0008	0.0003
Serum IgM (mg/dL)	n=79	3355.05 (1970.16)	n=5	2030.80 (1575.57)	n=11	973.00 (1576.88)	<0.0001	0.0004

Values are mean (SD).
*Non-parametric ANOVA
**ANCOVA. Age at diagnosis and gender were adjusted.

Discussion

We developed an allele-specific real-time PCR assay for detection of MYD88-L265P using commercially available PCR mix. This method provides necessary sensitivity and specificity and can be easily implemented in each laboratory with real-time PCR technology. Analysis of this larger cohort confirmed our previous finding that the MYD88-L265P allele is frequently mutated in WM patients and, in combination with clinical features, represents a reliable molecular marker for this disease. Interestingly, the WM patients with positive MYD88-L265P tended to have greater bone marrow disease burden and higher serum IgM compared to MYD88-L265P negative patients. Quantification of MYD88-L265P by real-time PCR allows the detection of minimal residual disease and monitoring therapeutic effect.