

# Highly Recurrent Copy Number Alterations and Insight Into The Origins Of 6q Deletions In Waldenström's Macroglobulinemia Revealed By Whole Genome Sequencing

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## Abstract

### Background

Over 90% of patients with Waldenström's Macroglobulinemia (WM), and 50-80% of patients with the precursor condition, IgM MGUS, express MYD88 L265P. These findings suggest that other mutations may support progression of IgM MGUS to WM. Chromosomal aberrations including large losses in 6q are commonly present in WM patients, though the gene loss accounting for WM pathogenesis remains unclear. We therefore sought to delineate copy number alterations (CNA) and structural variants using whole genome sequencing (WGS) in order to more clearly define other important gene alterations in WM.

### Methods

DNA from CD19+ bone marrow lymphoplasmacytic lymphoma cells (LPC) and CD19-depleted peripheral blood mononuclear cells from 10 WM patients was used for paired tumor/germline analysis by WGS. Coverage in the tumor sample was divided by the coverage in the paired germline sample for each matching position, resulting in coverage ratios for each 100Kb window. Statistically significant windows within each genome were then analyzed across the cohort by randomizing the coverage positions to assess the probability of observing the given frequency of a CNA by random chance. TaqMan quantitative polymerase chain reaction (qPCR) copy number assays were used to validate findings and to assess the CNA status for 8 of our top findings in an independent cohort of 30 patients. Sanger sequencing across the breakpoint, including flanking sequences, was used to validate translocations.

### Results

Functional annotation for identified CNAs was undertaken using Ingenuity Pathway Analysis that revealed a significant enrichment for pathways dysregulated in B-cell malignancies. Iteratively randomizing the genomic position of CNAs not related to the chromosome 6 deletions revealed a greater than 3 fold increase in the targeting of COSMIC genes than expected by chance ( $p < 0.001$ ) (Figure 1). Affected genes in the COSMIC census were BTG1 (9/10; 90%), FOXP1 (7/10; 70%), FNBP1 (7/10; 70%), CD74 (7/10; 70%), TOP1 (6/10; 60%), MYB (5/10; 50%), CBLB (5/10; 50%), ETV6 (5/10; 50%), TNFAIP3 (5/10; 50%), FBXW7 (5/10; 50%), PRDM1 (5/10; 50%), TFE3 (4/10; 40%), JAK1 (4/10; 40%), MAML2 (4/10; 40%), FAM46C (4/10; 40%), EBF1 (4/10; 40%), STL (4/10; 40%), and BIRC3 (4/10; 40%). Other affected genes of interest included PRDM2 (8/10; 80%), HIVEP2 (8/10; 80%), ARID1B (7/10; 70%) as well as LYN (7/10; 70%).

There were no singular regions of statistical significance in though neither of the previously suspected target genes for 6q loss, PRDM1 and TNFAIP3, was included in the regions of highest statistical significance (Figure 2). While no recurrent translocations were noted in this study, 2 or the 5 (40%) of the 6q deletions corresponded with validated translocation events. In one case, this was a result of chromothripsis focused on 6q (Figure 3) while in the other case, a t(6;X) translocation linked to the amplification of Xq was identified.

Validation studies confirmed presence of somatic deletions in by qPCR. As some CVAs were subclonal, we validated the correlation between the PCR relative copy number and WGS coverage predictions (Spearman's rho = .926;  $p = 2.2 \times 10^{-16}$ ). To establish the frequency of these CNAs, we validated these eight findings in an independent cohort of 30 WM patients revealing somatic losses in PRDM2 (28/30; 93%) at 1p36.21, BTG1 (26/30; 87%) in 12q21.33, HIVEP2 (23/30; 77%) at 6q24.2, MKLN1 (23/30; 77%) at 7q32, PLEKHG1 (21/30; 70%) at 6q25.1, LYN (18/30; 60%) at 8q12.1, ARID1B (15/30; 50%) at 6q25.1, and FOXP1 (11/30; 37%) at 3p13, and (Figure 4). There were fewer median validated deletions in CXCR4 mutated patients compared to WT ( $p=0.002$ ) and with the median total number of 6q CNAs compared to WT ( $p=0.007$ ). This was also true for any combination of two of the three validated 6q genes ruling out possible biasing by a single gene ( $p < 0.023$  for all).

Disclosures: There are no relevant conflicts of interest to disclose.

## Results

Category	Functions Annotation	p-Value
Cancer	lymphohematopoietic cancer	6.07E-13
Hematological System Development and Function	quantity of B lymphocytes	1.65E-12
Cellular Growth and Proliferation	proliferation of immune cells	2.90E-12
Hematopoiesis	arrest in differentiation of lymphocytes	6.39E-12
Cellular Growth and Proliferation	proliferation of lymphocytes	8.96E-12
Hematological System Development and Function	quantity of blood cells	1.23E-11
Hematological System Development and Function	quantity of leukocytes	3.49E-11
Hematological Disease	hematological neoplasia	3.64E-11

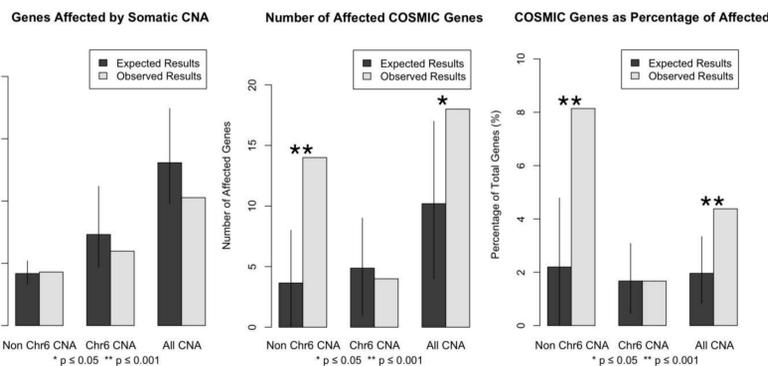


Figure 1: Characterization of somatic copy number alterations (CNA) in WM

Functional annotation for genes affected by CNA found outside of chromosome 6. The list is ordered by statistical significance and filtered only for duplicated functional annotations matching more than one category. Deletions of matching size were randomly distributed across the genome in 10,000 trials. The number of affected total RefSeq and COSMIC genes was calculated for each group. Results represent mean values with empirical 95% confidence intervals.

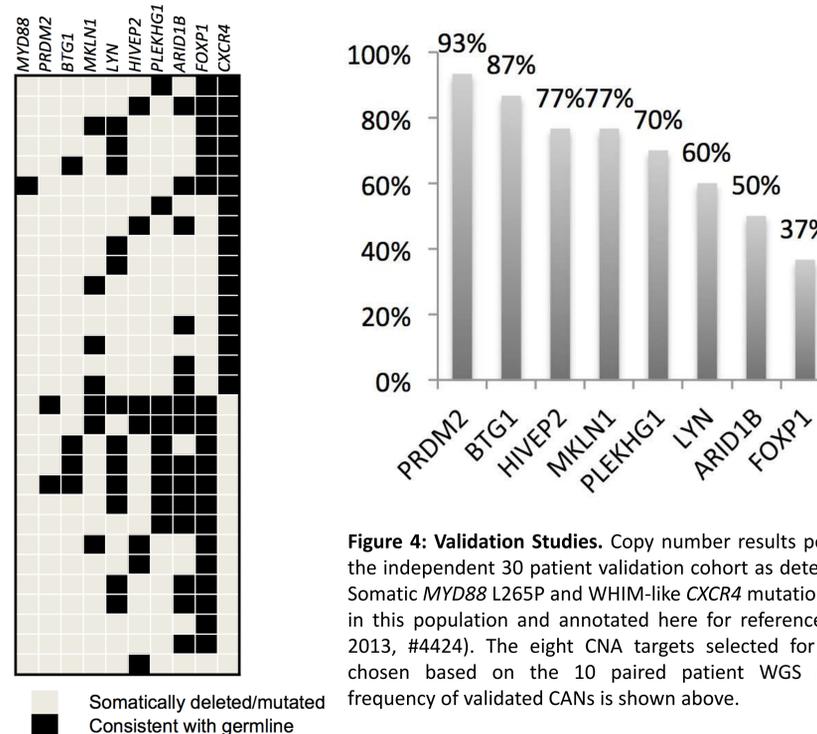


Figure 4: Validation Studies. Copy number results per patient for the independent 30 patient validation cohort as determined by qPCR. Somatic MYD88 L265P and WHIM-like CXCR4 mutations were assessed in this population and annotated here for reference (Cao et al ASH 2013, #4424). The eight CNA targets selected for validation were chosen based on the 10 paired patient WGS analysis. Overall frequency of validated CNAs is shown above.

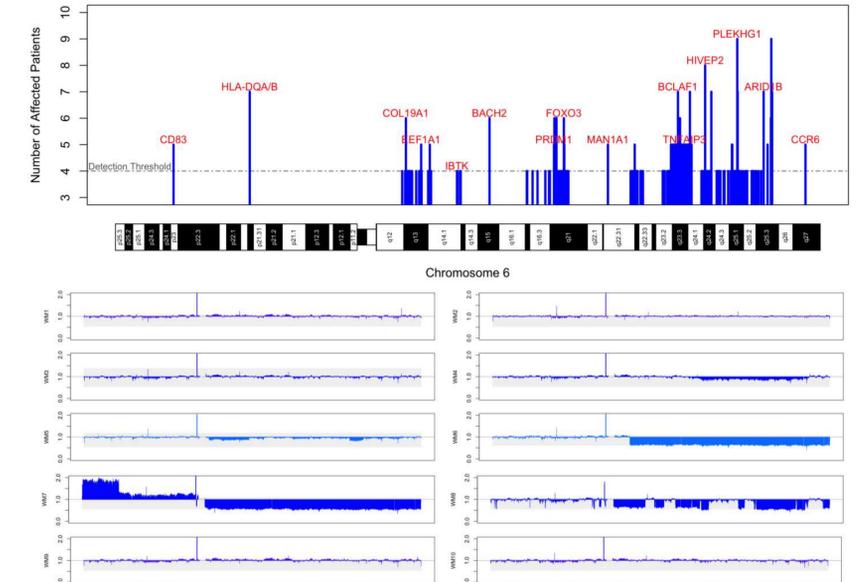


Figure 2: Somatic deletions identified on chromosome 6 by WGS in WM patients

Frequency of statistically significant chromosome 6 deletions from the 10 paired patients highlighting genes of interest. The positions of the deletions are mapped against chromosome 6 cytogenetic bands. Relative coverage across chromosome 6 for each of the 10 paired samples demonstrates large scale 6q deletions in 5 patients.

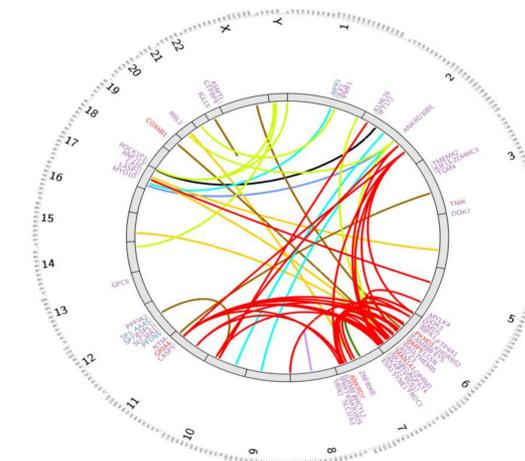


Figure 3: Summary of copy number and structural variants results identified in the 10 paired WM patients who underwent WGS.

All high confidence structural variants are colored coded by the sample in which they were detected. Genes disrupted by structural variants are listed in purple and potential gene fusions in red.

## Summary

- Highly recurrent copy number losses are present in WM LPCs that include genes with critical regulatory roles in lymphocytic growth and survival signaling.
- Validated translocations were associated with 6q deletion breakpoints in 6q deleted WM patients.
- CXCR4 WHIM like mutations inversely associated with 6q deletions, as well as frequency of copy number losses.