

The Genomic Landscape of Plasma Cells in Systemic Light chain Amyloidosis

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TO THE EDITOR:

The key event in the pathogenesis of Systemic light chain amyloidosis (AL) is an unstable misfolded secondary or tertiary structure of a monoclonal immunoglobulin (IG) light chain, that precipitates in the extracellular compartments.¹ The plasma-cell disorder underlying AL, is likely to lie within the common spectrum of plasma-cell diseases, but limited confirmatory data analysing the genetic architecture of AL are available.²

To address this, we prospectively included twenty-four newly diagnosed histologically proven AL samples from the National Amyloid Centre, University College London, UK, prior to any treatment. AL was confirmed by immunohistochemistry or mass spectrometry. CD138+ cells and peripheral blood were isolated, DNA extracted and used in an exome capture protocol and sequenced as previously published.³ This AL dataset was compared to previously published MGUS⁴ and MM^{5,6} datasets and filtered similarly to ensure comparability of mutation numbers. Detailed methods and Patient's characteristics may be found in the **Supplemental-Methods and Supplemental-Table 1.**

In terms of mutational burden, the median number of exonic, non-IG, non-synonymous mutations per sample that had a tumour variant allelic fraction greater than 5% was 39 (interquartile range (IQR): 5-185) which is more than was seen in MGUS⁴ (20 (IQR: 1-41), $p=0.002$) but not statistically different from MM⁶ (35 (IQR: 3-74), $p=0.4$), **Figure 1A**. When analysing the individual genes, we identified a total of 1491 genes that were mutated in our dataset with 236 of them being mutated more than once. As expected, there was no unifying mutation in AL. The dataset was too small to identify any significantly mutated genes.⁷ Among the recurrently mutated genes (**Supplemental-Table 2**), mutations in *IL7R*, a gene that encodes for a cell surface receptor known to regulate V(D)J recombination by altering the accessibility of DNA substrates to the recombinase in pro-B-cells,⁸ were noted. Thirty-seven percent of samples ($n=9$) had a mutation in one of the 63 previously described MM-driver genes.⁹ This is less than MM (84.1%)⁹ and similar to MGUS (36%).⁴ The number of mutated driver-genes per sample ranged from zero to five. Among the 63 driver-genes, 13 were mutated, **Table 1**. Most of these mutations were sub-clonal with IG-adjusted estimated tumour fractions ranging from 18% to 100% (**Supplemental-Table 3**). We identified cases with MM hotspot mutations in *NRAS* (Q61R and Q61H) but not in *KRAS*. Even though a trend for fewer *KRAS* mutations in AL was observed, there was no significant difference in the incidence of driver mutations between MM, MGUS, and AL, **Figure 1B**. Interestingly there were mutations in some of the other driver-genes such as *EGR1* (Q95R), *DIS3* (D479E,

M667L), *IRF4* (S332G), and *TRAF3* (K99_sp). Only the same *TRAF3* (K99_sp) and *DIS3* (D479E, M667L) mutations were seen in the MM dataset, **Supplemental-Figure 1A-E**.⁹ In MM, *IRF4* mutations are associated with t(11;14), and in this AL dataset the *IRF4* mutation (S332G) occurred in a non t(11;14) patient and was neither at the K123R hotspot seen in MM⁶ nor the L116R seen in CLL.¹⁰ There were no mutations in the previously reported adverse prognostic genes such as *ATM*, *ATR*, *ZFHX4* and *TP53*, nevertheless mutations in *BRCA2* (P1088T, N372H) and the driver-gene *EP300* (I997V) were seen suggesting DNA-repair pathway involvement. Copy-number analysis did not reveal any copy number changes at these loci suggesting the absence of bi-allelic inactivation. Finally, evidence would suggest the presence of NF-κB pathway activation, with not only mutations in the driver-genes (such as *TRAF3*, *IRF4*) but also mutations in kinases (such as *LYN* (I165T)), downstream transducing molecules (*CARD11* (R1077V)) and inhibitors (*NFKBIE_sp*), **Supplemental-Figure 2**. We compared the mutational landscape of AL to previously sequenced MM (n=1273)⁹ and MGUS (n=33)⁴ samples. Overall, there were 101 genes in common between AL, MGUS, and MM. Ninety-three percent of the AL-mutated genes (n=1386) were shared with either MM or MGUS. Only 7% of these genes (n=105) had not previously been reported in MM or MGUS, **Supplemental-Figure 3 and Table 4**. None of these mutations were recurrent. A Gene enrichment analysis of the 105 genes that were mutated in AL only¹¹ did not reveal any specific pathway enrichment suggesting they are random. There were no mutations in the ribosome sub-unit genes.¹² Among the 68 differentially expressed genes (Paiva et al.), we only identified a mutation in *TIAM2* previously reported in MM.² Of note, we did identify a mutation in the 8th exon of *PSMA2* gene (P223S) that was among the differentially expressed genes found by Abraham et al.¹³ In a hierarchical clustering approach of driver mutation based on previously published data,^{4,5,14,15} AL clustered with MGUS and was closely related to MM, **Figure 1C**. In terms of translocations, we identified seven t(11;14) encompassing 30% of cases. The breakpoints, located 2-600 kb upstream of *CCND1*, were consistent with those seen in other lymphoid malignancies, **Supplemental-Figure 4**. They were all generated via class switch recombination with breakpoints occurring in the *IGHA1* (2/7) and *IGHM* (5/7) switch regions. There was no evidence of other canonical translocation but we identified a t(1;14) involving *IGHG4* and the *RCCI* gene in a t(11;14) patient, **Supplemental-Figure 5**. There was no evidence of inter-chromosomal translocations involving *MYC*; one patient had an 8q24 gain, 5' to *MIR1208*, suggesting *MYC* rearrangements also occur in AL. Regarding other cytogenetic abnormalities, there was no difference in the incidence of copy number changes with the exception of del(1p), and

del(14q) that was lower than expected in MM (n=1, 4%, p=0.008 and n=0, p<0.0001 respectively) but similar to MGUS, **Supplemental-Figure 6**.

This is the largest dataset of whole exome sequencing (WES) of AL to date and combined, these data suggest the underlying disease in AL resembles other plasma-cell disorders such as MGUS and MM. The number of mutations in AL was comparable to MM suggesting AL is a more complex disease than MGUS. Like previously published AL cases that had undergone WES,² we failed to identify any unifying mutation. We were nonetheless able to detect MM-defined driver mutations. They occurred at similar frequencies to MGUS and were less common than in MM. They were predominantly sub-clonal suggesting they occurred late during disease progression. There was evidence of mitogen activated protein kinase (MAPK) activation with *NRAS* mutations (8%) in keeping with previously published data by Rossi et al¹⁶, NF-κB activation (17%) and DNA-repair pathway alterations (12%). Unlike previous reports, none of the *NRAS* mutated patients had classical CRAB criteria but one had another myeloma defining event (SFLC ratio>100 with an involved chain greater than 1000 mg/L).¹⁷ These data are consistent with observations from MGUS and SMM where *NRAS* mutations may be found with lower frequencies than in MM.^{4,16,18} There was a 93% overlap in mutated genes indicating a common spectrum of mutations between AL and other plasma-cell disorders. Attempts to cluster patients based on MM-driver genes placed AL close to MM and MGUS and not with other lymphoid malignancies.

Overall AL showed a similar mutation burden to MM but resembled MGUS in terms of copy number changes and driver-gene mutations suggesting AL lies within the continuous spectrum from MGUS to MM. Given that the similarities with MGUS and MM are outweighing the differences, it is unlikely that the plasmacell biology *per se* explains the clinical presentation of AL, however, these data support the ongoing use of myeloma-based therapy for this disease.

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Authorship contributions

Conception and design of the study: EMB, DR, BAW, GJM, ADW

Acquisition of data: EMB, DR, BAW, SS, ADW, PP

Analysis of data: EMB, CA, YW, CPW, MAB, NW, BAW, MFK, DCJ, JRJ, CP, TF, CD, CS, FED, GJM

Writing of manuscript: EMB, BAW, GJM, ADW

Review or revision of manuscript: all.

Conflicts of Interest

The authors have no relevant conflicts of interest to disclose.

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Tables

Table 1: Driver mutations found in AL

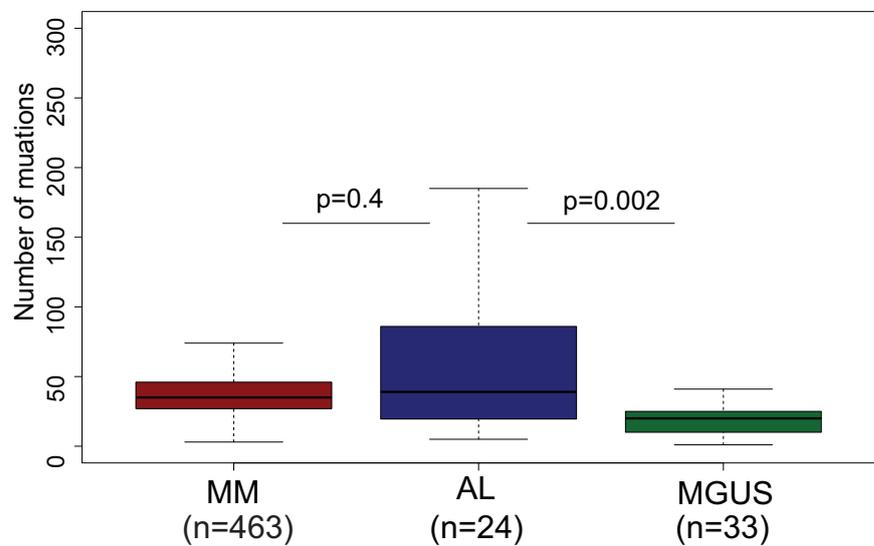
Gene	Number of mutations	Number of patients	Variants
<i>DIS3</i>	2	1	D479E, M667L
<i>DUSP2</i>	1	1	Splice donor variant
<i>EGR1</i>	1	1	Q95R
<i>EP300</i>	1	1	I997V
<i>IRF4</i>	1	1	S332G
<i>KLHL6</i>	1	1	V337M
<i>KMT2B</i>	1	1	P204H
<i>NRAS</i>	2	2	Q61R; Q61H
<i>SAMHD1</i>	1	1	F363L
<i>TET2</i>	1	1	P363L
<i>TRAF3</i>	1	1	Splice donor variant
<i>ZFP36L1</i>	1	1	L82S
<i>ZNF292</i>	1	1	I1740V

Figure Legends

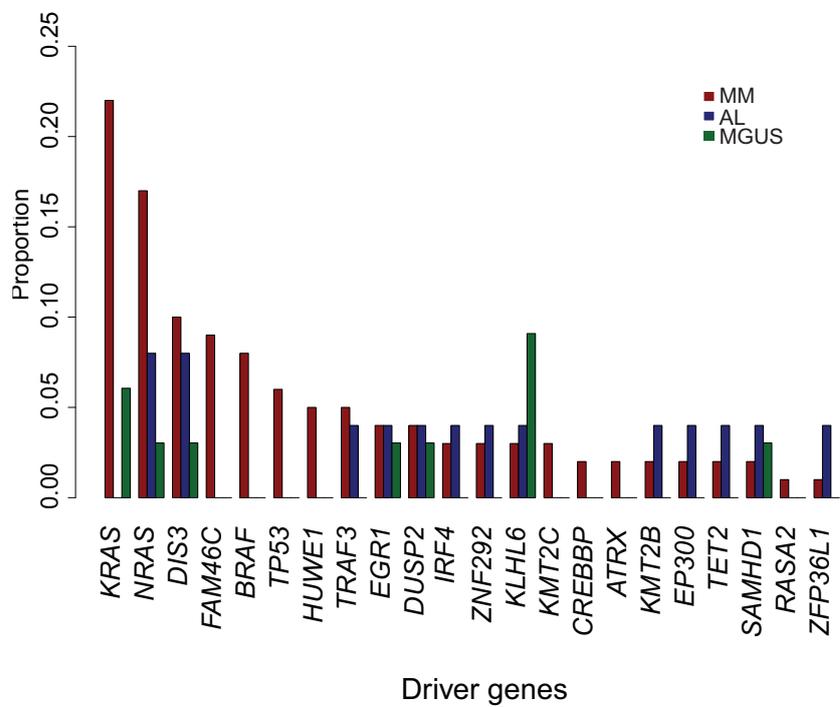
Figure 1: The mutational spectrum of amyloidosis resembles MM and MGUS. A: Boxplot suggesting AL has more mutations than MGUS but not a statistically different number than MM. **B:** Frequencies of select driver mutations in AL, MGUS and MM. **C:** Clustering of driver-mutation frequencies

Figure 1

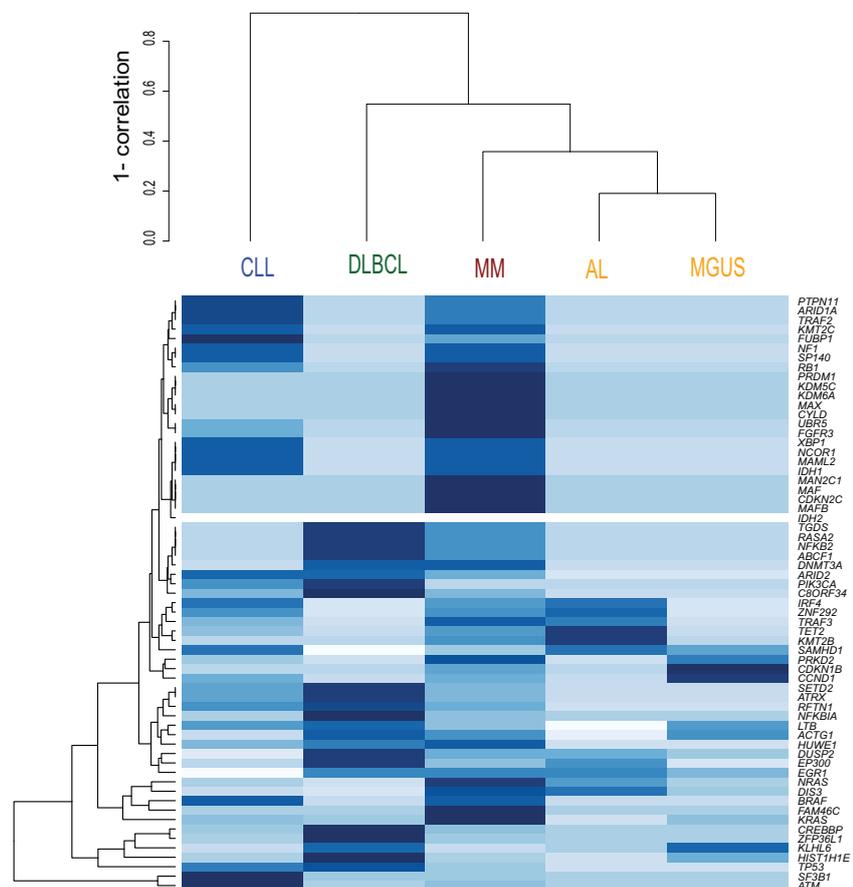
A.



B.



C.





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