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IGVL gene region usage correlates with distinct clinical presentation in IgM vs non-IgM light chain amyloidosis

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Key Points

- IGVL gene and family usage differs significantly in patients with IgM amyloidosis vs non-IgM amyloidosis.
- Differential IGVL gene usage may explain the distinct organ involvement and clinical presentation seen in patients with IgM AL amyloidosis.

Patients with immunoglobulin M (IgM) light chain (AL) amyloidosis have a distinct clinical presentation compared with those with non-IgM amyloidosis. We hypothesized that differential immunoglobulin light-chain variable region (IGVL) gene usage may explain the differences in organ involvement, because IGVL usage correlates with organ tropism. IGVL usage was evaluated by mass spectrometry of amyloid deposits (IgM, n = 45; non-IgM, n = 391) and differed across the 2 groups. In the λ family, *LV2-08* (13% vs 2%; $P < .001$) and *LV2-14* (36% vs 10%; $P < .001$) usage was more common in IgM vs non-IgM amyloidosis, whereas *LV1-44* (0% vs 10%; $P = .02$) and *LV6-57* (2% vs 18%; $P = .004$) usage was less common. In the κ family, there was a trend toward higher *KV4-01* (11% vs 4%; $P = .06$) usage in IgM amyloidosis. IGVL usage correlated with disease characteristics/organ tropism. *LV2-14* (more common in IgM amyloidosis) has historically been associated with peripheral nerve involvement and lower light chain burden, which were more frequent in IgM amyloidosis. *LV1-44* (less common in IgM), associated with cardiac involvement, was less frequent in IgM patients. *LV6-57* (less common in IgM) is associated with t(11;14), which was less frequent in IgM patients. In conclusion, IGVL gene usage differs in patients with IgM vs non-IgM amyloidosis and may explain the distinct clinical presentation.

Introduction

The clinical presentation and frequency of organ involvement are quite distinct in patients with immunoglobulin M (IgM) light chain (AL) amyloidosis compared with patients with non-IgM AL amyloidosis.[1-3] Patients with IgM amyloidosis have less heart involvement and more nerve, soft tissue, and lung involvement compared with patients with non-IgM AL amyloidosis.[1-3] Further, we recently demonstrated that patients with IgM AL amyloidosis have distinct subtypes of disease; 2 main subtypes are a pure plasma cell disorder (PPCD) subtype and a lymphoplasmacytic lymphoma (LPL)-like subtype.[2] Patients with these subtypes had distinct morphologic features on bone marrow biopsy and unique genetic features; however, organ involvement was not different between these 2 subtypes.[2] Therefore, despite gaining more understanding of the disease biology of IgM AL amyloidosis, the biological underpinnings behind the distinct organ involvement patterns in IgM amyloidosis remain unclear.

It has been observed that immunoglobulin light-chain variable region (IGVL) gene and gene family usage of the plasma cell clone impacts phenotypic manifestations in AL amyloidosis, particularly organ tropism.[4-9] Given the variability in clinical features among patients with IgM vs non-IgM AL amyloidosis,

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we hypothesized that IGVL gene usage would be different between these 2 groups and may explain the distinct organ involvement observed in IgM amyloidosis.

Methods

Patients with newly diagnosed IgM and non-IgM amyloidosis seen at the Mayo Clinic from January of 2006 to December of 2015 were identified. Subcutaneous fat aspirate or formalin-fixed paraffin-embedded tissue biopsy specimens were used for mass spectrometry (MS)-based typing of the deposits. MS analysis was performed at the time of clinical diagnosis or at a later date using archived diagnostic biopsy specimens.[10,11] IGVL gene usage was assessed by liquid chromatography tandem MS, as previously described.[6,10,11] The method does not obtain a complete amino acid sequence of the pathogenic light chain variable region. It uses consensus sequences of the framework regions of the LCV genes to find the LCV gene of the pathogenic clone. The success rate of MS in determining IGVL gene usage was 98% (436/446 patients for whom IGVL data were evaluated on MS). Results were ambiguous in 7 cases, and IGVL gene usage could not be identified in 3 cases. Testing for the $MYD88^{L265P}$ mutation was done on archival samples, as previously described.[2]

Organ involvement was defined by consensus criteria.[12] Patients with IgM AL amyloidosis were categorized into LPL and PPCD subtypes, as previously described.[2] Statistical analyses were carried out using JMP 14 software (SAS Institute, Cary, NC). The χ^2 test and Fischer's exact test were used for univariate analysis for categorical variables, and the Wilcoxon rank-sum/Kruskal-Wallis tests were used for continuous variables. Survival analysis was carried out using the Kaplan-Meier method, and the log-rank test was used to compare survival curves. Overall survival (OS) was defined as the time from diagnosis of systemic AL amyloidosis to death.

Results

Overall, 436 patients met the inclusion criteria and had successful IGVL gene usage identification on MS, including 45 patients with IgM AL amyloidosis and 391 patients with non-IgM AL amyloidosis.

As expected, we observed differences in baseline characteristics and organ involvement in patients with IgM vs non-IgM AL amyloidosis (supplemental Table 1). Patients with IgM amyloidosis were older (68 vs 64 years; $P = .07$) and were less likely to have λ as the involved light chain (64 vs 75%; $P = .15$), although the difference did not reach statistical significance. The difference in involved and uninvolved free light chains (dFLC) was lower in the IgM group (14.6 vs 25.8 mg/dL; $P = .006$), and N-terminal pro–B-type natriuretic peptide (NTProBNP) levels were also lower (median, 1890 vs 2655 pg/mL; $P = .01$) compared with non-IgM amyloid patients. The presence of t(11;14) was less common by fluorescence in situ hybridization (29% vs 48%; $P = .09$), although the difference did not reach statistical significance. We have previously shown that patients with LPL-type IgM amyloidosis typically do not have t(11;14) (seen in 0% of patients).[2] In contrast, patients with plasma cell–type IgM amyloidosis have a similar frequency of t(11;14) as do patients with non-IgM amyloidosis (approximately half of patients in each group).[2] Similar to our prior study, no patient (0/10) with LPL morphology IgM AL amyloidosis in the current cohort had t(11;14) compared with 60% (6/10) of patients with PPCD IgM AL. A total of 86% patients (18/21) with LPL-type IgM AL amyloidosis were $MYD88^{L265P}$ positive. This percentage is similar to that observed in Waldenström's macroglobulinemia/LPL without AL amyloidosis.[13] In contrast, none of the patients with PPCD subtype IgM AL amyloidosis (0/9) had MYD88 mutation ($P < .001$).

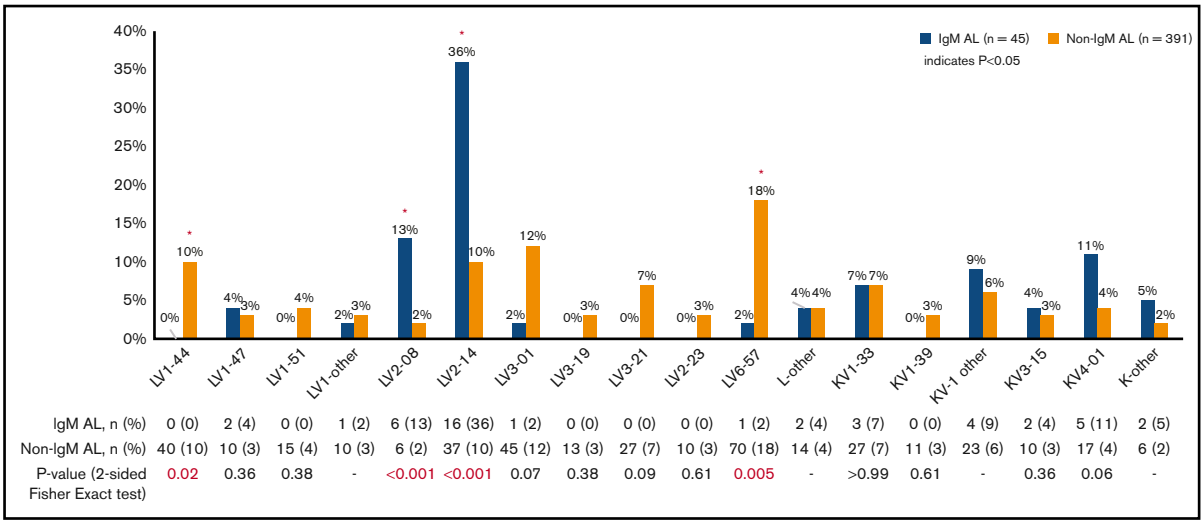
Patterns of organ involvement were different; heart involvement showed a trend toward being less frequent in IgM patients (62% vs 76%; $P = .055$), whereas peripheral nerve (31% vs 17%; $P = .04$), soft tissue (40% vs 21%; $P = .007$), and lung involvement (7% vs 3%; $P = .18$) were more common in patients with IgM vs non-IgM AL amyloidosis. There was no difference in liver (9% vs 15%), kidney (49% vs 51%), gastrointestinal (22% vs 25%), or autonomic nervous system involvement (18% vs 14%). We did not observe any significant differences in treatment among patients with IgM and non-IgM amyloidosis, with the exception of frequent rituximab use in combination chemotherapy in the IgM cohort (supplemental Table 2).

IGVL gene usage

As shown in Figure 1, IGVL usage differed across the 2 groups. In the λ group, *LV2-08* (13% vs 2%; $P < .001$) and *LV2-14* (36% vs 10%; $P < .001$) usage was more common in patients with IgM amyloidosis, whereas *LV1-44* (0% vs 10%; $P = .02$) and *LV6-57* (2% vs 18%; $P = .005$) usage was less common in the IgM amyloidosis group compared with the non-IgM amyloidosis cohort. There was also a trend toward less frequent involvement of *LV3-01* (2% vs 12%; $P = .07$) in IgM amyloid patients. In the κ group, there was a trend toward higher *KV4-01* (11% vs 4%; $P = .06$) gene usage in patients with IgM amyloidosis. Interestingly, IgM heavy chain deposition in the amyloid proteome was noted in 42% (n = 19) of patients with IgM amyloidosis. Heavy chain deposition could not be evaluated in the non-IgM group because of the limitations described before, mainly the lack of a robust method to exclude IgG heavy chain contamination by serum IgG.[6]

Correlation between IGVL gene usage and clinical features

IGVL gene usage correlated with known disease characteristics and organ tropism across the 2 groups. *LV2-14* usage, which was more common in the IgM group, was previously shown to be associated with a higher frequency of peripheral nerve involvement in AL amyloidosis and lower dFLC.[6] Peripheral nerve involvement is more common in IgM amyloidosis, and this group is also known to have lower dFLC levels, as seen in the current cohort and prior studies.[1-3,14] In the current study of patients with IgM AL amyloidosis, peripheral nerve involvement was seen in 38% (6/16) of patients with *LV2-14* usage and in 28% (8/29) of patients without *LV2-14* usage, although the difference was not statistically significant ($P = .5$). *LV1-44* has been associated with a higher likelihood of cardiac involvement in prior studies.[6,8] Several studies have shown that patients with IgM amyloidosis have lower rates of heart involvement.[1-3,14] *LV1-44* was seen less frequently in our IgM amyloid cohort; rates of cardiac involvement were lower than in the non-IgM amyloidosis group. Historically, *LV6-57*, which was less frequent in the IgM group, has been associated with higher rates of t(11;14).[6] We recently demonstrated that patients with IgM amyloidosis have lower rates of t(11;14) vs non-IgM amyloidosis, with t(11:14) seen in 27% vs 50% of patients, respectively ($P = .008$).[2] Key data on IGVL gene usage and clinical



	LV1-44	LV1-47	LV1-51	LV1-other	LV2-08	LV2-14	LV3-01	LV3-19	LV3-21	LV2-23	LV6-57	L-other	KV1-33	KV1-39	KV-1 other	KV3-15	KV4-01	K-other
IgM AL, n (%)	0 (0)	2 (4)	0 (0)	1 (2)	6 (13)	16 (36)	1 (2)	0 (0)	0 (0)	0 (0)	1 (2)	2 (4)	3 (7)	0 (0)	4 (9)	2 (4)	5 (11)	2 (5)
Non-IgM AL, n (%)	40 (10)	10 (3)	15 (4)	10 (3)	6 (2)	37 (10)	45 (12)	13 (3)	27 (7)	10 (3)	70 (18)	14 (4)	27 (7)	11 (3)	23 (6)	10 (3)	17 (4)	6 (2)
P-value (2-sided Fisher Exact test)	0.02	0.36	0.38	-	<0.001	<0.001	0.07	0.38	0.09	0.61	0.005	-	>0.99	0.61	-	0.36	0.06	-

Figure 1. IGVL gene usage in IgM vs non-IgM AL amyloidosis. *Red *P* values denote 2-sided $P < .05$.

correlation in the entire cohort are described in supplemental Table 3.

IgM AL amyloidosis subtypes

Among patients with IgM AL amyloidosis, there was no statistically significant difference in IGVL gene usage noted based on plasma cell morphology vs LPL morphology, although the sample size in each group was small (supplemental Table 4).

Correlation between IGVL gene usage and survival outcomes

In the IgM AL amyloidosis group, those with *LV2-08* gene usage had inferior OS (median OS, 8 vs 32 months; $P = .01$), likely as a result of differences in baseline prognostic factors and hematologic response rates, because this was not an independent prognostic factor after adjusting for Mayo 2012 stage and response. Among patients with IgM AL, those with *LV2-08* had numerically higher cardiac involvement (83% vs 59%; $P = .38$). There was no difference in hematologic response rates based on LV2-08 gene usage (supplemental Figure 1; supplemental Table 5). There was no statistically significant association between survival and the 2 other common IGVL genes expressed in IgM AL patients, *LV2-14* (median OS, 21 vs 31 months; $P = .3$) and KV04-01 (median OS, 83 vs 22 months; $P = .9$).

Discussion

In summary, IGVL gene usage differed significantly in patients with IgM vs non-IgM amyloidosis, correlating with known differences in disease characteristics and organ involvement. To our knowledge, this is the first study to report IGVL gene usage differences in IgM vs non-IgM AL amyloidosis. Earlier reports from our group and from other investigators demonstrated that IGVL gene usage correlates with clinical presentation and, to a certain extent, with outcomes in patients with AL amyloidosis.[15,16] Although studies evaluating IGVL gene usage have been done using various methods,[5,7,8,15,17] MS has become the method of choice for accurately typing amyloidosis and characterizing the amyloid proteome, including IGVL gene usage analysis.[6,10,11,18] A recent study demonstrated preferential IGVL gene usage in cystic light chain deposition disease of the lung, suggesting that IGVL gene usage may explain organ involvement in paraproteinemic disorders beyond AL amyloidosis.[19] Patient comorbidities or other mechanisms, such as the presence of anti-MAG antibodies in IgM disorders, may be associated with neuropathy in our cohort, although it is often difficult to determine the exact cause of neuropathy clinically when >1 risk factor is present. Data on these confounding variables was not systemically available in the entire cohort.

Our data provide further biological insight into IgM amyloidosis, which is a rare disorder. It is worth noting that two thirds of IgM amyloid patients had λ light chain disease, which is seen in only one fourth of IgM monoclonal disorders without amyloidosis. Therefore, presence of the λ light chain should raise suspicion for amyloidosis. There was a trend toward higher KV4-01 usage in patients with IgM amyloidosis, but this was not statistically significant. A lack of differences in κ family gene usage is likely due to the small number of patients with κ light chain amyloidosis. A strength of this study is the large number of patients evaluated for IGVL gene usage. Despite the unique differences noted in the 2 groups, there was overlap in IGVL gene usage in patients with IgM and non-IgM AL amyloidosis, suggesting that additional factors may contribute to the distinct clinical characteristics in patients with IgM AL amyloidosis. For example, it is unknown whether deposition of the IgM heavy chain in the amyloid deposits, seen in 42% of patients in the IgM group, impacts clinical manifestations. These questions should be examined in future studies. In a previous report by our group,[6] non-IgG heavy chains were present in amyloid deposits in 12% of patients with localized AL amyloidosis and only 2% of patients with systemic AL amyloidosis. Similarly, in a large independent cohort of >16 000 patients with 21 subtypes analyzed by MS,[20] heavy chain deposits were present in a minority of light chain amyloidosis patients (3.4%), with IgM being the most common heavy chain deposited. In this study, 59% of all amyloid cases were the AL type (light chain–only amyloidosis); these cases usually do not have a heavy chain in the deposit. A total of 2.3% of all amyloid cases (or 3.4% of the light and light+heavy chain amyloid cases) have a heavy chain. Among cases with a heavy chain

deposit, the percentages of cases with μ, α, γ, or δ heavy chain isotypes were 44%, 28%, 20%, and 6%, respectively.

Because our data for IGVL gene usage are derived by laser microdissecting the amyloid deposits from organ biopsies and subjecting them to MS, an inherent limitation of this method is the inability to compare IGVL gene usage in our cohort to IGVL gene usage in plasma cell dyscrasias that do not have amyloid deposits in tissues, such as LPL without amyloidosis or IgM myeloma. Given this technical limitation of the amyloid-specific method for IGVL gene usage identification, we are also unable to assess IGVL gene usage in polytypic plasma cells or the B-cell repertoire. The informatics method is also built on the assumption that the clonality observed in the amyloid deposit is low (ie, monoclonal or at most a few clones). Hence, the method itself cannot characterize all light chain variable regions present in a polyclonal population. Hence, as applied, the IGVL gene usage assessment in our study reflects the IGVL gene usage of the amyloidogenic clone (plasma cells and, in LPL patients, the lymphoplasmacytic component, including B cells), because we isolated amyloid deposits prior to IGVL characterization.

In conclusion, IGVL gene and family usage differs significantly in patients with IgM amyloidosis vs non-IgM amyloidosis; this may explain, in part, the distinct organ involvement and clinical presentation that are seen in patients with IgM AL amyloidosis. Because this is the first report of differences in IGVL gene usage in patients with IgM vs non-IgM amyloidosis, our results should be validated in future studies.

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Authorship

Contribution: S.S., S.D., and M.A.G. designed the study; S.D. performed MS IGVL analyses; S.S. performed the data analysis and wrote the first draft of the manuscript; and all authors critically reviewed the manuscript.

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