

CLINICAL TRIALS AND OBSERVATIONS

Clarifying immunoglobulin gene usage in systemic and localized immunoglobulin light-chain amyloidosis by mass spectrometry

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Key Points

- Mass spectrometry is a high-throughput, low-resource technique that can identify immunoglobulin variable region gene from tissue specimens.
- IGVL gene usage is restricted and different between systemic and localized AL and only partially explains organ tropism in this disease.

The goal of this study was to investigate the frequency of use of light-chain variable region (IGVL) genes among patients with systemic (AL_S) and localized (AL_L) amyloidosis and to assess for associations between IGVL gene usage and organ tropism. We evaluated clinic charts from 821 AL patients seen at the Mayo Clinic who had bone marrow, fat pad, and solid organ tissue samples typed by liquid chromatography tandem mass spectrometry (LC-MS). We identified 701 patients with AL_S and 120 with AL_L. Overall, we were able to identify an IGVL gene in 87 (72%) patients with AL_L and 573 (82%) patients with AL_S. When compared with AL_L, LV6-57 was more common, whereas KV3-20 and heavy-chain codeposition were less common in AL_S. In this large series of AL_S, characteristics particular to specific genotypes became apparent. LV6-57 patients were more likely to have renal involvement and to harbor a translocation 11;14. LV3-01 patients were less likely to have advanced cardiac disease and renal involvement. LV2-14 patients were more likely to have peripheral nerve involvement, an intact circulating immunoglobulin, and lower circulating dFLC. LV1-44 patients were more likely to have cardiac involvement. KV1-33 patients had more liver involvement and higher circulating dFLC. Finally, KV1-05 was associated with inferior overall survival but not independently of cardiac stage. IGVL gene usage appears to provide clues about disease pathophysiology and tissue tropism. LC-MS is a high-throughput and low-resource technique that can be used to identify IGVL gene from clinical tissue specimens. (*Blood*. 2017;129(3):299-306)

Introduction

Systemic immunoglobulin light-chain amyloidosis (AL_S) is the most common form of systemic amyloidosis, and despite significant advances in the field, it remains a fatal disease with a worse prognosis compared with that of multiple myeloma.^{1,2} AL_S is caused by the progressive deposition of structurally unstable, clonal immunoglobulin light chains that form systemic amyloid deposits. Outcomes of patients with AL amyloidosis are primarily dictated by the type and extent of organ involvement, especially cardiac involvement.³ Little is known about what regulates organ tropism in this disease.

Sometimes, amyloid deposition may be localized to the site of amyloid precursor protein production by a neoplastic plasma cell clone. These patients are labeled as having localized AL amyloidosis (AL_L) and have a much better prognosis because vital organs like heart, liver, and kidney are not compromised. These patients do not require systemic treatment of their AL amyloidosis. For these reasons, accurate distinction between the two is very important.

In an effort to elucidate mechanisms of organ tropism in AL_S, prior authors hypothesized that organ tropism may be a function of light-chain variable region (IGVL) gene and gene family of the clone.⁴⁻⁹

These contributions have been seminal because they helped establish important trends in IGVL gene rearrangements that confer a higher risk for specific organ involvement. These studies involved a small number of patients because they relied on the use of time-consuming, polymerase chain reaction–based sequencing of bone marrow plasma cells to identify IGVL genes and have not been translated to the clinical practice because of complexity of the analytical process.

We have established a proteomics-based clinical assay for identifying amyloid types using clinical tissue sample proteomic analysis by liquid chromatography/tandem mass spectrometry (LC-MS).¹⁰⁻¹² This method has been in routine use in a CAP/CLIA-compliant clinical laboratory in our institution since 2008. This method was expanded to identify clonotypic peptides from AL deposits and identify the IGVL gene and gene family with 100% specificity.¹¹ Here we apply this methodology in the largest cohort of patients with AL_S and AL_L in an effort to definitively characterize the relationship between the IGVL gene repertoire and organ tropism in this disease. We propose that the ability to identify the amyloidogenic clone directly from clinical tissue specimens using a proteomics-based methodology is a novel and potentially powerful clinical and research tool.

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Methods

Patient population and clinical data

We included patients with AL_L and AL_S seen at the Mayo Clinic between August of 1991 and July of 2013. The Mayo Foundation Institutional Review Board approved the study and only patients who consented to having their medical records reviewed for research purposes were included. The diagnosis of AL amyloidosis was predicated on the presence of a biopsy specimen that stained positive by Congo red and exhibited green birefringence under polarized light and was documented to be AL amyloidosis by typing with LC-MS. Individual patients' records were reviewed to abstract clinical organ involvement and systemic vs localized involvement. Patients without a circulating monoclonal protein (detected by immunofixation of serum and urine and serum-free light-chain assay), a bone marrow without clonal plasma cells (detected by immunohistochemistry and/or flow cytometry), and a fat aspirate negative for Congo red–positive deposits were classified as AL_L. Further tests to rule out systemic organ involvement in suspected AL_L were done as clinically indicated. Organ involvement for AL_S was defined using established criteria.¹³ All patients with cardiac involvement had either a positive endomyocardial biopsy or a suggestive echocardiographic picture in addition to elevation of at least one cardiac biomarker (ie, B-type natriuretic peptide [BNP], N-terminal pro B-type natriuretic peptide [NT-proBNP], or troponin). No patient without biopsy-confirmed cardiac involvement had normal cardiac biomarkers. Isolated cardiac or renal involvement was defined as isolated involvement of only the heart or kidneys, respectively, without other organs being clinically involved. Accurate abstraction of absence of organ involvement was not possible for some patients based on the limited information provided in their medical records, leaving 679 (97%) patients with cardiac involvement and 664 (95%) patients with renal involvement evaluable for these analyses. Extent of cardiac involvement was graded according to the Mayo 2004 staging system,³ which defines stage I as troponin T <0.035 ng/mL and NT-proBNP <332 ng/mL, stage III as both biomarkers equal to or above the respective thresholds, and stage II as either biomarker equal to or above the respective thresholds. Extent of renal involvement was graded according to the recently published renal staging system.¹⁴

Identification of immunoglobulin variable region gene usage and heavy-chain codeposition

The proteomics method for amyloid typing has been previously described both for solid tissue specimens and fat aspirates.^{10–12} Briefly, for patients with solid tissue specimens, laser microdissection (LMD) was performed on 3 to 4 Congo red–positive areas of formalin-fixed paraffin-embedded tissue blocks. The dissected tissue was digested into tryptic peptides and analyzed by LC-MS. For each fat aspirate specimen, 3 to 4 spicules were individually solubilized and digested in trypsin without undergoing LMD.¹² Tandem MS spectra present in each sample's raw file were identified with 3 different database search engines: Sequest,¹⁵ X!Tandem,¹⁶ and Mascot,¹⁷ all of which had been augmented with known protein sequences from human IGVL genes and families. The method for identifying IGVL gene was validated in an independent population (n = 30) by comparing it with mRNA sequencing performed on bone marrow samples,¹¹ and it was found to be 100% specific.

All peptides identified from a patient's sample were combined and filtered using the Scaffold software (Proteome Software, Portland, OR). Peptide identifications were accepted if they could be established with a >95% probability, at a 90% confidence interval. A spectral counting approach was used, and a count ≥5 was considered to be clinically significant. For every case, we created a proteomics profile that lists all of the confident protein identifications present in each sample along with their respective spectral counts. The IGVL gene was identified based on abundance of spectral counts.

We also considered the presence or absence of heavy chains in tissue specimens. Because we do not have a robust method to identify contamination of samples by serum immunoglobulins, we excluded all IgG-class immunoglobulins for all heavy-chain analyses because these are found in high concentrations in human serum. We included all detectable IgM, IgA, and IgD types. Furthermore, because our sequence libraries are not enriched to allow identification of IGH variable genes, we only examined IGH as a dichotomous

Table 1. Demographics and clinical characteristics of patients

Characteristic (N = 821)	AL _S (N = 701)	AL _L (N = 120)
Male	445 (64%)	46 (38%)
Age (y), median (range)	63 (36–89)	60 (32–86)
Seen at Mayo within 90 d of diagnosis	530 (76%)	61 (50%)
Circulating monoclonal protein in the serum or urine*		
κ	187 (27%)	
IgG	270 (39%)	
IgA	40 (6%)	
IgM	42 (6%)	
IgD	5 (0.7%)	
Light chain only	284 (41%)	
Biclonal	5 (0.7%)	
No circulating monoclonal protein	18 (3%)	
No data on monoclonal protein	37 (5%)	
Organ involvement		
Cardiac	457 (65%)	0
Mayo 2004 cardiac stage†		NA
I	76 (19%)	
II	150 (38%)	
III	169 (43%)	
Renal	357 (51%)	0
Renal stage‡		NA
I	64 (42%)	
II	70 (46%)	
III	17 (12%)	
Peripheral and autonomic nervous system	112 (16%)	0
Hepatic	56 (8%)	0
Gastrointestinal system	174 (25%)	18 (16%)
Skin	23 (3%)	36 (30%)
Other soft tissue	100 (14%)	37 (31%)
Lung parenchymal	43 (6%)	31 (26%)
Lymph nodes	13 (2%)	1 (0.8%)
Bladder or ureter	5 (1%)	18 (15%)
Tracheobronchial	0	5 (4%)

*Identified by immunofixation of serum or urine or by the serum-free light-chain assay.

†Of 701 patients, 395 (56%) had sufficient data available to assign cardiac stage.

‡Of 350 patients with renal involvement, 151 (43%) had enough data available to assign renal stage.

variable based on the presence or absence of IGH-constant regions in amyloid specimens.

Statistical analysis

The Fisher exact test and the Kruskal-Wallis test were used to ascertain differences between categorical and continuous variables, respectively. For gene groups that contained at least 30 patients, differences between those with and without the gene were sought for multiple characteristics including: presence or absence of organ involvement, plasma cell clone characteristics (dFLC, bone marrow plasma cells, fluorescence in situ hybridization [FISH]/cytogenetics), and differences in markers of organ damage (proteinuria, cardiac stage, renal stage). *P* values <.01 were considered significant to correct for multiple comparisons. *P* values between .01 and .05 were noted to be possible trends and worthy of future exploration with larger sample sizes of patients. Overall survival (OS) from diagnosis was calculated using the Kaplan-Meier method. All statistics were done using JMP software (SAS, Cary, NC).

Results

IGVL gene distribution and heavy-chain codeposition in AL_L and AL_S

The patients' baseline characteristics for AL_S and AL_L are shown in Table 1. We identified 120 patients with AL_L and 701 patients with

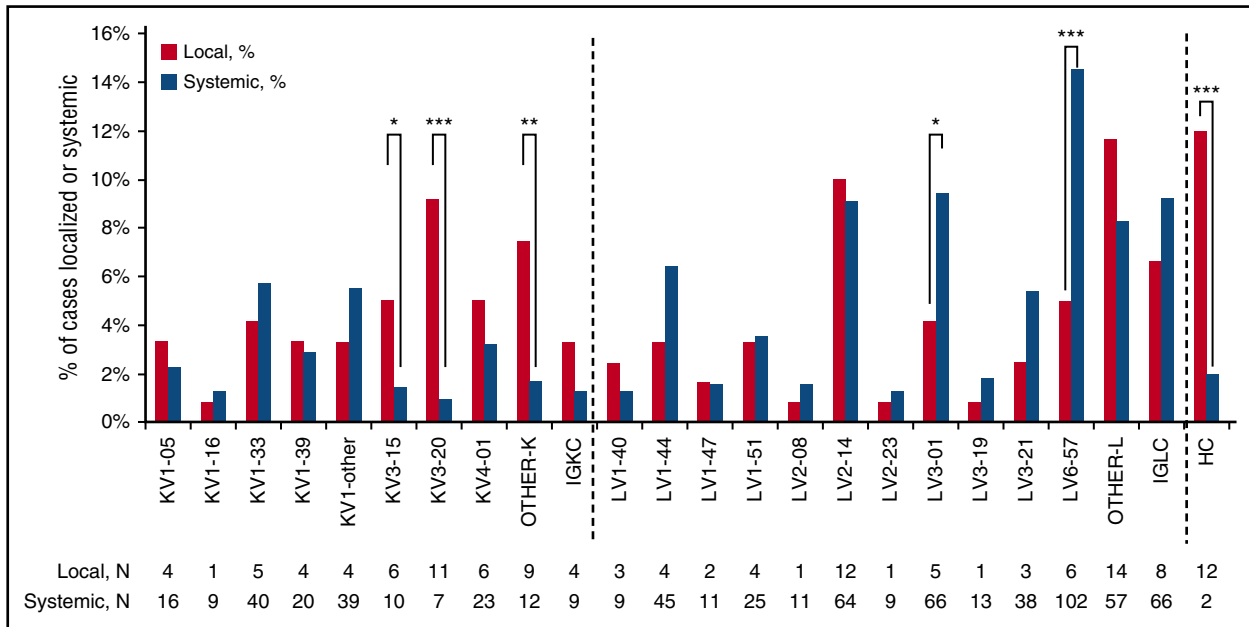


Figure 1. Immunoglobulin variable region gene usage in systemic and localized amyloidosis. *KV1-other*: includes rare KV1-genes (KV1-02, 06, 08, 09, 12, 13, 17, and 27) and cases where only KV1 family could be determined. *Other κ/λ*: includes rare κ genes (n = 8, KV2-28, 30, 38, KV3-01, 07, 11, KV6-21, rare KV1 excluded because included in KV1-other); rare λ genes (n = 29, LV1-01, 36, 52, 57, LV2-08, 11, 18, LV3-09, 10, 20, 25, LV4-60, 69, and LV10-54), cases where only family could be determined (n = 49); *IGKC and IGLC*: cases that no gene or family could be determined (n = 86). HC, non-IgG heavy chain. **P* < .05, ***P* < .01, ****P* < .001.

AL_S. Patients with AL_L were younger and less likely to be male. The number of κ AL_L and AL_S were 54 (45%) and 187 (27%), respectively. Overall, we were able to identify an IGVL gene in 86 (72%) patients with AL_L and in 573 (82%) patients with AL_S (*P* = .01). There was no difference in identifying IGVL gene between κ- and λ-restricted cases. We could not identify an IGVL gene or family in 16 (13%) AL_L cases and 79 (11%) AL_S cases (Figure 1).

KV1-33 was the most common κ IGVL, and the KV1 family overall was the most common κ family identified in both AL_S (16% of cases) and AL_L (12% of cases) (Figure 1). KV3-20 was underrepresented in AL_S compared with AL_L (1% vs 9%, respectively; *P* < .001), and LV6-57 was overrepresented (15% vs 5%, respectively; *P* < .001), in addition to being the most common IGVL gene in AL_S. Overall, the LV6-57, LV3-01, and LV2-14 genes and the KV1 family constituted 49% of clones identified in AL_S.

Non-IgG heavy chains (IGH) were present in the amyloid deposits of 14 (12%) patients with AL_L (5 IgA, 3 IgD, and 6 IgM) and 11 (2%) of patients with AL_S (1 IgA, 10 IgM) (*P* < .001) (Figure 1). Of the 11 systemic patients, 7 had a circulating heavy chain with the same restriction as the one deposited (6 with IgM and 1 with IgA). To exclude biopsy site as a source of bias, we examined heavy-chain codeposition across various tissue types. The proportion of samples with heavy-chain codeposition in AL_S was similar among bone marrow, fat aspirate, and nonmarrow/fat samples.

IGVL usage and organ tropism

Although this is the largest series of patients of AL IGVL, given the diversity of the repertoire of gene use in patients with AL, the actual numbers of patients using any given IGVL is small. The groups with at least 30 patients are shown in Table 2. As mentioned in the methods, *P* values < .01 were considered significant, but *P* values < .05 were considered highly suggestive of potential trends. Of 701 AL_S patients, 679 (97%) had enough information to ascertain presence or absence of

cardiac involvement and 664 (95%) to ascertain presence or absence of renal involvement.

Not surprisingly, renal involvement was more common in patients with LV6-57 compared with those without, and was less common in patients with LV3-21 compared with those without (Table 2). When considering less common genes, KV1-05 patients had a trend toward less renal involvement compared with those without: 18% vs 53%, *P* = .02. IGH deposition was not different between patients with and without renal involvement. When examining the 78 patients with and without isolated renal involvement, no significant difference in IGVL gene usage or heavy-chain codeposition was found.

Cardiac involvement was very prevalent and was present in 66% of patients. In spite of this, the likelihood of cardiac involvement was particularly high among patients with certain genes: 92% for patients with LV3-19 and 92% for patients with KV1-05 (Figure 2). However, these genes were identified in only 2% of AL_S cases. The rate of cardiac involvement for patients with these genes and with LV1-44 (Table 2) compare to those without and were as follows: LV3-19 (92% vs 65%, *P* = .02), KV1-05 (92% vs 65%, *P* = .03), and LV1-44 (80% vs 65%, *P* = .03; Table 2). IGH deposition was not different between patients with and without cardiac involvement. When comparing the 84 patients with isolated cardiac involvement (ie, no other organ involvement) to those without (ie, with cardiac but also with other organ involvement), no significant difference in IGVL gene usage or heavy-chain codeposition was found.

When considering organs other than heart and kidneys, there was a trend for patients with LV2-14 to have a higher rate of peripheral neuropathy (Table 2). KV1-33 was more likely to be associated with liver involvement and less likely to be associated with peripheral nerve involvement (Table 2).

Among the 120 patients with AL_L, LV2-14 was overrepresented in those with gastrointestinal involvement (Table 3), but otherwise no

Table 2. Notable patient characteristics by IGVL gene usage for most abundant clones*

Gene	Organ involvement	dFLC levels/amyloidogenicity	Organ toxicity	FISH abnormalities
LV6-57 (n = 102)	↑ Renal involvement: 79% vs 48%, <i>P</i> = .0004	No differences	24-h urine protein >5 g 56% vs 34%, <i>P</i> = .02†	↑t(11;14) 55% vs 40%, <i>P</i> < .05 ↓Trisomies 9.5% vs 22%, <i>P</i> < .05
LV3-01 (n = 66)	↓ Renal involvement: 41% vs 54%, <i>P</i> < .05	No differences	↓ Mayo stage III, 26% vs 43%, <i>P</i> = .02†	No differences
LV2-14 (n = 64)	↑ In gastrointestinal AL _L	↓ dFLC 9.47 vs 24.5 mg/dL, <i>P</i> = .025	No differences	No differences
	↑ Peripheral nerve involvement: 25% vs 14%, <i>P</i> < .05	↑ Intact circulating Ig 80% vs 57%, <i>P</i> = .002		
LV1-44 (n = 45)	↑ Cardiac involvement: 80% vs 65%, <i>P</i> = .03	No differences	No differences	↓Trisomies 4% vs 22%, <i>P</i> < .05
KV1-33 (n = 40)	↑ Liver involvement 27% vs 7%, <i>P</i> = .0002	↑ dFLC 64.8 mg/dL vs 22.2 mg/dL, <i>P</i> < .002	No differences	No differences
	↓ Peripheral nerve involvement 2.7% vs 15.6%, <i>P</i> = .017			
LV3-21 (n = 38)	↓ Renal involvement 29% vs 54%, <i>P</i> = .0025		No differences	↑ Monosomy 13q/13q 61% vs 33%, <i>P</i> = .017

BMPC, bone marrow plasma cells.

Characteristics tested for significance included: dFLC, circulating intact immunoglobulin, bone marrow plasma cells, presence of t(11;14), deletion 13q or trisomies by FISH, renal involvement, cardiac involvement, nerve involvement, liver involvement, Mayo 2004 cardiac stage, urine total protein, and estimated glomerular filtration rate.

*Comparison group for each row is patients not harboring this IGVL gene.

†Independent of baseline dFLC levels.

other statistically significant relationships were identified including non-IgG heavy-chain codeposition.

IGVL association with amyloidogenicity and plasma cell clone characteristics

Among other variables, we examined whether there were any differences in baseline dFLC levels according to IGVL gene, hypothesizing

that more amyloidogenic clones could cause clinical organ involvement at lower dFLC levels compared with less amyloidogenic ones. Of 701 AL_S patients, 463 (66%) had dFLC levels available at diagnosis. Finally, because bone marrow FISH has been associated with organ involvement and prognosis in AL_S,¹⁸⁻²⁰ we examined whether there was any differential IGVL gene usage according to bone marrow FISH.

Patients with KV1-33 were more likely to have higher and patients with LV2-14 lower baseline dFLC levels, respectively, compared with

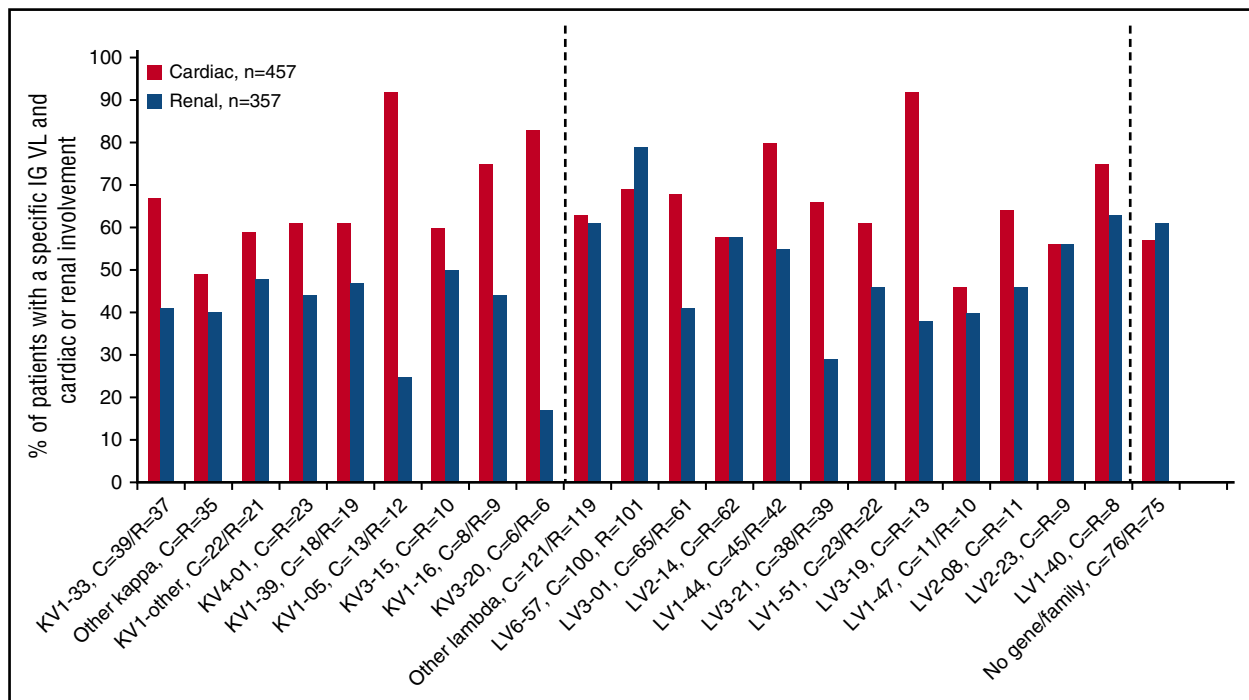


Figure 2. Cardiac and renal involvement according to immunoglobulin variable region gene usage. Other κ/λ: includes rare κ genes (KV2-28, 30, 38, KV3-01, 07, 11, KV6-21, rare KV1 excluded); rare λ genes (LV1-01, 36, 52, 57, LV2-08, 11, 18, LV3-09, 10, 20, 25, LV4-60, 69, and LV10-54); cases where only family could be determined (n = 39); and cases that no gene or family could be determined (n = 77); KV1-other: includes rare KV1-genes (KV1-02, 06, 08, 09, 12, 13, 17 and 27). *P* = nonsignificant across groups within patients with cardiac involvement. *P* < .0001 across gene groups within patients with renal involvement. C, cardiac; R, renal.

Table 3. Light-chain variable region gene distribution in localized amyloidosis according to organ involvement

Ig V gene	Skin, N = 36	Lung parenchyma, N = 31	Urothelial, N = 18	Gastrointestinal, N = 17
KV1-33	2 (6%)	1 (3%)	0	2 (11%)
Other κ	5 (14%)	5 (16%)	0	2 (11%)
KV1-other	1 (3%)	0	0	0
KV4-01	1 (3%)	1 (3%)	2 (11%)	1 (5%)
KV1-39	3 (8%)	0	0	0
KV1-05	3 (8%)	1 (3%)	0	0
KV3-15	1 (3%)	1 (3%)	2 (11%)	1 (5%)
KV1-16	1 (3%)	0	0	0
KV3-20	2 (6%)	5 (16%)	1 (6%)	2 (11%)
Other λ	6 (17%)	8 (26%)	5 (28%)	1 (5%)
LV6-57	0	3 (10%)	2 (11%)	0
LV3-01	2 (6%)	1 (3%)	0	1 (5%)
LV2-14*	2 (6%)	0	2 (11%)	5 (26%)
LV1-44	1 (3%)	1 (3%)	1 (6%)	1 (1%)
LV3-21	2 (6%)	0	0	1 (5%)
LV1-51	1 (3%)	2 (7%)	0	1 (5%)
LV3-19	0	1 (3%)	0	0
LV1-47	1 (3%)	0	1 (6%)	0
LV2-08	0	0	1 (6%)	0
LV2-23	1 (3%)	0	0	0
LV1-40	1 (3%)	1 (3%)	1 (6%)	0
No gene/family	1 (3%)	3 (10%)	4 (22%)	1 (5%)
Codeposited heavy chain	2 (6%)	1 (3%)	0	2 (11%)

Other κ/λ: includes rare κ genes (KV2-28, 30, 38, KV3-01, 07, 11, KV6-21, rare KV1 excluded); rare λ genes (LV1-01, 36, 52, 57, LV2-08, 11, 18, LV3-09, 10, 20, 25, LV4-60, 69, and LV10-54); cases where only family could be determined; and cases that no gene or family could be determined. KV1-other: includes rare KV1-genes (KV1-02, 06, 08, 09, 12, 13, 17, and 27).

* $P < .01$.

patients without these IGVL genes (Table 2). Patients with a LV2-14–producing clone were also more likely to have an intact circulating immunoglobulin (Table 2). There were no differences in IGVL gene usage between patients with very low dFLC levels (<5 mg/dL) and patients with dFLC (≥ 5 mg/dL).

We noted that patients with LV6-57 were more likely to have more advanced renal disease (24-hour urine protein >5 gr) independently of baseline dFLC levels (odds ratio [OR] 2.5, 95% confidence interval [CI] 1.2–5.4; $P = .02$). Patients with LV3-01 had a trend toward lower (OR 2.6, 95% CI 1.1–6.6; $P = .03$) and patients with KV1-05 had a trend toward higher (OR 4.4, 95% CI 1.03–30; $P = .045$) rates of stage III cardiac disease, respectively, an effect independent of baseline dFLC levels.

There was a trend for patients with LV6 to be more likely to harbor the t(11;14) translocation and less likely to harbor trisomies. When considering patients with LV1-44, trisomies appeared to be less common (Table 2). Finally, LV3-21 patients had a trend of higher bone marrow plasmacytosis and higher frequency of monosomy 13 (Table 2).

IGVL and overall survival

We performed limited exploratory analyses evaluating IGVL usage and OS mostly because we felt there were a lot of missing data to draw safe conclusions in regards to OS, which is influenced by several factors we were not able to accurately capture in this study (hematologic response, organ response, host factors, delayed diagnosis). Of 701 patients with AL_S, first-line treatment data were available for 385 (55%) patients, hematologic response to first-line treatment was available

for 361 (51%) patients, and only 103 (23%) patients with cardiac involvement and 143 (40%) patients with renal involvement had at least 12 months of follow-up for organ response.

Only KV1-05 appeared to be associated with a decreased OS compared with patients harboring other IGVL genes: median survival 12 months vs 59 months, $P = .003$ (supplemental Figure 1, available on the *Blood* Web site). This effect, however, was not independent of cardiac stage on a multivariate model including KV1-05, IGVL type and cardiac stage.

Discussion

This is the largest study to date reporting on IGVL gene and family usage in patients with systemic and localized AL amyloidosis. For the first time, we demonstrate that IGVL gene usage is different between AL_S and AL_L and that non-IgG heavy-chain deposition is more common in AL_L compared with AL_S. We identify new potential predilections for IGVL gene usage in AL_S patients with cardiac, renal, and hepatic involvement, and AL_L patients with gastrointestinal involvement. Finally, we identify new clones that are more likely to be associated with different levels of organ damage in AL_S.

Figure 3 provides a comparison between our results and those that have been published in the literature,^{6–9} and those that can be found in AL Base.²¹ Of the 29 to 33 functional IGV λ genes and the 31 to 36 functional IGV κ genes in the human genomic repertoire, consistent with previous publications, LV6-57 is the most common IGVL gene identified among patients with AL_S.^{8,22} Approximately half of the light-chain variable gene repertoire in AL_S is comprised of LV6-57, LV3-01, and LV2-14 genes and the KV1 family (particularly KV1-33). Not only are these genes the most commonly observed in AL_S, but they and their gene families are also conspicuously more common in AL_S than in the normal B-repertoire.^{21,23,24} In contrast, the distribution of IGVL genes and families for patients with AL_L was very similar to that seen in the normal B-cell repertoire, with LV6 found less commonly and the KV3 family (and the KV3-20 gene in particular) more commonly.⁸ The potential mechanisms underlying the differences between IGVL gene usage in AL_S, AL_L, nonamyloidogenic plasma cell dyscrasias, and normal controls are not clear. In the case of AL_L, the bias of IGVL gene usage might be explained by different antigenic stimuli. AL_L is often associated with autoimmune diseases²⁵ and lymphoproliferative disorders that may be autoimmune driven (ie, MALT lymphomas).²⁶

When considering patients with cardiac involvement, some of our results are consistent with those of Perfetti and colleagues.⁹ We also found a greater than twofold higher rate of cardiac involvement among patients with LV1-44. In their study of 99 patients with AL_S, Perfetti and colleagues observed trends for a lower risk for patients with LV3-01 and LV6-57 to have “dominant” cardiac involvement.⁹ In our study, rates of cardiac involvement for these two genotypes were no different from those found in other genotypes, but patients with LV3-01 were more likely to have less severe cardiac involvement by Mayo stage. Our study also revealed provocative trends among cardiac patients that will need to be reproduced in larger cohorts. For example, $>90\%$ of patients with KV1-05 and LV3-19 had cardiac involvement; the significance of this finding is tempered by the fact that there were only 16 and 13 patients with these variable genes in our systemic

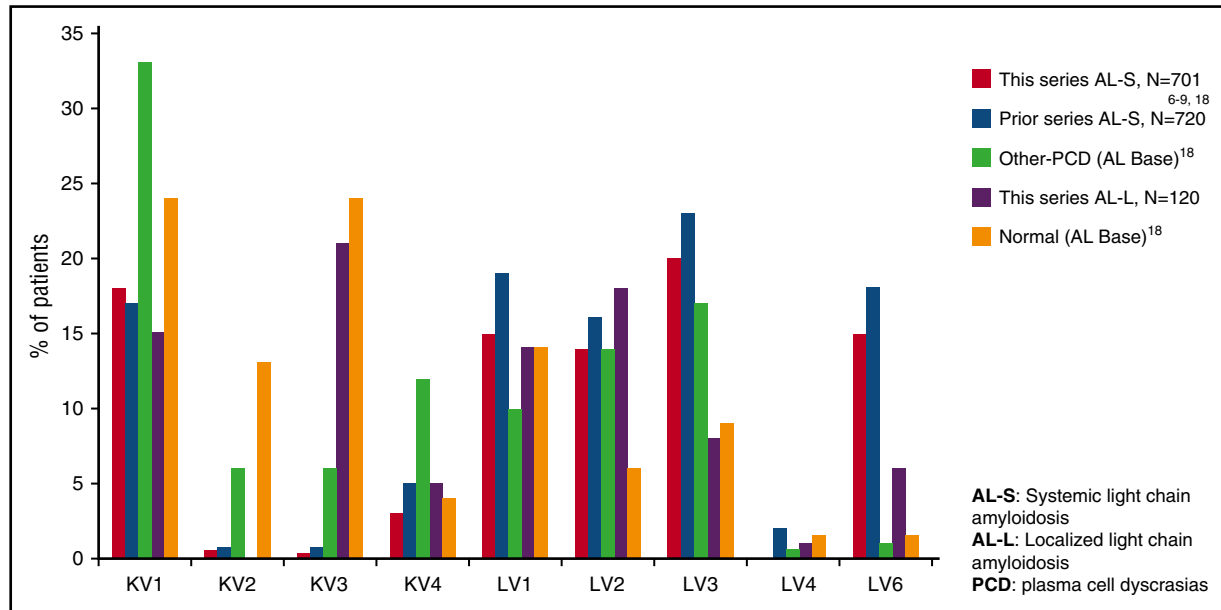


Figure 3. Variable region family usage in light-chain amyloidosis, other plasma cell dyscrasias, and normal subjects. Data for the prior ALs series are pooled from references 6-9,18 and data for both the other plasma cell dyscrasias and normal B cells are derived from AL Base.²¹

cohort. KV1-05 was also the only IGVL associated with worse overall survival, although this effect appeared to be dependent on cardiac stage.

In patients with renal involvement, LV6-57 was more commonly encountered, which is similar to prior reports.^{6,7} In addition, our observations that renal involvement was less common in patients with LV3-01 and LV3-21 are novel. Furthermore, LV6-57 was associated with higher levels of proteinuria independent of baseline dFLC levels, suggesting that LV6-57 might be more nephrotoxic. LV6-57 was more commonly associated with translocation 11;14, which is the most common FISH abnormality found in AL amyloidosis but also in other monoclonal gammopathies of renal significance²⁷ and multiple myeloma patients presenting with renal damage.²⁸

Other interesting novel observations included increased and decreased rates of peripheral neuropathy in patients with LV2-14 and KV1-33, respectively. Finally, patients with hepatic involvement have previously been shown to be more commonly κ restricted,^{6,29} although the dominance of KV1-33 is a novel finding in our study.

In general, the KV1 family appeared to be less amyloidogenic *in vivo*, because it required higher levels of circulating free light chains (ie, dFLC) to lead to clinically significant organ involvement. This has been supported by biophysical experiments.³⁰⁻³² In contrast, some of the increased amyloidogenicity seen with LV2-14 could be attributed to the higher burden of nonconservative mutations—which have been associated with increased amyloidogenicity—within the λ family.^{33,34} Our results regarding extent of organ involvement and IGVL amyloidogenicity are novel and potentially hypothesis-generating but at the same time exploratory and require further validation.

In our study, heavy chains were more commonly codeposited in AL_L. Furthermore, our study probably underestimated the codeposition of IGH in AL_L, because we had to exclude all IgG-class IGH for the lack of a robust mechanism to exclude sample contamination by serum IgG. Nonetheless, IGH codeposition has been noted in both AL_L and AL_S in the past.^{26,35-38} It needs to be noted, however, that we could not identify the variable heavy-chain region in codeposited IGH, and therefore we could not differentiate between monoclonal or polyclonal IGH

deposits. The fact that in most AL_S patients the heavy chain isolated in the amyloid deposits was also detected in the serum suggests that a systemic deposition mechanism exists at least for the IgM and IgA immunoglobulin subclasses in AL_S.

Our results suggest that the IGVL gene and family restriction is only part of the puzzle that constitutes clinical organ tropism and amyloid toxicity. Other, as yet unidentified, factors, either intrinsic to the structure of the light-chain protein or related to the micro-environment of the amyloid plaque,³⁵ as well as the composition of the codeposited proteins within the amyloid plaque itself, have also been postulated to play a role and should be the focus of future research.

Limitations of our study include the small number of patients harboring specific IGVL clones, which limits the power of some of our observations. Furthermore, there is still room for improvement in terms of the sensitivity of this assay, which will improve as our IGVL sequence template libraries and peptide identification algorithms improve. The number of cases included in this study is proven to be powerful enough to uncover significant trends, but further study will be required. Extent of organ involvement and amyloid toxicity is a function of other factors in addition to baseline dFLC, which could not always be captured with accuracy in this retrospective study (delay in diagnosis, host factors). Finally, we did not perform comprehensive OS or any organ response analyses primarily because of missing data. Furthermore, organ response and survival are influenced by several variables that we were not able to accurately capture in this retrospective study (eg, depth and especially duration of hematologic response, host factors, disease stage at presentation). Some of these exploratory analyses have been published in abstract form and no association was found.³⁹

Strengths of the study include the size of the cohort, the inclusion of a large number of AL_L patients, as well as the methodology itself, which is a fast and inexpensive method to evaluate IGVL genes from data already generated as part of the clinical typing assay.

In conclusion, we have clarified IGVL gene and family usage in a large cohort of patients with systemic and localized AL amyloidosis, using a novel methodology that relies on MS of clinical tissue

specimens. We show for the first time that IGVL gene and family repertoire is different between AL_S and AL_L. Our data show that IGVL gene family usage does not entirely explain clinical organ tropism, but they can provide a platform upon which future research exploring how other aspects of the amyloid proteome relate to organ tropism and clinical outcome. Moreover, our observations about propensity of certain genes being more amyloidogenic and associated with particular presentations could have direct clinical implications, especially as the methodology for top down sequencing of circulating monoclonal proteins moves into the mainstream. For example, individuals with an apparent monoclonal gammopathy of undetermined significance with KV3 gene usage are unlikely to have or develop AL_S; in contrast, patients with LV6-57 monoclonal gammopathy should be comprehensively evaluated for AL_S and followed closely, because LV6-57 is more common in AL_S than in the normal B-cell repertoire. In this fashion, the results presented in this

study could be applied in AL_S prevention, a major bottleneck in improving patient outcomes.⁴⁰

Authorship

Contribution: T.V.K., S.D., P.J.K., and A.D. conceived and designed the study; T.V.K., S.D., and A.D. analyzed and interpreted data; and all authors provided study materials of patients, collected and assembled data, and wrote and gave final approval of the manuscript.

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References

- Kumar SK, Rajkumar SV, Dispenzieri A, et al. Improved survival in multiple myeloma and the impact of novel therapies. *Blood*. 2008;111(5):2516-2520.
- Kumar SK, Gertz MA, Lacy MQ, et al. Recent improvements in survival in primary systemic amyloidosis and the importance of an early mortality risk score. *Mayo Clin Proc*. 2011;86(1):12-18.
- Dispenzieri A, Gertz MA, Kyle RA, et al. Serum cardiac troponins and N-terminal pro-brain natriuretic peptide: a staging system for primary systemic amyloidosis. *J Clin Oncol*. 2004;22(18):3751-3757.
- Prokaveva T, Spencer B, Kaut M, et al. Soft tissue, joint, and bone manifestations of AL amyloidosis: clinical presentation, molecular features, and survival. *Arthritis Rheum*. 2007;56(11):3858-3868.
- Bellavia D, Abraham RS, Pelliikka PA, et al. Utility of Doppler myocardial imaging, cardiac biomarkers, and clonal immunoglobulin genes to assess left ventricular performance and stratify risk following peripheral blood stem cell transplantation in patients with systemic light chain amyloidosis (AL). *J Am Soc Echocardiogr*. 2011;24(4):444-454.
- Comenzo RL, Zhang Y, Martinez C, Osman K, Herrera GA. The tropism of organ involvement in primary systemic amyloidosis: contributions of Ig V(L) germ line gene use and clonal plasma cell burden. *Blood*. 2001;98(3):714-720.
- Abraham RS, Geyer SM, Price-Troska TL, et al. Immunoglobulin light chain variable (V) region genes influence clinical presentation and outcome in light chain-associated amyloidosis (AL). *Blood*. 2003;101(10):3801-3808.
- Perfetti V, Casarini S, Palladini G, et al. Analysis of V(lambda)-J(lambda) expression in plasma cells from primary (AL) amyloidosis and normal bone marrow identifies 3r (lambdall) as a new amyloid-associated germline gene segment. *Blood*. 2002;100(3):948-953.
- Perfetti V, Palladini G, Casarini S, et al. The repertoire of λ light chains causing predominant amyloid heart involvement and identification of a preferentially involved germline gene, IGLV1-44. *Blood*. 2012;119(1):144-150.
- Vrana JA, Gamez JD, Madden BJ, Theis JD, Bergen HR III, Dogan A. Classification of amyloidosis by laser microdissection and mass spectrometry-based proteomic analysis in clinical biopsy specimens. *Blood*. 2009;114(24):4957-4959.
- Dasari S, Theis JD, Vrana JA, et al. Proteomic detection of immunoglobulin light chain variable region peptides from amyloidosis patient biopsies. *J Proteome Res*. 2015;14(4):1957-1967.
- Vrana JA, Theis JD, Dasari S, et al. Clinical diagnosis and typing of systemic amyloidosis in subcutaneous fat aspirates by mass spectrometry-based proteomics. *Haematologica*. 2014;99(7):1239-1247.
- Gertz MA, Comenzo R, Falk RH, et al. Definition of organ involvement and treatment response in immunoglobulin light chain amyloidosis (AL): a consensus opinion from the 10th International Symposium on Amyloid and Amyloidosis, Tours, France, 18-22 April 2004. *Am J Hematol*. 2005;79(4):319-328.
- Palladini G, Hegenbart U, Milani P, et al. A staging system for renal outcome and early markers of renal response to chemotherapy in AL amyloidosis. *Blood*. 2014;124(15):2325-2332.
- Eng JK, McCormack AL, Yates JR. An approach to correlate tandem mass spectral data of peptides with amino acid sequences in a protein database. *J Am Soc Mass Spectrom*. 1994;5(11):976-989.
- Fenyő D, Beavis RC. A method for assessing the statistical significance of mass spectrometry-based protein identifications using general scoring schemes. *Anal Chem*. 2003;75(4):768-774.
- Perkins DN, Pappin DJ, Creasy DM, Cottrell JS. Probability-based protein identification by searching sequence databases using mass spectrometry data. *Electrophoresis*. 1999;20(18):3551-3567.
- Warsame R, Kumar SK, Gertz MA, et al. Abnormal FISH in patients with immunoglobulin light chain amyloidosis is a risk factor for cardiac involvement and for death. *Blood Cancer J*. 2015;5:e310.
- Bochtler T, Hegenbart U, Kunz C, et al. Prognostic impact of cytogenetic aberrations in AL amyloidosis patients after high-dose melphalan: a long-term follow-up study. *Blood*. 2016;128(4):594-602.
- Bochtler T, Hegenbart U, Kunz C, et al. Translocation t(11;14) is associated with adverse outcome in patients with newly diagnosed AL amyloidosis when treated with bortezomib-based regimens. *J Clin Oncol*. 2015;33(12):1371-1378.
- Bodi K, Prokaveva T, Spencer B, Eberhard M, Connors LH, Seldin DC. AL-Base: a visual platform analysis tool for the study of amyloidogenic immunoglobulin light chain sequences. *Amyloid*. 2009;16(1):1-8.
- Solomon A, Frangione B, Franklin EC. Bence Jones proteins and light chains of immunoglobulins. Preferential association of the V lambda VI subgroup of human light chains with amyloidosis AL (lambda). *J Clin Invest*. 1982;70(2):453-460.
- Farner NL, Dörner T, Lipsky PE. Molecular mechanisms and selection influence the generation of the human V lambda J lambda repertoire. *J Immunol*. 1999;162(4):2137-2145.
- Ozaki S, Abe M, Wolfenbarger D, Weiss DT, Solomon A. Preferential expression of human lambda-light-chain variable-region subgroups in multiple myeloma, AL amyloidosis, and Waldenström's macroglobulinemia. *Clin Immunol Immunopathol*. 1994;71(2):183-189.
- Mahmood S, Bridoux F, Venner CP, et al. Natural history and outcomes in localized immunoglobulin light-chain amyloidosis: a long-term observational study. *Lancet Haematol*. 2015;2(6):e241-e250.
- Grogg KL, Aubry MC, Vrana JA, Theis JD, Dogan A. Nodular pulmonary amyloidosis is characterized by localized immunoglobulin deposition and is frequently associated with an indolent B-cell lymphoproliferative disorder. *Am J Surg Pathol*. 2013;37(3):406-412.
- Kourelis TV, Nasr SH, Dispenzieri A, et al. Outcomes of patients with renal monoclonal immunoglobulin deposition disease. *Am J Hematol*. 2016;91(11):1123-1128.
- Greenberg AJ, Rajkumar SV, Therneau TM, Singh PP, Dispenzieri A, Kumar SK. Relationship between initial clinical presentation and the molecular cytogenetic classification of myeloma. *Leukemia*. 2014;28(2):398-403.
- Russo P, Palladini G, Foli A, et al. Liver involvement as the hallmark of aggressive disease in light chain amyloidosis: distinctive clinical features and role of light chain type in 225 patients. *Amyloid*. 2011;18(Suppl 1):92-93.
- Kaplan B, Ramirez-Alvarado M, Sikkink L, et al. Free light chains in plasma of patients with light chain amyloidosis and non-amyloid light chain deposition disease. High proportion and heterogeneity of disulfide-linked monoclonal free light chains as pathogenic features of amyloid disease. *Br J Haematol*. 2009;144(5):705-715.
- Stevens FJ. Four structural risk factors identify most fibril-forming kappa light chains. *Amyloid*. 2000;7(3):200-211.
- Blancas-Mejía LM, Ramirez-Alvarado M. Systemic amyloidoses. *Annu Rev Biochem*. 2013;82:745-774.
- Poshusta TL, Sikkink LA, Leung N, Clark RJ, Dispenzieri A, Ramirez-Alvarado M. Mutations in specific structural regions of immunoglobulin light chains are associated with free light chain levels

- in patients with AL amyloidosis. *PLoS One*. 2009; 4(4):e5169.
34. Ramirez-Alvarado M. Amyloid formation in light chain amyloidosis. *Curr Top Med Chem*. 2012; 12(22):2523-2533.
35. D'Souza A, Theis J, Quint P, et al. Exploring the amyloid proteome in immunoglobulin-derived lymph node amyloidosis using laser microdissection/tandem mass spectrometry. *Am J Hematol*. 2013;88(7):577-580.
36. Kaplan B, Martin BM, Boykov O, et al. Co-deposition of amyloidogenic immunoglobulin light and heavy chains in localized pulmonary amyloidosis. *Virchows Archiv*. 2005;447(4): 756-761.
37. Sethi S, Theis JD, Leung N, et al. Mass spectrometry-based proteomic diagnosis of renal immunoglobulin heavy chain amyloidosis. *Clin J Am Soc Nephrol*. 2010; 5(12):2180-2187.
38. Klein CJ, Vrana JA, Theis JD, et al. Mass spectrometric-based proteomic analysis of amyloid neuropathy type in nerve tissue. *Arch Neurol*. 2011;68(2):195-199.
39. Taxiarchis Kourelis SD, Kurtin PJ, Ramirez-Alvarado M, et al. Immunoglobulin variable region family usage and outcomes of patients with systemic light chain amyloidosis [abstract]. *Blood*. 2014;124(21). Abstract 3402.
40. Kourelis TV, Kumar SK, Go RS, et al. Immunoglobulin light chain amyloidosis is diagnosed late in patients with preexisting plasma cell dyscrasias. *Am J Hematol*. 2014;89(11): 1051-1054.