Genome-wide association study of clinical parameters in immunoglobulin light chain amyloidosis in three patient cohorts

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SUPPLEMENTARY MATERIAL

Genome-wide association study of clinical parameters in immunoglobulin light chain amyloidosis in three patient cohorts

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Association analysis of the clinical profiles

Among Ig profiles, the λ/κ LCO and the λ LCO profiles showed a strong association with SNP rs9344 (**Supplementary Table 1**). The OR for rs9344 in the λ/κ LCO profile was 1.62 ($p=1.99x10^{-12}$) and in the λ LCO profile it was 1.70 ($p=1.29x10^{-11}$). The weakest association was noted for the IgG profile (1.20, $9.69x10^{-3}$), with non-overlapping 95%CIs to the LCO profiles. For overall AL amyloidosis, the OR was 1.35 and for MM it was 1.06, as reported earlier [1]. Z-scores are also listed because they will be used in a figure to be shown later. The median dFLC values were independent of the rs9344 genotypes: A/A, N=147, 211 mg/L; A/G, N=560, 192 mg/L; G/G, N=400, 217 mg/L.

For the IgG profile, rs10507419 reached genome-wide significance with an OR of 1.49 and p-value of $5.63x10^{-8}$. The two isotypes IgG λ and IgG κ showed similar ORs (1.57 and 1.51, respectively) and IgG λ reached genome-wide significance of $2.90x10^{-8}$ (**Supplementary Table 2**). ORs of profiles λ/κ LCO (0.90), λ LCO (0.91), liver (0.98) and κ any (1.00) differed significantly (non-overlapping 95%Cs) from rs10507419. Among MM isotypes, the OR for IgG MM was 1.01 while the ORs for both IgG λ and IgG κ were 1.00.

Genome-wide association was found for SNP rs6752376 in the heart & kidney profile (OR 1.54, $p=2.88 \times 10^{-8}$) (**Supplementary Table 3**). The profiles for kidney and heart only reached ORs of 1.24 and 1.27, respectively. ORs for liver (0.98) and κ any (1.04) profiles differed significantly from the heart & kidney profile. The OR for MM was 1.00.

The liver profile rs7820212 reached genome-wide significance even with a small patient number (194) (OR 1.86, $p= 1.86 \times 10^{-8}$) (**Supplementary Table 4**). ORs of all other clinical profiles differed significantly from the liver profile. The OR for AL amyloidosis overall was 1.07 and for MM it was 1.04.

Of note, there was no or at most moderate heterogeneity for any genome-wide significant associations in **Supplementary Tables 1 to 4** between the 3 AL amyloidosis cohorts as indicated by I^2. However, there were SNPs of lower significance with higher I^2 values indicating either random variation or population-specific differences which could lead to false negative results. The ORs of the significant associations did not change when stratified for age and sex.

We assessed the associations of the previously described 10 putative candidate SNPs from the combined AL amyloidosis cohorts with each of the 9 profiles [1]. With the exception of SNP rs9344 (**Supplementary Table 1**), no other SNP reached a genome-wide significance with AL amyloidosis defined by a clinical profile. However, several SNPs reinforced the above dichotomy for rs9344 and rs10507419 between the λ/κ LCO and IgG profiles noted above. Non-overlapping 95%CIs between these profiles were noted for rs60544959, rs4672160, rs57082053, rs10507419 and rs9634914 (detailed data not shown).

Z-scores are plotted for the 4 SNPs in the 9 clinical profiles (and overall AL amyloidosis and MM) in **Supplementary Figure 1** based on the data shown in Supplementary Tables 1 to 4. Positive scores are shown in red segments and negative ones are shown in green. The Figure illustrates the high scores of the lead SNPs, and exemplifies the dichotomy for rs9344 and rs10507419 in the LCO and IgG profiles.

Functional annotation

For the IgG profile, promoter capture Hi-C data are lacking for the most significant SNP rs10507419 on chromosome 13q13.2 but data are available for the linked SNPs rs9529347 and rs619472921 (r^2=1.00), showing interaction within the *NBEA* gene promoter (**Supplementary Figure 2**). The yellow line links the SNPs with the promoter, $p=10^{-10}$.

Liver profile SNP rs7820212 on chromosome 8q11.23 maps 28kb 3' of *FAM150A*, a ligand for receptor tyrosine kinases. Promoter capture Hi-C data are lacking for rs7820212 but data are available for the linked SNP rs1837633 and other 3 SNPs (r^2=0.97-0.99) shown in **Supplementary Figure 3**. These SNPs interact with the *RB1CC1* gene promoter (yellow line, $p=10^{-11}$).

REFERENCES

1. da Silva Filho MI, Försti A, Weinhold N, et al. Genome-wide association study of immunoglobulin light chain amyloidosis in three patient cohorts: comparison to myeloma Leukemia 2017. In press.

Supplementary Table **1**. Summary statistics for the λ/κ LCO risk allele G of rs9344 among clinical profiles

Profiles	Number of cases	Odds ratio	95% CI [a]	P-value [b]	I^2 [c]	Z-score
Overall AL	**1129**	**1.35**	**1.23-1.48**	**7.80×10^{-11}**	**0.36**	6.51
IgG	447	1.20	1.05-1.38	9.69×10^{-3}	0.00	2.59
λ any	**930**	**1.40**	**1.27-1.55**	**9.28×10^{-11}**	**0.00**	6.48
κ any	265	1.33	1.11-1.59	2.03×10^{-3}	0.00	3.09
λ/κ LCO	**535**	**1.62**	**1.42-1.85**	**1.99×10^{-12}**	**0.00**	7.04
λ LCO	**404**	**1.70**	**1.46-1.98**	**1.29×10^{-11}**	**0.00**	6.77
Kidney	844	1.34	1.20-1.48	6.89×10^{-8}	0.20	5.40
Heart	**835**	**1.39**	**1.24-1.54**	**2.91×10^{-9}**	**0.49**	5.94
HK	426	1.31	1.14-1.52	2.14×10^{-4}	0.38	3.70
Liver	194	1.40	1.14-1.73	1.63×10^{-3}	0.00	3.15
Overall MM	3790	1.06	1.00-1.12	4.00×10^{-2}	0.61	2.09
MM LCO κ[d]	123	1.01	0.78-1.30	0.95		
MM LCO λ[d]	89	1.03	0.75-1.30	0.87		

[a] CI, confidence interval

[b] P-value based on the meta-analysis of the three patient cohorts in AL amyloidosis, and two patient cohorts in multiple myeloma

[c] I^2 proportion of total variance due to heterogeneity

[d] Based on German MM data.

Genome-wide significant associations are indicated in bold

Supplementary Table **2**. Summary statistics for the IgG profile risk allele A of rs10507419 among clinical profiles

Profiles	N cases	Odds ratio	95% CI [a]	P-value [b]	I^2 [c]	Z-score
Overall AL	1129	1.13	1.03-1.25	1.15×10^{-2}	0.00	2.53
IgG	**447**	**1.49**	**1.29-1.72**	**5.63×10^{-8}**	**0.49**	5.43
IgG λ	**345**	**1.57**	**1.34-1.85**	**2.90×10^{-8}**	**0.42**	5.55
IgG κ	85	1.51	1.21-1.89	2.39×10^{-4}	0.67	3.68
λ any	930	1.18	1.06-1.32	2.20×10^{-3}	0.00	3.06
κ any	265	1.00	0.82-1.22	9.88×10^{-1}	0.00	0.02
λ/κ LCO	535	0.90	0.78-1.04	1.63×10^{-1}	0.00	-1.39
λ LCO	404	0.91	0.77-1.07	2.46×10^{-1}	0.35	-1.16
Kidney	844	1.18	1.06-1.32	2.69×10^{-3}	0.00	3.00
Heart	835	1.16	1.04-1.30	1.02×10^{-2}	0.00	2.57
HK	426	1.33	1.15-1.55	1.81×10^{-4}	0.38	3.75
Liver	194	0.98	0.78-1.24	9.00×10^{-1}	0.00	-0.13
Overall MM	3790	1.06	1.00-1.13	4.47×10^{-2}	0.03	2.00
IgG MM	748	1.01	0.88-1.15	9.35×10^{-1}	-	0.08
IgG λ MM	200	1.00	0.80-1.26	9.56×10^{-1}	-	0.06
IgG κ MM	548	1.00	0.86-1.16	9.40×10^{-1}	-	0.07

[a] CI, confidence interval

[b] P-value based on the meta-analysis of three patient cohorts in AL amyloidosis, and two patient cohorts in multiple myeloma; the IgG profiles of MM are based on only German cohort

[c] I^2 proportion of total variance due to heterogeneity

Genome-wide significant associations are indicated in bold

Supplementary Table **3**. Summary statistics for the HK profile risk allele T of rs6752376 among clinical profiles

Profiles	Number of cases	Odds ratio	95% CI [a]	P-value [b]	I^2 [c]	Z-score
Overall AL	1129	1.17	1.06-1.28	9.96×10^{-4}	0.75	3.29
IgG	447	1.20	1.04-1.39	1.12×10^{-2}	0.33	2.54
λ any	930	1.24	1.12-1.38	5.20×10^{-5}	0.67	4.05
κ any	265	1.04	0.87-1.25	6.59×10^{-1}	0.10	0.44
λ/κ LCO	535	1.20	1.05-1.37	7.78×10^{-3}	0.46	2.66
λ LCO	404	1.25	1.08-1.46	3.52×10^{-3}	0.54	2.92
Kidney	844	1.24	1.11-1.38	8.62×10^{-5}	0.71	3.93
Heart	835	1.27	1.14-1.42	1.50×10^{-5}	0.30	4.31
HK	**426**	**1.54**	**1.32-1.79**	**2.88×10^{-8}**	**0.07**	5.55
Liver	194	0.98	0.80-1.21	8.86×10^{-1}	0.00	-0.14
Overall MM	3790	1.00	0.94-1.06	9.25×10^{-1}	0.37	-0.09

[a] CI, confidence interval

[b] P-value based on the meta-analysis of three patient cohorts in AL amyloidosis, and two patient cohorts in multiple myeloma

[c] I^2 proportion of total variance due to heterogeneity

Genome-wide significant associations are indicated in bold

Supplementary Table **4**. Summary statistics for the liver profile risk allele A of rs7820212 among clinical profiles

Profiles	Number of cases	Odds ratio	95% CI [a]	P-value [b]	I^2 [c]	Z-score
Overall AL	1129	1.07	0.98-1.17	1.40×10^{-1}	0.19	1.48
IgG	447	1.00	0.87-1.15	9.63×10^{-1}	0.00	0.05
λ any	930	1.10	0.99-1.21	6.32×10^{-2}	0.52	1.86
κ any	265	0.97	0.81-1.16	7.49×10^{-1}	0.05	-0.32
λ/κ LCO	535	1.13	0.99-1.29	6.67×10^{-2}	0.26	1.83
λ LCO	404	1.14	0.98-1.32	8.30×10^{-2}	0.61	1.73
Kidney	844	1.10	0.99-1.22	8.33×10^{-2}	0.36	1.73
Heart	835	1.09	0.98-1.21	1.07×10^{-1}	0.00	1.61
HK	426	1.02	0.88-1.17	8.04×10^{-1}	0.06	0.25
Liver	**194**	**1.86**	**1.50-2.31**	$\mathbf{1.86 \times 10^{-8}}$	**0.04**	5.63
Overall MM	3790	1.04	0.98-1.10	1.81×10^{-1}	0.00	1.34

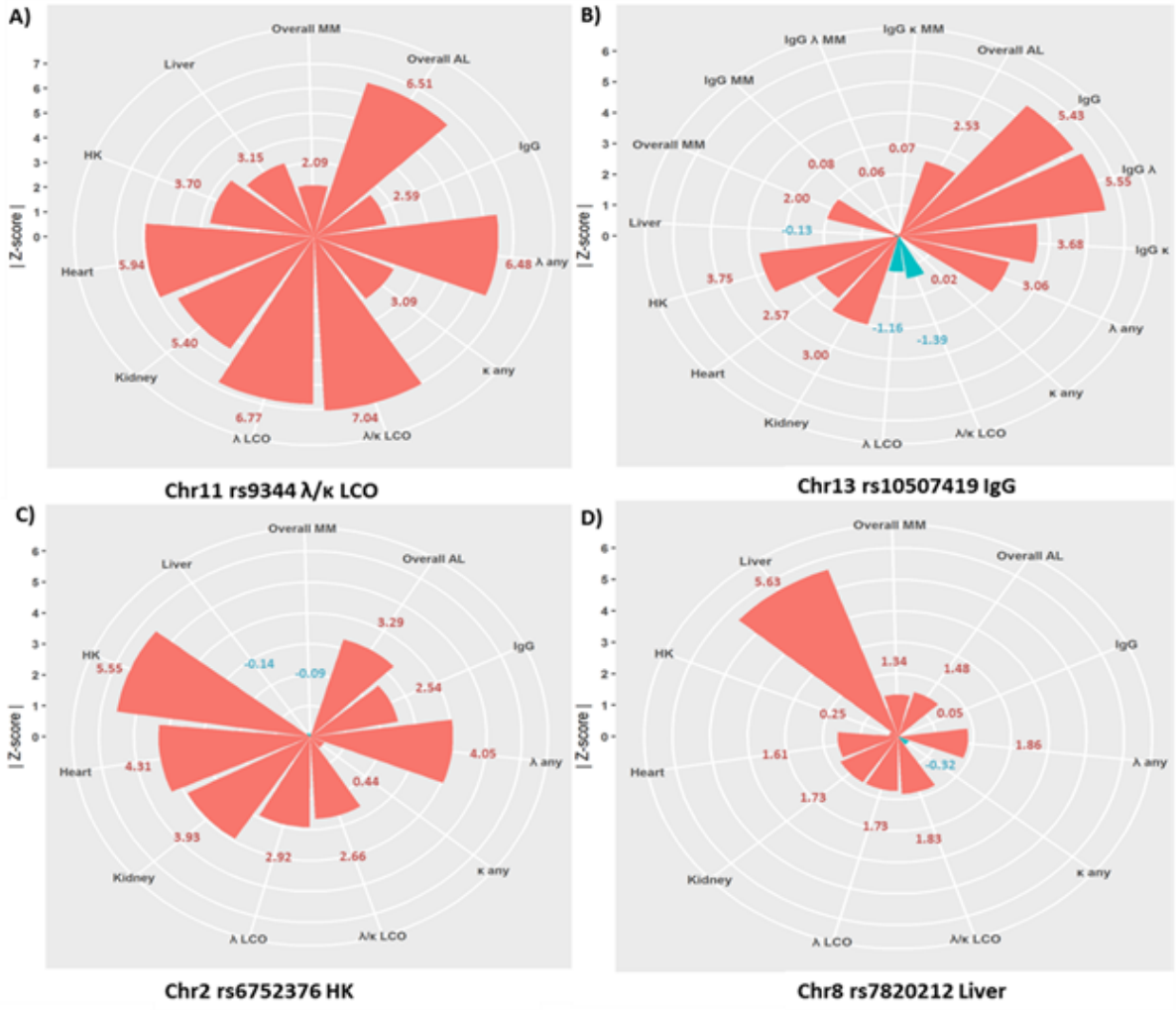
[a] CI, confidence interval

[b] P-value based on the meta-analysis of three patient cohorts in AL amyloidosis, and two patient cohorts in multiple myeloma

[c] I^2 proportion of total variance due to heterogeneity

Genome-wide significant associations are indicated in bold

Supplementary Figure S1. The coxcomb plots correspond to the absolute values of the Z-scores (beta/s.e.) for each clinical profile, and the color indicates whether the SNP is protective (green) or risk-associated (red) for each profile.
A) λ/κ LCO profile; B) IgG profile; C) heart & kidney profile; D) liver profile



Supplementary Figure S2. Promoter-capture long-range interactions of IgG profile SNPs rs61947292 and rs9529347 with the promoter of NBEA in GM12878 cells based on Misfud et al data 18. These SNPs are at LD ($r^2 = 1$) with the lead SNP rs10507419 on chromosome 13 (see Fig. 1 B). In GWAS of the IgG profile these SNPs displayed p= 6.05x10-8 and 6.16x10-8. Interactions of the promoter of NBEA are displayed as links across the circle. The yellow link highlights interaction of the NBEA promoter with the locus of the significant associations in the IgG profile. The interaction score is estimated at 9.98 (p=10-10). In the circle, genes are shown in green, RNA genes in orange. The below blue box represents the bait fragment, which is the promoter of NBEA. The red box represents the target fragment for the promoter interaction. On the top in the boxes a segment of the Manhattan plot of the IgG profile is shown with the relevant SNPs. Tracks further down show the locations of the genes with their symbols. The track with a black round symbol on the left shows chromatin segmentation in GM12787 cells, colored by state (grey quiescent, red transcribed, orange flanking transcribed). The bottom scale shows the chromosomal location.

Supplementary Figure S3. Promoter-capture long-range interactions of liver profile SNPs rs1837633 and other 3 SNP shown in red box with the promoter of RB1CC1 in GM12878 cells based on Misfud et al data 18. These SNPs are at LD (r^2 =0.97-0.99) with the lead SNP rs7820212 on chromosome 8 (see Fig. 1D). The interaction score is 11.19 (p=10-11). For explanation, see the legend of Supplementary Figure 2. In the circle, genes are shown in blue. In the boxes, dark grey color in chromatin segmentation indicates heterochromantin.