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

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ABSTRACT

INTRODUCTION

Immunoglobulin light chain (AL) amyloidosis is a progressive plasma cell dyscrasia which is characterized by deposition of amyloid fibers derived from immunoglobulin light chain systemically in many organs [1]. Amyloidogenic light chains are secreted by clonal plasma cells and, because of the immunoglobulin variability, they are unique to each patient [2, 3]. Characteristics of amyloids related to disease severity and sequelae, including the target organs where amyloids accumulate, such as the heart, kidney, liver and peripheral nerves [3]. Heart failure is usually the critical life-threatening condition; the median survival time depends on the extent of amyloid interference with the critical organ function and survival may range from some months to some years [1, 4]. The incidence in AL amyloidosis is estimated to be somewhat over 3 per million [5, 6]. Monoclonal gammopathy of undetermined significance (MGUS) is often the precursor disease for AL amyloidosis and the related disease multiple myeloma (MM) [7]. It has been reported that some 10 to 15% of multiple myeloma patients have AL amyloidosis [8, 9]; conversely, some 10% of AL amyloidosis patients have MM at the time of diagnosis [10]. AL amyloidosis and MM share genetic risk loci and the 7 single nucleotide polymorphism (SNPs) initially described for MM were replicated in 443 AL amyloidosis patients with a nominal significance of $p<0.05$ [11].

We have recently characterized 10 putative genetic risk loci (at significance level of $<10^{-5}$) for AL amyloidosis using a genome-wide association study (GWAS) approach on a total of 1351 German, UK and Italian patients [12]. In the present study we carry out a systematic association study on the GWAS identified loci and the available clinical data including the affected organs and the type of serum immunoglobulin (Ig).

METHODS

The patient populations and GWAS analysis have been described elsewhere [12]. Shortly, the German amyloidosis patients (595 passed the GWAS quality control) were ascertained through the Amyloidosis Center at University Clinic Heidelberg. The UK samples (474) were obtained from the National Amyloidosis Centre, London and the Italian samples (282) came from the Amyloidosis Research and Treatment Center,

Pavia. The diagnostic criteria used were as described [13]. DNA was genotyped using Illumina Human OmniExpress-12 v1.0 and related arrays. Local control populations included 2,107 Germans, 5637 Britons 465 Italians.

A total of 9 clinical profiles were selected among organ involvement (kidney, heart, heart & kidney and liver, irrespective of whether other organs were involved) and Ig profiles (IgG with intact Ig, λ any, κ any, λ/κ light chain only (LCO), and λ LCO).

Analysis of the GWAS data was performed using imputed data as described [12]. All SNPs having a minor allele frequency (MAF) <1% were excluded. The association test between imputed SNPs and AL amyloidosis was performed in SNPTESTv2.5. The three data sets were combined in meta-analysis and heterogeneity was assessed by the I^2 statistic (interpreted as low <0.25, moderate 0.50 and high >0.75). Genomic locations are given in NCBI Build 37/UCSC hg19 coordinates. For genome-wide significance, a limit of $p< 5x10^{-8}$ was used.

In order to test homogeneity of the results between AL amyloidosis and MM ASSET analysis was performed [14]. This method explores all possible subsets for negative, positive, or null associations, identifying the subset with the strongest association signal; it also accounts for the multiple tests required by the subset analysis.

To investigate chromatin state segmentation profiles (ChromHMM) and 3-dimentional interactions (Hi-C) at risk loci we made use of the ENCODE project data on cell lines, including lymphoblastoid cells (GM12878). We also used HaploReg v4.1 (www.broadinstitute.org/mammals/haploreg) to evaluate the regulatory nature and the possible functional effects of SNPs and their proxies $r^2 \geq 0.8$, or 0.95 [15]. All relevant data available in HaploReg were considered in the SNP search but when multiple cell types were listed data on hematologic cell types were reported. Association profiles were visualized using the Locuszoom [16] in conjunction with the UCSC genome browser [17]. Z score calculated for SNPs as log OR divided by standard error [18].

RESULTS

We selected 9 clinical profiles for a specific analysis of GWAS data (Table 1). Among Ig related profiles, λ any (with or without heavy chains) was the most common one, found in 930 patients. λ/κ LCO was found in 535 patients. Kidney and heart profiles were the largest organ profiles, including over 800 patients each, and liver profile was the smallest with only 194 patients. The median diagnostic ages differed minimally, from 62 to 66 years. The male-female ratio was 1.37 overall and it did not appreciably differ between the profiles. In the bottom of Table 1 data on the MM cohorts are given.

Association analysis and comparison with MM

We carried out a systematic association analysis of each of the 9 clinical profiles against controls in each of the 3 cohorts. Manhattan plots are shown for joint analysis in 4 clinical profiles with genome-wide associations. In the liver profile a genome-wide association, based on imputed SNPs, was noted in chromosome 11 but it had a MAF of 1%; thus few individuals had the variant allele and the association was considered no further.

Among Ig profiles, the λ/κ LCO and the λ LCO profiles showed a strong association with SNP rs9344 (Table 2). The OR for rs9344 OR 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 [12]. Z-scores are also listed because they will be used in figures to be shown later.

For the IgG profile, rs10507419 reached genome-wide significance with an OR of 1.49 and p-value of $5.63x10^{-8}$. The two subgroups IgG λ and IgG κ showed similar ORs (1.57 and 1.51, respectively) and IgG λ reached genome-wide significance of $2.90x10^{-8}$ (Table 3). 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 the IgG profile. Among MM subtypes, 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, $p=2.88 \times 10^{-8}$) (Table 4). 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 x 10^{-8}) (Table 5). 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 Tables 2 to 5 between the 3 AL amyloidosis cohorts as indicated by I^2. 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 [12]. With the exception of SNP rs9344 (Table 2) no other SNP associated specifically with AL amyloidosis defined by a clinical profile.

Biological interference

Regional plots of association are shown in Fig. 2 for the genome-wide significant SNPs in 4 clinical profiles. For the λ/κ LCO profile, rs9344 on chromosome 11q13.3 maps to a splice site in the *cyclin D1* gene as shown previously (Fig. 2A) [12]. For the IgG profile, SNP rs10507419 on chromosome 13q13.2 maps within the *LINC00457* gene (*long intergenic non-protein coding RNA 457*) of unknown function and resides 330 kb 5' of *NBEA* (*neurobeachin*) (Fig, 2B). ENCODE Hi-C data are lacking for rs10507419 but data are available for the linked SNP (r^2=1.00) rs9529341, 1 kb away, showing long-range association within the *NBEA* gene (Supplementary Fig. 1, not included). The SNP changes motif for transcription factor Pax-4. STEFAN: WE NEED DATA ON 13q deletion for this point.

The heart & kidney profile risk SNP rs6752376 on chromosome 2p25.2 is located between two RNA genes, 63 kb from *LINC01247* (*long intergenic non-protein coding RNA 1247*) and 9.9 kb 3' of *ACO17053.1* (not shown in Fig. 2C); functions of both of these are unknown. rs6752376 is a moderate expression quantitative

trait locus (eQTL) ($3.7x10^{-5}$) to human metabolic profile relating to serum concentration of pantothenate [19]. According to HaploReg the SNP alters motifs for 3 transcription factors, Nkx2, Nkx3, PLZF. Liver profile SNP rs7820212 on chromosome 8q11.23 maps 28kb 3' of *FAM150A (family with sequence similarity 150 member A*). 116 kb away is the locus for *RB1CC1 (RB1 inducible coiled-coil 1).* The SNP changes motif for transcription factor CEBPB.

DISCUSSION

The recent GWAS on these 3 AL amyloidosis cohorts reported 4 SNPs reaching (or almost reaching) a genome-wide significance [12]. With the exception of the most significant SNP, rs9344, none of the other 3 were associated with the defined 9 clinical profiles, probably because of decreased patient numbers. Interestingly, 3 completely new profile-specific genetic loci were identified with homogeneous results from the 3 cohorts. Independent associations of rs9344 with the two LCO profiles and of rs10507419 with the two IgG profiles show internal consistency.

The preferential association of rs9344 with LCO profiles could possibly be explained by the association of this SNP with translocation (11;14) and the resulting disturbance of IgH production in AL amyloidosis and MM [12, 20, 21]. However no light chain excess has been reported in t(11;14) AL amyloidosis [22, 23]. Data on MM cell line have suggested that compromised production of IgH leads to excess production of free light chains [24]. How rs9344 could interfere with IgH production independent of t(11;14) remains enigmatic. Risk allele G at the splice site of *cyclin D1* encodes a full length cyclin D1 which has many functions, including involvement in double-strand repair with RAD51, BRCA1 and BRCA2 and thus a possible interference with the class switch recombination for *IgH* [25, 26]. Curiously, while the LCO profiles were strongly associated with rs9344, the weakest association was noted for the IgG profile. Conversely, rs10507419 was strongly associated with the IgG profiles while weakly opposite associations were found with this SNP and the LCO profiles. rs10507419 on chromosome 13q13.2 maps close to the *NBEA* locus (13q13.3) which is a fragile site causing deletion of the telomeric end of chromosome 13q in patients with MM, MGUS and AL-amyloidosis [22, 27-29]. We found in Hi-C data that rs10507419 shows long-range association with the *NBEA* locus. Occasionally *NBEA* is fused with the tip of chromosome 8q24 containing *PVT1* [30]. The translocation may interfere with expression of *RB1* which is located at 13q14.2 [29].

The possible functions of rs6752378 SNP associated with heart & kidney profile are unknown as are those for the adjacent RNA genes *LINC01247* and *ACO17053.1*. The SNP may influence serum concentration of pantothenate but how this might be related to the heart & kidney profile remain unknown [19]. Liver profile SNP rs7820212 on chromosome 8q11.23 maps close to *FAM150A*, which is a ligand for receptor tyrosine kinases leukocyte tyrosine kinase (LTK) and anaplastic lymphoma kinase (ALK). These belong to the insulin receptor superfamily, and their aberrant activation has been described in many cancers, such as non-small lung cancer and neuroblastoma in which *ALK* mutations are common [31, 32]. Fusion genes of *ALK* are often found in lymphomas with resulting downstream activation of the Ras/Raf/MEK/ERK pathway [33]. rs7820212 is adjacent to the *RB1CC1* gene which encodes a protein interacting with pathways involved in regulation of cell growth, proliferation, apoptosis, autophagy, and cell migration [34, 35]. It has tumor suppressor properties in enhancing *RB1* (*retinoblastoma 1)* gene expression in cancer cells and promoting senescence [36]. The SNP changes the binding motif for CEBPB, which is an important transcription factor regulating the expression of genes involved in immune and inflammatory responses. CEBPB may regulate osteoclast activity in MM and through redundant functions with CEBPA it may be involved in multiple cellular processes in hematopoietic cells [37, 38].

In conclusion, 4 SNPs reached genome-wide associations in clinical profile-specific AL amyloidosis. While the associations were internally consistent and homogeneous between the 3 cohorts the underlying mechanisms remain speculative but tangible. For rs9344 the preference for LCO amyloidosis is another lead to mechanistic understanding.

CONFLICT OF INTEREST STATEMENT

The authors declare no competing financial interests.

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Supplementary Information accompanies this paper on the Leukemia website

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Table 1. Number of AL amyloidosis and multiple myeloma patients according to clinical profiles

Clinical profiles		German	British[c]	Italian	Joined	Median age (range) in years[a]	Sex-ratio[b]
Overall AL amyloidosis		562	410	257	1129	64 (30-87)	1.37:1
Ig profiles [c]	IgG	194	157	96	447	66 (30-87)	1.19:1
	IgG λ	160	116	69	345	66 (30-87)	1.16:1
	IgG κ	34	24	27	85	66 (40-85)	1.30:1
	λ any	438	304	188	930	64 (30-87)	1.38:1
	κ any	122	74	69	265	65 (38-87)	1.28:1
	λ/κ LCO	312	96	127	535	62 (37-84)	1.49:1
	λ LCO	231	84	89	404	62 (37-84)	1.59:1
Organ profiles	Kidney	358	320	166	844	64 (30-87)	1.30:1
	Heart	396	239	200	835	64 (34-87)	1.44:1
	HK	180	140	106	426	63 (38-87)	1.39:1
	Liver	105	57	32	194	63 (34-87)	1.49:1
Overall multiple myeloma		1508	2282	-	3790	63 (27-89)	1.41:1
Ig profiles	IgG MM	748	-	-	-	57 (30-72)	1.44:1
	IgG λ MM	200	-	-	-	58 (33-72)	1.17:1
	IgG κ MM	548	-	-	-	57 (30 -72)	1.55:1

a: Median age of the joined cohort.

b: Sex-ratio is calculated as male:female ratio for the joined cohort.

c: Data on some clinical profiles were missing in the British cohort.

Table **2**. Summary statistics for the λ/κ LCO risk allele G of rs9344 in 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

[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

Genome-wide significant associations are indicated in bold

Table **3**. Summary statistics for the IgG profile risk allele A of rs10507419 of in 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 cohc 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

Table **4**. Summary statistics for the HK profile risk allele T of rs6752376 in 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**	$\mathbf{2.88 \times 10^{-8}}$	**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

Table **5**. Summary statistics for the liver profile risk allele A of rs7820212 in 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**	**1.86×10^{-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

LEGENDS TO FIGURES

Figure 1. Manhattan plots of association analysis for AL amyloidosis clinical profiles with genome-wide significant results. A) λ/κ LCO profile; B) IgG profile; C) heart & kidney profile; D) liver profile. The x-axis shows the chromosomal position and the y-axis is the significance (–log10 P; 2-tailed) of association derived by logistic regression. The red line shows the genome-wide significance level (5×10^{-8}) and the blue line shows suggestive significance level (1×10^{-5}). The significant/top SNPs are labeled.

Figure 2. Regional association plots showing the significant/top SNPs in the four AL amyloidosis clinical profiles. A) λ/κ LCO profile; B) IgG profile; C) heart & kidney profile; D) liver profile. The x-axis shows the chromosomal position as Mb and the mapped genes annotated from the UCSC genome browser. The y-axis shows the significance (–log10 P; 2-tailed) on the left and recombination rates (light blue lines) on the right. The reference SNP is labeled and colored purple, the rest of the SNPs are colored based on their r^2 to the reference SNP, on a shown scale, based on pairwise r^2 values from HapMap CEU. Square-shaped SNP symbols represent genotyped SNPs and circle-shaped SNPs represent imputed ones.

Supplementary Figure : Z-diagrams

Supplementary Figure : Hi-C

Supplementary Figure : Forest plot for each SNP considering amyloidosis and multiple myeloma results.