Genome-wide association study of immunoglobulin light chain amyloidosis in three patient cohorts: comparison to myeloma

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ABSTRACT

Immunoglobulin light chain (AL) amyloidosis is characterized by tissue deposition of amyloid fibers derived from immunoglobulin light chain. AL amyloidosis and multiple myeloma (MM) originate from monoclonal gammopathy of undetermined significance. We wanted to characterize germline susceptibility to AL amyloidosis using a genome-wide association study (GWAS) on 1229 AL amyloidosis patients from Germany, UK and Italy, and 7526 healthy local controls. For comparison with MM, recent GWAS data on 3790 cases were used. For AL amyloidosis, single nucleotide polymorphisms (SNPs) at 10 loci showed evidence of an association at $p<10^{-5}$ with homogeneity of results from the 3 sample sets; some of these were previously documented to influence MM risk, including the SNP at the IRF4 binding site. In AL amyloidosis, rs9344 at the splice site of *cyclin D1*, promoting translocation (11;14), reached the highest significance, $p = 7.80 \times 10^{-11}$; the SNP was only marginally significant in MM. SNP rs79419269 close to gene *SMARCD3* involved in chromatin remodeling was also significant ($p=5.2\times10^{-8}$). These data provide evidence for common genetic susceptibility to AL amyloidosis and MM. Cyclin D1 is a more prominent driver in AL amyloidosis than in MM, but the links to aggregation of light chains need to be demonstrated.

INTRODUCTION

Immunoglobulin light chain (AL) amyloidosis is a progressive disease often with a fatal outcome which affects around 3 per million of the population in Western countries [1, 2]. The disease is characterized by the deposition of amyloid fibers derived from immunoglobulin light chain in multiple tissues leading to progressive organ failure. Characteristics of the amyloids relate to the 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 which may ensue in a few months or years after diagnosis [4]. Amyloidogenic light chains which contain the whole light chain or part of the variable domain are generally derived from clonal plasma cells unique to each patient [3, 5]. Poor survival from AL is often related to late diagnosis [6]. Monoclonal gammopathy of undetermined significance (MGUS) is the precursor condition for AL amyloidosis and multiple myeloma (MM) [7]. About 60% of MGUS constitute progressive disease, leading in 90% towards MM or other B-cell neoplasms and in 10% towards AL amyloidosis [7, 8]; AL amyloidosis may also occur outside the context of MGUS.

Three recent genome-wide association studies (GWASs) on MM identified 8 single nucleotide polymorphisms (SNPs) which were associated with the disease, including loci mapping to 2p23.3 (rs6746082), 3p22.1 (rs1052501), 3q26.2 (rs10936599), 5q15 (rs56219066), 6p21.33 (rs2285803), 7p15.3 (rs4487645), 17p11.2 (rs4273077) and 22q13.1 (rs877529) [9-11]. The 7 SNPs (except the one at 5q15) were tested on 443 AL amyloidosis patients and all were replicated at a nominal $p<0.05$ [12]. Translocation t(11;14) which was earlier shown to be influenced by the *cyclin D1* splice site polymorphism (rs603965, also known as rs9344 which will be used here) in MM, was also found in AL amyloidosis with results identical to MM [12, 13]. Importantly, t(11;14) is detected in more than 50% of the patients with AL amyloidosis [14-17] but in 20% or less of MM [13]. Recently a large international pooling of GWAS data on MM was published, increasing the number of associated loci to 17 [18].

To explore the relationship between genetic susceptibility and the development of AL amyloidosis we performed a GWAS on 1229 German, UK and Italian patients and 7526 local

controls. The results show that some genetic changes were shared between AL amyloidosis and MM but also suggest some unique genetic risk factors for AL amyloidosis.

METHODS

German AL amyloidosis patients were ascertained through the Amyloidosis Center at University Clinic Heidelberg. The UK samples were obtained from the National Amyloidosis Centre, London and the Italian samples came from the Amyloidosis Research and Treatment Center, Pavia. Informed consent and the appropriate approvals of the ethical review boards of the respective universities were obtained for all subjects. Blood samples were drawn on newly diagnosed patients. IgM cases were not included due to their different biology. A total of 1351 blood samples were available for analysis. The diagnosis of AL amyloidosis was made in accordance with standard criteria as described [19]. Interphase fluorescence in situ hybridization (iFISH) data were available only in the German population. The methods for the assessment of IgH translocations t(11;14), t(4;14), t(14;16) and translocation with an unknown partner have been described [20].

DNA was genotyped using Illumina Human OmniExpress-12 v1.0 arrays. General genotyping quality control assessment was carried out as previously described and all SNPs and samples presented in this study passed the set thresholds, including exclusion of population outliers by principal component analysis; however this was necessary only on German samples, adjusted for 2 principal components [9, 10]. The patient numbers after quality control were 562 (Germany), 410 (UK) and 257 (Italy) (Table 1). Genotype frequencies were compared with control genotype data generated by the Heinz-Nixdorf Recall (HNR) study on 2147 German individuals, also used in the above MM studies; DNA was genotyped using Illumina HumanOmni1-Quad_v1 (707 individuals) or Human OmniExpress -12v1.0 (1508 individuals) array. Italian healthy controls (n=413) were typed with the former array [21]. The UK controls came from the UK Wellcome Trust Case Control Consortium 26 study on 5029 individuals typed using Illumina Human 1.2M-Duo Custom_v1 [9, 10].

Genotypes for common variants across the genome in the three cohorts were imputed using data from the 1000 Genomes Project (phase 3, Oct. 2014) with IMPUTE2 v2.3.0 [22] after pre-phasing with the SHAPEIT v2 r software [23]. We set thresholds for imputation quality to retain both potential common and rare variants for validation. Specifically, poorly imputed SNPs defined by an information metric < 0.30 were excluded. All SNPs having a minor allele frequency (MAF) <1% were also excluded. The association test between imputed SNPs and AL amyloidosis was performed in SNPTESTv2.5 using the expected genotype counts [24]. All three data sets were combined through an inverse variance meta-analysis under a fixed-effects model using the software GWAMA [25]. All reported SNPs were confirmed by individual genotyping. All genomic locations are given in NCBI Build 37/UCSC hg19 coordinates. For genome-wide significance, a limit of $p< 5x10^{-8}$ was used; a suggestive significance level of 10^{-5} was also considered.

In order to test homogeneity of the results between AL amyloidosis and MM we performed a combined analysis using ASSET [26]. 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. The previously reported MM GWAS described the patient and control populations [10]; these included 1508 German cases and 2107 controls, and 2282 UK cases, independent from the AL cohorts, and 5197 controls.

To investigate chromatin state segmentation profiles (ChromHMM) at risk loci we made use of the ENCODE project data on cell lines, including lymphoblastoid cells (GM12878) [27]. 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 [28]. 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 [29] in conjunction with the UCSC genome browser [27].

RESULTS

Association analysis and comparison with MM

A total of 1229 AL amyloidosis cases and 7526 controls passed the quality control filters for GWAS; the main characteristics of the patients did not essentially differ between the three centers (Table 1). Fig. 1 shows a Manhattan plot of the association meta-analysis with rs numbers for 10 SNPs in loci for which more than one SNP reached a p-value below 10^{-5} (blue line in Fig. 1; red line is for genome-wide significance of $5x10^{-8}$). The most significant association was found on chromosome 11, represented by SNP rs9344. Other SNPs showing evidence of genome-wide significant associations were rs4487645 and rs79419269 both mapping to chromosome 7. The locus reaching a genome-wide significance level on chromosome 6 was based entirely on imputed SNPs and this was not considered further.

For the 10 SNPs shown in Fig. 1 the risk alleles, ORs and heterogeneity of the results between the three centers (P_{het} and I^2) are shown in Table 2. The OR was 1.35 for rs9344 ($p= 7.80x10^{-11}$) on chromosome 11q13.3 and the results showed no heterogeneity between the three centers, also true for the other listed SNPs. Chromosome 7p15.3 SNP rs4487645 had an OR of 1.35 ($p=1.80x10^{-9}$) and 7q36.1 SNP rs79419269 had an OR of 1.41 ($p=5.2x10^{-8}$). rs1005300 on chromosome 22q13.1 showed an OR of 1.29 ($p=8.07x10^{-8}$). Some of the loci have already been published for MM: rs142802669 on chromosome 3p22.1 maps to the *ULK4* gene, rs4487645 on chromosome 7p15.3 maps to the binding site of IRF4, rs9344 is the splice site variant of *cyclin D1* associated with the translocation t(11;14), rs4792800 on chromosome 17p11.2 maps to the intron of *TNFRSF13B* and rs1005300 on chromosome 22 maps to the *CBX7* locus [9, 10, 13, 30]. The present chromosome 17 and 22 SNPs were not the same as the SNPs reported for MM but they were close and in linkage disequilibrium (LD, r^2 0.96 and 0.56, respectively) with those reported. According to HaploReg, rs4792800 on chromosome 17p11.2 was only 2kb away and in high LD (r^2 0.92) with rs34562254 which is a missense variant in *TNFRSF13B*; however, this variant is predicted to be tolerated/benign in all transcripts by SIFT/PolyPhen.

Table 2 shows also the respective SNP associations for MM among the German-UK MM patients (N = 3790) and controls (N = 7304). All ORs were higher among AL amyloidosis than MM patients, and the AL amyloidosis associated SNPs on chromosomes 1p36.13, 11q24.1 and 14q13.2 were not significant in MM. For these SNPs and those at 7q36.1 and 11q13.3 the 95%

confidence intervals (CIs) did not overlap between the AL amyloidosis and MM cohorts. We refer to these 5 SNPs operationally as 'AL amyloidosis-specific associations'.

We analyzed the homogeneity of the AL amyloidosis (3 data sets) and MM (2 data sets) cohorts using ASSET (Supplementary Figure 1). Chromosome 11q13.3 (rs9344) scored positive for AL amyloidosis data only. All associations which were earlier found in MM (chromosomes 3p22.1, 7p15.3, 17p11.2 and 22q13.1) scored positive for all samples in the three amyloidosis sets, except that for the chromosome 17p11.2 SNP the German results were considered null. For the remaining SNPs, results in at least two amyloidosis sets were positive and no more than a single MM result was positive. The exception was the SNP at 11q24.1 (rs11219122) for which only the German amyloidosis result was positive.

The newly reported 8 MM-related SNPs were tested for association in AL amyloidosis in Supplementary Table 1 [18]. For rs7781265 at 7q36.1 the OR and significance were higher for AL amyloidosis than for MM (1.41, $7.6x10^{-8}$ vs 1.20, $1.82x10^{-7}$; this SNP became genome-wide significant for MM after additional replication sets were included).

Cytogenetic data were available only for 380 German patients on whom association analysis for rs9344 was performed separately on all patients and on those with and without t(11:14) and all IgH translocations (Table 3). t(11;14) accounted for 58% of all AL amyloidosis cases. The OR was 1.70 ($p=4.7x10^{-7}$) for the risk allele G with t(11;14) and it was lower 1.50 ($p=6.6x10^{-6}$) for cases with any IgH translocation. The OR was 1.14 (p=0.41) for IgH translocation other than t(11;14). We tested the associations of all other 9 SNPs from Table 2 with t(11:14) specific AL amyloidosis but no associations were found (data not shown). Homozygous GG individuals with t(11;14) accounted for 24% (93/380) of AL amyloidosis patients; among t(11;14) cases the OR was 3.52 for GG homozygotes against AA homozygotes compared to control genotypes.

Biological interference

HaploReg data on biological mechanisms for the 10 SNPs we summarized in Table 4. Additionally, regional plots of association results and ENCODE functional annotation are shown

in Fig. 2 for 5 SNPs. Among amyloidosis-specific SNPs, rs10799599 on chromosome 1p36.13 is located between two phospholipase genes, within a region of high LD spanning the 3'end of the *PLA2G2A* gene (*phospholipase A2, group IIA*). This SNP and 7 highly linked SNPs (r^2>0.8) are expression quantitative trait loci (eQTLs) in the liver for *PLA2G2A* at a p-value of 10^{-8}, thus suggesting a possible mechanistic clue (footnote to Table 3). rs79419269 on chromosome 7q36.1 maps to an intron of the *SMARCD3* gene (*SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily D, member 3*), and it affects promotor and enhancer histone marks in many tissues and changes motifs of 5 transcription factors, including Foxp1 and HDAC2. According to ChromHMM the SNP is weakly transcribed in GM12878 cells and located next to a weak or poised enhancer. rs11219122 on chromosome 11q24.1 maps next to an RNA gene *MIR4493* of unknown function. The SNP changes motifs for 10 transcription factors. As shown in Fig. 2 the SNP is located at a weakly transcribed locus next to a weak or poised enhancer.

rs2273156 on chromosome 14q13.2 maps to a 100 kb linkage region and the SNP is intronic to an RNA gene (*RP11-85K15.2*) which is antisense to *SRP54* (*signal recognition particle 54kDa*) (Fig. 2 and Table 4). It is in high linkage with 67 SNPs of which 1/3 are intronic to *SRP54.* According to HaploReg rs2273156 is a modest eQTL to *SRP54* in transformed fibroblasts (p-value ~10^{-5}) but it is a stronger eQTL in lymphoblastoid cells and whole blood to *PPP2R3C* (p-value up to 10^{-14}), the *protein phosphatase 2 regulatory subunit B* gene (footnote to Table 3) . It is also an eQTL in lymphoblastoid cells to the next distal gene *KIAA0391* (p-values up to 10^{-10}), which is a part of mitochondrial ribonuclease P and functions in mitochondrial tRNA maturation. According to ChromHMM rs2273156 is located in a weakly transcribed sequence or at a transcriptional transition/elongation site depending on the cell line. SNPs linked to rs2273156 (r^2>0.95) are listed in Supplementary Table 2. rs12890307 is in the 5'UTR and at an active promoter site of SRP54. This SNP is highly conserved and exhibits a multitude of functional changes. rs12890307 is eQTL to the above three genes, strongest to *PPP2R3C* (whole blood p-value 10^{-198})[31].

rs35629860 on chromosome 16p11.2 spans a linkage region of 200 kb and contains many genes with limited functional data (Fig.2, Table 4). rs35629860 is located at strong enhancer/active

promoter sites in many cell types, including lymphoblastoid cells (Fig.2). The SNP is intronic to *FBRS* (*fibrosin*) and next to *PRR14* (*proline rich 14*). The linkage region includes *RNF40* (*ring finger protein 40, E3 ubiquitin protein ligase*), *SRCAP* (*Snf2-related CREBBP activator protein*) and more distal *BCL7C* (*B-cell CLL/lymphoma 7C*). The SNP displays histone marks in many tissues and is an eQTL in 31 tissues and cell types; expression of *RNF40* is affected in 10 tissues, including blood (p-value 10^{-7}); the expression of *PRR14* is affected in the skin (p-value 10^{-7}) and of *BCL7C* in lymphoblastoid cells (p-value 10^{-5})(footnote to Table 4). rs35629860 is also an eQTL in several tissues to *KAT8* (*lysine acetyltransferase 8*) which is located 0.5 Mb centromeric from the SNP. rs35629860 is flanked 5 kb away by rs3747481 (r^2 0.95), a missense variant in *PRR14*, influencing expression of *PRR14* in blood (p-value 10^{-11}) and also an almost equally strong eQTL for *RNF40* in blood (p-value 10^{-10}) (Supplementary Table 3). The missense variant is predicted by SIFT to be deleterious and by PolyPhen to be benign.

DISCUSSION

AL-amyloidosis is a rare disease for which genetic etiology is poorly understood, except that all the 7 SNPs first described in MM were replicated in AL amyloidosis with nominal p-values of <0.05 [12]. In the present study we identified 10 clusters of SNPs with p-values $<10^{-5}$ for which the data from the three centers showed no evidence on heterogeneity and no confounding factors are known. When these were tested in the MM cohort all ORs were higher in AL amyloidosis than in MM and the 95%CIs for 5 SNPs did not overlap ('AL-amyloidosis specific associations'), suggesting the existence of biological differences. However, for the 3 SNPs reaching a genome-wide significance of $5x10^{-8}$ only rs9344 was unique to AL amyloidosis. The OR for rs9344 (G/A870) on chromosome 11q13.3 was 1.35 (p-value $7.80x10^{-11}$) in AL amyloidosis vs. 1.06 in MM (p-value 0.04).

The present data showed that the association of rs9344 was strongest (OR 1.70) in patients with t(11;14) while for other IgH aberrations the OR was only 1.14. Thus the high overall risk of rs9344 in AL amyloidosis is mainly explained by the high prevalence (58%) of t(11;14) compared to MM (20%) [13]. Because of the high prevalence of t(11;14) and of the risk allele G (54%) there is a substantial group (24%) of AL amyloidosis patients with a combined high risk

genotype (GG homozygosity and t(11;14)). Among t(11;14) specific AL amyloidosis the OR for GG homozygosity was 3.52 compared AA homozygosity. SNP rs9344 maps to a splice site in cyclin D1 for which the G allele is an optimal splice donor, encoding the full length protein D1a whereas allele A partially hinders splicing resulting in a shorter protein D1b [32, 33]. In a previous study we showed that the levels of D1a and D1b transcript correlated with the GG, GA and AA genotypes in CD138-selected plasma cell [13]. It is intriguing that the G allele encoding the full length D1a would be associated with AL amyloidosis or MM because it has a low transforming capacity compared to D1b [34]. Cyclin D1 activation and translocation (11;14) are early pre-MGUS events in plasma cell dyscrasias [35, 36]. As the G allele is the risk factor for t(11;14) in AL amyloidosis and MM the D1a protein will promote the translocation which in turn would drive cyclin D1 expression by the IGH enhancers at the translocation locus. D1a may also act through separate pathways of promoting chromosomal aberrations because it is associated with double-strand repair complexes of RAD51, BRCA1 and BRCA2 [37]. The influence of this splice site variant G/A870 on the risk of cancer has been tested in numerous case-control studies on many cancer types and most positive studies support a small risk for the A-allele in line with its transforming potential [38, 39]. The present data suggest that the transformation pathway in plasma cell dyscrasias is routed via allele G and cyclin D1 activation with an important contribution by t(11;14), particularly in AL amyloidosis.

Among the SNPs associated with both AL amyloidosis and MM rs4487645 on chromosome 7p15.3 mapped within the binding site for *interferon regulatory factor 4* (*IRF4*, alias *myeloma oncogene 1*) which is a strong enhancer for the adjacent gene *CDCA7L* [30]. IRF4 is a critical transcriptional regulator in B-cell development, plasma cell survival and transformation through MYC [40-43]. The SNPs on chromosomes 17p11.2 and 22q13.1 were in high LD with the ones reported for MM [10]. rs4792800 on 17p11.2 mapped to the intron of *TNFRSF13B, tumor necrosis factor receptor superfamily member 13B* (alias *TACI),* a key regulator of B-cell development and homeostasis [10]. rs1005300 on chromosome 22q13.1 is part of a 40kb LD region encompassing *CBX7*, the chromobox homolog 7 gene, which encodes a polycomb protein forming a part of polycomb repressive complex 1 [10]. Targeting *CBX7* expression in the mouse lymphoid compartment induced the formation of aggressive B-cell lymphomas, in cooperation

with MYC [44]. CBX7 regulates crucial genes involved in cancer progression and in epithelial-mesenchymal transition, including *osteopontin* and *E-cadherin* [44].

Among the AL amyloidosis-specific associations only rs79419269 on chromosome 7q36.1 with an OR of 1.41 reached a genome-wide significance of $5x10^{-8}$. However, this locus was marked by another SNP rs7781265 (r^2=0.85) in the international pooling study on MM and the OR was somewhat higher (1.20) than that (1.14) for rs79419269 in MM in the present study (Supplementary Table 1) [18]. rs79419269 mapped to a 50 kb linkage region encompassing three genes *ABCF2*, a ATP-binding membrane transporter, *CHPF2*, chondroitin polymerizing factor and an RNA gene *MIR671*. It included also the 3' part of the *SMARCD3* gene, which encodes a member of the SWI/SNF family of proteins. These influence ATP dependent nucleosome remodeling, display helicase activity and may regulate transcription. SMARCD3 is involved in epithelial-mesenchymal transition by inducing Wnt5a signaling [45]. In a GWAS on glycosylation levels of immunoglobulin G (IgG), which are thought to influence IgG effector functions, the present locus including *SMARCD3* was one of the candidates [46].

For the other AL amyloidosis associated SNPs we have to be cautious in speculating mechanisms because they did not reach a genome-wide significance. rs10799599 on chromosome 1p36.13 displayed an eQTL in the liver for *PLA2G2A*, suggesting that this gene could be a functional explanation. The gene encodes an enzyme which has been associated with intestinal cancers and, more recently, as an inflammation-related prognostic marker in many tumors, including MM [47]. AL amyloidosis-specific SNP (rs11219122) on chromosome 11q24.1 mapped next to an RNA gene *MIR4493* of unknown function.

Another amyloidosis-specific SNP (rs2273156) on chromosome 14q13.2 was in LD with *SRP54* which is a key signal recognition particle (SRP) protein escorting nascent secretory proteins from ribosomes to endoplasmic reticulum for secretion [48, 49]. The SRP complex consists of a 7S RNA and 6 protein subunits: SRP9, SRP14, SRP19, SRP54, SRP68, and SRP72. Whether the SRP system functions in the secretion of M-protein in AL amyloidosis or MM is not known but signal recognition particle-dependent pathways are used in cells engineered to produce immunoglobulins [50]. Moreover, the secretion of immunoglobulins from ribosomes to

endoplasmic reticulum is obviously highly overloaded process in plasma cell dyscrasias [42, 51]. rs2273156 and the linked SNPs displayed eQTL for *SRP54* but also genes outside the linkage region, particularly for the *PPP2R3C* gene which encodes a regulatory subunit of the serine/threonine phosphatase, protein phosphatase 2. Homozygous knockout mice for *PPP2R3C* exhibit impaired proliferation of B cells suggesting that the gene is required for the B-cell receptor-mediated proliferation [52]. This protein is also able to regulate the P-glycoprotein ATP-binding cassette transporter through its phosphatase activity and influence drug responses to vincristine and doxorubicin [53].

Many genes are in LD with rs35629860 on chromosome 16p11.2, including *PRR14* which encodes a protein which tethers heterochromatin to nuclear lamina [54]. Somatic mutations in *PRR14* are found in human and animal tumors [55]. Although rs35629860 display eQTL for several genes, including *PRR14*, *RNF40* and *KAT8*, the linked missense variant in *PRR14* may favor this gene as the mechanistic link. The adjacent *SRCAP* gene encodes a catalytic component of the chromatin-remodeling SRCAP complex. Srcap protein is an ATPase which functions by exchanging histone H2AZ/H2B dimers for nucleosomal H2A/H2B dimers. It is also an activator in Notch-mediated, CREB-mediated and steroid receptor-mediated transcription. Many cancer cell lines have mutations in *SRCAP*; Floating-Harbor syndrome and polycystic kidney disease are associated with germline mutations in this gene [56, 57]. rs35629860 is in LD (r^2=0.89) with rs8058578 which was found to provide suggested evidence for MM[18].

In conclusion, these data provide further evidence for shared inherited susceptibility between AL amyloidosis and MM. The association at *cyclin D1* splice site variant G/A870 was much stronger in AL amyloidosis compared to MM which is explained by the higher prevalence of t(11;14)-defined cases in AL amyloidosis and highlight the role of *cyclin D1* and perhaps also of t(11;14) in the pathogenesis of AL amyloidosis. Two of the amyloidosis associated SNPs mapped close to chromatin remodeling genes, *SMARCD3* on chromosome 7q36.1 and *SRCAP* or *PRR14* on chromosome 16p11.2, which together with *CBX7* on chromosome 22q13.1 suggest a functional importance. On chromosome 14q13.2 both candidates *SRP54* and *PPP2R3C* could mediate B-cell related functions. Even if the present results define AL amyloidosis germline architecture akin to MM, the intriguing clue to the pathophysiology of AL amyloidosis may be the strong association

with rs9344 at *cyclin D1*. While *cyclin D1* is a driver in a majority of AL amyloidosis cases through t(11;14) the puzzling mechanistic question is whether the resulting stoichiometric excess of light chains promotes their aggregation.

CONFLICT OF INTEREST

The authors declare no competing financial interests.

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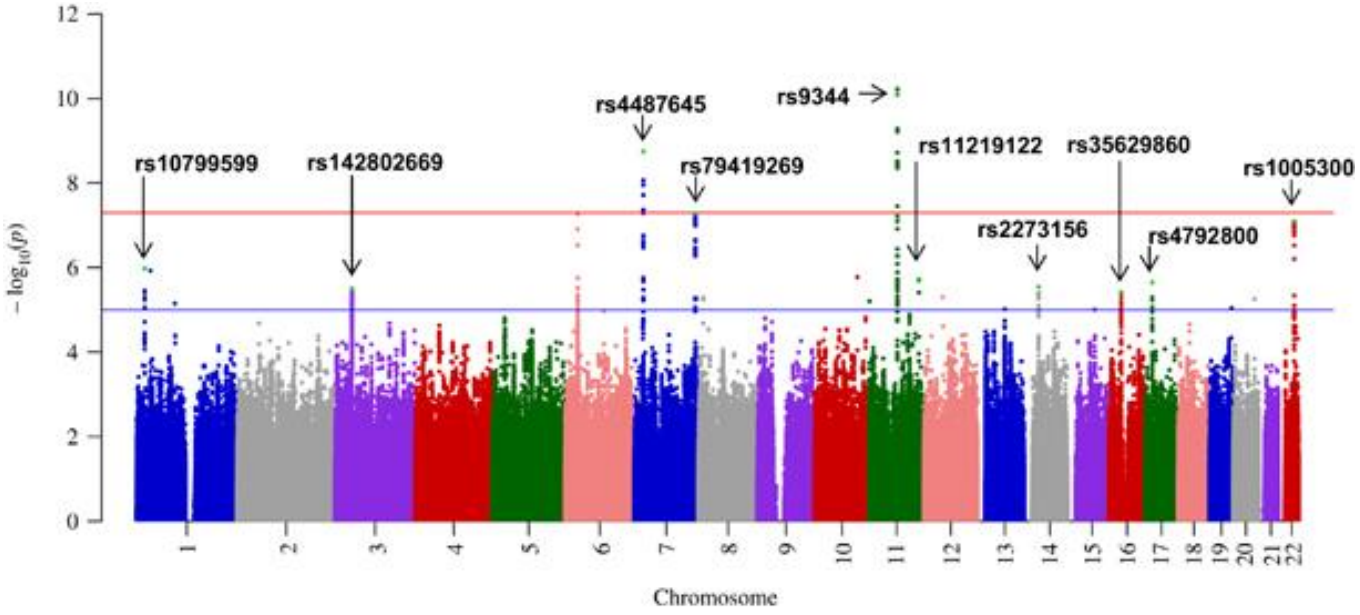
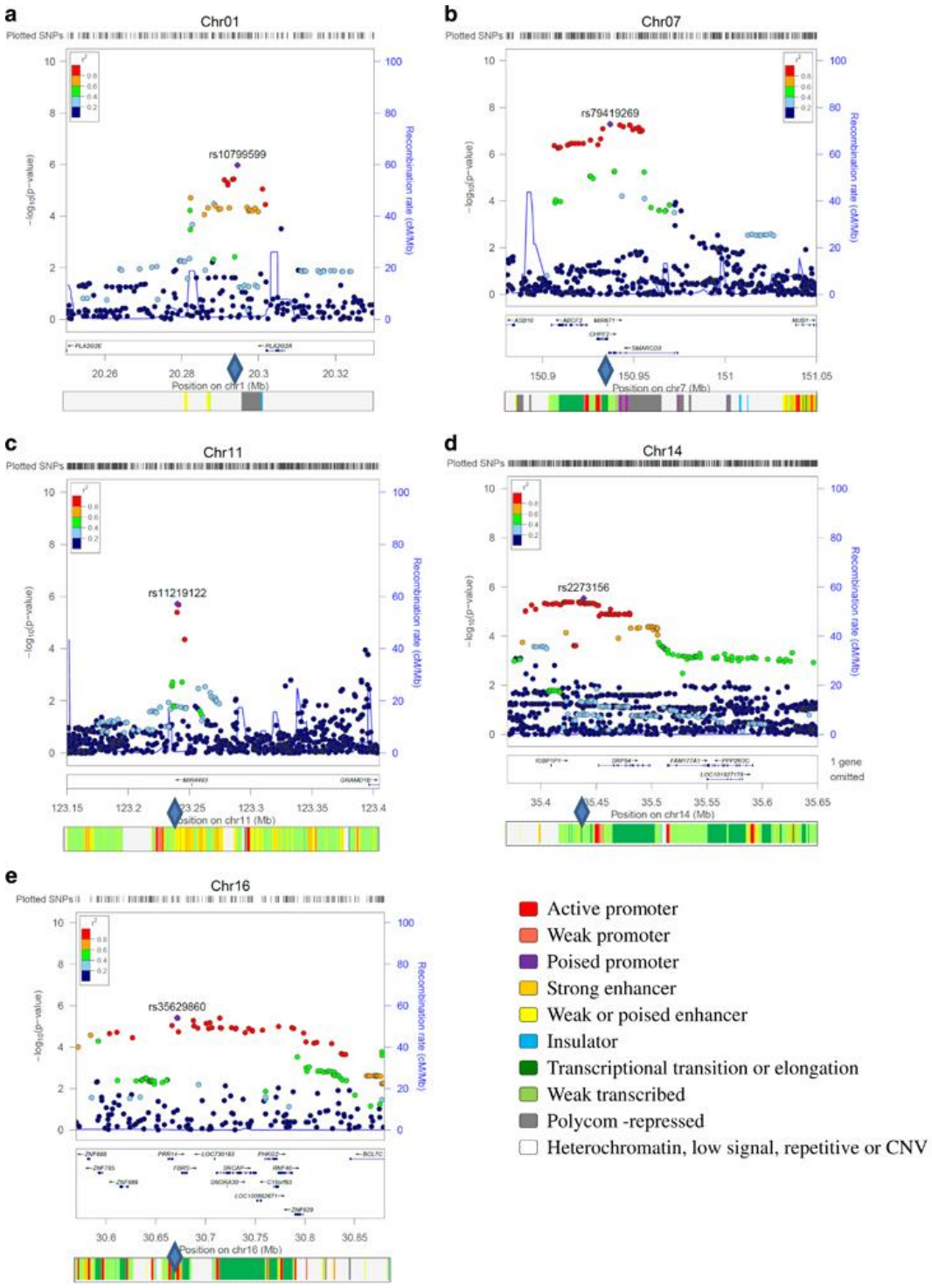
FIGURES

Fig. 1. Manhattan plot on meta- analysis of the 1230 AL-amyloidosis cases and 7589 regionally matched controls. rs numbers are shown for 10 SNPs in peaks with p-value below 10^{-5}.
The red horizontal line represents the significance thresholds of $p = 5.0 \times 10^{-8}$ and the blue horizontal line represents the significance thresholds of $p = 1.0 \times 10^{-5}$.



Fig, 2. Regional plots of association and chromatin state segmentation profile for the SNPs associated with AL amyloidosis (a-e). Plots show results for 1p36.13 (rs10799599, a), 7q36.1 (rs79419269, b), 11q24.1 (rs11219122, c), 14q13.2 (rs2273156, d) and 16p11.2 (rs35629860, e). The y axes show the $-\log_{10}$ (p-values) of the SNPs and the x axes show their chromosomal positions. The top associated SNP is shown as a purple diamond and is labeled by its rsID. The color intensity of each symbol represents the strength of LD with the top SNP. Genetic recombination rates (cM/Mb) are indicated with a light blue line. The lower track shows the chromatin-state segmentation track (ChromHMM) for lymphoblastoid cells from the HapMap ENCODE Project.



TABLES

Table 1: Demographic and clinical characteristics of the AL amyloidosis patients from the three centers.

	German	British	Italian	All populations
Sample size, *N*	562	410	257	1229
Age at diagnosis				
Median age (years; range)	62 (34.3-84.8)	65 (29.5-86.8)	65 (38.1-87.1)	64 (29.5-87.1)
Gender				
Males	324 (58%)	241 (59%)	146 (57%)	710 (58%)
Females	239 (42%)	170 (41%)	111 (43%)	520 (42%)
Number of organs involved				
No Organ	27 (5%)	17 (4%)	5 (2%)	49 (4%)
One organ	254 (45%)	198 (48%)	116 (45%)	568 (46%)
Two organs	237 (42%)	167 (41%)	126 (49%)	530 (43%)
Three organs	45 (8%)	28 (7%)	10 (4%)	83 (7%)
Type of organ involved[a]				
Kidney	359 (64%)	320 (78%)	166 (65%)	845 (69%)
Heart	397 (71%)	239 (58%)	200 (78%)	836 (68%)
Liver	106 (19%)	57 (14%)	32 (12%)	195 (16%)
Missing Data	-	18	-	18
Antibody isotypes				
Lambda (L)	438 (78%)	304 (80%)	188 (73%)	930 (78%)
Kappa (K)	123 (22%)	74 (20%)	69 (27%)	266 (22%)
Light-chains only: L or K	312 (55%)	95 (29%)	127 (49.5%)	534 (47%)
Light-chains & heavy-chains	249 (44%)	233 (71%)	130 (50.5%)	612 (53%)
Missing data on light-chains	1	32	-	33
Missing data on heavy-chains	1	82	-	83

[a]: **The sum of the percentages exceeds 100 because in many patients multiple organs were involved.**

Table 2: Top SNPs in the meta-analysis of amyloidosis considering German (DE), UK and Italian (IT) GWAS. Also shown are respective associations for MM.

SNP	CHR	BP	A	B[a]	Amyloidosis Meta-analysis (DE + UK + IT)					Meta Myeloma DE + UK		
					RAF[t]	OR (95% CI)[c]	P	P_{het}[d]	I^{2}[e]	RAF[t]	OR (95% CI)[c]	P
rs10799599	1p36.13	20294500	G	C	0.62	1.26 (1.15 - 1.39)	1.05×10^{-6}	0.21	0.36	0.62	1.01 (0.95 - 1.07)	0.71
rs142802669	3p22.1	41869427	A	C	0.17	1.30 (1.16 - 1.45)	3.15×10^{-6}	0.34	0.08	0.17	1.28 (1.19 - 1.38)	1.62×10^{-11}
rs4487645	7p15.3	21938240	A	C	0.67	1.35 (1.22 - 1.49)	1.80×10^{-9}	0.42	0.00	0.70	1.28 (1.21 - 1.36)	1.56×10^{-15}
rs79419269	7q36.1	150937076	T	C	0.12	1.41 (1.25 - 1.60)	5.20×10^{-8}	0.96	0.00	0.12	1.14 (1.05 - 1.24)	2.44×10^{-3}
rs9344	11q13.3	69462910	A	G	0.56	1.35 (1.23 - 1.48)	7.80×10^{-11}	0.21	0.36	0.54	1.06 (1.00 - 1.12)	4.00×10^{-2}
rs11219122	11q24.1	123240173	T	C	0.35	1.25 (1.14 - 1.37)	1.91×10^{-6}	0.05	0.66	0.35	1.00 (0.94 - 1.06)	0.97
rs2273156	14q13.2	35438799	T	C	0.19	1.29 (1.16 - 1.44)	2.88×10^{-6}	0.14	0.50	0.18	1.02 (0.95 - 1.10)	0.55
rs35629860	16p11.2	30671532	G	A	0.26	1.26 (1.14 - 1.39)	3.87×10^{-6}	0.99	0.00	0.26	1.13 (1.06 - 1.20)	2.45×10^{-4}
rs4792800	17p11.2	16845167	A	G	0.12	1.36 (1.20 - 1.54)	2.27×10^{-6}	0.12	0.53	0.12	1.25 (1.15 - 1.36)	3.12×10^{-7}
rs1005300	22q13.1	39547891	C	G	0.59	1.29 (1.17 - 1.41)	8.07×10^{-8}	0.95	0.00	0.61	1.25 (1.18 - 1.32)	5.81×10^{-14}

a - B is the risk allele; b - risk allele frequency; c – OR odds ratio, CI confidence interval; d - p-value derived from Cochran's Q heterogeneity test; e - proportion of total variance that is due to heterogeneity (I^{2}).

Table 3: Risk of AL amyloidosis in Germans, overall and by cytogenetic subtype, considering rs9344 genotypes.

Cytogenetic subtypes	RAF[a]	Cases				Controls				OR (95% CI)[b]	P
		AA	GA	GG	total	AA	GA	GG	total		
AL amyloidosis	0.56	71	282	209	562	459	1049	639	2147	1.39 (1.22 - 1.60)	2.00×10^{-6}
t11;14 positive	0.55	19	110	93	222	459	1049	639	2147	1.70 (1.38 - 2.09)	4.70×10^{-7}
IgH positive	0.55	33	160	121	313	459	1049	639	2147	1.50 (1.26 - 1.79)	6.60×10^{-6}
t11;14 negative	0.54	27	80	51	158	459	1049	639	2147	1.14 (0.91 - 1.44)	0.22
IgH negative	0.54	13	30	23	67	459	1049	639	2147	1.15 (0.81 - 1.62)	0.38
IgH positive and t11;14 negative	0.54	14	49	28	91	459	1049	639	2147	1.14 (0.84 - 1.54)	0.41

a – frequency of risk allele G; b – OR odds ratio, CI confidence interval

Table 4. HaploReg v4.1 annotation of the 10 candidate SNPs for AL amyloidosis.

SNP	CHR	SNPs r^2>0.8[a]	Promoter histone marks[b]	Enhancer histone marks[c]	DNAse	Proteins bound	Motifs changed	Selected eQTL hits	GENCODE genes	dbSNP func annot
rs10799599	1	7	muscle	5 tissues	8 tissues		4 altered motifs	liver[d]	3kb 5' of Metazoa_SRP	
rs142802669	3	2						31 hits	ULK4	intronic
rs4487645	7	9	2 tissues	10 tissues	10 tissues	IRF4,PU1		blood	DNAH11	intronic
rs79419269	7	-	9 tissues	13 tissues	skin		5 altered motifs		SMARCD3	intronic
rs9344	11	-	14 tissues	15 tissues	9 tissues	PAX5C20,POL2	4 altered motifs		CCND1	synonymous splice site
rs11219122	11	4					10 altered motifs		12kb 3' of MIR4493	
rs2273156	14	67	lung	3 tissues			Foxj1,STAT	28 hits[e]	RP11-85K15.2	intronic
rs35629860	16	22	18 tissues	23 tissues	18 tissues	5 bound proteins	CCNT2	31 hits[f]	FBRS	
rs4792800	17	17	3 tissues	4 tissues	11 tissues		12 altered motifs		TNFRSF13B	intronic
rs1005300	22	24	16 tissues	18 tissues	9 tissues	HNF4A	5 altered motifs	2 hits	CBX7	intronic

[a] based on 1000 Genomes Phase 1 data.
[b] enrichment of H3K4me3/H3K9ac histone marks.
[c] enrichment of H3K4me1/H3K27ac histone marks.
[d] eQTL in the liver for *PLA2G2A* at $p=2.5x10^{-8}$.
[e] eQTL in transformed fibroblasts for *SRP54* at $p=1.6x10^{-5}$, in whole blood for *IGBP1P1* at $p=8.9x10^{-7}$, in whole blood for *PPP2R3C* at $1.1x10^{-14}$, and in lymphoblastoid cells for *PPP2R3C* at $p=1.3x10^{-5}$-$3.8x10^{-11}$ and for *KIAA0391* at $p=6.3x10^{-6}$-$5.1x10^{-10}$.
[f] eQTL in blood for *RNF40* at $p=9.8x10^{-8}$, in skin for *PRR14* at $p=2.9x10^{-7}$, in lymphoblastoid cell for *BCL7C* at $p=1.5x10^{-5}$, in thyroid for *c16orf93* (*CCDC193)* at $p=7.8x10^{-14}$ and in artery wall for *KAT8* at $p=6.1x10^{-10}$.

Supplementary information

Supplementary Table 1. Results for 8 SNPs identified in a new meta-analysis for multiple myeloma (Mitchell et al. 2016, ref. 18).

SNP	CHR	BP	A	B[a]	Amyloidosis Meta-analysis (DE + UK + IT)					Meta Multiple Myeloma		
					RAF[b]	OR (95% CI)[c]	*P*-value	P_{het} [d]	I^{2} [e]	RAF[b]	OR (95% CI)[c]	*P*-value
rs34229995	6p22.3	15244018	C	G	0.02	1.41 (1.09 - 1.84)	0.01	0.88	0.00	0.03	1.40 (1.25 - 1.58)	1.76×10^{-8}
rs9372120	6q21	106667535	T	G	0.20	1.11 (0.99 - 1.24)	0.06	0.47	0.00	0.22	1.20 (1.14 - 1.26)	8.72×10^{-14}
rs7781265	7q36.1	150950940	G	A	0.12	1.41 (1.24 - 1.59)	7.6×10^{-8}	0.81	0.00	0.13	1.20 (1.12 - 1.29)	1.82×10^{-7}
rs1948915	8q24.21	128222421	T	C	0.33	1.03 (0.94 - 1.14)	0.48	0.48	0.00	0.35	1.14 (1.10 - 1.19)	3.14×10^{-10}
rs2811710	9q21.3	21991923	T	C	0.64	1.10 (1.00 - 1.21)	0.04	0.23	0.32	0.66	1.14 (1.09 - 1.19)	6.5×10^{-10}

rs2790457	10p12.1	28856819	A	G	0.73	1.05 (0.95 - 1.16)	0.34	2.4×10^{-3}	0.83	0.74	1.12 (1.07 - 1.17)	8.44×10^{-7}
rs7193541	16q23.1	74664743	C	T	0.59	1.12 (1.02 - 1.22)	0.02	0.13	0.50	0.59	1.12 (1.08 - 1.17)	1.14×10^{-8}
rs6066835	20q13.13	47355009	T	C	0.08	1.12 (0.95 - 1.31)	0.17	0.43	0.00	0.08	1.24 (1.16 - 1.33)	1.16×10^{-9}

[a] - allele B is the risk allele; [b] - risk allele frequency; [c] - 95% confidence interval; [d] - p-value derived from Cochran's Q heterogeneity test; [e] - proportion of total variance that is due to heterogeneity (I^2).

Supplementary Table 2. HaploReg v4.1 annotation of SNPs in high LD (r^2 >0.95) with rs2273156 at 14q13.2.

SNPs	pos (hg38)	LD (r^2)	Ref	Alt	EUR freq	Promoter histone marks	Enhancer histone marks	DNAse	Proteins bound	Motifs changed	Selected eQTL hits	GENCODE genes	dbSNP func annot
rs35106382	34965085	0.98	T	C	0.19	BLD, MUS[a]	BLD, SKIN	BLD		ERalpha-a,LRH1,Maf	29 hits	*RP11-85K15.2*	intronic
rs8014327	34965530	0.98	C	T	0.19	BLD, MUS	3 tissues	HRT		Foxj2,Maf	32 hits	*RP11-85K15.2*	intronic
rs12894365	34966185	0.98	C	A	0.19	3 tissues	4 tissues	BLD	POL2	Pou6f1	32 hits	*RP11-85K15.2*	intronic
rs12894820	34966319	0.98	G	A	0.19	3 tissues	5 tissues		GATA1	LUN-1	32 hits	*RP11-85K15.2*	intronic
rs2273156	34969593	1	T	C	0.19	LNG	4 tissues			Foxj1,STAT	28 hits	*RP11-85K15.2*	intronic
rs12893021	34971474	0.98	A	C	0.19	3 tissues	11 tissues	BRST,SKIN	CFOS	7 altered motifs	28 hits	*RP11-85K15.2*	intronic
rs17102904	34971843	0.97	G	T	0.19	3 tissues	12 tissues	5 tissues		CCNT2,TAL1,TCF4	28 hits	*RP11-85K15.2*	intronic
rs76472781	34973178	0.98	C	G	0.19		5 tissues				29 hits	*RP11-85K15.2*	intronic
rs12895051	34974864	0.98	G	A	0.19		BLD			GR	33 hits	*RP11-85K15.2*	intronic
rs35090385	34975229	0.96	T	G	0.19	BRST	BLD, SPLN			Pdx1	28 hits	*RP11-85K15.2*	intronic
rs71421920	34977539	0.98	T	C	0.19	BLD	4 tissues			6 altered motifs	28 hits	*RP11-85K15.2*	intronic
rs8019179	34978589	0.98	T	C	0.19	BLD, ESC	4 tissues	LNG		4 altered motifs	30 hits	*RP11-85K15.2*	intronic
rs17102923	34980633	0.98	G	A	0.19	19 tissues	23 tissues			Evi-1,Hand1,SIX5	30 hits	*RP11-85K15.2*	intronic
rs34678475	34981897	0.96	T	A	0.19	24 tissues	23 tissues	36 tissues	CTCF,POL2	Pou5f1,Sox	30 hits	*RP11-85K15.2*	intronic
rs12890307	34982926	0.95	G	A	0.19	24 tissues	23 tissues	53 tissues	34 bound proteins	4 altered motifs	34 hits[b]	*SRP54*	5'-UTR

[a] Abbreviations: BLD blood, MUS muscle, LNG lung, BRST breast, ESC esophagus, SPLN spleen, HRT heart.

[b] eQTL in blood for *PPP2R3C* at $p=9.8x10^{-198}$, for *SRP54* at $p=2.0x10^{-27}$ and for *KIAA0391* at $p=4.0x10^{-6}$-$3.7x10^{-10}$.

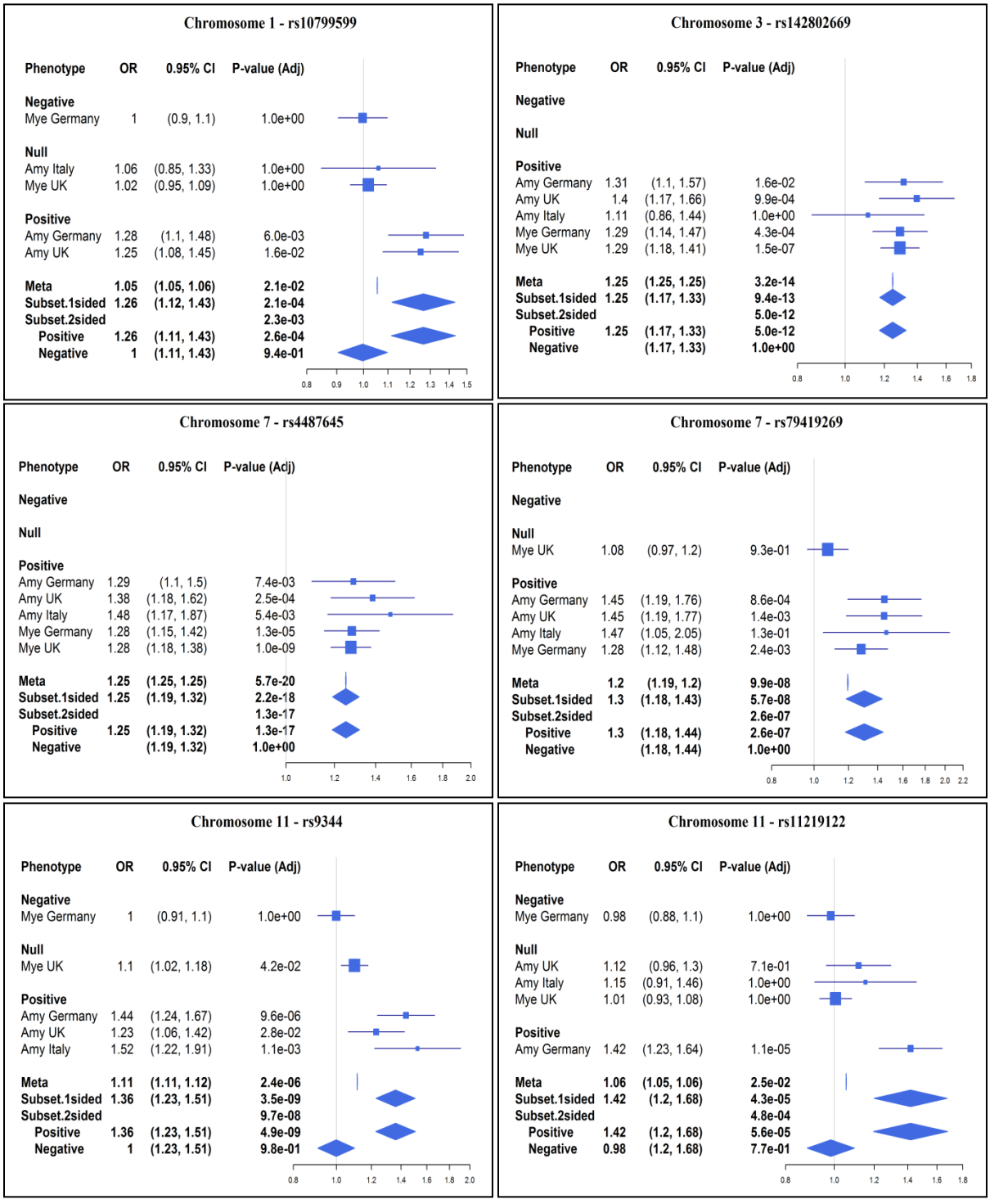
Supplementary Table 3. HaploReg v4.1 annotation of SNPs in high LD (r^2 >0.95) with rs35629860 at 16p11.2.

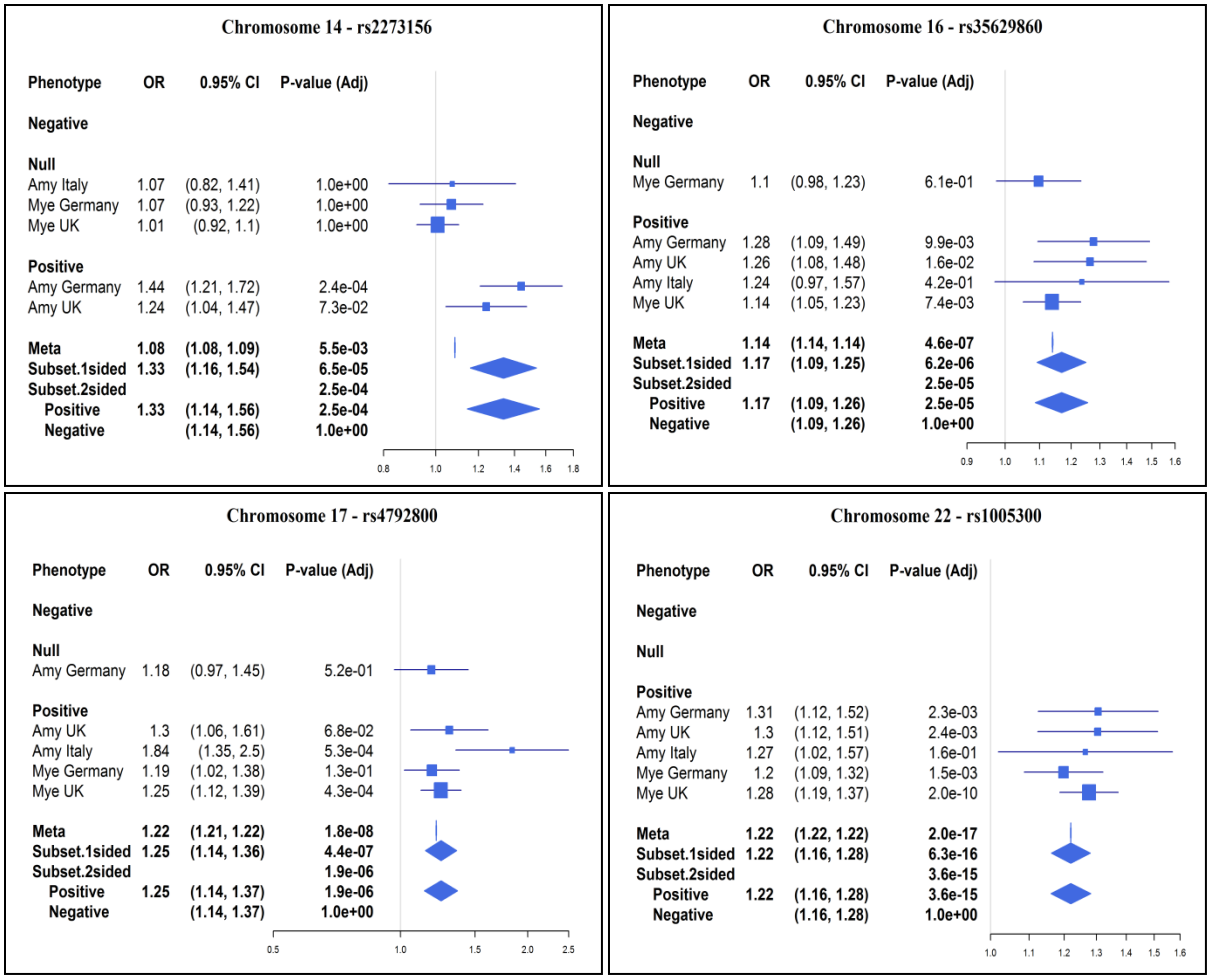
SNPs	pos (hg38)	LD (r^2)	Ref	Alt	EUR freq	Promoter histone marks	Enhancer histone marks	DNAse	Proteins bound	Motifs changed	Selected eQTL hits	GENCODE genes	dbSNP func annot
rs3747481	30655046	0.95	C	T	0.28		13 tissues	Blood		8 altered motifs	38 hits[a]	*PRR14*	missense
rs35629860	30660211	1	G	A	0.27	24 tissues	23 tissues	18 tissues	5 bound proteins	CCNT2	31 hits	*FBRS*	
rs67128646	30660776	1	A	C	0.27	19 tissues	24 tissues	13 tissues	6 bound proteins	Foxi1,Foxj2,VDR	30 hits	*FBRS*	

[a] eQTL in blood for *PRR14* at $p=7.2x10^{-11}$ and in blood and other tissues for e.g., *RNF40, C16orf93 (CCDC193)* and *KAT8*.

Supplementary Figure 1.

SNP ASSET analysis of SNPs considering amyloidosis by the 3 centers and multiple myeloma results from Germany and UK.




Chromosome 14 - rs2273156
Phenotype OR 0.95% CI P-value (Adj)
Negative
Null
Amy Italy 1.07 (0.82, 1.41) 1.0e+00
Mye Germany 1.07 (0.93, 1.22) 1.0e+00
Mye UK 1.01 (0.92, 1.1) 1.0e+00
Positive
Amy Germany 1.44 (1.21, 1.72) 2.4e-04
Amy UK 1.24 (1.04, 1.47) 7.3e-02
Meta 1.08 (1.08, 1.09) 5.5e-03
Subset.1sided 1.33 (1.16, 1.54) 6.5e-05
Subset.2sided 2.5e-04
Positive 1.33 (1.14, 1.56) 2.5e-04
Negative (1.14, 1.56) 1.0e+00
0.8 1.0 1.2 1.4 1.6 1.8
Chromosome 16 - rs35629860
Phenotype OR 0.95% CI P-value (Adj)
Negative
Null
Mye Germany 1.1 (0.98, 1.23) 6.1e-01
Positive
Amy Germany 1.28 (1.09, 1.49) 9.9e-03
Amy UK 1.26 (1.08, 1.48) 1.6e-02
Amy Italy 1.24 (0.97, 1.57) 4.2e-01
Mye UK 1.14 (1.05, 1.23) 7.4e-03
Meta 1.14 (1.14, 1.14) 4.6e-07
Subset.1sided 1.17 (1.09, 1.25) 6.2e-06
Subset.2sided 2.5e-05
Positive 1.17 (1.09, 1.26) 2.5e-05
Negative (1.09, 1.26) 1.0e+00
0.9 1.0 1.1 1.2 1.3 1.4 1.5 1.6
Chromosome 17 - rs4792800
Phenotype OR 0.95% CI P-value (Adj)
Negative
Null
Amy Germany 1.18 (0.97, 1.45) 5.2e-01
Positive
Amy UK 1.3 (1.06, 1.61) 6.8e-02
Amy Italy 1.84 (1.35, 2.5) 5.3e-04
Mye Germany 1.19 (1.02, 1.38) 1.3e-01
Mye UK 1.25 (1.12, 1.39) 4.3e-04
Meta 1.22 (1.21, 1.22) 1.8e-08
Subset.1sided 1.25 (1.14, 1.36) 4.4e-07
Subset.2sided 1.9e-06
Positive 1.25 (1.14, 1.37) 1.9e-06
Negative (1.14, 1.37) 1.0e+00
0.5 1.0 1.5 2.0 2.5
Chromosome 22 - rs1005300
Phenotype OR 0.95% CI P-value (Adj)
Negative
Null
Positive
Amy Germany 1.31 (1.12, 1.52) 2.3e-03
Amy UK 1.3 (1.12, 1.51) 2.4e-03
Amy Italy 1.27 (1.02, 1.57) 1.6e-01
Mye Germany 1.2 (1.09, 1.32) 1.5e-03
Mye UK 1.28 (1.19, 1.37) 2.0e-10
Meta 1.22 (1.22, 1.22) 2.0e-17
Subset.1sided 1.22 (1.16, 1.28) 6.3e-16
Subset.2sided 3.6e-15
Positive 1.22 (1.16, 1.28) 3.6e-15
Negative (1.16, 1.28) 1.0e+00
1.0 1.1 1.2 1.3 1.4 1.5 1.6