ARTICLE

Genome-wide association study identifies variation at 6q25.1 associated with survival in multiple myeloma

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Survival following a diagnosis of multiple myeloma (MM) varies between patients and some of these differences may be a consequence of inherited genetic variation. In this study, to identify genetic markers associated with MM overall survival (MM-OS), we conduct a meta-analysis of four patient series of European ancestry, totalling 3,256 patients with 1,200 MM-associated deaths. Each series is genotyped for ∼600,000 single nucleotide polymorphisms across the genome; genotypes for six million common variants are imputed using 1000 Genomes Project and UK10K as the reference. The association between genotype and OS is assessed by Cox proportional hazards model adjusting for age, sex, International staging system and treatment. We identify a locus at 6q25.1 marked by rs12374648 associated with MM-OS (hazard ratio = 1.34, 95% confidence interval = 1.22–1.48, $P = 4.69 \times 10^{-9}$). Our findings have potential clinical implications since they demonstrate that inherited genotypes can provide prognostic information in addition to conventional tumor acquired prognostic factors.

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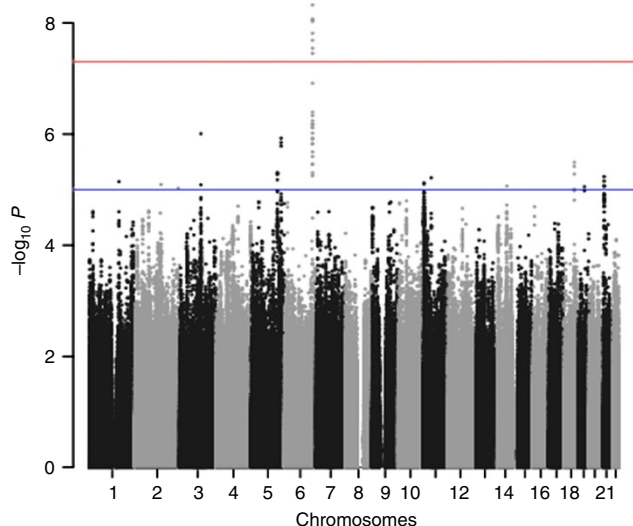
Multiple myeloma (MM) is a malignancy characterized by the infiltration of clonal plasma cells in the bone marrow[1,2]. There is considerable heterogeneity in the survival outcomes among MM patients and a variety of clinical features and tumour biomarkers have been shown to be predictive of prognosis[3–8].

As a potential prognostic factor, the concept of germline variation imparting inter-individual variability in tumour development and progression is receiving increasing attention[9–12]. This observation is exemplified by breast cancer where a more accurate assessment of prognosis can be made by taking into account genetic information to improve therapeutic decision making, opening up the possibility of patient-tailored drug selection decisions[13]. In addition, detecting genes with prognostic relevance has the potential to aid the identification of pathways that could be targeted for novel therapeutic interventions.

Here to identify germline variation influencing patient outcome following a diagnosis of MM we have pooled genotype data from four independent genome-wide association studies (GWAS) of MM[14–17] and linked these to survival time data. Our findings are consistent with the hypothesis that an individual's prognosis following treatment for MM is influenced by germline variation. Specifically, we identified a locus at 6q25.1 (rs12374648) associated with MM-OS that was statistically significant. Moreover, the association at 6q25.1 was consistently seen in each of the four patient cohorts and was not confined to a specific molecular subtype of MM.

Results

Genome-wide association study. After applying quality control measures, genotype data were available on 3,256 cases from the GWAS series, Supplementary Figs 1 and 2. The inflation factor four λ for each of the studies ranged from 0.99 to 1.03 and for the overall analysis was 1.02 (Supplementary Figs 3 and 4). We identified eight single nucleotide polymorphisms (SNPs) associated with MM-OS at P values $\leq 5.0 \times 10^{-8}$; proportional hazards model. All eight SNPs were located on chromosome 6q25.1 and were in linkage disequilibrium ($r^2 = 0.58$–1.0), Figs 1

and 2. The strongest association was provided by rs12748648 ($P = 4.89 \times 10^{-9}$, hazard ratio (HR) = 1.34, 95% confidence interval (CI) = 1.22–1.48, risk allele frequency = 0.19); Table 1, Fig. 2 and Supplementary Fig. 5. The association was consistent across the four series and there was very little between-study heterogeneity ($P = 0.34$, $I^2 = 11\%$; test of heterogeneity). Homozygosity for rs12748648 GG was associated with median survival time of 26.7 (UK-My9), 42.8 (GER-German-speaking Myeloma Multicenter Study Group (GMMG)) and 80 (US-University of Arkansas for Medical Sciences (UAMS)) months as compared with 60, 92.2 and 137 months, respectively, for patients homozygous for AA genotype (Fig. 3). To address the possibility that the impact of rs12748648 on MM-OS is a consequence of its association with known cytogenetic risk factors we performed a multivariate analysis on the 1,165 patients of the UK-My9 cohort including rs12748648, high-risk IgH translocations, gain(1q21) and del(17p). This showed that 6p25.1 (rs12374648) independently impacted MM-OS (Supplementary Table 1). Patients with complete remission after autologous cell transplant (ASCT) tend to have a better survival. We examined whether the 6q25.1 association for OS was confined to patients with or without complete remission and we found the association was apparent in both patient groups ($P = 0.84$; test of heterogeneity).

Overall survival risk allele for myeloma at 6p25.1. rs12748648 maps within a 49.2-Kb ($r^2 > 0.2$) region of linkage disequilibrium intergenic to *MTHFD1L* and *AKAP12* genes (Fig. 2). The genomic region contains multiple enhancer marks and the SNP localizes to a predicted enhancer element that is bound by TCF4 (TCF7L2), Supplementary Fig. 5. Analysis of eQTL data did not demonstrate a relationship between rs12748648 genotype and expression of *MTHFD1L*, *AKAP12* or distantly flanking genes (Supplementary Figs 6 and 7). Examining encyclopedia of DNA elements (ENCODE) CHIP-seq data in the lymphoblast cell line GM12878 of the 6p25.1 showed that rs12748648 maps to region enriched for H3K27me3, a polycomb repressive mark. As DNA methylation can have a role in access of such polycomb repression[18], we undertook a meQTL analysis of the region around rs12748648. We found an association between rs12748648 risk genotype and reduced methylation of both *MTHFD1L* and *AKAP12* genes ($P = 0.0077$ and 0.0097, respectively, log-linear regression; Supplementary Fig. 8). In addition to the 6q25.1 (rs12748648) association for MM-OS we identified suggestive associations (that is $P < 10^{-5}$; proportional hazards model) at 1q23.3 (rs1934908), 19q13.11 (rs1974807), 5q31.3 (rs2906053), 3q13 (rs4682170), 18q21 (rs57942319) and 2q22 (rs61070260; Supplementary Table 2 and Supplementary Figs 9–20). All of the SNPs defining these associations mapped to genomic regions with regulatory marks (Supplementary Table 3), with rs1934908 impacting *FCLRA* expression in plasma cells (Supplementary Fig. 21).

All of the SNP associations noted above for MM-OS showed a consistent effect on progression-free survival, Supplementary Table 4. It is possible that some of the inherited genetic variants that impact on the risk of developing MM[14,16,17,19], may also impact on MM-OS. To address this possibility we examined the relationship between previously published risk SNPs and MM-OS. None of the nine validated risk SNPs for MM were associated with MM-OS, that is, $P > 0.05$, proportional hazards model; Supplementary Table 5.

Discussion

Our findings suggest a hypothesis that an individual's prognosis following treatment for MM is influenced by germline variation.

Figure 1 | Association plot for combined analyses for MM-OS. The P-values of the association between each SNP and myeloma survival were obtained by Cox regression analyses with adjustment and then combined. The y axis shows the $-\log_{10} P$-values of each SNP analysed, and the x axis shows their respective chromosome position. The red horizontal line corresponds to $P = 5.0 \times 10^{-8}$. All statistical tests were two-sided.
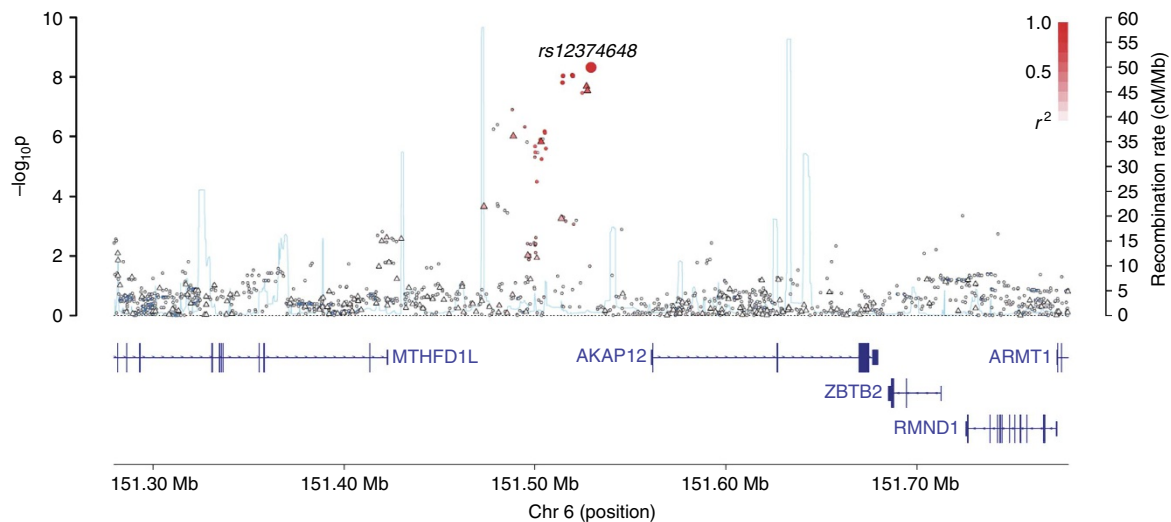
Figure 2 | Regional plot of association results and recombination rates for the rs12374648 (6q25.1) MM-OS risk locus. Plots show association results of both genotyped (triangles) and imputed (circles) SNPs in the GWAS samples and recombination rates. $-\log_{10} P$-values (y axes) of the SNPs are shown according to their chromosomal positions (x axes). The top genotyped SNP in each combined analysis is shown as a large diamond and is labelled by its rsID. The colour intensity of each symbol reflects the extent of LD with the top genotyped SNP, white ($r^2 = 0$) through to dark red ($r^2 = 1.0$). Genetic recombination rates, estimated using HapMap samples from Utah residents of western and northern European ancestry (CEU), are shown with a light blue line. Physical positions are based on NCBI build 37 of the human genome. Also shown are the relative positions of genes and transcripts mapping to the region of association. Genes have been redrawn to show their relative positions; therefore, maps are not to physical scale. Below each plot is a diagram of the exons and introns of the genes of interest.

Table 1 | Summary result for the combined analysis of 6q25.1 (rs12374648) and MM-OS.

| SNP	Base pair	Risk allele	Study	RAF	N	Events	GG	AG	AA	HR (95% CI)	P-value
rs12374648 (6q25.1)	151529369	G					GG	AG	AA		
			UK-My9	0.19	1,163	510	38	367	758	1.45 (1.24–1.68)	1.69×10^{-6}
			UK-My11	0.19	871	201	28	283	560	1.39 (1.10–1.75)	0.006
			GER-GMMG	0.20	508	156	18	164	326	1.09 (0.82–1.44)	0.55
			US-UAMS	0.20	702	330	30	218	454	1.28 (1.06–1.54)	0.009
			Combined							1.34 (1.22–1.48)	4.69×10^{-9}
											$P_{het} = 0.34$, $I^2 = 11\%$

RAF, risk allele frequency.
The P-values of the association between each SNP and myeloma survival were obtained by Cox regression analyses with adjustment and then combined.

In our analysis we adjusted for the influence of treatment on survival within studies, and report associations consistent across the four studies, thus our findings are likely to relate to underlying biology impacting the survival of the myeloma clone. The treatment used in all of the studies included ASCT, the GMMG studies used a tandem transplant, younger patients in the Medical Research Council (MRC) study received a single ASCT in both the Myeloma IX (UK-My9) and Myeloma IX (UK-My11) cases. The UAMS (US-UAMS) cases received predominantly tandem transplants together with combination induction and consolidation therapy. Prognostic factors generated from these studies are generally applicable to patients treated with ASCT and the use of novel agents including lenalidomide and bortezomib used as both induction and maintenance.

The 6q25.1 locus that associates with MM-OS spans a region of LD intergenic to *MTHFD1L* and *AKAP12* genes. While variation at 6q25.1 has previously been linked to coronary heart disease[20,21] (rs6922269) and late-onset Alzheimer disease[22] (rs11754661) the risk SNPs for these diseases are not correlated with rs12574648 (respective LD metrics–$r^2 = 0.002$, $D' = 0.08$ and $r^2 = 0.046$, $D' = 0.65$). *MTHFD1L* is involved in mitochondrial

tetrahydrofolate (THF) synthesis[23,24]. One-carbon substituted forms of THF are important for the *de novo* synthesis of purines and thymidylate supporting cellular methylation by regenerating methionine from homocysteine. There have been no previous reports of associations of cancer risk or overall survival (OS) with variation at *AKAP12*, but *AKAP12* has been shown to be a tumour suppressor, acting through CyclinD1 (ref. 25). *AKAP12* is regulated by methylation in a number of cancers[26–33] and is epigenetically repressed in MM, where its expression can be upregulated following treatment with DNA demethylation compounds such as trichostatin and/or 5-aza-2′-deoxycytidine[34].

Intriguingly, although rare, 6q is a site of recurrent deletion in lymphoid tumours, that includes homozygous deletions at 6q25.3 (*AR1D1B/WTAP*)[35] and mutations at 6q21 (*PRDM1/BLIMP1*)[36–38]. It is however unlikely that any somatic mutations in this regions are responsible for the MM-OS signal. While the functional basis for the rs12374648 remains to be established the SNP maps to a binding site for the transcription factor TCF7L2, alias TCF4. Binding of TCF7L2 correlates with hypomethylation and contributes to formation of differentially methylated regions of the genome[39]; TCF7L2 is commonly bound
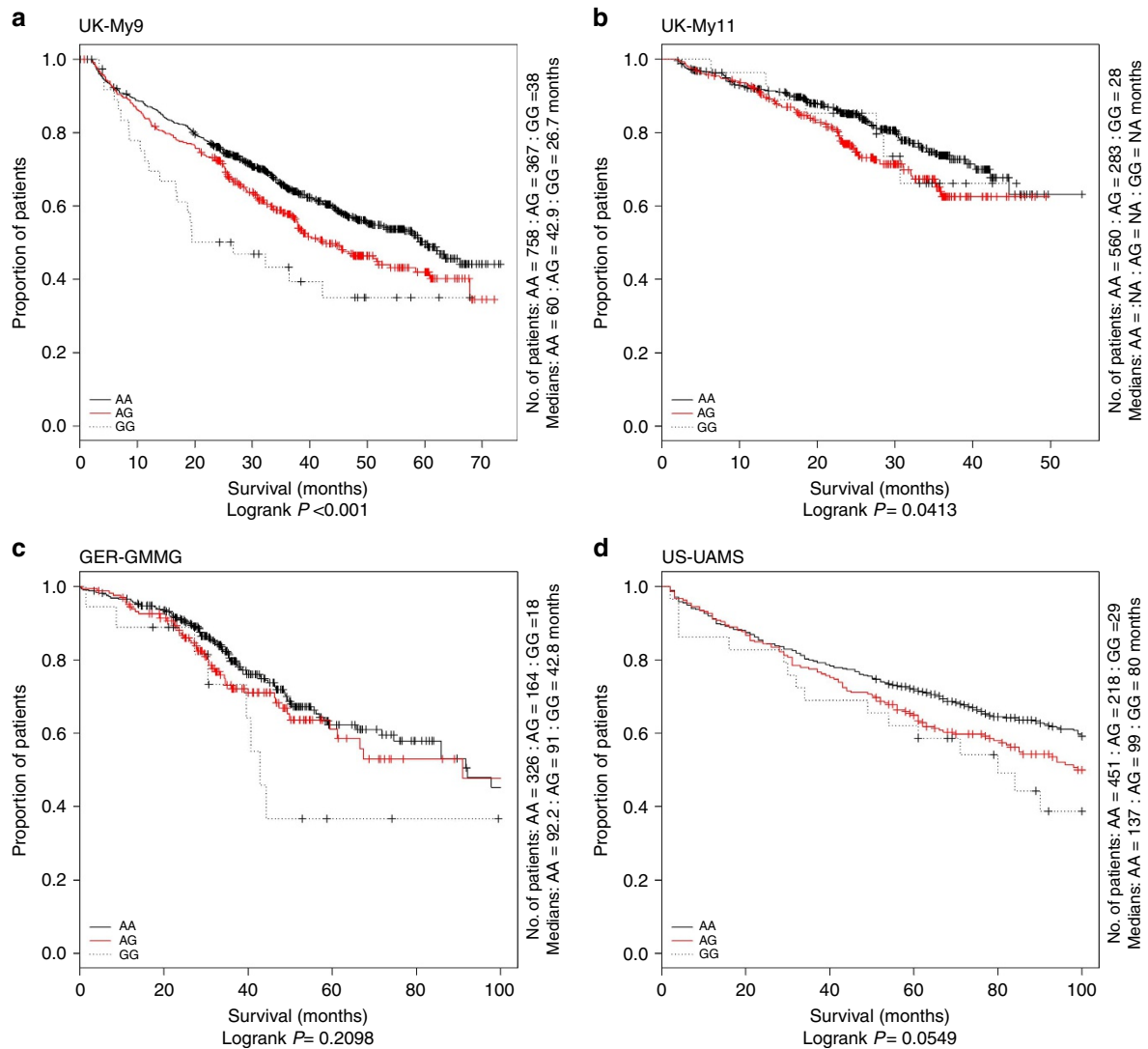
Figure 3 | Kaplan–Meier curves for MM-OS at 6q25.1 (rs12374648). Survival curves for the AA homozygotes are shown as a solid line. The red line depicts the survival curve for the AG heterozygotes, and the dashed line depicts the survival curve for the rare homozygotes GG. Vertical ticks indicate censored data points.

close to loci that are demethylated during differentiation[40,41]. Although speculative, we note that the rs12374648 risk genotype was associated with hypomethylation at the genomic region encompassing *MTHFD1L* and *AKAP12*, suggesting a possible mechanistic basis for the 6q25.1 association.

In addition to variation at 6q25.1, we identified suggestive associations at six other loci, a number of which annotate genes having strong *a priori* evidence for having a role in MM. Notably at 1q23.3, a region commonly amplified in MM, an association with rs1934908 genotype is also seen to be an eQTL for *FRCLA*, a gene with regulatory influence on IgG levels[42]. In contrast to these suggestive associations we did not find any evidence to support the recent claim that variation at 16p13 defined by the rare SNP rs72773978 influences the risk of MM-OS[43], $P_{combined} = 0.92$, proportional hazards model; Supplementary Table 6.

GWASs have been successful in identifying variants influencing susceptibility for most cancers. Notably, nine common variants have thus far been shown to be associated with MM risk[14–17,19]. Paradoxically variants for cancer prognosis have been elusive and in this study we have only identified one variant for myeloma survival at genome-wide statistical significance. A major reason for the disparity is study power. Despite the size of our study, the power to detect association with MM-OS is at best only modest. All of the common susceptibility alleles for MM are associated with relative risks of ~1.15 and such alleles can be identified through large case-control series, in contrast our survival analysis, based on 1,200 myeloma related deaths, had only 80% power to detect alleles with a HR greater ≥1.5 and minor allele frequency (MAF) >0.2.

Our findings support the hypothesis that germline variation influences outcome following treatment of MM. These results provide insight into the molecular mechanisms of tumour progression implying that germline markers of prognosis have the potential to enhance risk stratification. However, it is clear that trials larger than ours are required to identify additional loci associated with OS. Genotyping samples from future clinical trials, is likely to be especially informative and to offer the prospect of establishing the relationship between inherited variants, molecular subtypes and specific therapies.

Methods

Myeloma patient samples. We pooled data from four independent MM case series[14–17] in populations of European ancestry with existing high-density SNP genotyping (Supplementary Fig. 1): (i) UK-My11-GWAS comprising 877 MM cases from the UK MRC Myeloma IX trial. (ii) UK-My9-GWAS comprising 1,165

Table 2 | Clinical characteristics in the four patient cohorts.

	UK-My9	UK-My11	GER-GMMG	US-UAMS
Number of cases	1,165	877	511	703
Median age at diagnosis	64	66	57	59
Median follow-up (months)	46.7	29.8	45.2	114
	48.0 (ASCT)	29.8 (ASCT)		
Gender				
Male	694	520	294	444
Female	471	357	217	259
ISS				
I	246	231	232	332
II	475	373	168	208
III	444	273	111	163
WHO performance stage				
0	302	316	177	NA
1	529	360	147	NA
2	210	139	24	NA
≥ 3	119	46	6	NA
NA	5	16	157	NA
Deceased				
Yes	653	201	156	335
No	512	676	355	368
Disease progression				
Yes	900	465	358	252
No	265	412	153	451
Autologous cell transplant	504	516	511	703
Study	Intensive: 504	Intensive: 516	HD3: 81	TT2: 248
	Non-intensive: 661	Non-intensive: 361	HD4: 275	TT3: 183
			Non-trial: 155	TT3B: 104
				TT4: 95
				Non-trial: 73
Treatment received	CTD: 358	CTD: 265	PAD: 175	DPACE-VAD: 128
	CTDa: 215	CTDa: 184	TAD: 52	DTPACE: 151
	CVAD: 346	CRD: 250	VAD: 263	MVTDPACE: 96
	MP: 237	CRDa: 178	Other: 21	VDTPACE: 11
	Other: 9			VDTPACEa: 182
				Other: 31
Heavy chain paraprotein				
IgG	635	464	297	393
IgA	226	221	116	150
IgD	23	17	4	5
LCO	134	112	82	129
None	8	6	7	11
NA/other	139	57	5	15
Light chain paraprotein				
Lambda	354	260	165	417
Kappa	672	560	340	258
No light chain	0	5	0	6
NA/other	139	52	6	22
t4;14				
Yes	72	81	49	NA
No	593	504	370	NA
NA	500	292	92	NA
Gain 1q21				
Yes	206	161	152	NA
No	360	297	245	NA
NA	599	419	114	NA
del p53				
Yes	60	41	37	NA
No	583	417	366	NA
NA	522	419	108	NA

CRD, cyclophosphamide, lenalidomide, dexamethasone; CTD, cyclophosphamide, thalidomide, dexamethasone; CVAD, cyclophosphamide, vincristine, doxorubicin, dexamethasone; DPACE, dexamethasone, cisplatin, doxorubicin, cyclophosphamide and etoposide; DTPACE, exametthasone, thalidomide, cisplatin, doxorubicin, cyclophosphamide and etoposide; MP, melphalan, prednisone; MVDTPACE, melphalan, bortezomib, thalidomide, dexamethasone, cisplatin, doxorubicin, cyclophosphamide, etoposide; NA, not applicable; PAD, bortezomib, adriamycin, dexamethasone; TAD, thalidomide, doxorubicin, dexamethasone; TT, total therapy; VAD, vincristine, doxorubicin and dexamethasone; VDTPACE, bortezomib, thalidomide, dexamethasone, cisplatin, doxorubicin, cyclophosphamide, etoposide.

MM cases from the UK MRC Myeloma IX trial; (iii) German-GMMG-GWAS, comprising 511 MM patients recruited by the GMMG, coordinated by the University Clinic, Heidelberg; (iv) US-UAMS-GWAS comprising 703 newly diagnosed MM patients treated at the Myeloma Institute at UAMS. All of these trials have been reported previously but importantly all include a single or double autologous stem cell transplant as one component of the treatment[44–47] (Table 2). All studies were approved by the relevant institutional review boards, and all participants provided written informed consent.

Genotyping and quality control. Cases were genotyped using Illumina Human OmniExpress-12 v1.0 arrays according to the manufacturer's protocols (Illumina, San Diego, USA). Standard quality control was performed on all scans, excluding individuals with low call rate ($<90\%$) and extremely high or low heterozygosity ($P < 1.0 \times 10^{-4}$, test of heterogeneity), as well as all individuals evaluated to be of non-European ancestry (using the HapMap version 2 CEU, JPT/CHB and YRI populations as a reference; Supplementary Fig. 2). A summary of the number of genotyped SNPs and the number of SNPs passing QC is detailed in Supplementary Fig. 1.

Imputation. Genotypes for common variants across the genome were imputed using 1000 Genomes Project (phase 1 integrated release 3, March 2012) and UK10K as reference in conjunction with IMPUTE2 v2.1.1 (ref. 48) after pre-phasing with SHAPEIT software[49]. Poorly imputed SNPs defined by an information measure (Is) <0.90 were excluded from the analyses. All genomic locations are given in NCBI Build 37/UCSC hg19 coordinates.

Statistical analysis. The primary end point was MM-specific OS (MM-OS). Time-to-event was calculated from date of recruitment (left censoring) to avoid bias from the inclusion of prevalent cases. In the German series, the follow-up was started from transplantation, shortly after diagnosis. Follow-up was right censored on the date of death if death was other than MM, or the date last known alive if death did not occur. Kaplan–Meier survival curves according to genotype were generated and the homogeneity of the survival curves between genotypes was examined. Cox regression analysis was used to estimate genotype-specific HR and associated 95% CI. Where necessary we controlled for cryptic population sub-structure by including a variable number of principal components as covariates for each data set. For each SNP HRs were generated using common allele homozygotes as the reference group. P-values presented correspond to the significance of a test difference among all three of the genotype groups (common allele homozygote, heterozygote and rare allele homozygote). For SNPs where fewer than five minor allele homozygotes were observed, minor allele homozygote genotypes were combined with the heterozygotes. For the statistically significantly associated SNPs, we ran multivariable Cox models adjusting for ISS, age and treatment where appropriate. We confined our analysis to SNPs with MAF $>5\%$ because of extreme value of the test statistics. Overall statistical significance tests for each SNP were performed by combining the results for each data set using a fixed-effects meta-analysis. All statistical tests were two-sided. Inflation of the test statistics, λ, was estimated by dividing the 45th percentile of the test statistic by 0.357—the 45th percentile for a χ^2 distribution on 1 degree of freedom. Heterogeneity between studies was quantified using the I^2 statistic. Associations were regarded as statistically significant at a P value $\leq 5.0 \times 10^{-8}$ (that is, genome-wide significance). Multivariable stepwise variable selection was performed using a standard backward-elimination approach, variables were retained at a level of significance $P < 0.05$. All statistical analyses were performed using R (v3.1.3) software[50,51].

eQTL analysis. Expression quantitative trait locus (eQTL) analyses were performed for all genes in the 1 MB region spanning the MM-OS associated SNPs using Affymetrix Human Genome U133 + 2.0 array data for plasma cells from 184 MRC Myeloma IX trial patients, 658 GMMG patients and 604 UAMS patients, analysis as previously described[52]. In addition, we surveyed the effect of genotype on expression in other tissues using the publicly accessible GTEx (ref. 53), Blood eQTL (ref. 54), MuTHER studies[55], Framingham heart study[56] and SCAN (ref. 57) databases.

meQTL analysis. Methylation quantitative trait locus analyses of adjacent genes to SNPs of interest using probe-level DNA methylation data generated using Illumina 450 K methylation arrays on plasma cells from 365 MRC myeloma XI trial patients (UK-My11). Association between SNP genotype and normalized methylation levels was tested by linear regression.

Translocation detection. Conventional cytogenetic studies of MM cells were conducted using standard karyotyping methodologies, and standard criteria for the definition of a clone were applied. Fluorescence *in situ* hybridization and ploidy classification of UK samples was conducted using methodologies previously described[58,59]. Fluorescence *in situ* hybridization and ploidy classification of GMMG samples was performed as previously described. The XL IGH Break Apart probe (MetaSystems, Altlussheim Germany) was used to detect any IGH translocation in GMMG[60].

Bioinformatics. To explore the epigenetic profile of association signals, we used chromatin state segmentation in lymphoblastoid cell lines (LCL) data generated by the ENCODE project. The states were inferred from ENCODE histone modification data (histone H4 Lys20 methylation (H4K20me1), H3 Lys9 acetylation (H3K9ac), H3K4me3, H3K4me2, H3K4me1, H3K36me3, H3K27me3, H3K27ac and CCCTC-binding factor (CTCF)) binarized using a multivariate hidden Markov model. We used HaploReg and RegulomeDB[61,62] to examine whether any of the

SNPs or their proxies (that is, $r^2 > 0.8$ in the 1000 Genomes EUR reference panel) annotating putative transcription factor binding or enhancer elements. We assessed sequence conservation using Genomic Evolutionary Rate Profiling scores[63].

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Additional information

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