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Expression quantitative trait loci of genes predicting outcome are associated with survival of multiple myeloma patients

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Abstract

Gene expression profiling can be used for predicting survival in multiple myeloma (MM) and identifying patients who will benefit from particular types of therapy. Some germline single nucleotide polymorphisms (SNPs) act as expression quantitative trait loci (eQTLs) showing strong associations with gene expression levels. We performed an association study to test whether eQTLs of genes reported to be associated with prognosis of MM patients are directly associated with measures of adverse outcome. Using the Genotype-Tissue Expression (GTEx) portal, we identified a total of 16 candidate genes with at least one eQTL SNP associated with their expression with $p < 10^{-7}$ either in EBV-transformed B-lymphocytes or whole blood. We genotyped the resulting 22 SNPs in 1,327 MM cases from the International Multiple Myeloma rESEarch (IMMEnSE) consortium and examined their association with overall survival (OS) and progression free survival (PFS), adjusting for age, sex, country of origin and disease stage. Three polymorphisms in two genes (*TBRG4*-rs1992292, *TBRG4*-rs2287535, *ENTPD1*-rs2153913) showed associations with (OS) at $P < 0.05$, with the former two also associated with PFS. The associations of two polymorphisms in *TBRG4* with OS were replicated in 1277 MM cases from the International Lymphoma Epidemiology (InterLymph) Consortium. A meta-analysis of the data from IMMEnSE and InterLymph (2579 cases) showed that *TBRG4*-rs1992292 is associated with OS (HR=1.14, 95% C.I. 1.04–1.26, $p=0.007$). In conclusion, we found biologically a plausible association between a SNP in *TBRG4* and OS of MM patients.

Keywords

Multiple myeloma; overall survival; progression-free survival; genetic polymorphisms; eQTL

Introduction

Multiple myeloma (MM) is a malignancy of terminally differentiated plasma cells, which are primarily resident in the bone marrow. MM is the second most common haematological

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Author role

FC, AM and DC conceived and designed the study. AM and CP performed labwork. AM, CP and AC drafted the manuscript. AM, CP, FC; DC and AC performed data quality control and statistical analyses. All other authors provided samples and data. All authors critically read, commented and approved the manuscript.

Conflicts of interest

Authors declare no conflict of interests.

malignancy, with an annual crude incidence rate of 6.5 and 8 new cases per 100,000 inhabitants in Europe and in the United States of America, respectively¹.

The advances in therapy made in the last decade have resulted in a considerable increase in patient survival. However, MM remains an incurable disease for most patients, who eventually relapse. The clinical course of MM is characterized by a high degree of heterogeneity, with long-term responders to therapy who survive long enough to eventually die of other causes, and patients who are refractory to any therapy and succumb very quickly to the disease².

Gene expression profiling (GEP) is being widely used for tumor classification and prognosis and can effectively identify patients with very poor outcome. Numerous prognostic gene signatures have been identified in the past years; some of them were identified agnostically from direct comparison of patients with different survival while others were informed by genes relevant to the biology of MM^{3–11}. Moreover, GEP has been able to classify patients based on their response to certain kinds of therapy, which could be valuable to personalize treatments given the vast heterogeneity of treatments and drug combinations^{12,13}.

Over recent years single nucleotide polymorphisms (SNPs) have been found associated with MM survival, through candidate^{14–18} or genome wide association studies (GWAS)^{19,20}. However, the influence of germline variants on MM outcome is still a poorly explored field and few studies have identified SNPs associated with a different response to specific therapies²¹.

Recent evidence derived from large projects such as the Genotype-Tissue Expression (GTEx) database have identified SNPs as expression quantitative trait loci (eQTLs), strongly associated with gene expression²². eQTLs have been successfully used as surrogates of direct measurement of gene expression to study disease etiology^{23,24}. In most cases they are located in physical proximity to the genes whose expression they influence (“cis-eQTLs”, usually mapping to promoter or enhancer regions), while some eQTLs are located in a different chromosomal region, or even on a different chromosome from the gene whose expression is affected (“trans-eQTLs”). Interestingly, it has been shown that polymorphisms associated with complex traits in GWAS, including risk of many cancers, are enriched in eQTLs^{24,25}. Considering that the expression of several genes is associated with MM prognosis we hypothesized that SNPs that affect expression levels of those genes might also be associated with prognosis.

We performed an association study within the International Multiple Myeloma rESEarch (IMMEnSE) consortium to examine SNPs that act as eQTLs for genes included in expression signatures that have been previously shown to influence MM survival. We hypothesize that these eQTLs could be used as markers of outcome. We attempted to replicate the top associations in the International Lymphoma Epidemiology (InterLymph) consortium and performed a meta-analysis of results from both consortia.

Materials and Methods

Study samples

International Multiple Myeloma rESEarch (IMMEnSE) consortium.—The first phase of the association study was performed in the IMMEnSE consortium, which has been described elsewhere²⁶. Each collaborating institution retrospectively collected clinico-pathological data from medical records on age, sex, country of origin, disease stage (Durie-Salmon and/or International Staging System), and type of first-line therapy, response to first-line therapy, progression and vital status. We analyzed 1,302 MM patients with staging information for the Durie-Salmon system and 1,064 subjects with staging information for the International staging system (ISS), while 1,050 patients had data for both (table 1). MM cases were diagnosed according to the IMWG criteria from 2001 to 2015 and 640 were treated with bortezomib/immunomodulatory drugs which we defined as “recent therapies”.

The IMMEnSE study protocol was approved by the Ethics Committee of the Medical Faculty of the University of Heidelberg (reference number: S-004/2020). Following the guidelines of the Declaration of Helsinki, written informed consent was obtained from each participant.

International Lymphoma Epidemiology (InterLymph) consortium.—Multiple myeloma studies from InterLymph consisted of 9 participating studies of European ancestry (EA) with genotype and phenotype information (2,434 cases and 3,446 controls), which was pooled to perform genome-wide association studies (GWAS) for risk and survival. In total, the primary InterLymph dataset had 885 cases with stage information (ISS). A secondary InterLymph survival dataset consisting of 392 patients diagnosed with MM with follow-up and disease stage available from The University of Texas/MD Anderson Cancer Center (MDACC) and University of California San Francisco in the United States was added.

Contributing studies were approved by local ethics review committees, and all participants provided written, informed consent.

Characteristics of study participants are summarized in table 1.

SNP selection

We selected a comprehensive list of genes whose expression levels were associated with poor MM prognosis in the literature^{3–5,8,9,27}. We also identified GEP signatures associated with differential response to therapy¹³. From this review, we assembled a list of 283 genes and searched for eQTLs associated with the expression levels of those genes using the browser of the genotype-tissue expression project GTEx (<http://www.gtexportal.org>)²⁴. For our study, the cis window established from the browser was 1 megabase up- and down-stream of the transcriptional start site of each gene. We performed these queries using the expression data on the tissues represented in GTEx that are closest to the cells of interest for MM, i.e. EBV-transformed B-lymphocytes (from 114 samples) and whole blood (from 338 samples). We ranked the eQTLs according to p-values of association with gene expression. Ten of these genes (*RPS28*, *YWHAZ*, *CNDP2*, *TBRG4*, *HLA-DPA1*, *DHFR*, *RAB2A*, *SERPINB1*, *HLA-DRB1* *IKZF1*) have significant eQTLs in both tissues while six

other genes (*ACTR2*, *HELLS*, *ENTPD1*, *CCND2*, *CCND1*, *ANK3*) had eQTLs in at least one of the tissues analysed. For each gene we selected at least one eQTL while considering the linkage disequilibrium (LD) among eQTLs. The final list included 22 independent SNPs from these 16 genes (table 2).

Genotyping and quality control

IMMEnSE.—Genomic DNA was extracted from peripheral blood using the QIAampR 96 DNA QIAcubeR HT Kit and stored at -20°C till use. All the genotyping assays were carried out in 384-well format, with 10 ng of DNA from each subject using TaqMan (Thermo Fisher Scientific Inc.) or KASP (LGC Genomics) SNP genotyping assays. For quality control about 5% of the samples were interspersed in the plates as duplicated. Samples with a call rate lower than 80% (N=184) were discarded.

InterLymph GWAS (primary and secondary).—Samples were genotyped using the Affymetrix 6.0 and Illumina (610 Quad, Human660W-quad Beadchip, Omni5, OmniExpress Beadchip, Oncoarray) platforms. Each of the GWAS was subjected to rigorous standardised quality control independently prior to imputation, which was performed via the Michigan imputation server (<https://imputationserver.sph.umich.edu/>) based on the Haplotype Reference Consortium (HRC)²⁹. After imputation, each site was filtered to include only imputed variants with information score >0.6 and further quality controls checks were implemented (genotype rate $>95\%$, minor allele frequencies >0.01 , and Hardy-Weinberg equilibrium (HWE) $>10^{-5}$ in controls). Finally, the data were pooled and final quality control was performed on the pooled GWAS set including checks for missingness, duplicates, sex mismatch, abnormal heterozygosity, cryptic relatedness, population outliers (principal components analyses: Eigenstrat), and genomic inflation ($\lambda > 1.00$). Additional information on the MM GWAS studies contributing in the InterLymph consortium are showed in supplementary table 1.

Statistical and bioinformatic analyses

Survival analysis in IMMEnSE was performed with Cox proportional hazards regression, calculating hazard ratios (HR) and 95% confidence intervals (CI), using overall survival (OS) and progression-free survival (PFS) as endpoints. OS was defined as the time interval between MM diagnosis and death or last follow-up. PFS was defined as the time interval between the ASCT (autologous stem cell transplantation) or high-dose treatment (for patients not eligible for ASCT) until documented progression or until the last progression-free examination. All analyses were adjusted for age at diagnosis, sex, country of origin, MM stage (calculated with the Durie-Salmon or ISS system) and type of first-line therapy, defined as treatment based on bortezomib/immunomodulatory drugs (“recent therapies”) or any other regimen (such as vincristine/adriamycin/dexamethasone or melphalan/prednisone, “chemotherapy based only therapies”). The statistical analysis was performed using per-allele and co-dominant models. We considered the threshold of statistical significance, using a Bonferroni correction, to be $p < 0.0023$ ($0.05/22$ SNPs). A stratified analysis by type of first line therapy was also performed for the six polymorphisms selected from the signature of Terragna et al.¹³ In addition, we performed the same analysis adjusted by bone lesions for the two polymorphisms in *TBRG4* since this gene is implicated in bone-related disease³⁰.

The InterLymph survival GWAS data were analyzed using Gwasurvivr, an R package for genome-wide survival analysis³¹ with Cox-proportional hazard models adjusting for age, sex, site, 10 principal components from the GWAS, and ISS stage.

Results from IMMEnSE and InterLymph (primary and secondary) GWAS were meta-analysed according to a fixed effect model. The results of the single SNPs were not adjusted for type of first line therapy which was available only in IMMEnSE but not in InterLymph.

To identify the regulatory potential of selected SNPs and the regions nearby we used HaploReg (<https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php>)³¹ and RegulomeDb (<http://regulome.stanford.edu>)³³.

Results

For IMMEnSE, the overall genotyping call rate was 92.3%, the minimum call rate observed was 91.9% (rs2972572) and the maximum 98.2% (rs1992292). The concordance between duplicates was of 99.9%. Five of the selected SNPs (*HLA-DQBI*-rs141471663, *HLA-DQBI*-rs1130456, *HLA-DRBI*-rs66859861, *SERPINBI*-rs62392542, *HLA-DPAI*-rs116102562) were not in Hardy-Weinberg equilibrium (HWE). All those SNPs were located in chromosome 6 where the MHC complex is located as well. It is well known that this particular genomic region is not neutral from the point of view of natural selection and is also known to contain duplicated sequences and copy number variants (CNVs),^{34,35} and deviation from HWE may be expected³⁴. Considering also that some of these SNPs (rs1140347 and rs62391542) are also not in HWE in the 1000 Genomes Project, and, on the other hand, that concordance of genotypes of duplicated samples in our study was 100% for these SNPs, we included them in further statistical analyses.

Discovery phase (IMMEnSE results).

The most significant association was seen for *TBRG4*-rs1992292 which showed an association with OS when adjusted for ISS disease stage system (table 3). The C/T genotype is associated with a worse OS in our set of patients (HR=1.59, 95% C.I.= 1.18–2.15, p=0.0024) in the co-dominant model of inheritance. Additionally, rs2289375, another independent SNP in the same gene, showed weaker evidence of association in the same direction of *TBRG4*-rs1992292 (HR=1.33, 95% C.I. =1.06–1.67, p=0.013). These 2 SNPs were also nominally associated with a worse PFS (table 4). Results were similar regardless of the staging system used for adjustment (Durie-Salmon or ISS).

Additionally, we found several associations with different end-points at the nominal level of p<0.05. Namely, the *ENTPDI*-rs2153913 SNP showed associations with OS when considering all cases (HR=0.71, 95% C.I.=0.54–0.94, p=0.017, for the heterozygotes in the codominant model) and cases treated with new therapies (HR=0.61, 95% C.I.=0.37–0.98, p=0.043), but not cases treated with the old therapies (HR=0.78, 95% C.I.=0.55–1.11, p=0.168).

Both polymorphisms in *TBRG4* showed associations with OS when adjusting by bone lesions. In particular, the strongest association was observed for *TBRG4*-rs1992292 for the

codominant model of inheritance ($HR_{het}=2.21$, 95% C.I.=1.49–3.28, $p=0.0001$). All results for these analyses are reported in supplementary table 2.

All the results presented and tables 3 and 4 were adjusted for ISS, while the results adjusted using Durie-Salmon staging are showed in supplementary tables 3 and 4.

Replication phase (InterLymph).

Survival analysis in the InterLymph datasets was performed on the top three associations seen in IMMEnSE ($p<0.05$): *ENTPDI*-rs2153913, *TBRG4*-rs1992292, *TBRG4*-rs2289375. Associations with both polymorphisms in *TBRG4* replicated in the primary InterLymph dataset with OS: rs1992292 showed an $HR=1.16$, 95% C.I.=1.01–1.33, $p=0.046$ and rs2289375 an $HR=1.24$, 95% C.I.=1.06–1.47, $p=0.008$, considering the allelic model. The association with *ENTPDI*-rs2153913 was not replicated in this set ($HR=1.03$, 95% C.I.=0.88–1.19, $p=0.731$). None of the above-mentioned associations replicated in the additional set of cases from the secondary InterLymph dataset.

A total of 2579 cases were used for the meta-analysis and the polymorphism *TBRG4*-rs1992292 showed to be significantly associated with OS, with no heterogeneity between the three groups ($HR=1.14$ 95% C.I. 1.04–1.26, $p=0.007$) and a forest plot for this analysis is shown in figure 1. No evidence of association was observed for *ENTPDI*-rs2153913 ($HR=0.93$, 95% C.I. 0.84–1.04, $p=0.211$).

Kaplan-Meier curves for the survival of MM patients according to genotype at the two *TBRG4* SNPs are shown separately for IMMEnSE and the primary InterLymph dataset (supplementary figure 1 and 2).

Discussion

The investigation of germline variants that act as expression quantitative trait loci (eQTL) for genes whose expression is known to affect MM prognosis could be used to identify predictors of patient outcome. Based on this rationale, we tested whether eQTLs of genes included in expression signatures that define MM patients with poor prognosis are associated with adverse outcome and therefore could be used as genetic markers of prognosis.

Our results suggest that the minor alleles of the *TBRG4*-rs1992292 and *TBRG4*-rs2289375 SNPs are associated with a worse survival. *TBRG4* encodes for a regulator of transforming growth factor beta (TGF- β), which is involved in various cellular pathways, including the regulation of hematopoiesis, an important process for myeloma cell proliferation and survival²⁶. Increased levels of TGF- β in the bone marrow microenvironment induce an increase of IL-6 and VEGF secretion, major cytokines involved in cancer cell proliferation and angiogenesis²⁵. Moreover it has been reported that down-regulation of *TBRG4* contributes to arrest of cell cycle in the G1 phase, which ultimately leads to a better outcome in MM²⁸. This gene was selected initially because it was reported that its higher expression is associated with a shorter survival in MM patients⁸.

TBRG4-rs1992292 is in strong LD ($r^2=0.935$ in European population (CEU) of the 1000 Genomes project) with rs6967730, that has a rank of 1f in RegulomeDB, indicating that it is likely to affect the binding of additional transcription factors and it is linked to expression of *TBRG4*. In this regard, it has been reported that rs6967730 is located within a transcription factor binding site for CTCF (CCCTC-binding factor), a highly conserved zinc finger protein. CTCF can function as a transcriptional activator, a repressor or an insulator protein, blocking the communication between enhancers and promoters³⁹. Therefore, rs6967730 could be responsible for changing the expression of *TBRG4* by modifying the binding site of CTCF. Even though the information provided by our and other studies is relevant, in-depth analysis of the biological role of the *TBRG4*-rs1992292 SNP in modulating MM survival, including mechanistic insights, is still needed.

According to our results also *TBRG4*-rs2289375 is associated with a worse survival of MM patients. This SNP has a RegulomeDB rank of 2b and is in LD ($r^2=1$) with rs3757573, which has a rank of 1f indicating that it could have a strong functional role in affecting the expression of *TBRG4*. The GTEx portal reports that the TT genotype is associated with a higher expression of *TBRG4* in both the tissues we considered, in line with our results where carriers of this genotype have a worse survival (figure 2).

Finally, although *ENTPDI*-rs215391 did not replicate in the InterLymph datasets, this SNP, according to our bioinformatics analysis, has a clear biological link with MM. Indeed, GTEx reports that the C allele of *ENTPDI*-rs215391 decreases the expression of the *ENTPDI* gene, which translates into a lower production of adenosine which, in turn, results in a less active adenosine-mediated immunosuppressive pathway increasing the anticancer monitoring immune system. Considering the above, we cannot exclude a potential contribution of *ENTPDI*-rs215391 in MM outcome.

The study has some weaknesses: data on PFS and type of first line treatment were not available for InterLymph cases; therefore, we could not confirm the result obtained with PFS as endpoint. Another limitation is the lack of karyotype data which is involved in the heterogeneity observed in patient prognosis. Moreover, the selection of the eQTLs was limited to one or two eQTLs for each region that we selected (the ones showing associations with gene expression levels with the lowest p-values in GTEx) and therefore we could not exclude the possibility of having missed additional associations. Our results, however, represent a proof of principle that eQTLs could be used as MM survival markers and offer a starting point to further investigate in this direction alongside other known prognostic markers.

Standard eQTL analysis, which involves a direct association test between markers of genetic variation with gene expression levels, has many advantages. The main one is that the genotypes are not influenced by sample manipulation or by environmental variables since invariable throughout life of an individual. eQTL analysis can be performed *in silico* using available GWAS dataset and free bioinformatic tools as GTEx, which makes this kind of analysis basically costless compared to GEP which involves the use of expensive equipment and reagents.

The main strengths of the study are that our results were confirmed in two of three independent datasets with a large overall sample size with information on overall survival and stage.

In conclusion, we found biologically plausible associations between SNPs in *TBRG4* and OS of MM patients that should be investigated more deeply. eQTLs are a valid surrogate for GEP, and are much easier to measure than GEP itself.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Abbreviation:

ASCT	autologous stem cell transplantation
CI	confidence intervals
CTCF	CCCTC-binding factor
EA	European ancestry
eQTLs	expression quantitative trait loci
GEP	Gene expression profile
GWAS	genome wide association studies
HR	hazard ratios
HWE	Hardy-Weinberg equilibrium
ISS	International staging system
LD	linkage disequilibrium
MM	multiple myeloma
OS	overall survival
PFS	progression free survival
SNPs	single nucleotide polymorphisms

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What's new?

Gene expression profile (GEP) is widely used for tumor classification and prognosis of MM patients. SNPs so called expression quantitative trait loci (eQTLs) have been strongly associated with gene expression variation and have been successfully used as surrogates of direct measurement of gene expression to study disease etiology.

Herein we sought to test whether eQTLs of genes reported to be associated with prognosis of MM patients are directly associated with measures of adverse outcome.

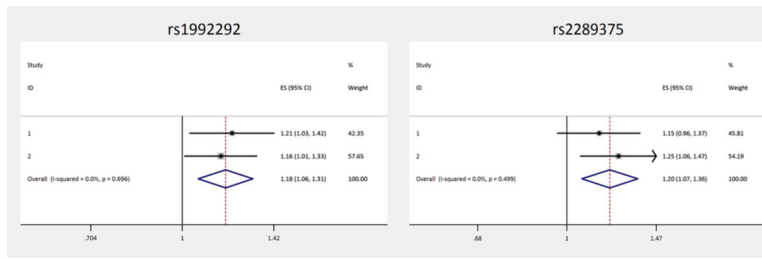


FIGURE 1.
Forest plot for TBRG4-rs1992292

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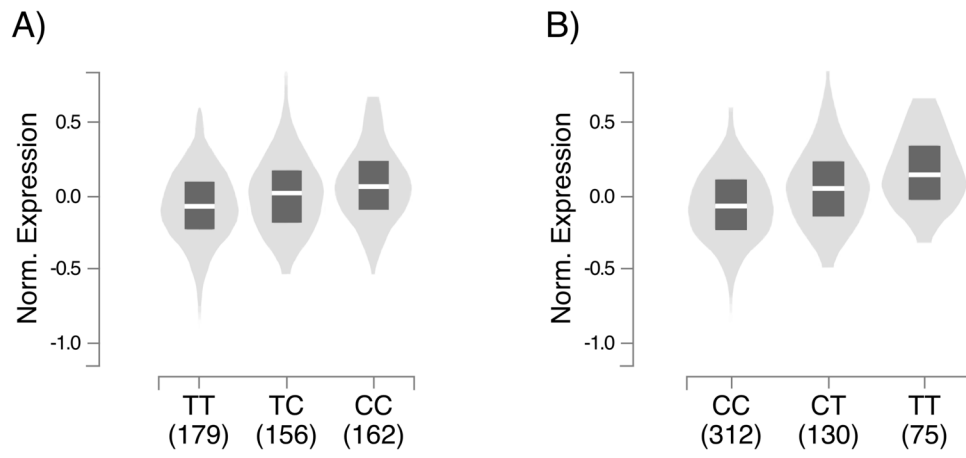


FIGURE 2.

A, box plot of the association between rs1992292 and TBRG4 expression; B, box plot of the association between rs2289375 and TBRG4 expression. The data used for the analyses described in this manuscript were obtained from: GTEx Analysis Release V8, accessed on 10/10/2019334.

Table 1.

Study populations

	IMMENSE	InterLymph	
		Primary	Secondary
Country of origin			
Italy	124		
Poland	793		
Spain	103		
Portugal	30		
Denmark	260		
Hungary	17		
USA		765	392
Canada		120	
Total	1,327	885	392
Median age (25%-75% percentiles)	61 (54–67)	61 (54–68)	60 (53–67)
Gender			
Males	52%	63%	56%
Females	48%	37%	44%
Disease stage Durie-Salmon ^a			
1	186	71	-
2	320	83	-
3	808	419	-
Total	1,316	574	-
Disease stage ISS ^a			
1	323	178	156
2	347	466	127
3	393	241	109
Total	1,064	885	392
First line therapy ^{a,b}			
New	640	-	-
Old	687	-	-
Total	1,327	-	-
Median overall survival months (25%-75% percentiles)	39 (20.5–69.47)	60 (31–93)	55 (28–81)
Median progression-free survival months (25%-75% percentiles)	23 (11.70–43.72)	NA	NA

^aThe sum does not add up to the total of subjects due to missing data.

^bNew therapies are those based on proteasome inhibitors and/or immunomodulating drugs; old therapies are all others.

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Table 2.

List of selected SNPs.

Gene	Gene signature	SNP	Alleles Major/minor	GTEEx p-value ^a
<i>RPS28</i>		rs2972572	A/G	7.9×10 ⁻⁴²
<i>DHFR</i>	Kuiper 2012 ¹¹	rs2560424	C/T	1.4×10 ⁻¹⁶
		rs7387	T/A	1.4×10 ⁻¹⁶
<i>RAB2A</i>		rs948421	T/C	1.2×10 ⁻¹⁰
<i>HLA-DQB1</i>		rs1140347	T/C	2.3×10 ⁻³⁰
		rs1063355	T/C	2.1×10 ⁻²⁹
<i>HLA-DRB1</i>	Moreaux 2013 ³	rs66859861	C/T	1.7×10 ⁻¹⁸
		rs9270917	G/T	4.7×10 ⁻²⁹
<i>SERPINB1</i>		rs62391542	C/T	8.6×10 ⁻⁰⁸
<i>HLA-DPA1</i>		rs116102562	T/C	9.3×10 ⁻¹⁶
		rs1054026	G/C	4.2×10 ⁻¹⁵
<i>YWHAZ</i>		rs3134353	A/T	4.5×10 ⁻¹⁸
<i>TBRG4</i>	Shaughnessy 2007 ⁸	rs1992292	T/C	3.5×10 ⁻⁰⁸
		rs2289375	C/T	3.1×10 ⁻¹⁰
<i>CNDP2</i>	Decaux 2008 ⁴	rs8084058	A/G	8.8×10 ⁻⁰⁹
		rs4891557	C/T	4.4×10 ⁻⁰⁹
<i>ACTR2</i>		rs4671647	C/T	6.9×10 ⁻⁷
<i>HELLS</i>		rs7100415	G/C	5.4×10 ⁻⁶
<i>ENTPD1</i>	Terragna 2016 ¹³	rs2153913	G/C	1.3×10 ⁻²¹
<i>CCND2</i>		rs3217860	A/G	1.4×10 ⁻⁶
<i>CCND1</i>		rs7102758	A/G	1.1×10 ⁻⁶
<i>ANK3</i>		rs7072106	C/G	2.7×10 ⁻¹²

^aP-values of association between SNP genotypes and level of expression of the respective gene. The data used for the analyses described in this manuscript were obtained from: GTEEx Analysis Release V7, accessed on 10/10/2017.

Table 3.

Results of the association between OS of MM patients and eQTLs in the IMMEnSE population.

SNP	Alleles (M/m) ^b	N of patients	N of deceased patients	Allelic model		Codominant model		P	P
				HR _{het} (95% CI)	P	HR _{het} (95% CI)	P		
rs2972572	A/G	864	214	1.06(0.87–1.28)	0.564	1.16(0.84–1.60)	0.369	1.11(0.75–1.63)	0.604
rs1063355	T/G	836	206	1.06(0.90–1.25)	0.454	1.10(0.78–1.55)	0.563	1.13(0.81–1.56)	0.477
rs1140347	G/A	894	249	0.94(0.82–1.07)	0.376	1.01(0.64–1.59)	0.96	0.89(0.68–1.16)	0.394
rs66859861	C/T	890	240	0.98(0.84–1.15)	0.83	1.20(0.84–1.73)	0.311	1.00(0.73–1.39)	0.973
rs3134353	T/A	984	265	1.16(0.97–1.39)	0.103	1.22(0.84–1.79)	0.297	1.38(0.93–2.04)	0.106
rs8084058	G/A	970	262	0.97(0.81–1.15)	0.755	0.93(0.66–1.32)	0.695	0.93(0.65–1.34)	0.705
rs4891557	C/T	966	260	0.94(0.76–1.17)	0.586	0.87(0.66–1.13)	0.298	1.09(0.62–1.92)	0.743
rs1992292	T/C	984	265	1.23(1.05–1.45)	0.012	1.59(1.18–2.15)	0.002	1.52(1.08–2.16)	0.017
rs2289375	C/T	956	259	1.16(0.96–1.38)	0.106	1.33(1.02–1.73)	0.034	1.19(0.79–1.81)	0.408
rs1054026	G/C	984	263	0.87(0.70–1.08)	0.223	0.70(0.37–1.33)	0.275	0.64(0.35–1.19)	0.163
rs2560424	C/T	982	265	0.79(0.64–0.96)	0.023	0.81(0.62–1.04)	0.105	0.59(0.34–1.03)	0.065
rs116102562	T/C	935	243	0.97(0.65–1.44)	0.886	1.06(0.33–3.41)	0.922	0.99(0.37–2.66)	0.982
rs948421	T/C	978	265	1.17(0.97–1.39)	0.094	1.31(0.90–1.90)	0.152	1.42(0.96–2.10)	0.076
rs62391542	C/T	941	254	1.00(0.85–1.17)	0.984	1.33(0.90–1.96)	0.146	1.08(0.76–1.52)	0.657
rs7387	T/A	982	267	1.26(1.03–1.54)	0.027	1.37(0.78–2.41)	0.273	1.68(0.97–2.91)	0.065
rs4671647	C/T	1014	276	1.28(0.73–1.06)	0.18	0.87(0.68–1.12)	0.291	0.77(0.50–1.18)	0.239
rs7100415	G/C	981	272	0.99(0.84–1.18)	0.954	1.00(0.77–1.31)	0.961	0.98(0.68–1.40)	0.909
rs2153913	G/C	1022	277	0.88(0.75–1.05)	0.146	0.71(0.54–0.94)	0.017	0.79(0.58–1.08)	0.149
rs3217860	A/G	1012	272	0.87(0.71–1.06)	0.181	0.82(0.63–1.05)	0.13	0.90(0.53–1.52)	0.7
rs7102758	A/G	1018	277	0.95(0.73–1.24)	0.718	0.92(0.70–1.22)	0.589	1.02(0.73–1.43)	0.891
rs7072106	C/G	1017	272	1.05(0.88–1.27)	0.556	1.09(0.85–1.40)	0.484	1.07(0.67–1.0)	0.787

^a adjusted for age, sex, country of origin, ISS disease stage and kind of first line therapy

^b M = major allele; m = minor allele

Table 4. Results of the association between PFS of MM patients and eQTLs in the IMMEnSE population.

Gene	SNP	Alleles (M/m) ^b	N of patients	N of deceased patients	Allelic model			Codominant model		
					HR _{het} (95% CI)	P	HR _{het} (95% CI)	P	HR _{hom} (95% CI)	P
<i>RPS28</i>	rs2972572	A/G	1072	298	1.03(0.88–1.20)	0.703	1.11(0.85–1.48)	0.429	1.06(0.77–1.46)	0.716
<i>HLA-DQB1</i>	rs1063355	T/G	1043	293	1.05(0.92–1.21)	0.468	1.09(0.82–1.44)	0.559	1.10(0.83–1.45)	0.493
<i>HLA-DQB1</i>	rs1140347	G/A	1098	332	0.96(0.85–1.08)	0.474	1.07(0.74–1.54)	0.726	0.92(0.73–1.17)	0.515
<i>HLA-DRB1</i>	rs66859861	C/T	1083	323	0.95(0.83–1.08)	0.430	0.95(0.70–1.29)	0.757	0.90(0.68–1.18)	0.44
<i>YWHAZ</i>	rs3134353	T/A	1201	355	1.05(0.90–1.22)	0.512	1.08(0.80–1.47)	0.602	1.12(0.81–1.54)	0.491
<i>CNDP2</i>	rs8084058	G/A	1179	345	0.99(0.85–1.16)	0.946	0.95(0.70–1.27)	0.718	0.97(0.71–1.33)	0.87
<i>CNDP3</i>	rs4891557	C/T	1174	348	0.91(0.76–1.10)	0.336	0.85(0.68–1.07)	0.184	0.98(0.61–1.60)	0.96
<i>TBRG4</i>	rs1992292	T/C	1196	350	1.18(1.03–1.37)	0.018	1.49(1.15–1.93)	0.002	1.40(1.04–1.90)	0.027
<i>TBRG4</i>	rs2289375	C/T	1165	345	1.17(1.00–1.37)	0.044	1.33(1.06–1.67)	0.013	1.23(0.85–1.76)	0.269
<i>HLA-DPA1</i>	rs1054026	G/C	1200	352	0.99(0.81–1.20)	0.923	0.89(0.49–1.60)	0.699	0.91(0.52–1.59)	0.732
<i>DHFR</i>	rs2560424	C/T	1202	356	0.85(0.71–1.01)	0.069	0.87(0.69–1.09)	0.225	0.68(0.42–1.10)	0.117
<i>HLA-DPA1</i>	rs116102562	T/C	1126	312	1.03(0.72–1.47)	0.872	1.14(0.40–3.28)	0.801	1.12(0.46–2.72)	0.802
<i>RAB2A</i>	rs948421	T/C	1192	351	1.10(0.94–1.28)	0.243	1.20(0.87–1.66)	0.253	1.25(0.89–1.74)	0.194
<i>SERPINB1</i>	rs62391542	C/T	1155	339	1.02(0.89–1.17)	0.773	1.34(0.97–1.86)	0.076	1.11(0.83–1.49)	0.468
<i>DHFR</i>	rs7387	T/A	1201	356	1.07(0.91–1.27)	0.411	1.13(1.13–1.76)	0.592	1.19(0.77–1.84)	0.431
<i>ACTR2</i>	rs4671647	C/T	1223	359	0.92(0.78–1.08)	0.319	0.85(0.68–1.06)	0.153	0.92(0.65–1.32)	0.67
<i>HELLS</i>	rs7100415	G/C	1185	351	0.98(0.84–1.15)	0.871	0.96(0.76–1.20)	0.702	0.97(0.71–1.33)	0.851
<i>ENTPD1</i>	rs2153913	G/C	1227	356	0.91(0.78–1.06)	0.219	0.80(0.63–1.02)	0.071	0.85(0.63–1.13)	0.261
<i>CCND2</i>	rs3217860	A/G	1223	355	0.89(0.75–1.06)	0.202	0.89(0.71–1.11)	0.286	0.80(0.51–1.27)	0.35
<i>CCND1</i>	rs7102758	A/G	1018	277	1.04(0.83–1.30)	0.736	1.06(0.83–1.35)	0.661	1.02(0.76–1.37)	0.907
<i>ANK3</i>	rs7072106	C/G	1017	272	1.07(0.91–1.26)	0.39	1.13(0.91–1.41)	0.261	1.10(0.73–1.64)	0.648

^a adjusted for age, sex, country of origin, ISS disease stage and kind of first line therapy

^b M = major allele; m = minor allele