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A pleiotropic variant in *DNAJB4* is associated with multiple myeloma risk

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Abstract

Pleiotropy, which consists of a single gene or allelic variant affecting multiple unrelated traits, is common across cancers, with evidence for genome-wide significant loci shared across cancer and noncancer traits. This feature is particularly relevant in multiple myeloma (MM) because several susceptibility loci that have been

Abbreviations: CI:, confidence interval; eQTL:, expression quantitative trait loci; GTEx:, genotype-tissue expression project; GWAS:, genome-wide association studies; HSC:, hematopoietic stem cell; HWE:, Hardy-Weinberg equilibrium; IMMEnSE:, International Multiple Myeloma rESEarch; IMWG:, International Myeloma Working Group; InterLymph:, International Lymphoma Epidemiology Consortium; LD:, linkage disequilibrium; lncRNA:, long non coding RNA; MM, multiple myeloma; OR, odds ratio; PC, principal component; SNP, single nucleotide polymorphism.

Marco Dicanio, Matteo Giaccherini and Alyssa Clay-Gilmour share the first position and Celine Vachon, Federico Canzian and Daniele Campa share the last position.

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identified to date are pleiotropic. Therefore, the aim of this study was to identify novel pleiotropic variants involved in MM risk using 28 684 independent single nucleotide polymorphisms (SNPs) from GWAS Catalog that reached a significant association ($P < 5 \times 10^{-8}$) with their respective trait. The selected SNPs were analyzed in 2434 MM cases and 3446 controls from the International Lymphoma Epidemiology Consortium (InterLymph). The 10 SNPs showing the strongest associations with MM risk in InterLymph were selected for replication in an independent set of 1955 MM cases and 1549 controls from the International Multiple Myeloma rESEarch (IMMEnSE) consortium and 418 MM cases and 147 282 controls from the FinnGen project. The combined analysis of the three studies identified an association between *DNAJB4*-rs34517439-A and an increased risk of developing MM (OR = 1.22, 95%CI 1.13-1.32, $P = 4.81 \times 10^{-7}$). rs34517439-A is associated with a modified expression of the *FUBP1* gene, which encodes a multifunctional DNA and RNA-binding protein that it was observed to influence the regulation of various genes involved in cell cycle regulation, among which various oncogenes and oncosuppressors. In conclusion, with a pleiotropic scan approach we identified *DNAJB4*-rs34517439 as a potentially novel MM risk locus.

KEYWORDS
genetic susceptibility, multiple myeloma, pleiotropy, pleiotropy scan, polymorphisms

What's new?

Genetic variants can have multiple, seemingly unrelated, effects. Often, these so-called "pleiotropic" variants play a role in cancer. Here, the authors set out to identify new pleiotropic variants involved in multiple myeloma (MM) risk. They analyzed 28,684 independent single nucleotide polymorphisms (SNPs) that had been identified in genome-wide association studies as having an effect on a human trait. This analysis revealed an association between increased MM risk and a variant called *DNAJB4*-rs34517439-A. That variant has been associated with changes in expression of a DNA- and RNA-binding protein that helps regulate cell cycle genes.

1 | INTRODUCTION

Multiple myeloma (MM) is an incurable hematological disease originating from plasma cells in the bone marrow.[1] MM is the third most common hematological tumor with a crude incidence rate of 6.8/100000 new cases per year in Europe[2] (https://gco.iarc.fr/today/home).

Several studies have investigated the genetic susceptibility of MM, using genome-wide association studies (GWAS)[3-10] or more targeted approaches.[8,11-15] Twenty-four risk loci have been found to be associated with MM risk through GWAS; however, these variants explain approximately only 16% of MM heritability.[3] Due to the large number of tests conducted in a GWAS, a stringent statistical significance threshold, $P < 5 \times 10^{-8}$, is employed. The associated increase in type II error rates means that many true associations may remain undetected and unreported.

One strategy to avoid this loss of information is to investigate pleiotropic variants. Pleiotropy is a genetic phenomenon consisting of a single gene or allelic variant affecting multiple, often unrelated traits.[16] GWASs have identified several loci associated with cancer and noncancer phenotypes. Recently, it was estimated that half of all the SNPs showing an association with a P_{value} threshold of 10^{-8} are pleiotropic, and 12.34% show associations with 10 or more phenotypes.[17]

Pleiotropy is frequent across cancers,[18-20] with a portion of genome-wide associated loci shared with other traits and several regions harboring single nucleotide polymorphisms (SNP) associated with multiple cancer types. For example, the *TERT* and 8q24 loci are associated with risk of multiple cancer types, including MM.[12,21-25] This feature is particularly evident in MM since we observed, using GWAS Catalog, that 17 of the 24 associated polymorphisms (~71%) are pleiotropic or in high linkage disequilibrium (LD) ($r^2 > 0.8$) with SNPs associated with other traits (https://www.ebi.ac.uk/gwas/), including telomere length,[26] BMI[27] and risk of various cancers, such as myeloproliferative neoplasms[28] and pancreatic cancer.[25] Therefore, the aim of this study was to test the impact of all the SNPs associated at genome-wide significant level with any human trait on risk of MM. This strategy has been employed for several cancer sites[18-20,29-31] but never for MM.

2 | MATERIALS AND METHODS

2.1 | Study populations

The study employed a two-step approach, consisting of a discovery phase in which the International Lymphoma Epidemiology Consortium (InterLymph) GWAS data were analyzed, and a validation phase performed using both summary statistics from the FinnGen project MM GWAS (https://www.finngen.fi/en/access_results) and cases and controls of the International Multiple Myeloma rESEarch (IMMEnSE) consortium.

InterLymph has been described elsewhere.[8] Briefly, InterLymph generated GWAS data, using multiple platforms, for 2434 MM cases and 3446 controls of European ancestry from the United States of America, Canada and Australia genotyped using the Affymetrix 6.0 and Illumina (610 Quad, Human660W-quad Beadchip, Omni5, OmniExpress Beadchip, Oncoarray) platforms.[8] Imputation was performed using the Haplotype Reference Consortium as reference panel, and the Michigan imputation server (https://imputationserver.sph.umich.edu/).[32] After imputation, each site was filtered to include only imputed variants with information score > 0.6 and further quality control checks were implemented (genotype rate > 95%, minor allele frequencies >0.01, and Hardy-Weinberg equilibrium [HWE] $P > 10^{-5}$ in controls). Finally, the data were pooled and final quality controls were performed on the pooled GWAS set, including checks for missingness, duplicates, sex mismatch, abnormal heterozygosity, cryptic relatedness, population outliers (through principal component [PC] analysis), and genomic inflation. After applying quality control measures to the imputed data, 5 864 648 SNPs remained for analysis.[8]

The FinnGen project[33] is a cohort of 176 899 Finnish individuals genotyped with Illumina and Affymetrix platforms (https://www.finngen.fi). Subjects with sex discrepancies, call rate < 95%, excess heterozygosity (+-4SD) and non-Finnish ancestry were removed. SNPs with call rate < 98%, deviation from HWE ($P < 10^{-6}$) and low minor allele count < 3 were removed. Subsequently, imputation was conducted using the SISu v3 reference panel with Beagle 4.1 (version 08Jun17.d8b). Imputed variants with information score < 0.7 were removed.[33] A GWAS testing association of 16 962 023 SNPs with 2444 endpoints (among which MM risk) was performed. Analyses were adjusted for age, sex, 10 PCs and for genotyping batch. A total of 418 MM cases and 147 282 controls who were cancer-free at recruitment were evaluated in this study. Summary statistics of genome-wide associations between SNPs and MM risk were obtained from https://r4.finngen.fi on April 22, 2021.

Information on IMMEnSE is reported in detail elsewhere.[34] Briefly, it consists of a multicentric study involving seven countries (Denmark, France, Hungary, Israel, Italy, Poland, Portugal and Spain). Patients had a confirmed diagnosis of MM in compliance with International Myeloma Working Group (IMWG) criteria,[35] while controls were from the same center or geographic region as the MM patients, including individuals from the general population, blood donors or patients hospitalized for diseases other than cancer. For each participant sex, age (at diagnosis for cases, at recruitment for controls) and country of origin were collected. For this study, 1955 MM cases and 1549 controls were included. Considering all of the studies, the total number of subjects analyzed was 4807 MM cases and 152 277 controls (Table 1).

2.2 | SNP identification and selection

We downloaded a list of all the SNPs associated with at least one human phenotype at $P < 5 \times 10^{-8}$ from the GWAS Catalog web site (https://www.ebi.ac.uk/gwas/). The list was downloaded in January 2020 and all SNPs without "rs" identifier (n = 1667) were excluded, leaving 66 296 unique SNPs for analysis.

2.3 | Sample preparation, genotyping and quality control in IMMEnSE

DNA samples from IMMEnSE consortium participants were extracted from whole blood and genotyped using TaqMan (Thermo Fisher Applied Biosystems, Waltham MA, USA) assay technology, according to the manufacturer's recommendations. Genotyping was conducted in 384 well plates, including n = 203 duplicate samples (6%) for quality control purpose (concordance rate was higher than 98%). The distribution of cases and controls was unknown to the operator performing the genotyping. The fluorescent emission of the genotyping assay was detected by a QuantStudio 5 Real-Time PCR system (Thermofisher) and the genotyping calls were made with QuantStudio software (Thermofisher). The average call rate of the SNPs was 86.30%. Subjects with a call rate < 70% (331 cases and 216 controls) were excluded from further analysis. Pearson's chi-square test (χ^2) was performed to assess if genotype frequencies were in HWE. The analysis, restricted to controls, was performed overall and separately for each country. All SNPs were in HWE except for rs10187103

TABLE 1 Study populations

	InterLymph	IMMEnSE	FinnGen	Total
Diagnosis				
MM cases	2434	1955	418	4807
Controls	3446	1549	147 282	152 277
Total	5880	3504	147 700	157 084
Median age (25%-75%)[a]				
MM cases	61 (26-90)	61 (54-67)	–	–
Controls		51 (43-61)	–	–
Sex				
Male	61%	52%	–	–
Female	39%	48%	–	–

Note: Details on age and sex distribution of FinnGen individuals are not available.

[a]Median age values of MM cases and controls with 25th and 75th percentile.

($P = 4.99 \times 10^{-8}$), rs1063348 ($P = 9.27 \times 10^{-4}$) and rs465530 ($P = 8.91 \times 10^{-6}$) in the controls from Denmark and therefore we excluded the genotypes of Danish subjects for these three SNPs from further analysis.

2.4 | Statistical analysis

Unconditional logistic regression analysis was performed using InterLymph data to assess the association between the pleiotropic SNPs and risk of developing MM, reporting odds ratios (ORs) with 95% confidence intervals (CIs). Analyses were adjusted for age at diagnosis/recruitment, sex, study site and for the first five PCs. LD pruning ($r^2 = 0.8$) was applied to eliminate SNPs representing the same locus or variants in LD with known MM susceptibility regions. All independent variants that showed an association at $P < 10^{-4}$ (arbitrary threshold) were then analyzed in the validation populations. Summary statistics were used for FinnGen, whereas for IMMEnSE, additive and co-dominant unconditional logistic regression analysis was performed, adjusted for age at diagnosis/recruitment, sex and country of origin.

Finally, random-effects meta-analysis was performed with the PLINK software to combine results of the two phases. Heterogeneity was quantified with the I^2 metric and evaluated with the Cochran's Q statistic test for each SNP. To account for multiple testing, we considered LD ($r^2 > 0.8$) among the SNPs used in the discovery phase to obtain a list of independent variants (n = 28 684). The threshold for statistical significance was therefore set to $P = 0.05/28684 = 1.74 \times 10^{-6}$ using Bonferroni's correction.

2.4.1 | Annotation of functional effect of the SNPs

Several bioinformatic tools were used to evaluate possible functional features of the SNPs associated with MM. The occurrence of regulatory motif alterations was investigated through HaploReg (https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php)[36] and RegulomeDB (https://regulomedb.org/regulome-search).[37] The Genotype-Tissue Expression project (GTEx) portal[38] (https://www.gtexportal.org/home/) was used to assess if selected SNPs are expression quantitative trait loci (eQTLs), that is, their alleles are associated with differential expression of genes in cis.

3 | RESULTS

3.1 | Discovery

Among the 66 296 variants selected for testing, 6438 were not genotyped or imputed in the InterLymph MM GWAS and therefore could not be analyzed. Of the 59 858 remaining SNPs, 2997 SNPs were associated with risk of developing MM at $P < 0.05$. Within that group, 56 SNPs with $P < 10^{-4}$ were selected for the validation studies (Table S1). Forty SNPs already known to be associated with the risk of

TABLE 2 Combined analysis using InterLymph, IMMEnSE and FinnGen results

SNP	Region	(Alleles) M/m[a]	InterLymph		IMMEnSE		FinnGen		Combined analysis				
			OR (95%CI)	P_{value}	OR (95%CI)	P_{value}	OR (95%CI)	P_{value}	OR (95%CI)	P_{value}	I^{2b}	Q^b	Q-P_{value}
rs34517439	1p31.1	C/A	1.30 (1.15-1.48)	4.70×10^{-5}	1.09 (0.89-1.34)	.402	1.21 (1.09-1.34)	.071	**1.22 (1.13–1.32)**	**4.81×10^{-7}**	**7.30%**	**2.25**	**.325**
rs6674512	1p22.3	G/A	1.40 (1.21-1.62)	8.71×10^{-6}	1.10 (0.88-1.36)	.402	1.04 (0.91-1.20)	.765	1.17 (0.96-1.43)	.115	77.50%	9.74	.008
rs10187103	2q24.3	C/T	0.84 (0.77-0.91)	6.06×10^{-5}	0.99 (0.88–1.12)	.915	0.99 (0.90-1.09)	.924	0.95 (0.83–1.08)	.397	81.10%	10.38	.006
rs1022206	3q13.13	C/T	1.18 (1.09-1.28)	8.61×10^{-5}	1.11 (1.00–1.24)	.061	0.99 (0.92-1.07)	.923	1.09 (0.97-1.22)	.136	80.00%	10.25	.006
rs4143832	5q31.1	G/T	1.24 (1.12-1.37)	4.66×10^{-5}	0.95 (0.83–1.08)	.439	0.91 (0.84-0.99)	.275	1.02 (0.84-1.25)	.819	91.30%	22.96	1.03×10^{-5}
rs537930	5q31.1	G/T	0.83 (0.76-0.91)	5.09×10^{-5}	0.97 (0.86–1.09)	.571	0.96 (0.88-1.04)	.596	0.92 (0.83-1.01)	.088	70.10%	6.51	3.80×10^{-2}
rs1063348	6p21.32	G/A	1.19 (1.09-1.30)	7.44×10^{-5}	0.95 (0.85–1.07)	.398	NA[c]	NA	1.07 (0.87-1.32)	.534	89.6%	14.49	.001
rs13252276	8p23.1	C/T	0.77 (0.68-0.87)	3.10×10^{-5}	1.01 (0.91–1.12)	.870	1.03 (0.96-1.11)	.711	0.93 (0.79–1.10)	.415	88.00%	17.50	2.00×10^{-4}
rs465530	12q14.3	G/T	1.18 (1.09–1.28)	4.94×10^{-5}	1.01 (0.89–1.13)	.926	0.95 (0.88-1.02)	.458	1.03 (0.90-1.19)	.657	87.80%	17.06	2.00×10^{-4}
rs8132680	21q22.12	T/C	1.21 (1.11-1.33)	2.96×10^{-5}	0.99 (0.88–1.12)	.924	1.04 (0.96-1.13)	.646	1.08 (0.96-1.21)	.197	77.40%	8.06	.018

[a]M = the most common allele in controls; m = the less common allele found in the control population has been used as reference and the other allele was defined as the exposure factor.
[b]Measures of heterogeneity between the combined analysis components.
[c]This polymorphism is not present in FinnGen, and therefore the combined analysis refers only to InterLymph and IMMEnSE populations.

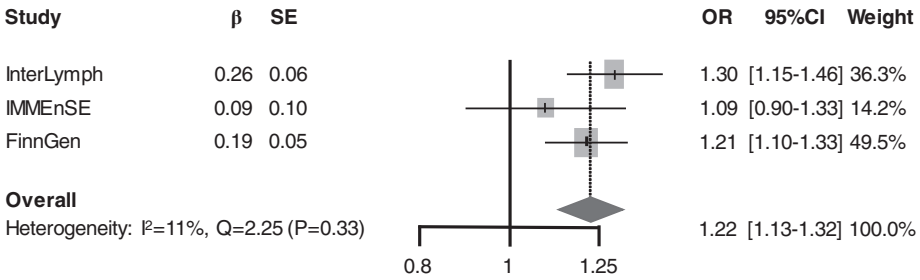

FIGURE 1 Forest plot of the association between *DNAJB4*-rs34517439-A and MM risk

developing MM or in LD ($r^2 \geq 0.8$) with these known SNPs were further excluded. Residual LD ($r^2 > 0.4$) between the remaining 16 SNPs was evaluated, resulting in the exclusion of an additional six SNPs. The remaining 10 SNPs were selected for evaluation in FinnGen and IMMEnSE. Figure S1 shows a flowchart of the process.

3.2 | Replication and combined analysis

We performed the combined analysis using exclusively the allelic model, since this was available for the FinnGen dataset. We found a statistically significant association (using $P < 1.74 \times 10^{-6}$) between the A allele of *DNAJB4*-rs34517439 and an increased risk of developing MM ($OR_{combined\text{-}analysis} = 1.22$, 95%CI 1.13-1.32, $P = 4.81 \times 10^{-7}$) with very low study heterogeneity ($I^2 = 7.30\%$, Q = 2.25) (Table 2 and Figure 1). However, we observed some degree of heterogeneity in the combined analysis for the SNPs that did not show any statistically significant results, that could be due to the different direction of the association (Table 2) or to differences of the allelic frequencies between Finnish and non-Finnish Europeans (Table S2).

Though not statistically significant, we observed an association between *LOC105374037*-rs1022206 and risk of developing MM in the IMMEnSE population when comparing homozygotes for the rare allele (T/T) to homozygotes for the common allele (C/C) ($OR_{homozygptes} = 1.29$, 95%CI 1.03-1.62, $P = .028$) and in the recessive model ($OR_{recessive} = 1.28$, 95%CI 1.04-1.57, $P = .019$) in the IMMEnSE population (Table 3).

3.3 | Functional effect of the SNPs

RegulomeDB assigned a rank of 5 to both SNPs, implying a possible influence on a transcription factor binding site. In addition, GTEx and HaploReg indicated that *DNAJB4*-rs34517439 is an eQTL affecting far upstream element binding protein 1 gene (*FUBP1*) expression in several human tissues. This included a statistically significant correlation between the A allele of this SNP and a reduced expression of *FUBP1* in cultured fibroblasts ($P = 1.10 \times 10^{-4}$). GTEx also indicated an association between the T allele of *LOC105374037*-rs1022206 and increased expression of the nectin cell adhesion molecule 3 (*NECTIN3*) gene in cultured fibroblasts ($P = 2.10 \times 10^{-20}$).

4 | DISCUSSION

Pleiotropic variants are commonly found associated with complex diseases, like cancer. To investigate the possible association between common SNPs and the risk of developing MM, we analyzed all the genetic variants associated with any human trait in 4807 patients and 152 277 controls. We observed a statistically significant association ($P < 1.74 \times 10^{-6}$) between *DNAJB4*-rs34517439-A and risk of developing MM. This SNP is pleiotropic since it is associated with numerous traits such as cutaneous melanoma, diastolic blood pressure, fat-free mass, hair color, hand grip strength, height, lung cancer, noncognitive aspects of educational attainment, psoriasis, serum alkaline phosphatase levels, smoking initiation and BMI, with a wide range of effect sizes and P_{values} as reported in Table S3. The association between this SNP and BMI is of particular interest since BMI is also associated with MM risk.[39] The A allele that was associated with increased MM risk in our study has previously been associated with increased risk of lung cancer.[40] This pleiotropic effect, especially in cancers, may be attributed to the LD of rs34517439 with the variants inside or in the proximity of DnaJ heat shock protein family (Hsp40) member B4 (*DNAJB4*), situated on chromosome 1. This protein is a molecular chaperone specifically recognizing wild-type from mutant E-cadherin protein. Since E-cadherin is an important element in the suppression of tumor invasion, the function of DNAJB4 represents a significant pleiotropic mechanism of tumoral invasion inhibition.[41,42] *DNAJB4* also acts as a co-chaperone, forming a complex with Hsp70 which participates in protein folding.[43] The expression and activity of heat shock proteins increases during cellular stress,[44] and DNAJB4 has been observed to reduce tumor metastasis and progression in several cancer types, including lung carcinoma and melanoma.[45,46] This mechanism could also be relevant to MM. In bioinformatic analysis, GTEx showed an association between *DNAJB4*-rs34517439-A and a reduced expression of the *FUBP1* gene in various tissues, including lung, skin (sun exposed and not sun exposed), adipose tissue, the arteries and the heart. The altered expression of the gene in these tissues mirrors the associations found in the GWASs (eg, altered expression in lung for lung cancer, or skin for melanoma). *FUBP1* encodes a multifunctional DNA and RNA-binding protein involved in cell cycle regulation and self-renewal of hematopoietic stem cells (HSCs).[47] *FUBP1* acts both as oncogene and as tumor suppressor, and its activity is tissue-dependent.[48] In various tumor types FUBP1 promotes the overexpression of the *MYC* oncogene,[48] whereas *FUBP1* silencing does not influence the expression of *MYC* in normal

TABLE 3 Associations between MM risk and the top 10 SNPs in IMMEnSE population

			Allelic model[b]			Codominant model[c]						Dominant model[d]			Recessive model[e]		
SNP	Region	(Alleles) M/m[a]	OR[f]	95% CI	P_{value}	OR het[f]	95% CI	P^{value}	OR hom[f]	95% CI	P^{value}	OR[f]	95% CI	P^{value}	OR[f]	95% CI	P^{value}
rs34517439	1p31.1	C/A	1.09	0.89-1.34	.402	1.10	0.88-1.38	.395	1.09	0.44-2.66	.853	1.10	0.88-1.37	.387	1.07	0.44-2.62	.878
rs6674512	1p22.3	G/A	1.10	0.88-1.36	.402	1.14	0.90-1.44	.267	0.74	0.24-2.28	.600	1.12	0.89-1.41	.320	0.73	0.24-2.24	.580
rs10187103	2q24.3	C/T	0.99	0.88-1.12	.915	0.90	0.75-1.07	.215	1.09	0.83-1.43	.540	0.93	0.79-1.10	.407	1.15	0.89-1.49	.287
rs1022206	3q13.13	C/T	1.11	1.00-1.24	.061	1.01	0.86-1.20	.889	**1.29**	**1.03–1.62**	**.028**	1.08	0.92-1.26	.348	**1.28**	**1.04–1.57**	**.019**
rs4143832	5q31.1	G/T	0.95	0.83-1.08	.439	0.94	0.79–1.10	.428	0.94	0.66-1.32	.709	0.94	0.80-1.10	.406	0.96	0.68-1.35	.807
rs537930	5q31.1	G/T	0.97	0.86-1.09	.571	0.86	0.73-1.00	.061	1.14	0.84-1.55	.385	0.90	0.77-1.05	.168	1.22	0.90-1.64	.198
rs1063348	6p21.32	G/A	0.95	0.85-1.07	.398	0.92	0.77-1.10	.368	0.92	0.72-1.17	.487	0.92	0.77-1.09	.336	0.96	0.77-1.20	.731
rs13252276	8p23.1	C/T	1.01	0.91-1.12	.870	1.02	0.86-1.21	.825	1.01	0.82-1.25	.895	1.02	0.87-1.19	.826	1.00	0.83-1.21	.973
rs465530	12q14.3	G/T	1.01	0.89-1.13	.926	0.83	0.67-1.01	.064	1.03	0.81-1.31	.820	0.89	0.73-1.07	.220	1.16	0.95-1.42	.147
rs8132680	21q22.12	T/C	0.99	0.88–1.12	.924	1.01	0.86-1.19	.869	0.96	0.72-1.28	.785	1.00	0.86-1.17	.960	0.96	0.72-1.27	.751

Note: In bold it is reported the statistically significant association.

[a]M = the more common allele in controls; m = the less common allele in controls. The more common allele found in the control population has been used as the reference and the other allele was defined as the exposure factor.

[b]Also termed quantitative additive (m vs M).

[c]Mm vs MM (het) and mm vs MM (hom).

[d]Mm + mm vs MM.

[e]mm vs Mm + MM.

[f]All the analyses were adjusted for age at diagnosis/recruitment, sex and country of origin. Results in bold show associations with $P < .05$.

fibroblasts, prostate and bladder cancer cells.[48] Moreover, FUBP1 is involved in the upregulation and downregulation of the cell cycle inhibitor *p21*.[49,50] The pleiotropic role of rs34517439 could be explained by its involvement in the altered expression of *FUBP1* in different tumors, that determines their development, by interacting with specific factors in each tissue. Specifically for hematopoietic lineages, the cooperation of FUBP1 with RUNX1 in promoting the expression of the *c-KIT* oncogene was reported.[51] In summary, a possible explanation for the *DNAJB4*-rs34517439 association with increased risk of developing MM could be that the A allele modifies the expression of *DNAJB4* leading to an incorrect folding of FUBP1. In turn, this mechanism could be responsible for the disruption of the HSCs homeostasis equilibrium, thus increasing the risk of developing MM. Although we lack an experimental validation of the proposed mechanism in MM cell lines, our hypothesis relies on the experimental and genomic data collected from GTEx, which are broadly used in the scientific community.

In addition, the T allele of *LOC105374037*-rs1022206 in homozygosity showed an increased risk of developing MM in the IMMEnSE population. LOC105374037 is an uncharacterized long noncoding RNA (lncRNA) for which possible functional roles are unknown. Interestingly, *LOC105374037*-rs1022206 is an eQTL for *NECTIN3* gene expression in cultured fibroblasts. Fibroblasts are the cells with the highest expression of NECTIN3 in humans.[52] Cancer-associated fibroblasts (CAFs) are one of the known cellular elements participating in MM tumoral microenvironment in the BM.[53] In fact, they show a bidirectional loop with myeloma cells providing chemotaxis, adhesion, apoptosis resistance and proliferation through cytokines, growth factors, angiogenetic factors and cell-cell contact.[54] However, considering the lack of data in the literature on the topic it is not clear how the increased expression of NECTIN3 mediated by LOC105374037-rs1022206 might be involved in MM risk. Furthermore, Nectin-3 is involved in cell survival through PDGF receptor signal and in inhibition of cell movement.[52,55] The SNP *LOC105374037*-rs1022206 is in LD with variants associated in with various traits: balding type 1, smoking initiation, self-reported math ability, feeling hurt and neuroticism. Although these traits apparently do not show a shared mechanism or pathway, the *LOC105374037* lncRNA could be the common element explaining the pleiotropic effect of the SNP on different cellular functions.

A possible limitation of this study could be that only the allelic model was available for all the datasets and therefore it was the only one assessed in the final combined analysis using InterLymph, IMMEnSE and FinnGen populations. In fact, we were not able to confirm the promising association detected in the IMMEnSE population for *LOC105374037*-rs1022206-T, that needs to be further investigated. Another possible limitation is the ethnic origin of the populations used in the replication phase, due to the known genetic differences between the Finnish and non-Finnish Europeans.[56,57] Specifically, for the 10 SNPs considered in the replication phase of our study, the average difference in allelic frequency is 6%, and for the two SNPs that show significant association this difference is lower than 5%. The heterogeneity value obtained in the combined analysis for *DNAJB4*-rs34517439 is very low ($I^2 = 7.30\%$) and not significant, and therefore, it is highly unlikely that differences in the allelic frequencies might have affected the results. However, we have observed some degrees of heterogeneity in the other SNPs. An additional limitation in our study is that the number of SNPs chosen for replication was limited. We chose a threshold of $P < 10^{-4}$ as a good compromise allowing us to select a suitably small number of variants to genotype in IMMEnSE and to maximize chances of finding significant results in the overall analysis. It would have not been worth choosing SNPs showing a weaker association in InterLymph, because we would not have sufficient statistical power to find genome-wide significant associations in the combined results.

Finally, considering that the P_{value} we observed, although statistically significant considering the Bonferroni correction for multiple testing, does not reach genome-wide significance, further studies are warranted to establish this SNP as a new MM risk locus.

In conclusion, our results suggest the involvement of a pleiotropic region on chromosome 1 in MM development and highlight pleiotropy as an approach to uncover additional risk variants in cancer susceptibility.

AUTHOR CONTRIBUTIONS

Daniele Campa conceived and designed the study. Marco Dicanio and Matteo Giaccherini performed the lab work, Marco Dicanio, Matteo Giaccherini and Alyssa Clay-Gilmour analyzed the data. All the authors contributed with the interpretation of the data. Marco Dicanio, Matteo Giaccherini and Daniele Campa wrote the first draft of the manuscript and all authors contributed to the writing and approve of the final version of the manuscript. The work reported in the paper has been performed by the authors, unless clearly specified in the text.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The primary data for this work will be made available to researchers who submit a reasonable request to the corresponding author, conditional to approval by the centers participating in this study. Data will be stripped from all information allowing identification of study participants.

ETHICS STATEMENT

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 For InterLymph, contributing studies were approved by local ethics review committees. Summary statistics were used from the FinnGen study, that was approved by the ethical Review Board of the Hospital District of Helsinki and Uusimaa. FinnGen participants provided written, informed consent.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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