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RESEARCH ARTICLE

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ULK4 and *CDKN2A* polymorphisms influence the risk of developing monoclonal gammopathy of undetermined significance

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Abbreviations: 500FG, 500 Functional Genomics; AL, amyloid light-chain amyloidosis; ARF, Alternative Reading Frame; BBMRI-NL, Biobanking and Biomolecular Resources Research Infrastructure—Netherlands; BMBF, German Federal Ministry of Education and Research (Bundesministerium für Bildung und Forschung); cQTL, cytokine quantitative trait locus; CRAB, hypercalcemia, renal insufficiency, anemia, bone lesions; DKFZ, German Cancer Research Center (Deutsches Krebsforschungszentrum); eQTL, expression quantitative trait locus; ERDF, European Regional Development Fund; GWAS, genome-wide association study; HFGP, Human Functional Genomics Project; HWE, Hardy–Weinberg equilibrium; IBERICAT, Iberian Multiple Myeloma and MGUS Study; ICD, International Classification of Diseases; IMMEnSE, International Multiple Myeloma rESEarch Consortium; IRB, Institutional Review Board; LD, linkage disequilibrium; LOCO, leave-one-chromosome-out; MAC, minor allele count; MAF, minor allele frequency; MDE, myeloma-defining events; MDM, monocyte-derived macrophages; MGUS, monoclonal gammopathy of undetermined significance; MM, multiple myeloma; OR, odds ratio; PBMC, peripheral blood mononuclear cell; QC, quality control; SNP, single nucleotide polymorphism; SPA, saddle-point approximation; UKBB, UK Biobank; WB, whole blood.

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Abstract

Monoclonal gammopathy of undetermined significance (MGUS) is a necessary precursor condition to multiple myeloma (MM). Given the role of autophagy in modulating MM risk, we investigated whether genetic variation in autophagy-related genes influences susceptibility to MGUS. We analyzed the association of 34,042 common autophagy-related single nucleotide polymorphisms (SNPs) with MGUS across six independent cohorts, five from Europe and one from North America, comprising 2317 MGUS cases and 282,358 controls. We also assessed their impact on immune parameters, including absolute counts of 91 blood-derived immune cell subsets and 103 circulating immunological proteins. Meta-analysis revealed a genome-wide significant association between the $ULK4_{rs6599175C}$ allele and increased MGUS risk ($p = 3.35 \times 10^{-8}$). Carriers of this allele showed reduced counts of memory B cell subsets (IgM+CD38+CD27+ and IgD+IgM+CD27+; $p = .0038$ and $p = .0056$, respectively) and natural effector B cells (CD24+CD38+IgD+IgM+ cells; $p = .0060$). Although these associations were not statistically significant after multiple testing correction, they suggest a role of *ULK4* in early B-cell differentiation. Additionally, the $CDKN2A_{rs2811710}$ variant showed a suggestive association with MGUS risk ($p = 2.17 \times 10^{-4}$), affecting transcription factor binding involved in B-cell proliferation and differentiation, although it lacked association with immune markers. In conclusion, we confirm a genome-wide significant association of the *ULK4* locus and MGUS risk, supporting its role in early B-cell differentiation, and identify *CDKN2A* as a candidate susceptibility locus warranting further investigation.

KEYWORDS
autophagy, blood-derived cell populations, genetic variants, MGUS, susceptibility

What's New?

Monoclonal gammopathy of undetermined significance (MGUS) is an asymptomatic precursor to multiple myeloma, sharing substantial genetic features with overt malignancy. Given evidence implicating autophagy in myeloma risk, this study examined whether genetic variations in autophagy-related genes influence MGUS susceptibility. Using large-scale genome-wide association meta-analysis integrated with immunogenomic data, the authors identified an association between MGUS risk and *ULK4*, which encodes a serine/threonine kinase involved in various biological processes. A variant of *CDKN2A* was additionally identified as a candidate susceptibility locus. The findings highlight genetic factors that potentially contribute to autophagy pathways and early B-cell differentiation in plasma cell disorders.

1 | INTRODUCTION

Monoclonal gammopathy of undetermined significance (MGUS) is an asymptomatic, pre-malignant plasma cell disorder defined by the presence of fewer than 10% clonal plasma cells in the bone marrow, serum monoclonal (M-) protein levels below 3 g/dL, and the absence of myeloma-defining end-organ damage such as hypercalcemia, renal insufficiency, anemia, or lytic bone lesions.[1–3] As patients with MGUS do not meet CRAB criteria or exhibit myeloma-defining events (MDE), routine surveillance is essential to detect disease evolution. MGUS is relatively common in adults over the age of 50, with a prevalence of 3%–4% and carries an annual risk of progression to multiple myeloma (MM) of approximately 1%.[2,4]

MGUS is a precursor to several malignant or systemic conditions, including MM, light chain amyloidosis, lymphoplasmacytic lymphoma, and Waldenström macroglobulinemia.[5–7] Risk stratification models divide MGUS into low- and high-risk categories based on clinical and biochemical parameters, informing follow-up schedules: patients with high-risk profiles require annual evaluation, often for life or until progression.[8] Understanding susceptibility to

MGUS requires an integrated view of genetic predisposition, environmental exposures, immune function, and demographic factors. Established non-modifiable risk factors include older age, male sex, African ancestry, and family history of monoclonal gammopathies.[9] Prevalence estimates vary geographically from 0.24% to 9%, reflecting the interplay of genetic background, environmental influences, and differences in healthcare access.[10] Modifiable factors such as chronic immune stimulation (due to infections or autoimmune disorders) and occupational exposure to substances like asbestos, pesticides, or industrial chemicals may also contribute to disease onset.[10,11]

At the genetic level, MGUS shares substantial overlap with MM.[12,13] Genome-wide association studies (GWAS) have identified some single-nucleotide polymorphisms (SNPs) associated with MGUS risk.[14–17] Polygenic risk scores built on MM-associated loci show predictive utility for MGUS as well, supporting a shared polygenic architecture.[13,15] The estimated genetic correlation between MGUS and MM is approximately 55%, underscoring the contribution of common heritable factors.[13] Despite these advances, the biological mechanisms linking genetic variation to disease onset remain incompletely understood.[18] Recent studies suggest that dysregulation of cellular stress responses, including endoplasmic reticulum stress and oxidative stress, may contribute to plasma cell transformation.[19–21] Additional processes implicated in this cellular reprogramming include heat shock protein chaperoning,[22] aggresome formation,[23] and autophagy.[24] Among these, autophagy has drawn increasing attention for its complex roles in immune regulation and cell fate. Autophagy influences B-cell development and proliferation,[25] governs plasma cell survival,[26,27] and can either promote or suppress tumorigenesis depending on context.[28–30]

To test this hypothesis, we investigated 40,089 common SNPs within 234 autophagy-related genes in four European GWAS cohorts (UK Biobank, and German, Czech, and Swedish populations). The top associations were replicated in two independent datasets (Mayo/MD Anderson and IMMEnSE/IBERICAT).[16,31] We then assessed the functional consequences of prioritized SNPs using a systems-level immunogenomic approach in a large healthy donor cohort (500 Functional Genomics; 500FG) from the Human Functional Genomics Project (HFGP). Specifically, we evaluated associations between these SNPs and a broad range of immune traits, including circulating concentrations of 103 plasma and serum inflammatory proteins and counts of 91 immune cell subsets in whole blood (WB), PBMCs, and monocyte-derived macrophages (MDM). This integrative framework has previously yielded key insights into the pathogenesis of B-cell malignancies,[32–35] solid tumors,[36–38] immune-related disorders[39] and infectious conditions.[40]

2 | MATERIALS AND METHODS

A workflow diagram of the study is included in Figure 1.

2.1 | Study populations (discovery cohorts)

The discovery population consisted of 243 MGUS patients and 1285 healthy controls from Germany, 288 MGUS cases and 600 controls (Czech), 461 MGUS patients and 1025 controls (Swedish) and 107 MGUS cases and 277,496 controls (UK Biobank). Detailed methodological descriptions for the German, Czech, and Swedish GWAS have been published previously.[14] For these European cohorts, genotype data underwent rigorous, site-specific quality control prior to imputation using either IMPUTE2 v2.3 or the Michigan imputation server, based on the Haplotype Reference Consortium panel,[41] respectively. After imputation, each site filtered the data to include only high-quality imputed variants (information score >0.8), and further quality-control checks were implemented including checks for missingness, duplicates, abnormal heterozygosity, cryptic relatedness, population outliers (evaluated by principal components analyses using Eigenstrat software), and genomic inflation ($\lambda = 1.00$).[13,42]

The UK Biobank, a large-scale biomedical resource comprising genetic and health information from approximately 500,000 individuals aged 40 to 69 years across the United Kingdom, was analyzed independently.[43] MGUS cases were identified through cancer registry data using ICD-10 code D47.2 and ICD-O-3 code 9765 (Data-Fields 40011 and 40006). Controls were selected by excluding participants with any history of cancer, as recorded in cancer registries, hospital episode statistics, or self-reported data. Further exclusions concerned individuals of non–white British ancestry, sex chromosome aneuploidy, discordance between genetic and reported sex, relatedness, and other standard sample-level quality control exclusions recommended by the UK Biobank for genetic association analyses.[43]

After quality control, a total of 107 MGUS cases and 277,496 controls remained for analysis. GWAS was conducted using REGENIE v3.2,[44] following a two-step approach. In brief, step 1 involved whole-genome ridge regression using non-imputed genotype data filtered for high quality (MAF >1%, MAC >5, genotyping rate >99%, HWE $p > 10^{-8}$, and <1% missingness), applying a leave-one-chromosome-out (LOCO) scheme. Step 2 performed variant-level association testing using logistic ridge regression, incorporating saddle-point approximation (SPA) corrections for variants with score test p-values <.01. Standard errors were derived from the effect estimates and likelihood ratio test p-values. Both steps included covariates: age (at diagnosis for cases, at recruitment for controls), sex, genotyping array, and the first 10 genetic principal components. The main demographic characteristics of discovery populations, including age, sex distribution and ancestry, are summarized in Table 1.

2.2 | Single nucleotide polymorphism selection and meta-analysis of the discovery cohorts

A total of 234 autophagy-related genes were selected based on their presence in the autophagy database (http://autophagy.lu/index.html; Table S1). Estimates of association with MGUS risk for all genotyped or imputed SNPs within or near these genes (5 kb up-stream and 3 kb

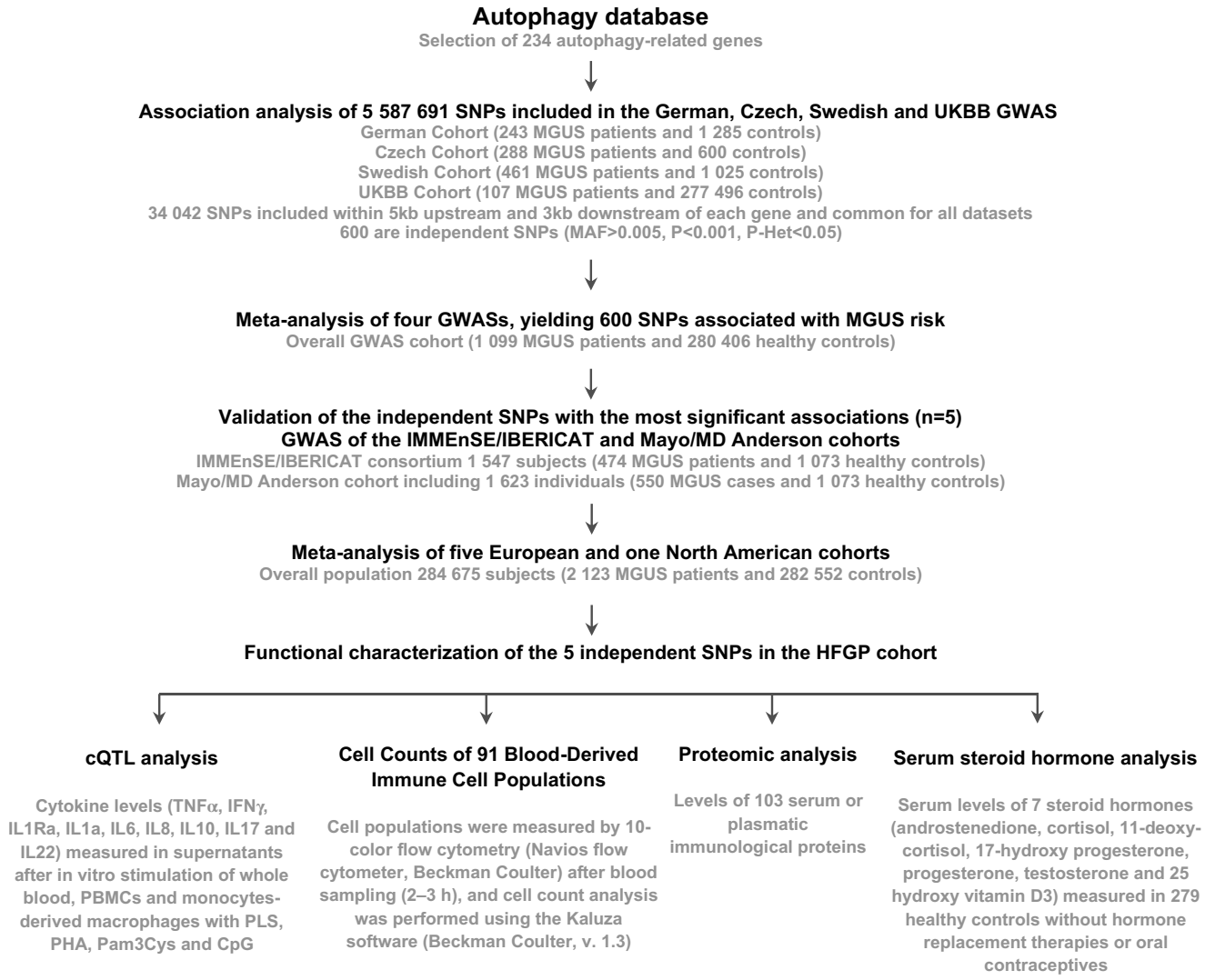

FIGURE 1 Flow diagram of the study.

downstream) were extracted from the four GWAS datasets and meta-analyzed using R studio v4.3.0[45] and METAL.[46] The I^2 statistic was used to assess statistical heterogeneity between the studies, and the pooled odds ratio (OR) was computed using the fixed-effect model for SNPs showing no significant heterogeneity. We obtained 40,089 SNPs, and, among them, 34,042 SNPs were present in all datasets. After the meta-analysis of the four GWAS cohorts, we selected for replication independent autophagy-related SNPs associated with MGUS risk ($n = 1303$). After excluding SNPs that showed a MAF <0.005 and a p-value≥.001 in the association analysis and a heterogeneity $p \leq .05$, we ended up with 600 SNPs that included 5 independent associations signals that were selected for further replication (Table S2). Pairwise linkage disequilibrium (LD) between SNPs was assessed using the LDlinkR package (v1.1.2) interfacing the LDlink API (https://ldlink.nci.nih.gov/) with data from European populations of the 1000 Genomes Project. Independent SNPs were defined as those showing an LD $r^2 < 0.1$.

2.3 | Replication cohorts, genotyping and overall meta-analysis

The SNPs selected for replication in the previous step were then tested for association with MGUS risk in the Mayo Clinic/MD Anderson cohort (744 MGUS cases and 879 healthy controls) and the International Multiple Myeloma rESEarch (IMMEnSE) consortium and IBERICAT (474 MGUS cases and 1073 healthy controls).[16] The Mayo Clinic/MD Anderson cohort is a case–control study comprising individuals diagnosed with MGUS and unrelated healthy controls recruited at the Mayo Clinic and The University of Texas MD Anderson Cancer Center, with cases diagnosed between 2002 and 2014, with diagnosis established according to standard International Myeloma Working Group (IMWG) criteria.[1] IMMEnSE is a retrospective case–control study that includes individuals diagnosed with MGUS, smoldering multiple myeloma, and multiple myeloma. IBERICAT is an on-going cohort study including all newly diagnosed MGUS, multiple myeloma and smoldering myeloma in four Catalan hospitals since

TABLE 1 Demographic characteristics of MGUS cases and controls across study cohorts.

Discovery cohorts[a]	MGUS cases (*n*)	Controls (*n*)	Ethnicity	Age, cases (mean ± SD)	Age, controls (mean ± SD)	Male cases (%)	Female cases (%)	Male controls (%)	Female controls (%)
Germany	243	1285	White Europeans	62.0 ± 11.0	60.0 ± 8.0	47.0	53.0	59.0	41.0
Czech Republic	288	600	White Europeans	62.0 ± 12.0	45.0 ± 13.0	51.0	49.0	48.0	52.0
Sweden (NSHDS/UFO)	461	1015	White Europeans	59.0 ± NR	56.0 ± NR	46.0	54.0	14.4	85.6
Validation cohorts	**MGUS cases (*n*)**	**Controls (*n*)**	**Ethnicity**	**Age, cases (mean ± SD)**	**Age, controls (mean ± SD)**	**Male cases (%)**	**Female cases (%)**	**Male controls (%)**	**Female controls (%)**
Mayo Clinic/ MD Anderson	744	879	White Europeans	65.4 ± 10.8	61.2 ± 10.4	57.8	42.2	45.4	54.6
IMMEnSE/ IBERICAT	474	1073	White Europeans	63.8 ± 12.7	50.2 ± 12.9	52.1	47.9	50.8	49.2

Abbreviation: NR, not reported.

[a]Individual-level demographic data for the UK Biobank cohort are not available due to data access restrictions. Therefore, age and sex distributions are not shown for this population. Only summary-level information provided by UK Biobank was used for association analyses.

2020. Genotyping data of selected SNPs were extracted from GWAS data. Demographic characteristics of the validation cohorts are summarized in Table 1.

All SNPs with a MAF >0.1 showed genotype frequencies in the control population similar to those found in the 1000 Genomes database (data not shown) and were in HWE. Finally, in order to analyze the overall impact of selected variants on MGUS risk, we performed an overall meta-analysis of the six independent studies using METAL.[46] As before, the I^2 statistic was used to assess heterogeneity among the studies, and the pooled odds ratio (OR) was computed using the fixed-effect model for SNPs showing no significant heterogeneity. Multiple testing significance threshold was set to 3.84×10^{-5} (0.05/1303 independent SNPs).

2.4 | Association of the autophagy-related variants with host immune responses

The five autophagy-related SNPs showing the most significant associations with MGUS risk were tested for their association with cytokine expression quantitative trait loci (cQTL) data after in vitro stimulation experiments. We also analyzed whether the SNPs were associated with absolute numbers of 91 blood-derived cell populations and 103 serum and plasmatic immunological proteins quantified in the approximately 550 volunteers of the 500 Functional Genomics (500FG) cohort from the Human Functional Genomics Project (http://www.humanfunctionalgenomics.org/site/).

cQTL data included cytokine levels (IFNγ, IL1β, IL6, TNFα, IL17, and IL22) after the stimulation of peripheral blood mononuclear cells (PBMCs), monocyte-derived macrophages (MDM), or whole blood from 408 healthy subjects with LPS (1 or 100 ng/mL; Sigma Aldrich, St. Louis, MO, USA), PHA (10 μg/mL, Sigma, St. Louis, MO, USA), Pam3Cys (10 μg/mL, EMC microcollections, Tübingen, Germany), CpG (100 ng/mL, InvivoGen, San Diego, CA, USA), *Borrelia burgdorferi* (ATCC 35210), *Escherichia coli* (ATCC 25922), *and Porphyromonas gingivalis* (ATCC 33277) for 24 or 48 h at 37°C and 5% CO_2. After log transformation, linear regression analyses adjusted for age and sex were used to determine the association of the selected SNPs with cQTL data. Details on PBMC isolation, macrophage differentiation, and whole blood stimulation assays have been reported elsewhere.[47]

To examine the impact of autophagy SNPs on cell-level variation, 91 blood-derived cell populations were measured by 10-color flow cytometry (Navios flow cytometer, Beckman Coulter, Miami, FL, USA) after blood sampling (2–3 h), and cell count analysis was performed using Kaluza software (Beckman Coulter, v.1.3) (Table S3). To reduce inter-experimental noise and increase statistical power, cell count analysis was performed by calculating parental and grandparental percentages, which were defined as the percentage of a certain cell type within the subpopulation of the cells from which it was isolated.[47] Detailed laboratory protocols for cell isolation, reagents, gating, and flow cytometry analysis have been reported elsewhere.[48]

Serum and plasma samples from the HFGP study were also subjected to targeted proteomic analysis. Circulating proteins were measured using the commercial Olink® Inflammation panel (Olink, Sweden) that resulted in the measurement of 103 different biomarkers (Table S4). Protein levels were expressed on a $\log_2$-scale as normalized protein expression values and normalized using bridging samples to correct for batch variation.

All analyses were performed using R software (http://www.r-project.org/) through custom scripts that implemented linear regression models using the lm() function from the stats package. Gene expression data were log10-transformed, and models were adjusted for age and sex as covariates. To account for multiple comparisons, we used Bonferroni corrected significance thresholds of 3.33×10^{-4} (0.05/5 SNPs/6 cytokines/5 stimulus), 1.10×10^{-4} (0.05/5 SNPs/91 cell-derived populations), and 9.71×10^{-5} (0.05/5 SNPs/103 proteins) for the cQTL, cell variability, and proteomic analyses, respectively.

2.5 | In silico functional analysis

Haploreg (http://www.broadinstitute.org/mammals/haploreg/haploreg.php)[49] and ENCODE annotation data (https://genome.ucsc.edu/ENCODE)[50] were also used to predict the functional role of the most interesting autophagy SNPs. We also analyzed whether selected SNPs could be expression quantitative trait loci (eQTL) for different cell types and tissues using data from public eQTL browsers such as GTEx portal (https://gtexportal.org/home/),[51] Blood eQTL browser (https://genenetwork.nl/bloodeqtlbrowser/).[52]

3 | RESULTS

3.1 | Association of autophagy-related polymorphisms with the risk of developing MGUS

This study included four European populations, comprising a total of 1099 MGUS patients and 280,406 healthy controls from the German, Czech, Swedish, and UKBB GWAS datasets used as discovery cohorts.

In the discovery phase, the meta-analysis identified several autophagy-related variants associated with MGUS risk. The strongest association was observed for *ULK4*$_{rs6599175}$ ($p = 2.04 \times 10^{-6}$). Four additional SNPs also showed relatively strong associations: *CDKN2A*$_{rs2811710}$ ($p = 3.40 \times 10^{-4}$), *ATG10*$_{rs1109846}$ ($p = 7.11 \times 10^{-4}$), *ITPR1*$_{rs11716177}$ ($p = 6.40 \times 10^{-4}$), and *EIF4G1*$_{rs56148883}$ ($p = 5.40 \times 10^{-4}$).

We then selected independent variants ($r^2 < 0.1$) with MAF >0.005 and $p \leq .001$ for replication in the Mayo Clinic/MD Anderson and IMMEnSE/IBERICAT cohorts. Replication in these two large, independent cohorts was critical, as it enabled us to demonstrate for the first time a genome-wide significant association between the *ULK4* locus and MGUS risk ($p < 5 \times 10^{-8}$; Table 2). The combined meta-analysis of all six populations confirmed the association of the *ULK4* locus with MGUS risk ($p = 3.35 \times 10^{-8}$). In addition, although not statistically significant after correction for multiple testing (p-Bonferroni $= 3.84 \times 10^{-5}$), we observed a suggestive association between the *CDKN2A*$_{rs2811710}$ variant and MGUS risk ($p = 2.17 \times 10^{-4}$).

3.2 | Association of autophagy-related SNPs with gene expression and host immune responses

Data from the GTEX portal provided functional relevance of *ULK4*$_{rs6599175}$ by showing that it strongly associates with *ULK4* mRNA expression in whole blood ($p = 2.55 \times 10^{-81}$; Figure S1).

To investigate the potential biological impact of the identified variants, we explored whether the *ULK4* polymorphism could modulate host immune responses, circulating immunological proteins, and blood-derived immune cell populations, using data from the 500FG cohort from the HFGP. Although the association of *CDKN2A*$_{rs2811710}$ with MGUS risk was modest and did not remain significant after correction for multiple testing, we included this variant in the functional

TABLE 2 Association analysis of autophagy-related SNPs representing susceptibility regions for MGUS.

			German GWAS		Czech GWAS		Swedish GWAS		UKBB GWAS		Discovery meta-analysis			Mayo/MD Anderson cohort		IMMENSE/IBERICAT cohort		Overall meta-analysis		
			$n = 1528$		$n = 888$		$n = 1486$		$n = 277{,}603$		$n = 281{,}505$			$n = 1623$		$n = 1547$		$n = 284{,}675$		
SNP	Gene	A1	(243 MGUS and 1285 healthy controls)		(288 MGUS and 600 healthy controls)		(461 MGUS and 1025 healthy controls)		(107 MGUS and 277,496 healthy controls)		(1099 MGUS and 280,406 healthy controls)			(744 MGUS and 879 healthy controls)		(474 MGUS and 1073 healthy controls)		(2317 MGUS and 282,358 healthy controls)		
			OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	P_{Het}	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	P_{Het}
rs6599175	*ULK4*	C	**1.52 (1.20–1.92)**	**5.70×10^{-4}**	**1.49 (1.16–1.92)**	**1.91×10^{-3}**	**1.21 (0.99–1.50)**	**6.71×10^{-2}**	1.17 (0.81–1.67)	.409	**1.35 (1.19–1.53)**	**2.04×10^{-6}**	.370	**1.28 (1.05–1.57)**	**.015**	1.18 (0.96–1.46)	.116	**1.29 (1.18–1.41)**	**3.35×10^{-8}**	.468
rs2811710[a]	*CDKN2A*	C	1.00 (0.81–1.25)	.975	**1.45 (1.15–1.83)**	**1.94×10^{-3}**	**1.29 (1.07–1.55)**	**7.89×10^{-3}**	1.29 (0.89–1.63)	.218	**1.23 (1.10–1.37)**	**3.40×10^{-4}**	.145	1.14 (0.96–1.36)	.146	1.07 (0.89–1.28)	.479	**1.17 (1.08–1.27)**	**2.17×10^{-4}**	.203
rs1109846	*ATG10*	C	1.21 (0.94–1.56)	.131	**1.35 (1.03–1.78)**	**3.09×10^{-2}**	**1.22 (1.01–1.48)**	**4.38×10^{-2}**	1.20 (0.83–1.74)	.332	**1.24 (1.10–1.41)**	**7.11×10^{-4}**	.927	0.83 (0.67–1.02)	.074	0.95 (0.76–1.19)	.648	1.08 (0.98–1.2)	.105	**.027**
rs11716177	*ITPR1*	T	1.13 (0.79–1.62)	.526	**1.40 (0.97–2.03)**	**7.55×10^{-2}**	**1.42 (1.03–1.97)**	**3.34×10^{-2}**	**2.03 (1.20–3.47)**	**8.80×10^{-3}**	**1.39 (1.15–1.68)**	**6.40×10^{-4}**	.348	0.86 (0.63–1.17)	.334	0.93 (0.60–1.43)	.751	**1.18 (1.01–1.37)**	**3.31×10^{-2}**	**.043**
rs56148883	*EIF4G1*	A	**1.39 (0.99–1.97)**	**5.96×10^{-2}**	1.19 (0.80–1.77)	.394	**1.34 (1.00–1.82)**	**5.36×10^{-2}**	**1.94 (1.15–3.28)**	**1.30×10^{-2}**	**1.38 (1.15–1.66)**	**5.40×10^{-4}**	.531	0.92 (0.68–1.24)	.577	**1.51 (1.09–2.09)**	**.013**	**1.27 (1.09–1.47)**	**2.15×10^{-3}**	.169

Note: $p < .05$ in bold.
Abbreviations: A1, effect-allele; *P*, *p*-value; SNP, single nucleotide polymorphism.
[a]rs2811710 is in LD with rs7036656 ($D' = 0.9407$ and $r^2 = 0.6325$).

analyses due to its well-established roles in the autophagy pathway and its prior role seen in MM.

Interestingly, we found that carriers of the *ULK4*$_{rs6599175C}$ allele showed reduced counts of memory B cell subsets (IgM+CD38+CD27+ and IgD+IgM+CD27+; $p = .0038$ and $p = .0056$, respectively; Figure 2A,B) and natural effector B cells (CD24+CD38+IgD+IgM+ cells; $p = .0060$; Figure 2C), suggesting that ULK4 may influence early B-cell differentiation in plasma cell dyscrasia. On the other hand, we failed to find positive associations between the *CDKN2A*$_{rs2811710}$ SNP and host immune parameters, which suggested that the *CDKN2A* locus does not modulate MGUS risk through the regulation of host immune responses. Additional functional studies are now warranted to decipher the mechanistic process by which this locus is affecting MGUS risk.

4 | DISCUSSION

This study fills a critical gap in the field by presenting the first systematic evaluation of autophagy-related genetic variation in MGUS. Through a large-scale meta-analysis across five European cohorts and one North American cohort, we identified, for the first time, a genome-wide significant association between the *ULK4* gene and MGUS susceptibility, marking a key advance in our understanding of early-stage plasma cell dyscrasias.

The rs6599175C allele in *ULK4* was associated with a 29% increased risk of MGUS, consistent with previously reported effect sizes in MM[14] and AL amyloidosis[53] (26% and 30%, respectively). This convergence suggests that *ULK4* may play a broader role in plasma cell dyscrasias, although its specific involvement in disease progression toward MM remains unclear. Previous studies examined this locus in broader or combined disease contexts (e.g., pooled MGUS/MM cohorts)[14,15,54] or through polygenic risk score approaches,[13] which may have lacked the resolution to detect locus-specific associations. Our findings provide genome-wide significant evidence supporting a potential role for *ULK4* in MGUS susceptibility and highlight the value of studying precursor conditions independently.

The *ULK4* gene is located on chromosome 3p22.1 and encodes for a member of the unc-51-like serine/threonine kinase (STK) family,

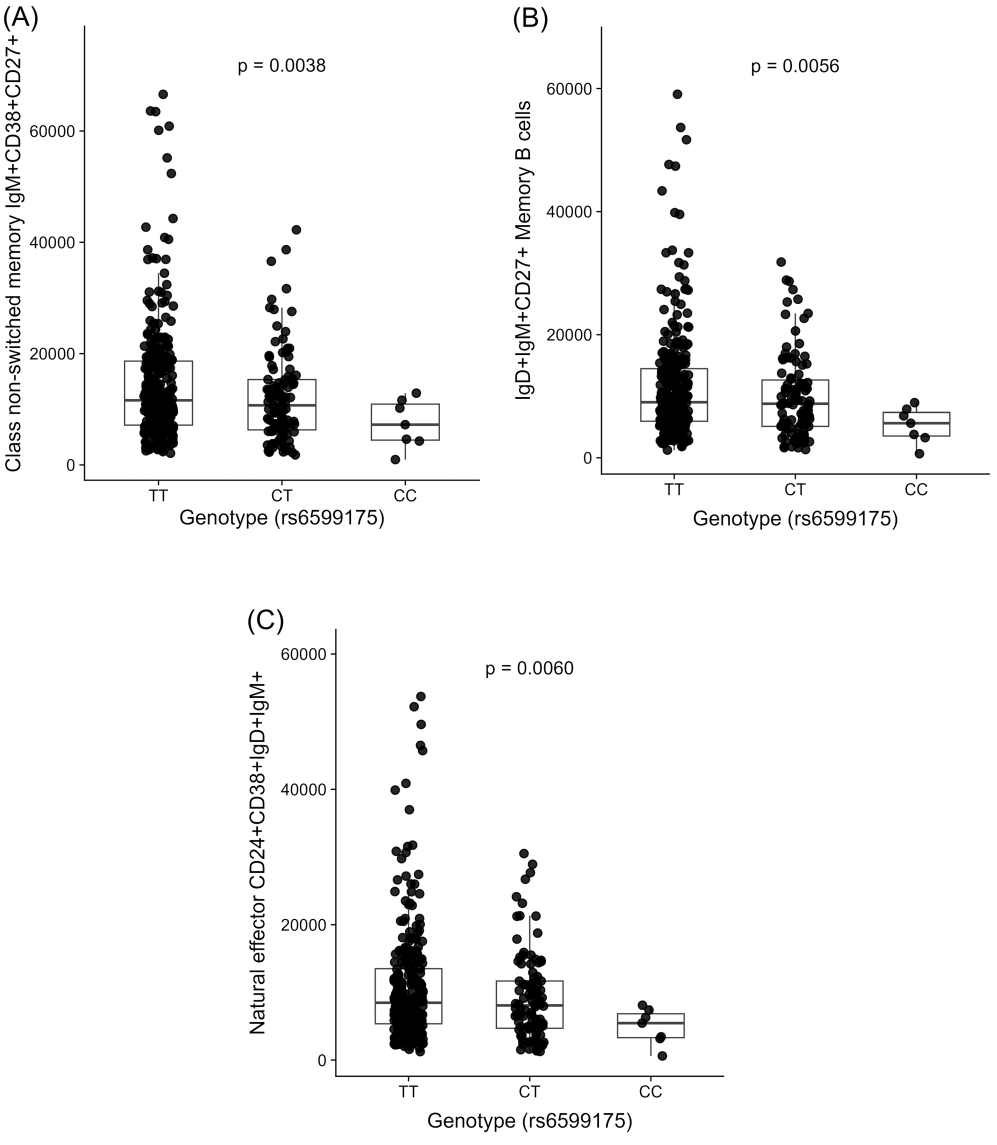

FIGURE 2 Association of the *ULK4*$_{rs6599175}$ SNP and numbers of different B cell subtypes. (A) Association between the *ULK4*$_{rs6599175}$ SNP and the number of class non-switched memory IgM+CD38+CD27+ cells; (B) Association between the *ULK4*$_{rs6599175}$ SNP and the number of IgD+IgM+CD27+ memory B cells; (C) Association between the ULK4rs6599175 SNP and the number of natural effector CD24+CD38+IgD+IgM + cells.

which is involved in various biological processes, including cytoskeletal remodeling and cell motility through regulation of mTOR-mediated autophagy. Furthermore, the identified variant has been associated with increased risk of hypertension,[55] cardiovascular disorders,[56] and other conditions.[57] According to data from the GTEx portal, *ULK4*$_{rs6599175}$ strongly associates with *ULK4* mRNA expression in whole blood ($p = 2.55 \times 10^{-81}$) and multiple tissues,[58] suggesting that this variant may directly influence *ULK4* protein concentrations. Additionally, our group has previously reported that the *ULK4*$_{rs6599175C}$ allele associates with serum concentrations of vitamin D3 in healthy donors,[59–61] further supporting a role for *ULK4* in modulating processes such as cell differentiation and proliferation, angiogenesis, immune responses and apoptosis, in both unaffected and malignant tissues. However, in our previous study, we did not observe an association between this variant and autophagic flux, suggesting that its effects may be mediated through alternative or indirect pathways. In support of this notion, we found suggestive associations of the *ULK4*$_{rs6599175C}$ allele with reduced counts of IgM+CD38+CD27+ and IgD+IgM+CD27+ cells and natural effector CD24+CD38+IgD+IgM+ B cells, suggesting that the *ULK4* gene may influence B-cell development and immune homeostasis relevant to MGUS initiation.

Beyond *ULK4*, we also observed that the *CDKN2A*$_{rs2811710}$ SNP showed a suggestive association with MGUS risk. Although this association did not remain statistically significant after correction for multiple testing, this may reflect limited statistical power due to modest effect sizes and the low prevalence of MGUS, as well as potential locus heterogeneity or context-dependent regulatory effects that are not fully captured in standard case–control GWAS. Nevertheless, it is reasonable to hypothesize that this variant may influence susceptibility to MGUS, given that *CDKN2A* has previously been implicated in multiple myeloma risk in family-based studies.[61] A previous study similarly failed to detect a statistically significant association after multiple testing correction but acknowledged that the lack of significance might have been due to limited statistical power.[13]

The *CDKN2A*$_{rs2811710C}$ allele was not associated with immunological parameters in the 500FG cohort; however, multiple lines of evidence support its functional effect. According to Haploreg data, the *CDKN2A*$_{rs2811710C}$ variant associates with *CDKN2A* and *STK16* mRNA expression levels in peripheral blood monocytes ($p = 1.24 \times 10^{-15}$ and $p = 5.48 \times 10^{-6}$, respectively)[52] and resides in histone-marked regions of regulatory chromatin across several blood cell types, including effect memory T cells, primary B cells and naïve and memory CD4+ and CD8+ T cells. In addition, this SNP alters transcription binding sites for key transcription factors, such as p300, Foxd3, HDAC2, HNF1, and OTX that modulate hematopoietic stem cell differentiation and proliferation[63–65] and mature B cell proliferation.[66–69] Overall, these findings suggest a functional role of this variant in modulating the risk of MGUS.

The *CDKN2A* gene, localized on chromosome 9p21.3, encodes a cyclin-dependent kinase inhibitor 2A, a well-established tumor suppressor protein.[70,71] This protein is involved in p53 stabilization, cell cycle arrest, inhibition of cell proliferation, induction of cellular senescence, and regulation of DNA methylation.[72–75] Recent evidence has also highlighted a role for *CDKN2A* in autophagy regulation and cellular senescence. Specifically, a conserved domain within exon 2 is required for ARF-mediated autophagy induction, a function that is disrupted by tumor-associated mutations.[76] Although it remains unclear whether the *CDKN2A* locus exerts its effects during the pre-tumoral stages or later during tumor progression, our findings support the role of this gene in determining MGUS predisposition, suggesting its involvement at the earliest stages of plasma cell neoplasia. Furthermore, previous studies have reported that germline mutations in *CDKN2A* are associated with overall survival in MM patients,[77] supporting the hypothesis that this locus is central in modulating susceptibility, and/or progression in plasma cell disorders such as MGUS and MM. While additional evidence is needed, *CDKN2A* could potentially serve as a biomarker for identifying individuals at increased risk of developing MGUS or for stratifying MGUS patients according to their likelihood of progression to MM. It may also represent a candidate for therapeutic exploration in the early stages of plasma cell dyscrasias.

This study has several strengths and limitations. It leverages the largest MGUS genetic dataset to date, comprising 284,675 individuals of European ancestry (2317 MGUS cases and 282,358 healthy controls), and evaluates inherited variation across 234 autophagy-related genes. The integration with immune phenotyping data from the 500FG cohort allowed us to explore functional consequences of the most relevant variants on immune cell populations and circulating inflammatory proteins. However, MGUS remains an asymptomatic condition, limiting the number of available cases and our ability to detect associations with rare variants. Although most cohorts were not systematically screened for subclinical MGUS, the majority of MGUS cases included in this study were clinically diagnosed by hematologists according to established diagnostic criteria. The UK Biobank cohort represents the exception, where MGUS cases were ascertained using registry-based ICD codes, which may be subject to misclassification, coding errors, and lack of confirmation by standardized hematological diagnostic criteria, and may therefore not fully capture clinically validated MGUS cases. Controls consisted of elderly individuals without a known clinical diagnosis of plasma cell dyscrasia. Any potential inclusion of undiagnosed MGUS among controls would be expected to bias effect estimates toward the null, rendering our observed associations conservative. The European-only ancestry of participants may also restrict the applicability of these findings to other populations. To address these challenges, future research should focus on building large, ethnically diverse, and longitudinal MGUS cohorts to improve statistical power and generalizability.

5 | CONCLUSIONS

This study demonstrates that genetic variation in the autophagy-related gene *ULK4* is associated with increased risk of MGUS. Our functional data indicated that the *ULK4*$_{rs1109846}$ variant may contribute to MGUS susceptibility by modulating *ULK4* mRNA expression levels and abundance of specific B cell subsets. Together with our previous findings showing that this SNP influences circulating

concentrations of vitamin D3, these results suggest a central role for *ULK4* in regulating vitamin D3-mediated B-cell immune responses. In addition, based on both genetic and functional analyses, we identified for the first time a SNP within the *CDKN2A* gene as a potential susceptibility marker for MGUS. The effect of the *CDKN2A* variant on MGUS risk might be mediated by its effect on mRNA transcription in monocytes and transcription factors possibly affecting cell differentiation.

AUTHOR CONTRIBUTIONS

José Manuel Sánchez-Maldonado: Writing – original draft; investigation; data curation; formal analysis. **Angelica Macauda:** Writing – review and editing; investigation. **Antonio José Cabrera-Serrano:** Investigation; data curation; formal analysis; writing – original draft. **Hauke Thomsen:** Writing – review and editing; resources; data curation. **Murat Güler:** Writing – review and editing; data curation. **Rob Ter Horst:** Writing – review and editing; resources; data curation; investigation. **Bethany van Guelpen:** Writing – review and editing; resources. **Pavel Vodicka:** Writing – review and editing; resources. **Stefano Landi:** Writing – review and editing; resources. **Subhayan Chattopadhyay:** Writing – review and editing; resources. **Pelin Ünal:** Writing – review and editing; resources. **Lucía Ruiz-Durán:** Resources; writing – review and editing. **Delphine Casabonne:** Writing – review and editing; resources. **Hartmut Goldschmidt:** Writing – review and editing; resources. **Istemi Serin:** Writing – review and editing; resources. **María Carretero-Fernández:** Writing – review and editing; resources. **Elena Cabezudo:** Writing – review and editing; resources. **Fernando Reyes Zurita:** Writing – review and editing; resources. **Aaron D. Norman:** Writing – review and editing; resources. **Ramón García-Sanz:** Writing – review and editing; resources. **Gabriele Capurso:** Writing – review and editing; resources. **Per Hoffmann:** Writing – review and editing; resources. **Ulrika Pettersson-Kymmer:** Writing – review and editing; resources. **Francisco Jiménez-Romera:** Writing – review and editing; resources. **S. Vincent Rajkumar:** Writing – review and editing; resources. **Niels Weinhold:** Writing – review and editing; resources. **Ludmila Vodickova:** Writing – review and editing; resources. **Christian Langer:** Writing – review and editing; resources. **Angelika Stein:** Writing – review and editing; resources; methodology. **Abdulkadir Karismaz:** Writing – review and editing; resources. **Víctor Moreno:** Writing – review and editing; resources. **Markus M. Nöthen:** Writing – review and editing; resources. **Karl-Heinz Jöckel:** Writing – review and editing; resources. **Francesca Tavano:** Writing – review and editing; resources. **Joaquín Martínez López:** Writing – review and editing; resources. **Shaji K. Kumar:** Writing – review and editing; resources. **Juan Francisco Gutiérrez-Bautista:** Writing – review and editing; resources. **Daniela Basso:** Writing – review and editing; resources. **Florentin Späth:** Writing – review and editing; resources. **Yolanda Benavente:** Writing – review and editing; resources. **Michelle A. T. Hildebrandt:** Writing – review and editing; resources. **Börge Schmidt:** Writing – review and editing; resources. **Tereza Sevcikova:** Writing – review and editing; resources. **Rui Manuel Vieira Reis:** Writing – review and editing; resources. **Yang Li:** Writing – review and editing; resources; investigation; data curation. **Miguel Ángel López-Nevot:** Writing – review and editing; resources. **Mihai G. Netea:** Writing – review and editing; resources; investigation; data curation. **Daniele Campa:** Resources; writing – review and editing. **Alyssa Clay-Gilmour:** Writing – review and editing; resources. **Susan Slager:** Writing – review and editing; resources. **Kari Hemminki:** Writing – review and editing; resources. **Celine Vachon:** Resources; writing – review and editing. **Asta Försti:** Conceptualization; writing – review and editing; data curation. **Federico Canzian:** Conceptualization; writing – review and editing; data curation. **Juan Sainz:** Conceptualization; writing – original draft; funding acquisition; supervision; resources; project administration; data curation; formal analysis.

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CONFLICT OF INTEREST STATEMENT

Hartmut Goldschmidt reports research funding and/or provision of investigational medicinal products from BMS/Celgene, Dietmar Hopp Foundation, Janssen, and Sanofi. He has received research support from Amgen, BMS, Celgene, GlycoMimetics Inc., GlaxoSmithKline (GSK), Heidelberg Pharma, Hoffmann-La Roche, Janssen Research and Development, Millennium, Novartis, Pfizer, Oncopeptides, and Sanofi. He has served on advisory boards for BMS, Janssen, Sanofi, and GSK. He has received honoraria from Amgen, BMS, GSK, Janssen, Sanofi, Pfizer, and Oncopeptides and travel support from Amgen, BMS, GSK, Janssen, Sanofi, Pfizer, and Oncopeptides. All other authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The UK Biobank is an open access resource and bona fide researchers can apply to use the UK Biobank dataset by registering and applying at https://www.ukbiobank.ac.uk/use-our-data/apply-for-access/. The summary statistics of the UK Biobank MGUS GWAS are available in Zenodo with: https://zenodo.org/records/10533713. Functional data used in this project have been meticulously catalogued and archived in the BBMRI-NL data infrastructure (https://hfgp.bbmri.nl/) using the MOLGENIS open-source platform for scientific data.[78] The genotype data used in the present study and further information are available by the corresponding authors upon reasonable request.

ETHICS STATEMENT

Each participant in the German, Czech, and Swedish GWAS, as well as in the UK Biobank GWAS and the Mayo/MD Anderson and IMMEnSE/IBERICAT studies, obtained approval from the respective institutional review board (IRB) and IRB certification permitting data sharing in accordance with the NIH Policy for Sharing of Data Obtained in NIH-Supported or NIH-Conducted Genome-Wide Association Studies. The IMMEnSE study protocol was approved by the Ethics Commission of the Medical Faculty of the University of Heidelberg (S-565/2015; last update on April 3, 2017) and the IBERICAT research protocol was approved by the Center Ethics Committee of Hospital de Bellvitge (Ref PR248/19). In accordance with the Declaration of Helsinki, written informed consent was obtained from each participant. The HFGP study was also approved by the Arnhem-Nijmegen Ethical Committee (no. 42561.091.12), and biological specimens were collected only after informed consent had been obtained.

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