Published in final edited form as:

Hematol Oncol. 2011 March ; 29(1): 42–46. doi:10.1002/hon.954.

Variation in innate immunity genes and risk of multiple myeloma

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Abstract

Multiple myeloma (MM) is a B-cell lymphoid malignancy suspected to be associated with immunologic factors. Given recent findings associating single-nucleotide polymorphisms (SNPs) in innate immunity genes with non-Hodgkin lymphoma, we conducted an investigation of innate immune gene variants using specimens from a population-based case-control study of MM conducted in Connecticut women. Tag SNPs (N=1,461) summarizing common variation in 149 gene regions were genotyped in non-Hispanic Caucasian subjects (103 cases, 475 controls). Odds ratios (OR) and 95% confidence intervals (CI) relating SNP associations with MM were computed using unconditional logistic regression, while the MinP test was used to investigate associations with MM at the gene level. We calculated permutation-adjusted *P*-values and false discovery rates (FDR) to account for the number of comparisons performed in SNP-level and gene-level tests, respectively. Three genes were associated with MM when controlling for a FDR of ≤10%: *SERPINE1* ($P_{\text{MinP}} < 0.0001$; FDR=0.02), *HGF* ($P_{\text{MinP}} = 0.0006$; FDR=0.06) and *CCR7* ($P_{\text{MinP}} = 0.001$; FDR=0.08). Two SNPs demonstrated robust associations: *SERPINE1* rs2227667 ($P = 2.1 \times 10^{-5}$, $P_{\text{permutation}} = 0.03$) and *HGF* rs17501108 ($P = 5.0 \times 10^{-5}$, $P_{\text{permutation}} = 0.07$). Our findings suggest that genetic variants in *SERPINE1* and *HGF*, and possibly *CCR7*, are associated with MM risk, and warrant further investigation in other studies.

Keywords

epidemiology; myeloma; genetics

INTRODUCTION

Multiple myeloma is an incurable B-cell malignancy characterized by plasma cell overproduction of monoclonal immunoglobulin (Ig) G, IgA, and/or light chains [1]. It accounts for approximately 10% of hematological malignancies and 1% of cancer deaths in Western countries [2]. The etiology of this malignancy is poorly understood, with only age and race (elevated risk in African Americans) having been established as risk factors to date [1]. Familial clusters of multiple myeloma and an increased risk of myeloma among

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Conflicts of Interest: The authors have no conflicts of interest to disclose.

individuals with a family history of myeloma or hematological malignancy have been reported, suggesting that genetic or shared environmental factors contribute to myeloma susceptibility [3–6]. Immunologic factors may also contribute to the development of myeloma, with reports of increased risk associated with chronic antigen stimulation, infections and autoimmune condition [7].

A small number of studies have investigated whether genetic polymorphisms in immunoregulatory genes influence multiple myeloma susceptibility; associations with variants in *TNF*, *IL4R*, *FCGR2* and *IL6* [8–10] have been reported, and warrant further investigation. Recent studies of non-Hodgkin lymphoma, another malignancy of predominantly B-cell origin, suggest that lymphomagenesis may be influenced by common variants in genes regulating innate immunity, a system of immune cells and mechanisms which recognizes and responds to infections by microbial pathogens in a non-adaptive manner [11–13]. To our knowledge, no studies to date have investigated whether innate immunity gene variants are associated with multiple myeloma. We genotyped 1,461 tag SNPs summarizing common genetic variation in 149 gene regions involved with the innate immune system in a case-control study of women conducted in Connecticut.

METHODS

The study methods have been previously described [14]. Histologically confirmed incident cases of multiple myeloma diagnosed between 1996 and 2000 among female Connecticut residents aged 21 to 84 years were identified through the Connecticut Tumor Registry. Of the 409 cases identified during this time period, 86 (21%) died prior to enrollment and 140 (34%) declined to participate, resulting in a total of 183 interviewed cases (57% of living cases; 45% overall participation rate). Controls aged 21–84 years were recruited as part of a parallel population-based case-control study of non-Hodgkin lymphoma. Controls below age 65 years were enrolled through random digit dialing methods, while Medicare files were used to select eligible control aged 65 years or older. A total of 717 controls participated in the study; the participation rate was 69% for those contacted by random-digit dialing and 47% for those identified through Medicare files. The study was approved by the Yale University School of Medicine's Human Investigations Committee, the Connecticut Department of Public Health, and the National Cancer Institute's Special Studies Institutional Review Board. Participation was voluntary, and written informed consent was obtained from all participants.

Following an in-person interview, subjects provided 10mL of peripheral blood or buccal cell cotton swab samples. Genomic DNA was extracted from peripheral blood and buccal cells by phenol-chloroform extraction (Gentra Systems, Minneapolis, MN). Samples from non-Hispanic Caucasian participants with blood-based DNA were genotyped at the NCI Core Genotyping Facility using the 1,536-SNP Illumina GoldenGate platform, using an oligonucleotide pool designed to tag 149 gene regions (210 genes) selected from known innate immune pathways (oxidative response, pattern recognition molecules and antimicrobials, integrins and adhesion molecules, complement, chemokines with their receptors and signaling molecules, and response genes and tissue factors). Tag SNPs were selected from the designable set of common SNPs (minor allele frequency ≥ 0.05) genotyped in the Caucasian (CEU) population sample of the International HapMap Project using the software application TagZilla (v1.1; <http://tagzilla.nci.nih.gov/>), which employs the pairwise binning algorithm of Carlson et al. [15]. For each gene, SNPs within the region 20kb 5' of the ATG-translation initiation codon and 10kb 3' of the end of the last exon were binned using a binning threshold of $r^2 > 0.80$, with greater weighting for SNPs with a design score of 1.1. Less than 5% of SNPs were forced into the panel, creating a negligible effect on the tagging algorithm. Samples from 103 cases and 475 controls were successfully genotyped.

After excluding assays with completion rates <90% or with genotype concordance rates <95%, 1,461 SNPs were available for analysis. A list of the gene, chromosomal position, and base change for each of the 1,461 SNPs in the final data set is provided in Supplementary Table 1.

SAS version 9.1 (SAS Institute Inc., Cary, NC, USA) was used to compute odds ratios (OR) and 95% confidence intervals (CI) for each SNP through unconditional logistic regression modeling with adjustment for age. Tests for trend were performed by modeling the number of rare alleles (0, 1 or 2) as a continuous variable. Gene-level statistical tests of association were conducted using the “MinP” test, which assess the true statistical significance of the smallest p-trend within each gene by permutation-based resampling methods (10,000 permutations) that automatically adjust for the number of tag SNPs tested within that gene and the underlying linkage disequilibrium pattern [16;17]. These analyses were performed using the MATLAB Statistics Toolbox™ 6.2 (The Mathworks, Inc., Natick, MA, USA).

We used different methods to assess the potential for our gene-level and SNP-level findings to have arisen due to multiple comparisons. For gene-level MinP tests we calculated the false discovery rate (FDR), the expected ratio of erroneous rejections of the null hypothesis to the total number of rejected hypotheses among all the variants assessed [18]. Statistically significant findings with a FDR of 10% or less were considered to be noteworthy. For SNP-level trend tests, we computed permutation-adjusted P-values (10,000 permutations) which account for the total number of evaluated SNPs [19].

We further investigated whether SNPs associated with multiple myeloma after permutation adjustment were also associated with expression levels of the gene of interest. To do this, we analyzed publicly available expression data (GENEVAR project, www.sanger.ac.uk/humgen/genevar/) from 90 Epstein-Barr virus-transformed lymphoblastoid cell lines derived from the HapMap CEU population sample [20]. Tests of association between SNP genotype and gene expression levels were performed by one-way ANOVA and by two-sample t-tests of dichotomized genotypes (homozygous rare allele / heterozygous vs. homozygous common allele).

RESULTS

One hundred and three cases and 475 controls were successfully genotyped for 1,461 SNPs in 149 gene regions (210 genes). The observed distribution of SNP trend test P-values did not differ from the expected distribution, suggesting no evidence of systematic bias between cases and controls (Supplementary Figure 1). Table 1 lists all of the investigated genes with MinP *P*-values <0.05 and summarizes the results of the SNP with the smallest trend test *P*-value within each gene. Three of the 210 investigated genes had MinP *P*-values that were noteworthy at a FDR of less than 10%: *SERPINE1* ($P_{\text{MinP}} < 0.0001$, FDR=0.02), *CCR7* ($P_{\text{MinP}} = 0.0006$, FDR=0.06) and *HGF* ($P_{\text{MinP}} = 0.001$, FDR=0.08). Within *SERPINE1*, the SNP rs2227667 was associated with multiple myeloma risk at a *P*-value of 2.1×10^{-5} ; other SNPs with *P*<0.05 included rs2227672 (*P*=0.0004), rs1050813 (*P*=0.03) and rs2227692 (*P*=0.049). One SNP within *CCR7* was significantly associated with risk (rs3136685, *P*=0.0001), as were two *HGF* SNPs (rs17501108, $P = 5.0 \times 10^{-5}$; rs1558001, *P*=0.009). After computing permutation-based *P*-values to account for all of the evaluated SNPs, noteworthy findings remained for *SERPINE1* rs2227667 ($P_{\text{permutation}} = 0.03$) and *HGF* rs17501108 ($P_{\text{permutation}} = 0.07$). Associations between multiple myeloma risk and genotypes of rs2227667, rs17501108 and rs3136685 are shown in Table 2. None of the three SNPs were found to be associated with expression levels of *SERPINE1*, *HGF* or *CCR7* in lymphoblastoid cell lines derived from the HapMap CEU samples (results not shown).

DISCUSSION

In this case-control study investigating 1,461 tag SNPs in 149 gene regions regulating innate immunity, genetic variants in the genes *SERPINE1*, *CCR7* and *HGF* were associated with myeloma risk. In particular, robust associations were observed for two SNPs, *SERPINE1* rs2227667 and *HGF* rs17501108.

SERPINE1, located on chromosome region 7q21, encodes plasminogen activator inhibitor type 1 (PAI-1), an important inhibitor of fibrinolysis. Multiple myeloma patients have previously been shown to have significantly elevated levels of PAI-1 compared to healthy patients [21], and are known to be particularly susceptible to deep vein thrombosis [22]. Moreover, *SERPINE1* overexpression has been found in cultured bone marrow mesenchymal stem cells (BMMSC) of multiple myeloma patients compared to cultured BMMSC from healthy donors [23]. Apart from its role in hemostasis, PAI-1 is suspected to influence tumor progression and metastasis for several types of cancer; reported effects of PAI-1 include inhibition of apoptosis [24;25] and increased angiogenesis [26;27], cell adhesion [28] and cell migration [29]. The *SERPINE1* polymorphism rs2227667 which was significantly associated with multiple myeloma risk in this study has been previously associated with plasma levels of PAI-1 [30] and the hemostatic marker D-dimer [31], although we did not observe an association between this SNP and *SERPINE1* expression within lymphoblastoid cell lines derived from the HapMap CEU samples. In total, these findings suggest that rs2227667 may have functional relevance, or is in linkage disequilibrium with a functional variant.

Our observed association with variation in *HGF*, located 19Mb centromeric of *SERPINE1* on 7q21, is intriguing given that its gene product, hepatocyte growth factor (HGF), is already known to play a role in multiple myeloma biology. In clinical studies, serum levels of HGF have been found to be elevated in MM patients compared to healthy individuals and to be associated with reduced survival [32–35]. Experimental investigations have demonstrated that HGF exerts strong proliferative and antiapoptotic effects in MM cells [36], promotes cell invasiveness [37], stimulates angiogenesis [38] and inhibits bone formation [39]. The SNP rs17501108, which was significantly associated with MM in our analysis, has also been associated with myopia in a previous study [40], although we did not observe an association between this SNP and *HGF* expression from the lymphoblastoid cell line data.

CCR7 encodes C-C chemokine receptor type 7 (CCR7), a receptor expressed on B- and T-lymphocytes that is involved in the trafficking of lymphocytes and dendritic cells to lymph nodes [41;42]. Although CCR7 expression in tumor cells from several cancer types has been associated with an increased risk of lymph node metastasis [43;44], CCR7 is typically not expressed in multiple myeloma [45]. The rs3136685 genotype was not associated with gene expression in the lymphoblastoid cell line data and, unlike with *SERPINE1* rs2227667 and *HGF* rs17501108, its association with multiple myeloma did not survive a permutation-based test accounting for the multiple comparisons. In light of this, and the absence of previous evidence linking it to MM, the findings for *CCR7* should be interpreted with caution.

An important strength of this study is its detailed assessment of innate immunity pathways, with 149 gene regions assessed. Given the large number of genes and SNPs evaluated in this analysis, we were careful to consider the potential for false-positive findings when interpreting our results. With that in mind, our findings for *SERPINE1* rs2227667 and *HGF* rs17501108 are particularly important given that they remained noteworthy following permutation-based tests accounting for the total number of evaluated SNPs, suggesting that

they are not due to chance. This study also has limitations. The sample size of this study is small, resulting in low power to detect genetic effects of modest magnitude as well as an increased probability of generating false-positive findings [46]. Another limitation is the low participation rate among cases and controls. While willingness to participate and provide blood is unlikely to be related to genotype differences [47], we cannot rule out the possibility that the results may reflect selection bias due to possible effects of the identified SNPs on survival. It is thus essential for these results to be replicated in other studies before meaningful inferences regarding causation are made.

CONCLUSION

In conclusion, our findings suggest that genetic variants in *SERPINE1* and *HGF*, and possibly *CCR7*, are associated with multiple myeloma risk, and warrant further investigation in other studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This project has been funded in whole or in part with federal funds from the National Cancer Institute, National Institutes of Health, under contract N01-CO-12400. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products or organizations imply endorsement by the US Government.

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Table 1
Summary of gene-level and SNP-level association test results for genes with $P_{\text{MinP}} < 0.05$

Gene	Location	# of tag SNPs	Gene MinP test:		Tag SNP within gene with smallest P_{trend} :				Raw P_{trend}	Permutation-adjusted P_{trend}^2
			P_{MinP}	FDR ¹	dbSNP ID	Minor allele	MAF _{Control}	MAF _{Case}		
<i>SERPINE1</i>	7q21.3-q22	8	<0.0001	0.02	rs2227667	G	0.25 / 0.13	0.25 / 0.13	2.1×10^{-5}	0.0336
<i>CCR7</i>	17q12-q21.2	4	0.0006	0.06	rs3136685	A	0.20 / 0.10	0.20 / 0.10	0.0001	0.1611
<i>HGF</i>	7q21.1	17	0.0012	0.08	rs17501108	T	0.08 / 0.17	0.08 / 0.17	5.0×10^{-5}	0.0740
<i>JAK3</i>	19p13.1	11	0.0087	0.46	rs3212711	A	0.33 / 0.22	0.33 / 0.22	0.0009	0.7058
<i>MAL</i>	2cen-q13	6	0.0207	0.64	rs1316873	G	0.11 / 0.05	0.11 / 0.05	0.0039	0.9913
<i>BAT5</i>	6p21.3	2	0.0226	0.64	rs1266071	T	0.11 / 0.06	0.11 / 0.06	0.0114	1.0000
<i>CARD4</i>	7p15-p14	12	0.0273	0.64	rs2256023	C	0.46 / 0.35	0.46 / 0.35	0.0026	1.0000
<i>XDH</i>	2p23-p22	20	0.0294	0.64	rs206849	A	0.39 / 0.50	0.39 / 0.50	0.0016	1.0000
<i>TLR10</i>	4p14	6	0.0362	0.64	rs11096955	G	0.40 / 0.30	0.40 / 0.30	0.0075	1.0000
<i>TLR6</i>	4p14	3	0.0378	0.64	rs1039559	G	0.43 / 0.53	0.43 / 0.53	0.0140	1.0000
<i>DEFA4</i>	8p23	6	0.0384	0.64	rs13251447	A	0.24 / 0.33	0.24 / 0.33	0.0081	1.0000
<i>CCL11</i>	17q21.1-q21.2	8	0.0461	0.64	rs4795896	C	0.10 / 0.16	0.10 / 0.16	0.0279	1.0000
<i>CD180</i>	5q12	12	0.0499	0.64	rs3756561	T	0.05 / 0.10	0.05 / 0.10	0.0056	1.0000

Abbreviations: SNP, single-nucleotide polymorphism; FDR, false discovery rate; MAF, minor allele frequency; OR, odds ratio; CI, confidence interval.

¹ False discovery rate threshold at which P-value retains level of significance (accounting for all 210 gene-level tests in the study).

Note: genes with MinP test findings that survive a false discovery rate threshold of 0.10 in bold-face type.

² Adjusting for all 1,461 evaluated SNPs.

Table 2

Associations with multiple myeloma for rs2227667, rs3136685 and rs17501108 genotypes

SNP	Genotype	N _{Control} (%)	N _{Case} (%)	OR ^I	(95% CI)	P
<i>SERPINE1</i> rs2227667	AA	255 (53.9)	76 (74.5)	1.00		
	AG	198 (41.9)	26 (25.5)	0.43	(0.26–0.70)	0.0006
	GG	20 (4.2)	0			
	AG / GG	218 (46.1)	26 (25.5)	0.39	(0.24–0.64)	0.0002
$P_{\text{trend}} = 2.1 \times 10^{-5}$						
<i>CCR7</i> rs3136685	GG	300 (63.2)	84 (81.6)	1.00		
	AG	158 (33.3)	18 (17.5)	0.39	(0.23–0.68)	0.0008
	AA	17 (3.6)	1 (1.0)	0.22	(0.03–1.68)	0.1433
	AG / AA	175 (36.8)	19 (18.5)	0.38	(0.22–0.64)	0.0004
$P_{\text{trend}} = 0.0001$						
<i>HGF</i> rs17501108	GG	401 (84.4)	69 (67.0)	1.00		
	GT	72 (15.2)	32 (31.1)	2.65	(1.62–4.35)	0.0001
	TT	2 (0.4)	2 (1.9)	6.91	(0.89–53.43)	0.0640
	GT / TT	74 (15.6)	34 (33.0)	2.75	(1.69–4.48)	4.6×10^{-5}
$P_{\text{trend}} = 5.0 \times 10^{-5}$						

Abbreviations: SNP, single-nucleotide polymorphism; N, number of subjects; OR, odds ratio; CI, confidence interval.

^I Odds ratios adjusted for age.