

Direct evidence for a polygenic etiology in familial multiple myeloma

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Key Points

- Although common risk alleles for multiple myeloma have been identified, their contribution to familial MM is unknown.
- We demonstrate an enrichment of common MM risk alleles in familial cases, providing the first direct evidence for a polygenic contribution.

Although common risk alleles for multiple myeloma (MM) were recently identified, their contribution to familial MM is unknown. Analyzing 38 familial cases identified primarily by linking Swedish nationwide registries, we demonstrate an enrichment of common MM risk alleles in familial compared with 1530 sporadic cases ($P = 4.8 \times 10^{-2}$ and 6.0×10^{-2} , respectively, for 2 different polygenic risk scores) and 10 171 population-based controls ($P = 1.5 \times 10^{-4}$ and 1.3×10^{-4} , respectively). Using mixture modeling, we estimate that about one-third of familial cases result from such enrichments. Our results provide the first direct evidence for a polygenic etiology in a familial hematologic malignancy.

Introduction

Multiple myeloma (MM) is the second most common hematologic malignancy. It is characterized by an uninhibited, clonal growth of plasma cells in the bone marrow, producing a monoclonal immunoglobulin ("M protein") or light chains that can be detected in peripheral blood.¹ It is preceded by monoclonal gammopathy of unknown significance (MGUS),^{2,3} a common condition (3% of ≥ 50 -year-olds) defined as a clonal growth of plasma cells that does not satisfy the criteria for MM, but progresses to MM at a rate of about 1% per year.^{4,5}

Since the 1920s, several authors have reported families with multiple cases of MM, including families both with Mendelian and non-Mendelian pedigrees.^{6,7} In the 2000s, systematic family studies, including in population-based registries, confirmed that first-degree relatives of patients with MM and its precursor condition, monoclonal gammopathy of unknown significance, have 2 to 4 times higher risk for MM,⁸⁻¹³ and a higher risk for lymphomas and certain solid tumors.^{14,15} According to estimates based on Swedish nationwide registries, about 2.4% of MM cases have a first-degree relative with MM.¹⁴

Recently, genome-wide association studies have identified single-nucleotide polymorphisms (SNPs) at 16 independent loci associated with MM risk.¹⁶⁻¹⁹ Although the discovery of risk alleles proves the existence of inherited susceptibility, the genetic background of familial MM remains unclear. The identified risk alleles are common, have modest effects, and have been identified by comparing unselected cases with controls. Although statistical predictions suggest that they account for somewhere on the order of 20% of the familial risk,¹⁸ no direct evidence for a polygenic etiology has been presented. It is still an open question whether familial MM is caused by aggregation of unusually high numbers of common risk alleles or by other factors (eg, rare variants with strong effects or exposure of family members to a common environmental factor).

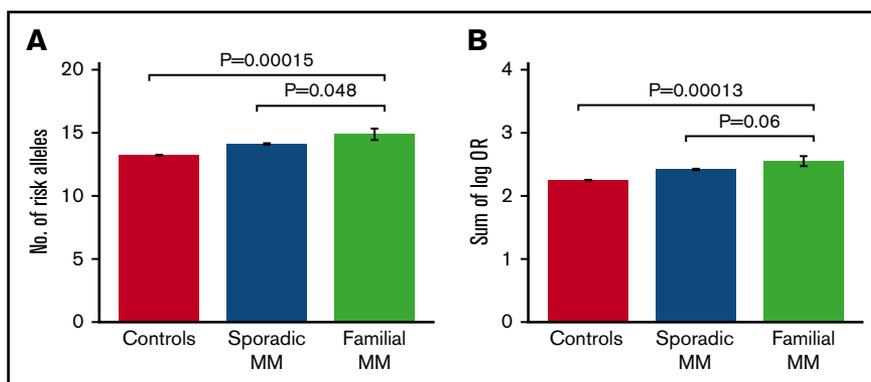


Figure 1. Risk allele burdens for familial MM, sporadic MM, and controls. Risk allele burdens for the 3 study groups, quantified as (A) number of risk alleles, and (B) sum of number of risk alleles per locus weighted by their log-transformed ORs. We detected an enrichment of MM risk alleles in familial cases compared with both sporadic cases and population-based controls. The indicated *P* values were obtained with 1-sided Student *t* test. Error bars indicate standard error of the mean. Of the 38 familial MM cases, 30 had a first-degree relative with MM and 8 had a second-degree relative. No further increase in average risk score was observed when excluding the cases with second-degree relatives (not shown).

Materials and methods

The study design is described in detail in the supplemental Methods. In essence, we carried out a nationwide search for patients with familial MM in Sweden. For this, we used the Swedish Cancer Registry and the Swedish Multi-generation Registry, which record cancer cases and familial relationships, respectively. Linking the registries, we identified 237 patients with MM diagnosed between 1958 and 2013 who had at least 1 first- or second-degree relative with MM. From the Swedish National Myeloma Biobank, we obtained samples from 36 of these patients, representing 24 families. We also obtained samples from 2 second-degree relatives with MM from Norway. In terms of clinical characteristics, the identified familial cases were comparable to sporadic cases (supplemental Tables 1 and 2).

The samples were analyzed using Illumina OmniExpress SNP microarrays. To increase the genotyping resolution, variants identified by whole-genome sequencing by the UK10K and 1000 Genomes consortia^{20,21} were imputed into the microarray data. We then extracted the genotypes of tag SNPs for the 16 risk loci that have been robustly associated with MM (supplemental Table 3) and quantified risk allele burden using 2 different scores: 1 calculated as the total number of risk alleles and 1 calculated by summarizing the number of risk alleles weighted by their log-transformed odds ratios (ORs) across all loci. For this, we used the ORs reported in Mitchell et al¹⁸ (supplemental Table 3). To avoid statistical inflation resulting from relatedness, gene scores from individuals from the same family were averaged. The corresponding scores were calculated for 2 preexisting sets of SNP microarray data: 1 representing 1530 sporadic MM cases from Sweden and Norway,¹⁹ and 1 representing 10 171 population-based controls from Sweden.²² All individuals included in our analyses were of

non-Finnish Scandinavian ancestry, as determined using the 2 top principal components calculated using the PLINK software²³ (supplemental Figure 1).

Results

Comparing the different study groups, we observed a borderline significant enrichment of risk alleles in familial cases compared with both sporadic cases (average number of risk alleles, 14.9 vs 14.1 [1-sided Student *t* test, $P = 4.8 \times 10^{-2}$]; sum of log OR, 2.55 vs 2.42 [$P = 6.0 \times 10^{-2}$]) and population-based controls (average number of risk alleles, 14.9 vs 13.2 [$P = 1.5 \times 10^{-4}$]; sum of log OR, 2.55 vs 2.25 [$P = 1.3 \times 10^{-4}$]; Figure 1; Table 1). In addition, the majority of the individual risk alleles occurred at higher frequencies in familial cases than in sporadic cases, although only 1 of the individual alleles reached statistical significance after correction for multiple testing (supplemental Table 4). The most overrepresented risk alleles were the ones at *CCAT1* (risk allele frequency, 0.55 vs 0.38; uncorrected Cochran-Armitage trend test $P = .002$; Bonferroni-corrected $P = .033$) and *ELL2* (risk allele frequency, 0.89 vs 0.77; uncorrected $P = .011$; corrected $P = .16$; supplemental Table 4). No difference in age of onset was observed between familial and sporadic cases (66.0 vs 68.4 years; *t* test $P = .22$). No differences in polygenic risk scores were observed between men and women within the 3 study groups (*t* test $P = .21-.75$). These results support that at least a subset of familial MM cases are characterized by an enrichment of known, common MM risk alleles.

We next sought to estimate the proportion of familial MM cases that associate with enrichment of common risk alleles. Theoretically, a polygenic risk score can be drawn from either of 2 distributions, depending on the etiology: cases that are caused by enrichment of common MM risk alleles will follow a right-shifted risk score distribution, and cases that have other causes (not reflected in our

Table 1. Comparisons of risk allele burdens between study groups, as well as standard errors of the means (SEM) and standard deviations (SD)

Comparison	Familial			Sporadic			Controls			Student <i>t</i> test <i>P</i> value
	Mean	SEM	SD	Mean	SEM	SD	Mean	SEM	SD	
Familial vs sporadic, number of risk alleles	14.9	0.451	2.26	14.1	0.0586	2.29				4.80e-2
Familial vs sporadic, sum of log (OR)	2.55	0.0792	0.396	2.42	0.0108	0.421				6.00e-2
Familial vs controls, number of risk alleles	14.9	0.451	2.26				13.2	0.0225	2.27	1.50e-4
Familial vs controls, sum of log (OR)	2.55	0.0792	0.396				2.25	0.0041	0.411	1.30e-4

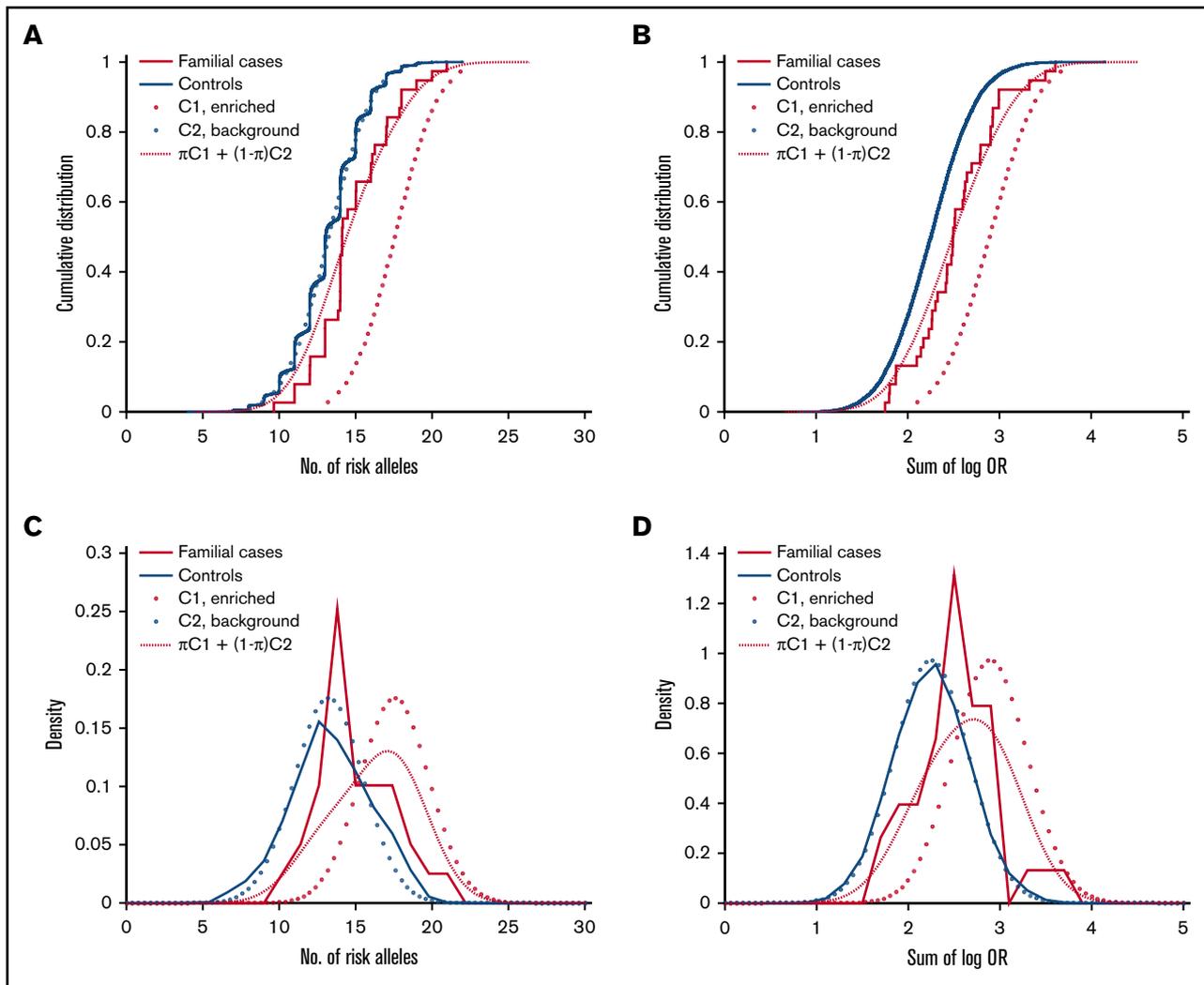


Figure 2. Estimation of the proportion of familial MM cases associated with enrichment of common MM risk alleles and other etiologies, respectively. Theoretically, the risk score of an individual with familial MM can be drawn from either of 2 underlying distributions, depending on the etiology: cases of familial MM associated with enrichment of common risk alleles are expected to follow a right-shifted distribution, whereas cases of MM on the basis of etiologies that are not quantified by our polygenic risk scores will follow the same distribution as population controls (solid blue). The risk score distribution for the group as a whole will be a mixture of these 2 components (C1 and C2; dotted) with proportions π and $1-\pi$, respectively. To estimate π , we fit this 2-component Gaussian mixture distribution (dashed red) to the observed risk scores (solid red): (A) with the number-of-risk-alleles score, the corresponding proportion was 30%, and mean of the enriched component was 17.6; (B) with the sum-of-log-OR score, we estimated π at 37%, and the mean of the enriched component was 2.91. These results support that about one-third of familial MM cases are caused by polygenic inheritance of a high risk allele burden. (C-D) Corresponding density distribution plots for the same data.

risk scores) will follow the same distribution as the background population. To estimate the proportions of these 2 underlying distributions, we fit a 2-component Gaussian mixture model to the observed risk scores of familial MM cases (Figure 2). With the number-of-risk-alleles score, the proportion of the component representing cases caused by a high risk allele burden was estimated at 32% (90% confidence interval, 19%-53%), with a mean of 17.6 (compared with 13.2 for controls; Figure 2A). With the sum-of-log-OR score, the corresponding proportion was estimated at 37% (90% confidence interval, 21%-65%) with a mean of 2.91 (compared with 2.25 for controls; Figure 2B). These results indicate that about one third of familial cases are a result of enrichments of common MM risk alleles.

Hypothetically, familial MM could be caused by rare mutations with strong effects that are not represented in the single-nucleotide

polymorphism microarray data. For completeness, we therefore analyzed 29 of the Swedish cases plus 6 cases (from 3 families) from Germany by whole-exome sequencing. As expected from the small sample size, we did not detect any variants with exome-wide significance. Looking specifically for rare variants in genes known to be associated with MM^{16-19,24} (supplemental Table 3) and genes frequently somatically mutated in MM plasma cells^{25,26} (supplemental Methods), we detected 10 nonsynonymous variants with minor allele frequency less than 1%. Only 2 of these were found in more than 1 case, and none of them were obviously pathogenic (supplemental Table 5).

Discussion

In conclusion, we describe the first direct association between a polygenic feature and familial MM. No similar evidence has been put

forward for any hematologic malignancy. Although the enrichment of common MM risk alleles among familial cases is of modest magnitude when calculated across the familial MM study group as a whole, our data indicate that polygenic risk scores for individuals with familial MM follow a bimodal distribution. We estimate that about one third of familial MM cases are caused by enrichments of risk alleles (Figure 2), whereas the rest follow the background distribution and likely have other causes. For example, the remaining two-thirds of cases could be caused by as yet undiscovered rare mutations or exposure to common environmental factors. Unfortunately, our sample size did not allow exome-wide screening for rare mutations. Another limitation of our study is that the Swedish registries do not record monoclonal gammopathy of unknown significance cases. We anticipate that future studies, including large-scale sequencing efforts, will provide a more detailed understanding of the genetic basis of familial MM and set the stage for clinical applications.

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Authorship

Contribution: B.-M.H., B.N., and M.H. designed the research; I.T., B.N., M.H., U.-H.M., E.A., H.N., A.-K.W., E.J., K.L., K.H., A.W., N.W., H.G., C.L., M.P., A.F., U.G., and T.G. organized and carried out the collection of samples; B.-M.H., B.N., E.J., C.C., A.N., and M.A. carried out genetic analyses; B.-M.H. and B.N. analyzed data and drafted the manuscript; and all authors contributed to data interpretation and the final manuscript.

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References

1. Rajkumar SV, Dimopoulos MA, Palumbo A, et al. International Myeloma Working Group updated criteria for the diagnosis of multiple myeloma. *Lancet Oncol*. 2014;15(12):e538-e548.
2. Landgren O, Kyle RA, Pfeiffer RM, et al. Monoclonal gammopathy of undetermined significance (MGUS) consistently precedes multiple myeloma: a prospective study. *Blood*. 2009;113(22):5412-5417.
3. Weiss BM, Abadie J, Verma P, Howard RS, Kuehl WM. A monoclonal gammopathy precedes multiple myeloma in most patients. *Blood*. 2009;113(22):5418-5422.
4. Kyle RA, Therneau TM, Rajkumar SV, et al. A long-term study of prognosis in monoclonal gammopathy of undetermined significance. *N Engl J Med*. 2002;346(8):564-569.
5. Kyle RA, Therneau TM, Rajkumar SV, et al. Prevalence of monoclonal gammopathy of undetermined significance. *N Engl J Med*. 2006;354(13):1362-1369.
6. Koura DT, Langston AA. Inherited predisposition to multiple myeloma. *Ther Adv Hematol*. 2013;4(4):291-297.
7. Morgan GJ, Johnson DC, Weinhold N, et al. Inherited genetic susceptibility to multiple myeloma. *Leukemia*. 2014;28(3):518-524.
8. Landgren O, Kristinsson SY, Goldin LR, et al. Risk of plasma cell and lymphoproliferative disorders among 14621 first-degree relatives of 4458 patients with monoclonal gammopathy of undetermined significance in Sweden. *Blood*. 2009;114(4):791-795.
9. Altieri A, Chen B, Bermejo JL, Castro F, Hemminki K. Familial risks and temporal incidence trends of multiple myeloma. *Eur J Cancer*. 2006;42(11):1661-1670.
10. Bourguet CC, Grufferman S, Delzell E, DeLong ER, Cohen HJ. Multiple myeloma and family history of cancer. A case-control study. *Cancer*. 1985;56(8):2133-2139.
11. Ogmundsdóttir HM, Einarsdóttir HK, Steingrimsdóttir H, Haraldsdóttir V. Familial predisposition to monoclonal gammopathy of unknown significance, Waldenström's macroglobulinemia, and multiple myeloma. *Clin Lymphoma Myeloma*. 2009;9(1):27-29.
12. Ogmundsdóttir HM, Haraldsdóttir V, Jóhannesson GM, et al. Familiality of benign and malignant paraproteinemias. A population-based cancer-registry study of multiple myeloma families. *Haematologica*. 2005;90(1):66-71.
13. Camp NJ, Werner TL, Cannon-Albright LA. Familial myeloma. *N Engl J Med*. 2008;359(16):1734-1735; author reply 1735.
14. Frank C, Fallah M, Chen T, et al. Search for familial clustering of multiple myeloma with any cancer. *Leukemia*. 2016;30(3):627-632.
15. Van Valkenburg ME, Pruitt GI, Brill IK, et al. Family history of hematologic malignancies and risk of multiple myeloma: differences by race and clinical features. *Cancer Causes Control*. 2016;27(1):81-91.
16. Broderick P, Chubb D, Johnson DC, et al. Common variation at 3p22.1 and 7p15.3 influences multiple myeloma risk. *Nat Genet*. 2011;44(1):58-61.
17. Chubb D, Weinhold N, Broderick P, et al. Common variation at 3q26.2, 6p21.33, 17p11.2 and 22q13.1 influences multiple myeloma risk. *Nat Genet*. 2013;45(10):1221-1225.

18. Mitchell JS, Li N, Weinhold N, et al. Genome-wide association study identifies multiple susceptibility loci for multiple myeloma. *Nat Commun.* 2016;7:12050.
19. Swaminathan B, Thorleifsson G, Jöud M, et al. Variants in ELL2 influencing immunoglobulin levels associate with multiple myeloma. *Nat Commun.* 2015;6:7213.
20. Abecasis GR, Auton A, Brooks LD, et al; 1000 Genomes Project Consortium. An integrated map of genetic variation from 1,092 human genomes. *Nature.* 2012;491(7422):56-65.
21. Walter K, Min JL, Huang J, et al; UK10K Consortium. The UK10K project identifies rare variants in health and disease. *Nature.* 2015;526(7571):82-90.
22. Ripke S, O'Dushlaine C, Chambert K, et al; Multicenter Genetic Studies of Schizophrenia Consortium; Psychosis Endophenotypes International Consortium; Wellcome Trust Case Control Consortium 2. Genome-wide association analysis identifies 13 new risk loci for schizophrenia. *Nat Genet.* 2013;45(10):1150-1159.
23. Chang CC, Chow CC, Tellier LC, Vattikuti S, Purcell SM, Lee JJ. Second-generation PLINK: rising to the challenge of larger and richer datasets. *Gigascience.* 2015;4:7.
24. Weinhold N, Johnson DC, Chubb D, et al. The CCND1 c.870G>A polymorphism is a risk factor for t(11;14)(q13;q32) multiple myeloma. *Nat Genet.* 2013;45(5):522-525.
25. Chapman MA, Lawrence MS, Keats JJ, et al. Initial genome sequencing and analysis of multiple myeloma. *Nature.* 2011;471(7339):467-472.
26. Lohr JG, Stojanov P, Carter SL, et al; Multiple Myeloma Research Consortium. Widespread genetic heterogeneity in multiple myeloma: implications for targeted therapy. *Cancer Cell.* 2014;25(1):91-101.