Published in final edited form as:
Leuk Res. 2009 December ; 33(12): 1609–1614. doi:10.1016/j.leukres.2009.03.013.

Genetic variation in cell cycle and apoptosis related genes and multiple myeloma risk

H. Dean Hosgood III[1], **Dalsu Baris**[1], **Yawei Zhang**[2], **Sonja I. Berndt**[1], **Idan Menashe**[1], **Lindsay M. Morton**[1], **Kyoung-Mu Lee**[1], **Meredith Yeager**[1], **Shelia H Zahm**[1], **Stephen Chanock**[1], **Tongzhang Zheng**[2], and **Qing Lan**[1]

[1] Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Department of Health and Human Services, Bethesda, MD

[2] Department of Epidemiology and Public Health, Yale School of Medicine, New Haven, CT

Abstract

Genetic variation may be an important risk factor for multiple myeloma. A hallmark of tumor formation and growth is cell cycle dysregulation and apoptosis avoidance. We previously reported the association of genetic variation in caspase genes, the apoptotic-regulating family, and multiple myeloma risk. To further examine if genetic variation in key cell cycle and apoptosis genes alters multiple myeloma risk, we genotyped 276 tag SNPs in 27 gene regions in a population-based case-control study of non-Hispanic Caucasian women (108 cases; 482 controls) in Connecticut. Logistic regression assessed the effect of each SNP on multiple myeloma risk and the minP test assessed the association at the gene region level. Three gene regions were significantly associated with risk of multiple myeloma (*BAX* minP = 0.018, *CASP9* minP = 0.025, and *RIPK1* minP = 0.037). Further explorations identified the most significant variant of *BAX*, *RIPK1*, and *CASP9* to be rs1042265, rs9391981, and rs751643, respectively. The *A* variant at rs1042265 (OR_{GA+AA} = 0.40, 95%CI = 0.21 – 0.78) and the *C* variant at rs9391981 (OR_{GC+CC} = 0.32, 95%CI = 0.12 – 0.81) were associated with a decreased risk of multiple myeloma. The *G* variant at rs7516435 was associated with an increased risk of multiple myeloma (OR_{AG} = 1.48, 95%CI = 0.94 – 2.32; OR_{GG} = 2.59, 95%CI = 1.30 – 5.15; p_{trend} = 0.005). Haplotype analyses supported the SNP findings. These findings suggest that genetic variation in cell cycle and apoptosis genes may play a key role in multiple myeloma and warrant further investigation through replication studies.

Keywords

multiple myeloma; caspase; BAX; RIPK1; cell cycle

Introduction

Multiple myeloma is a B-cell hematological malignancy that accounts for an estimated 19,900 incident cancer cases per year in the United States (1). Multiple myeloma cells affect the bone

Correspondence: H. Dean Hosgood, email (hosgoodd@mail.nih.gov), phone (301-594-4649), fax (301-402-1819), National Cancer Institute, Division of Cancer Epidemiology and Genetics, Occupational and Environmental Epidemiology Branch. 6120 Executive Blvd., EPS 8118, MCS 7240, Bethesda, Maryland 20892-7240.

No authors have any or potential conflicts of interest.

marrow microenvironment by adhering to extracellular matrix proteins, as well as bone marrow stromal cells. These adhesions made by the tumor cells stimulate cytokine-mediated tumor growth by activating numerous cell growth and survival pathways. Pathways that are activated include the extracellular signal-regulated kinase (ERK), the Janus kinase 2 / signal transducer and activator of transcription 3 (JAK2/STAT3), the phosphatidylinositol 3-kinase (PI3K/Akt), the Ras-Raf-MAPK kinase (MEK), and the nuclear factor κβ (NFκβ) pathways (2). As multiple myeloma cells localize in the bone microenvironment, the transforming growth factor β (TNFβ) and vascular epithelial growth factor (VEGF) are also upregulated, leading to further activation of cell growth and survival pathways. The downstream biologic effect of these pathways is the upregulation of cell cycle and apoptosis related genes, such as the pro-apoptotic gene, *BAX* (3) and the two main apoptotic pathways in humans, which both utilize the caspase enzyme cascade (4;5). Therefore, it is hypothesized that genetic variation in cell cycle and apoptosis genes could alter multiple myeloma susceptibility.

Although multiple myeloma initiation and progression are well characterized, there have been few known risk factors identified, besides race, for this incurable disease (6). Family history of multiple myeloma in a first-degree relative is also thought to contribute to the risk of myeloma (7), with several studies showing a 2-fold or more increased risk of multiple myeloma associated with family history of multiple myeloma (8;9). These studies also suggest that genetic variation may play a role in the etiology of myeloma.

The genetic susceptibility to multiple myeloma has not been extensively studied. While studies have evaluated polymorphisms in immune response (10-12), xenobiotic metabolism (13-15), and DNA repair genes (16;17), few studies have evaluated genetic variation in the cell cycle pathway. Our previous study evaluating the four key apoptosis related caspase genes and myeloma risk found significant associations with *CASP3* rs1049216 and *CASP9* rs1052576 (18). To extend these initial findings and to test our hypothesis that cell cycle polymorphisms may also play a role in multiple myeloma risk, we genotyped 276 tag single nucleotide polymorphisms (SNPs) in 27 cell cycle and apoptosis gene regions in a population-based case-control study.

Design and Methods

This population-based case-control study has been described previously (18). Briefly, female cases residing in Connecticut were histologically-confirmed incident multiple myeloma cases diagnosed between 1996 and 2000, aged 21 to 84 years, with no previous diagnosis of cancer, except non-melanoma skin cancer. Cases were alive at the time of interview and 183 interviews were obtained from the 323 eligible cases. Population-based female controls (n = 691) aged 21 to 84 years, were recruited for a parallel study of non-Hodgkin lymphoma, by random digit dialing methods for those below age 65 years and Health Care Financing Administration files for those 65 years and older. Participation rates for cases, controls below age 65, and controls 65 years and older were 57%, 69% and 47%, respectively.

Genomic DNA for genotyping was extracted from peripheral blood or buccal cells for 128 cases and 517 controls, using a phenol-chloroform extraction method (19). Three cases were excluded because of insufficient DNA. Tag SNPs were chosen from the designable set of common SNPs (minor allele frequency >5%) genotyped in the Caucasian population sample of the HapMap Project (Data Release 20/Phase II, NCBI Build 35 assembly, dpSNPb125) using Tagzilla (http://tagzilla.nci.nih.gov/), which implements a tagging algorithm based on the pairwise binning method (20). For each gene region, SNPs located within 20kb 5′ of the start of transcription (exon 1) and 10kb 3′ of the end of the last exon were grouped and selected using a binning threshold of r^2>0.8. When there were multiple transcripts available for the gene, the primary transcript was assessed. Tag SNPs were genotyped using a GoldenGate assay

(www.illumina.com), at the National Cancer Institute's Core Genotyping Facility (Gaithersburg, MD). Genes were included in this custom assay if they were previously reported to be associated with or hypothesized to be associated with hematopoietic diseases. Blinded duplicate and replicate samples were interspersed throughout the genotyping plates to assess quality control. All tag SNPs had concordance rates ≥ 95% and completion rates ≥ 90%. Subjects with a completion rate <90% were excluded (n = 2 controls). Thus, genotyping for the 290 cell cycle and apoptosis tag SNPs was successful for 125 cases and 515 controls. After restriction to only non-Hispanic Caucasian subjects to maximize study population homogeneity, 108 non-Hispanic Caucasian female cases and 482 non-Hispanic Caucasian female controls remained for analysis.

Of the 290 genotyped tag SNPs, 12 were excluded from analysis due to low minor allele frequency (<0.05). Hardy-Weinberg equilibrium (HWE) for each tag SNP was tested in controls with a Pearson χ^2 test or a Fischer's exact test if any of the cell counts were less than five. Fourteen tag SNPs (4.8%) deviated from HWE ($p \leq 0.05$), which was expected by chance. Two SNPs deviated substantially from HWE ($p \leq 0.001$) and were consequently removed from further analyses. Therefore, a total of 276 tag SNPs in 27 gene regions were analyzed. Unconditional logistic regression estimated the odds ratio (OR) and 95% confidence interval (CI) for the association between multiple myeloma risk and each tag SNP. The homozygote of the common allele was used as the reference group and the ORs and 95% CIs were adjusted for age (<50, 50–59, 60-69, and ≥70 years). Gene-dose effects were estimated by a linear trend test based on the number of variant alleles present (0, 1, 2). To assess the significance of the association between each gene region and multiple myeloma, MatLab was used to perform a minP test that assesses the significance of the minimal p-value in each gene region using a permutation-based resampling procedure (1,000 permutations) that takes into account the number of SNPs genotyped in each gene region and their underlying linkage disequilibrium structure (21).

Finally, haplotype blocks and structure were determined by the solid spine LD algorithm in Haploview (www.broad.mit.edu/mpg/haploview) using data from controls for the significant gene regions. Haplotype frequencies were estimated using the expectation-maximization algorithm (22). Haplotypes with frequencies less than 1% were excluded. The overall difference in haplotype frequencies between cases and controls was assessed using a global score test (23). Haplotype ORs and 95% CIs were estimated, adjusted for age (<50, 50–59, 60-69, and ≥70 years). All statistical analyses were performed with SAS unless stated otherwise.

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.

Results

Cases were slightly older than controls in our study population of non-Hispanic Caucasian females (p = 0.04) (Table I). Cases and controls had similar education, marital, and smoking statuses (p > 0.05).

Three of the 27 gene regions involved in the cell cycle and apoptosis pathways were significantly associated with risk of multiple myeloma (*BAX* minP = 0.018, *CASP9* minP = 0.025, and *RIPK1* minP = 0.037) (Table II). Of the 23 tag SNPs that were genotyped in these significant regions, four were significantly associated ($p_{trend} \leq 0.05$) with multiple myeloma risk (Table III). Supplemental table I provides the allele distribution among cases and controls, as well as, multiple myeloma risk associated with all genotyped tag SNPs. Although several

SNPs were associated with multiple myeloma risk in the *BLC2L10*, *CASP1/4/*5, and *BCL2A1* gene regions, those regions had a non-significant minP and thus those SNPs were thought to be more likely chance findings.

The most significant variant in the *BAX* region was the *A* variant at rs1042265, which was associated with a 60% decreased risk of multiple myeloma (OR_{GA+AA} = 0.40, 95%CI = 0.21 – 0.78) (Table III). The *C* variant at *RIPK1* rs9391981 was also associated with a decreased risk of multiple myeloma (OR_{GC+CC} = 0.32, 95%CI = 0.12 – 0.81). The *CASP9* genomic region had two significant tag SNPs, rs4646047 and rs7516435, which were moderately correlated (D' = 0.99, r^2 = 0.35). When including rs4646047 and rs7416435 in the same model, only rs7516435 remained significantly associated with multiple myeloma risk. The *G* variant at rs7516435 was associated with an increased risk of multiple myeloma (OR_{AG+GG} = 1.65, 95% CI = 1.08 – 2.53).

The linkage disequilibrium structures for the three genetic regions associated with risk of multiple myeloma (*BAX*, *CASP9*, and *RIPK1*) are shown in supplemental figure 1. The linkage disequilibrium plot for *CASP9* identified only one haplotype block and it was significantly associated with multiple myeloma risk ($P_{global\ test}$ = 0.011). Haplotype analyses for the *CASP9* block suggested that the increased risk associated with *CASP9* and multiple myeloma was driven by rs7516435 (Table IV). The addition of our previously reported *CASP9* rs1052576 to our haplotype analysis did not add further information. Haplotype analyses for *BAX* and *RIPK1* did not provide any additional information beyond the individual tag SNP analyses.

Discussion

Our exploratory analysis of genetic variation in the cell cycle and apoptosis pathways identified three gene regions that were significantly associated with multiple myeloma risk (*BAX*, *CASP9*, and *RIPK1*). Further explorations identified the most significant variant as rs1042265, rs7516435, and rs9391981 for *BAX*, *CASP9*, and *RIPK1*, respectively.

The two main apoptotic pathways in humans, the extrinsic or receptor-mediated pathway and the intrinsic or mitochondrial pathway, both utilize the caspase enzyme cascade (4;5). *CASP9* is a member of the intrinsic pathway, which is activated as a result of mitochondrial damage and cytochrome c release. After cytochrome c is released into the cytoplasm, the apoptosome is formed and the caspase-9 cascade is activated (24). Our previous study in this population found a significant inverse association between the *A* variant at *CASP9* rs1052576 and multiple myeloma risk (18). The *A* variant at *CASP9* rs1052576 was also associated with a decreased risk of non-Hodgkin's lymphoma in our parallel study (25). *CASP9* rs7516435 and rs1052576 were moderately correlated in our population (D' = 0.98; r^2 = 0.35). Our study found the *G* variant at *CASP9* rs7516435 to also be associated with risk of multiple myeloma and inclusion of both SNPs in the same model revealed that the association was driven by the rs7516435 variant. It should be noted that rs7516435 is an intron in *DNAJC16*, upstream of *CASP9*; therefore, this variant may affect *DNAJC16* as well as *CASP9*. DnaJ proteins play critical roles in many biological processes, including cell cycle regulation (26) and have been recently associated with breast and brain cancer etiology (27;28). If our findings in the *CASP9* gene region are replicated in another population, further research should determine if one of these SNPs or another SNP in linkage disequilibrium with these SNPs is functionally important.

Although not fully understood, the activation of *CASP9* by mitochondrial release of cytochrome c is believed to be regulated by the BCL-2-related protein family, through both anti-apoptotic (BCL-2) and the pro-apoptotic protein BAX, which are both regulated by p53 (3). Interestingly, our study also found genetic variation in the *BAX* gene region, particularly

in an exon located in the 3′UTR (rs1042265), to be associated with decreased risk of multiple myeloma. Previous studies evaluating genetic susceptibility to *BAX* polymorphisms have focused on another B-cell neoplasm, chronic lymphocytic leukemia (CLL). The *A* variant at *BAX* rs4645878 has been shown to be associated with decreased BAX expression and overall survival in CLL patients (29); however, a larger study did not replicate these findings (30). When evaluating acute lymphoblastic leukemia (ALL) and acute myeloblastic leukemia (AML) patients, overexpression of BAX mRNA suggested that alteration of BAX transcription may be involved with leukemogenesis (31).

RIPK1 is a member of a kinase family that are integral mediators of cellular response to stress (32). RIPK1 aids the cell in oxidative stress management by interacting with the cell surface receptor death domains, such as TNF, to activate NFκβ (33). This initiation of NFκβ leads to the activation of the caspase cascade and consequently cell death. Reports of genetic susceptibility to multiple myeloma involving the TNF pathway have focused on candidate polymorphisms in *TNF-α* (34-36), with one study finding a significant inverse association with the *A* variant at *TNF-α* -308 (34) and the other two studies founding no association with the *A* variant (35;36). Our study is the first, to the best of our knowledge, to suggest that genetic variation in a *RIPK1* intron (rs9391981) may be important to the risk of multiple myeloma.

Finally, the current analysis did not find a significant association between multiple myeloma risk and *CASP3*. Our previously reported *CASP3* rs1049216 (18) was highly correlated with *CASP3* rs2705897 (D' = 0.97; r2 = 0.94) from this tag SNP analysis; however, this analysis has a smaller sample and thus lower power to detect an association. Another possible explanation is that the previously reported association was a chance finding. Replication studies are needed to further evaluate this association.

Our study had several limitations. Our study had a modest sample size, which may have led to false positive and negative findings (37). We attempted to account for spurious findings by using the minP permutation method. The use of this gene-based permutation analysis identifies genes' true significance by comparing the observed association with the distribution of gene-disease associations seen in 1000 randomly generated populations. This robust method can be useful in identifying important genomic regions associated with multiple myeloma. However, it is possible that some associations significant at the SNP level but deemed non-significant at the gene level are indeed true positive findings. Similarly, it is possible some of our findings even if significant at the gene level are false positives. However, our results are biologically plausible, given in the importance of the cell cycle and apoptosis pathways in multiple myeloma pathogenesis. Another concern is that our study had a relatively low participation rate; however, bias in our results is unlikely, since genotyping frequencies have been found to be very similar based on participation status among controls in other studies (38). As our analysis included only non-Hispanic whites, the generalizability of our findings to other ethnic groups is limited. While the allelic frequency variation by ethnicity in HapMap is minimal for rs1042265 and rs9391981, the *G* allele at rs7516435 is only 22% among individuals of European descent, compared to 57% and 62% for Asian and African descent, respectively. Further, it is unknown if genetic variation at rs1042265, rs9391981, and rs7516435 alters the protein coding sequence, functionality, or expression levels of their respective genes. Finally, our findings may not generalize to all multiple myeloma cases since disease stage and aggressiveness were not available.

In conclusion, this population-based case-control study is the first to comprehensively evaluate the risk of multiple myeloma associated with genetic variation in the cell cycle and apoptosis pathways, to the best of our knowledge. Our study suggests that polymorphisms in the *BAX*, *CASP9*, and *RIPK1* gene regions may be important for the etiology of multiple myeloma. However, given that candidate gene analyses can hardly distinguish functional SNPs from

genetic markers, our results should be viewed as exploratory until they are replicated in larger studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

All authors had full access to all of the study data and have contributed to, seen, and approved the final version of the manuscript. TZ, QL, SZ, and SC designed this study, managed data collection, and participated in data processing. HDH participated in data processing, conducted most of the analyses, and was primarily responsible for writing the paper. IM was also involved with data processing. The analysis incorporated suggestions by DB, YZ, SB, LMM, KL, MY, and QL.

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 U.S. Government.

Reference List

1. Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Cancer statistics, 2007. CA Cancer J Clin 2007 Jan;57(1):43–66. [PubMed: 17237035]
2. Hideshima T, Mitsiades C, Tonon G, Richardson PG, Anderson KC. Understanding multiple myeloma pathogenesis in the bone marrow to identify new therapeutic targets. Nat Rev Cancer 2007 Aug;7(8): 585–98. [PubMed: 17646864]
3. Adams JM, Cory S. The Bcl-2 protein family: arbiters of cell survival. Science 1998 Aug 28;281(5381): 1322–6. [PubMed: 9735050]
4. Hajra KM, Liu JR. Apoptosome dysfunction in human cancer. Apoptosis 2004 Nov;9(6):691–704. [PubMed: 15505412]
5. Nicholson DW, Thornberry NA. Caspases: killer proteases. Trends Biochem Sci 1997 Aug;22(8):299–306. [PubMed: 9270303]
6. De Roos, A.; Baris, D.; Weiss, N.; Herrinton, L. Multiple Myeloma. In: Schottenfeld, D.; Fraumeni, JF., editors. Cancer epidemiology and prevention. Vol. 3rd. New York: Oxford University Press; 2006.
7. Alexander DD, Mink PJ, Adami HO, Cole P, Mandel JS, Oken MM, et al. Multiple myeloma: a review of the epidemiologic literature. Int J Cancer 2007;120:40–61. [PubMed: 17405120]
8. Landgren O, Linet MS, McMaster ML, Gridley G, Hemminki K, Goldin LR. Familial characteristics of autoimmune and hematologic disorders in 8,406 multiple myeloma patients: a population-based case-control study. Int J Cancer 2006 Jun 15;118(12):3095–8. [PubMed: 16395700]
9. Hemminki K, Li X, Czene K. Familial risk of cancer: data for clinical counseling and cancer genetics. Int J Cancer 2004 Jan 1;108(1):109–14. [PubMed: 14618624]
10. Zheng C, Huang D, Liu L, Bjorkholm M, Holm G, Yi Q, et al. Cytotoxic T-lymphocyte antigen-4 microsatellite polymorphism is associated with multiple myeloma. Br J Haematol 2001 Jan;112(1): 216–8. [PubMed: 11167807]
11. Zheng C, Huang D, Liu L, Wu R, Bergenbrant GS, Osterborg A, et al. Interleukin-10 gene promoter polymorphisms in multiple myeloma. Int J Cancer 2001 May 20;95(3):184–8. [PubMed: 11307152]
12. Brown EE, Lan Q, Zheng T, Zhang Y, Wang SS, Hoar-Zahm S, et al. Common variants in genes that mediate immunity and risk of multiple myeloma. Int J Cancer. 2007 Feb 21;
13. Lincz LF, Kerridge I, Scorgie FE, Bailey M, Enno A, Spencer A. Xenobiotic gene polymorphisms and susceptibility to multiple myeloma. Haematologica 2004 May;89(5):628–9. [PubMed: 15136237]
14. Kang SH, Kim TY, Kim HY, Yoon JH, Cho HI, Yoon SS, et al. Protective role of CYP1A1*2A in the development of multiple myeloma. Acta Haematol 2008;119(1):60–4. [PubMed: 18285692]

15. Ortega MM, Nascimento H, Melo MB, Teori MT, Costa FF, Lima CS. Polymorphisms of glutathione S-transferase mu1 (GSTM1) and theta1 (GSTT1) genes in multiple myeloma. Acta Haematol 2003;109(2):108–9. [PubMed: 12624497]

16. Velangi MR, Matheson EC, Morgan GJ, Jackson GH, Taylor PR, Hall AG, et al. DNA mismatch repair pathway defects in the pathogenesis and evolution of myeloma. Carcinogenesis 2004 Oct;25 (10):1795–803. [PubMed: 15142887]

17. Hayden PJ, Tewari P, Morris DW, Staines A, Crowley D, Nieters A, et al. Variation in DNA repair genes XRCC3, XRCC4, XRCC5 and susceptibility to myeloma. Hum Mol Genet 2007 Dec 15;16 (24):3117–27. [PubMed: 17901044]

18. Hosgood HD III, Baris D, Zhang Y, Zhu Y, Zheng T, Yeager M, et al. Caspase polymorphisms and genetic susceptibility to multiple myeloma. Hematol Oncol. 2008 Apr 1;

19. Garcia-Closas M, Egan KM, Abruzzo J, Newcomb PA, Titus-Ernstoff L, Franklin T, et al. Collection of genomic DNA from adults in epidemiological studies by buccal cytobrush and mouthwash. Cancer Epidemiol Biomarkers Prev 2001 Jun;10(6):687–96. [PubMed: 11401920]

20. Carlson CS, Eberle MA, Rieder MJ, Yi Q, Kruglyak L, Nickerson DA. Selecting a maximally informative set of single-nucleotide polymorphisms for association analyses using linkage disequilibrium. Am J Hum Genet 2004 Jan;74(1):106–20. [PubMed: 14681826]

21. Chen BE, Sakoda LC, Hsing AW, Rosenberg PS. Resampling-based multiple hypothesis testing procedures for genetic case-control association studies. Genet Epidemiol 2006 Sep;30(6):495–507. [PubMed: 16755536]

22. Excoffier L, Slatkin M. Maximum-likelihood estimation of molecular haplotype frequencies in a diploid population. Mol Biol Evol 1995 Sep;12(5):921–7. [PubMed: 7476138]

23. Schaid DJ, Rowland CM, Tines DE, Jacobson RM, Poland GA. Score tests for association between traits and haplotypes when linkage phase is ambiguous. Am J Hum Genet 2002 Feb;70(2):425–34. [PubMed: 11791212]

24. Shivapurkar N, Reddy J, Chaudhary PM, Gazdar AF. Apoptosis and lung cancer: a review. J Cell Biochem 2003 Apr 1;88(5):885–98. [PubMed: 12616528]

25. Lan Q, Zheng T, Chanock S, Zhang Y, Shen M, Wang SS, et al. Genetic variants in caspase genes and susceptibility to non-Hodgkin lymphoma. Carcinogenesis. 2006 Oct 27;

26. Helmbrecht K, Zeise E, Rensing L. Chaperones in cell cycle regulation and mitogenic signal transduction: a review. Cell Prolif 2000 Dec;33(6):341–65. [PubMed: 11101008]

27. Mitra A, Fillmore RA, Metge BJ, Rajesh M, Xi Y, King J, et al. Large isoform of MRJ (DNAJB6) reduces malignant activity of breast cancer. Breast Cancer Res 2008 Mar 7;10(2):R22. [PubMed: 18328103]

28. Lindsey JC, Lusher ME, Strathdee G, Brown R, Gilbertson RJ, Bailey S, et al. Epigenetic inactivation of MCJ (DNAJD1) in malignant paediatric brain tumours. Int J Cancer 2006 Jan 15;118(2):346–52. [PubMed: 16049974]

29. Starczynski J, Pepper C, Pratt G, Hooper L, Thomas A, Milligan D, et al. Common polymorphism G (-248)A in the promoter region of the bax gene results in significantly shorter survival in patients with chronic lymphocytic Leukemia once treatment is initiated. J Clin Oncol 2005 Mar 1;23(7):1514–21. [PubMed: 15735127]

30. Skogsberg S, Tobin G, Krober A, Kienle D, Thunberg U, Aleskog A, et al. The G(-248)A polymorphism in the promoter region of the Bax gene does not correlate with prognostic markers or overall survival in chronic lymphocytic leukemia. Leukemia 2006 Jan;20(1):77–81. [PubMed: 16307023]

31. Wojcik I, Szybka M, Golanska E, Rieske P, Blonski JZ, Robak T, et al. Abnormalities of the P53, MDM2, BCL2 and BAX genes in acute leukemias. Neoplasma 2005;52(4):318–24. [PubMed: 16059649]

32. Meylan E, Tschopp J. The RIP kinases: crucial integrators of cellular stress. Trends Biochem Sci 2005 Mar;30(3):151–9. [PubMed: 15752987]

33. Devin A, Cook A, Lin Y, Rodriguez Y, Kelliher M, Liu Z. The distinct roles of TRAF2 and RIP in IKK activation by TNF-R1: TRAF2 recruits IKK to TNF-R1 while RIP mediates IKK activation. Immunity 2000 Apr;12(4):419–29. [PubMed: 10795740]

34. Morgan GJ, Adamson PJ, Mensah FK, Spink CF, Law GR, Keen LJ, et al. Haplotypes in the tumour necrosis factor region and myeloma. Br J Haematol 2005 May;129(3):358–65. [PubMed: 15842659]

35. Zheng C, Huang DR, Bergenbrant S, Sundblad A, Osterborg A, Bjorkholm M, et al. Interleukin 6, tumour necrosis factor alpha, interleukin 1beta and interleukin 1 receptor antagonist promoter or coding gene polymorphisms in multiple myeloma. Br J Haematol 2000 Apr;109(1):39–45. [PubMed: 10848780]

36. Brown EE, Lan Q, Zheng T, Zhang Y, Wang SS, Hoar-Zahm S, et al. Common variants in genes that mediate immunity and risk of multiple myeloma. Int J Cancer 2007 Jun 15;120(12):2715–22. [PubMed: 17315188]

37. Wacholder S, Chanock S, Garcia-Closas M, El GL, Rothman N. Assessing the probability that a positive report is false: an approach for molecular epidemiology studies. Journal of the National Cancer Institute 2004 Mar 17;96(6):434–42. [PubMed: 15026468]

38. Bhatti P, Sigurdson AJ, Wang SS, Chen J, Rothman N, Hartge P, et al. Genetic variation and willingness to participate in epidemiologic research: data from three studies. Cancer Epidemiol Biomarkers Prev 2005 Oct;14(10):2449–53. [PubMed: 16214931]

Table I

Characteristics of multiple myeloma cases and controls

	Cases	%	Controls	%	*p*-value
Age (years)					**0.04**
< 50	8	7.4	91	18.9	
50 – 59	22	20.4	89	18.5	
60 – 69	30	27.8	109	22.6	
70 +	48	44.4	193	40.0	
Education					0.09
<12 years	15	13.9	52	10.8	
High School Graduate	37	34.3	119	24.7	
Vocational / some college	32	29.6	144	29.9	
College degree	15	13.9	97	20.1	
Graduate / professional degree	9	8.3	70	14.5	
Marital Status					0.20
Married / living as married	58	53.7	285	59.1	
Widowed	28	25.9	119	24.7	
Seperated / Divorced	13	12.0	60	12.4	
Never married	9	8.3	18	3.7	
Smoking (pack years)					0.59
Never smoker	57	52.8	221	45.9	
<6.3	11	10.2	65	13.5	
>6.3 - 16.5	10	9.3	61	12.7	
>16.5 - 33.0	13	12.0	60	12.4	
>33.0	14	13.0	74	15.4	

Table II

Cell cycle and apoptosis genomic regions associated with multiple myeloma

Genomic Region*	Gene Name	Genes (and # of SNPs) included	Total SNPs	minP**
BAX	Bcl2-associated X protein	*bax* (4), *dhdh* (3), *gys1* (2)	9	**0.018**
CASP9	caspase 9	*casp9* (4), *dnajc16* (1), *ela2b* (2)	7	**0.025**
RIPK1	receptor-interacting serine-threonine kinase 1	*ripk1* (6), *serpinbp1* (1)	7	**0.037**
BCL2L10	BCL2-like 10	*bcl2l10* (2), *gnb5* (3)	5	0.104
CASP1/4/5	caspase 1, 4, and 5	*casp1* (4), *casp4* (6), *casp5* (9), *loc440067* (2)	21	0.148
BCL2A1	BCL2-related protein A1	*bcl2a1* (8)	8	0.155
TP53I3	tumor protein p53 inducible protein 3	*tp53i3* (2), *pfn4* (1), *sf3b14* (1)	4	0.155
CCND1	cyclin D1	*ccnd1* (4)	4	0.236
FAS	TNF receptor superfamily, member 6	*fas* (20)	20	0.26
BCL2L1	BCL2-like 1	*bcl2l1* (3), *tpx2* (1)	4	0.417
BCL2L11	BCL2-like 11	*acoxl* (2), *bcl2l11* (10)	12	0.467
MDM2	p53 binding protein homolog	*mdm2* (3)	3	0.487
CASP2	caspase 2	*casp2* (3), *clcn1* (1), *flj90586* (1)	5	0.544
BCL10	B-cell CLL/lymphoma 10	*bcl10* (10), *c1orf52* (2)	12	0.552
CASP3	caspase 3	*casp3* (6), *flj33167* (3)	9	0.598
CASP14	caspase 14	*casp14* (6), *flj40365* (2)	8	0.603
CASP6	caspase 6	*casp6* (4), *flj20647* (3), *pla2g612a* (1)	8	0.647
CASP8AP2	caspase 8 associated protien 2	*casp8ap2* (9), *cx62* (4), *mdn1* (3)	16	0.691
RIPK2	receptor-interacting serine-threonine kinase 2	*ripk2* (6)	6	0.736
BCL2L2	BCL2-like 2	*pabpn1* (1), *ppp1r3e* (1)	2	0.769
FASLG	Fas ligand (TNF superfamily, member 6)	*faslg* (9)	9	0.772
CASP7	caspase 7	*casp7* (12)	12	0.809
BCL2	B-cell CLL/lymphoma 2	*bcl2* (56), *fvt1* (5)	61	0.89
MYC	v-myc myelocytomatosis viral oncogene homolog	*myc* (9)	9	0.924
TP53	tumor protein p53	*tp53* (1)	1	0.949
PIM1	pim-1 oncogene	*pim1* (1)	1	0.958
CASP8/10	caspase 8 and 10	*als2cr12* (1), *casp8* (9), *casp10* (3)	13	0.976

*defined as SNPs between 20kb 5′ of the start of transcription (exon 1) and 10kb 3′ of the end of the last exon were grouped using a binning threshold of r2>0.8;

** adjusted for age (<50, 50-59, 60-69, ≥ 70 years)

Table III

Risk of multiple myeloma associated with all genotyped tag SNPs of signficant cell cycle and apoptosis genes

		Alleles		Heterozygotes			Homozygotes			Dominant Model			
Gene Region	**SNP**	**Major**	**Minor**	**OR***	**95% CI***	**p**	**OR***	**95% CI***	**p**	**OR***	**95% CI***	**p**	**p - trend**
Bax	rs1805419	G	A	1.00	0.64-1.54	0.982	1.12	0.50-2.48	0.789	1.01	0.67-1.54	0.952	0.868
	rs11667200	T	A	0.56	0.32-1.00	**0.049**	1.18	0.12-11.60	0.886	0.58	0.33-1.02	0.057	0.077
	rs11667229	T	C	0.98	0.60-1.61	0.951	0.87	0.46-1.64	0.667	0.95	0.60-1.52	0.843	0.689
	rs11667351	T	G	0.56	0.33-0.97	**0.040**	0.77	0.09-6.69	0.809	0.57	0.33-0.98	**0.041**	0.051
	rs2270939	T	C	1.07	0.67-1.71	0.789	1.86	0.74-4.67	0.189	1.16	0.74-1.80	0.519	0.316
	rs3765148	G	A	1.24	0.70-2.19	0.460		not applicable		1.31	0.75-2.29	0.351	0.253
	rs4802527	C	G	0.77	0.48-1.24	0.285	1.26	0.34-4.73	0.728	0.80	0.50-1.27	0.347	0.475
	rs1042265	G	A	0.42	0.21-0.81	**0.010**		not applicable		0.40	0.21-0.78	**0.007**	**0.007**
	rs2270938	A	T	0.94	0.59-1.50	0.798	1.05	0.55-2.01	0.886	0.96	0.62-1.50	0.871	0.977
CASP9	rs2020902	A	G	0.81	0.49-1.32	0.396	0.39	0.05-3.08	0.372	0.77	0.48-1.26	0.303	0.243
	rs4646047	C	T	0.76	0.47-1.23	0.267	0.51	0.27-0.95	**0.034**	0.68	0.43-1.07	0.095	**0.033**
	rs4646092	C	T	0.89	0.57-1.38	0.589	0.73	0.24-2.20	0.578	0.87	0.57-1.33	0.517	0.472
	rs4661636	C	T	0.90	0.57-1.41	0.639	0.91	0.46-1.80	0.796	0.90	0.59-1.37	0.631	0.686
	rs7516435	A	G	1.48	0.94-2.32	0.090	2.59	1.30-5.15	**0.007**	1.65	1.08-2.53	**0.020**	**0.005**
	rs12130370	T	C	1.28	0.78-2.12	0.327	1.51	0.82-2.78	0.183	1.34	0.84-2.16	0.221	0.174
	rs3766160	G	A	1.22	0.77-1.91	0.396	2.34	1.05-5.23	**0.038**	1.35	0.88-2.06	0.165	0.059
RIPK1	rs10498658	C	T	0.99	0.64-1.55	0.981	1.40	0.63-3.12	0.409	1.05	0.69-1.60	0.818	0.598
	rs2326173	G	A	0.93	0.60-1.45	0.764	0.59	0.27-1.32	0.200	0.86	0.57-1.31	0.485	0.269
	rs6596945	C	A	1.21	0.77-1.88	0.410	1.33	0.47-3.73	0.587	1.22	0.79-1.87	0.366	0.360
	rs6920337	G	A	0.96	0.62-1.50	0.868	0.78	0.29-2.11	0.630	0.94	0.61-1.44	0.770	0.677
	rs7765221	G	A	1.49	0.87-2.58	0.149		not applicable		1.40	0.82-2.41	0.221	0.353
	rs9391981	G	C	0.34	0.13-0.86	**0.023**		not applicable		0.32	0.12-0.81	**0.017**	**0.016**
	rs7775816	G	A	1.34	0.85-2.10	0.208	1.43	0.39-5.31	0.592	1.34	0.87-2.09	0.188	0.196

*adjusted for age (<50, 50-59, 60-69, ≥ 70 years)

Table IV

Haplotype analyses for significant cell cycle and apoptosis genomic regions associated with multiple myeloma

Gene Region	Block	Haplotype	Frequency	OR*	95% CI*	Global test*	Order of SNPs
BAX	1	TG	0.74	1.00	reference	0.192	rs2270939 (T>C), rs3765148 (G>A)
		CG	0.18	1.29	0.89 - 1.86		
		TA	0.07	1.48	0.85 - 2.58		
	2	CT	0.82	1.00	reference	0.279	rs4802527 (C>G), rs11667200 (T>A)
		GA	0.11	0.64	0.37 - 1.12		
		GT	0.06	1.18	0.65 - 2.12		
		CA	0.01	0.37	0.05 - 2.95		
	3	TTGGT	0.37	1.00	reference	0.277	rs11667229 (T>C), rs11667351 (T>G), rs1805419 (G>A), rs1042265 (G>A), rs2270938 (A>T)
		CTGGA	0.29	1.22	0.83 - 1.79		
		TTAGA	0.14	1.17	0.41 - 3.28		
		CGAAA	0.09	0.47	0.23 - 1.00		
		CGAGA	0.03	1.34	0.53 - 3.37		
		TTGGA	0.02	1.17	0.41 - 3.28		
		CGGGA	0.01	0.87	0.18 - 4.11		
		CTAGT	0.01	2.57	0.80 - 8.26		
		CTGAA	0.01	0.47	0.06 - 3.79		
CASP9	1	GTCTTAA	0.34	1.00	reference	**0.011**	rs3766160 (G>A), rs12130370 (T>C), rs4646092 (C>T), rs4661636 (C>T), rs4646047 (C>T), rs2020902 (A>G), rs7516435 (A>G)
		ACCCCAG	0.21	1.37	0.92 - 2.03		
		GTCCTAA	0.15	0.60	0.34 - 1.05		
		GCTCCGA	0.13	0.89	0.53 - 1.49		
		GCTCCAA	0.07	1.10	0.57 - 2.12		
		GTCCCAG	0.06	1.95	1.01 - 3.74		
		GTTCCGA	0.01	0.44	0.06 - 3.38		
RIPK1	1	GC	0.63	1.00	reference	0.211	rs7775816 (G>A), rs6596945 (C>A)
		GA	0.20	1.27	0.87 - 1.86		
		AC	0.16	1.42	0.95 - 2.13		

Gene Region	Block	Haplotype	Frequency	OR*	95% CI*	Global test*	Order of SNPs
	2	GG	0.67	1.00	reference	0.547	rs6920337 (G>A), rs2326173 (G>A)
		AA	0.23	0.84	0.58 - 1.22		
		GA	0.09	0.78	0.45 - 1.35		
		AG	0.01	1.54	0.47 - 5.10		
	3	CG	0.66	1.00	reference	0.517	rs10498658 (C>T), rs7765221 (G>A)
		TG	0.26	1.15	0.82 - 1.63		
		TG	0.08	1.29	0.76 - 2.17		

* adjusted for age (<50, 50-59, 60-69, ≥ 70 years)