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Caspase polymorphisms and genetic susceptibility to multiple myeloma

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Abstract

Multiple myeloma is a haematological malignency, characterized by clonal expansion of plasma cells. However, little is known about the cause of multiple myeloma. Cancer cells must avoid apoptosis to ensure unregulated tumour formation and growth. The highly conserved caspase cascade is essential to the regulation of the apoptotic pathway. To examine if five single nucleotide polymorphisms (SNPs) in four caspase genes [*CASP3* Ex8-280 C >A (rs6948), *CASP3* Ex8 + 567 T >C (rs1049216), *CASP8* Ex14-271 A >T (rs13113), *CASP9* Ex5 + 32 G >A (rs1052576), *CASP10* Ex3-171 A >G (rs39001150)] alter multiple myeloma risk, we conducted a population-based case-control study of women (128 cases; 516 controls) in Connecticut. Compared to individuals with the TT genotype of *CASP3* Ex8 + 567 T >C, subjects with the CC genotype had a five-fold decreased risk of multiple myeloma (odds ratio (OR)_{CC} = 0.2, 95% confidence interval (CI) = 0.0–1.0). Further, individuals with the AG and AA genotypes of *CASP9* Ex5 + 32 G >A also experienced a decreased risk of multiple myeloma (OR_{AG} = 0.8, 95% CI = 0.5–1.3; OR_{AA} = 0.5, 95% CI = 0.3–0.9; *p*-trend = 0.02). While no previous study has evaluated the association between caspase genes and multiple myeloma, studies have found associations with lung, breast, esophageal, gastric, colorectal and cervical cancers. Our parallel study of non-Hodgkin lymphoma, which utilized the same controls, found strong evidence that caspase genes play a key role in lymphogenesis. The protective associations observed in two key caspase genes suggest that genetic variation in *CASP* genes may play an important role in the aetiology of multiple myeloma.

Keywords

multiple myeloma; caspase; variant; polymorphism; snp; risk factors

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Introduction

Multiple myeloma is a B-cell malignancy often characterized by the increased production and secretion of monoclonal immunoglobulins (IgG, IgA, IgD or IgE); however, an increase in Igs is not detectable in all cases [1]. Little is known about risk factors for multiple myeloma [2].

Apoptosis eliminates cells that jeopardize homeostasis and integrity of the organism [3]. A hallmark of cancer cells is their ability to avoid apoptosis and consequently persist and replicate unchecked in the body [4]. 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. The extrinsic pathway utilizes caspase-8 and -10 as initiator caspases, while the intrinsic pathway employs caspase-9. Both pathways converge to use caspase-3, -6 and -7 as the effector caspases, which lead to cell death by nuclear membrane breakdown, DNA fragmentation, chromatin condensation and the formation of apoptotic bodies [5,6].

Studies of microarray profiling of genetically identical twins found at least a two-fold change in expression of *CASP8* when comparing the gene expression of cells from the multiple myeloma patient and the healthy control [7]. Somatic mutations of *CASP3* have also been identified in multiple myeloma tumours [8]. While no population-based study has evaluated the association between caspase genes and multiple myeloma, such studies have found variants in caspase genes to be associated with lung [9–11], breast [11–13], esophageal [11], gastric [11], colorectal [11] and cervical cancers [11]. Our parallel study of non-Hodgkin lymphoma (*NHL*), which utilized the same controls, recently found strong evidence that variants in caspase genes play a key role in lymphogenesis [14]. We hypothesized the caspase variants we found to be associated with *NHL* or its subtypes would also be associated with genetic susceptibility to multiple myeloma. Therefore, we genotyped five single nucleotide polymorphisms (SNPs) in three initiator (*CASP8*, *CASP9* and *CASP10*) and one effector (*CASP3*) caspase genes in a population-based case-control study.

Materials and methods

This population-based case-control study utilized histologically confirmed incident multiple myeloma female cases. Cases were diagnosed between 1996 and 2000, aged 21–84 years, residing in Connecticut (United States), with no previous diagnosis of cancer except non-melanoma skin cancer, and alive at the time of interview, as previously described [15]. Case interviews were obtained from 183 of the 323 eligible cases, yielding a 57% case response rate. Controls were recruited for a parallel population-based case-control study of *NHL* [16]. Random digit dialling methods for those below age 65 years (69% participation rate) and Health Care Financing Administration files for those 65 years and older (47% participation rate) were used to select female population-based controls ($n = 691$), aged 21–84 years.

Biologic sample collection and genotyping methods have been previously described [17]. Briefly, peripheral blood and buccal cells were used to extract genomic DNA using a phenol–chloroform extraction method (Gentra Systems, Minneapolis, MN). Genotyping of the five SNPs [*CASP3* Ex8-280 C > A (rs6948), *CASP3* Ex8 + 567 T > C (rs1049216), *CASP8* Ex14-271 A > T (rs13113), *CASP9* Ex5 + 32 G > A (rs1052576), *CASP10* Ex3-171 A > G (rs39001150)] was successful for 128 cases and 516 controls. Candidate genes were selected *a priori* based on minor allele frequencies of greater than 5% in the SNP500Cancer database and plausibility of contribution to the aetiology of multiple myeloma. Genotyping was performed by real-time PCR on the ABI 7900HT sequence detection system (<http://snp500cancer.nci.nih.gov>). Blinded duplicate samples from 100 study subjects and 40 replicate samples from each of two blood donors were interspersed throughout the plates used for genotype analysis. A duplicate quality control sample analysis yielded a QC concordance rate of >99%.

All statistical methods were performed using SAS software, version 9.1 (SAS Institute, Inc., Cary, NC). Demographic characteristics were compared in cases and controls by the χ^2 test. Hardy–Weinberg equilibrium (HWE) for each SNP was tested with a Pearson χ^2 test among controls. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated by unconditional logistic regression, adjusting for age (<50, 50–9, 60–9, >70). Exact tests were used when cell counts were less than 5. Since further adjustment for race (Caucasian, African American) yielded similar results, results reported here are only age adjusted. Gene dosage effects were determined by assigning ordinal values for the subject's number of variant alleles [1,2,3] to a genotype trend variable. Haplotype frequencies for *CASP3* were estimated by the expectation-substitution approach and the global score test was used to determine the overall differences in haplotype frequencies between Caucasian cases and Caucasian controls, adjusted for age [18,19]. All haplotype analyses were performed with SAS genetics.

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 ($p = 0.02$) and had a higher percentage of black women (10.9%) than controls (2.7%) (Table 1). All SNPs were in HWE among controls ($p > 0.05$). *CASP9* Ex5 + 32A was significantly ($OR_{AG} = 0.8$, 95% CI = 0.5–1.3; $OR_{AA} = 0.5$, 95% CI = 0.3–0.9; p -trend = 0.02) and *CASP3* Ex8 + 567C was borderline ($OR_{CC} = 0.2$, 95% CI = 0.0–1.0; p -trend = 0.16) associated with a decreased risk of multiple myeloma when adjusting for age (Table 2). While not significant, *CASP8* Ex14-271T was suggestive of decreased risk. The two *CASP3* polymorphisms were in strong linkage disequilibrium (LD) ($D' = 0.98$) but moderately correlated ($R^2 = 0.37$). Haplotype analysis of the two *CASP3* SNPs yielded no significant association with multiple myeloma risk. No significant gene–gene interactions were observed for the five *CASP* SNPs.

Discussion

Literature on the genetic susceptibility to multiple myeloma is sparse. To the best of our knowledge, no published study has previously examined the association between variants in caspase genes and risk of multiple myeloma. The protective associations observed for polymorphisms in two of the four key caspase genes, *CASP3* Ex8 + 567 T > C and *CASP9* Ex5 + 32 G > A, suggest that genetic variation in *CASP* genes may play an important role in the aetiology of multiple myeloma.

Evidence from caspase-3 knock-out mouse models and caspase-3 null cell lines show that *CASP3* is essential to the regulation of B-cell homeostasis, through chromatin margination, DNA fragmentation, nuclear collapse and cleavage of many key players involved in apoptosis [20–24]. While caspase-3 appears to be the crucial player in apoptosis, some of the effects seen may be resulting from the activation of other unknown downstream components. For example, caspase-3 or a related protease is responsible for the cleavage of 42 of the 58 known caspase substrates [6,25]. Further research is needed to determine the functionality of the two *CASP3* variants in our study and to determine if our results could be explained by other variants in linkage disequilibrium with our variants that are associated with risk of multiple myeloma.

CASP9 was the only initiator caspase in our study to be associated with risk of multiple myeloma. *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, it complexes with APAF-1 and forms the apoptosome with procaspase-9 and

activates the caspase-9 cascade. Caspase-3 is then activated, leading to cell death [26]. Mouse models of mutant caspase-9 and caspase-9 knock-out mice have been associated with perinatal and postnatal lethality [27–29]. Caspase-8 and -10 are initiator caspases of the extrinsic apoptosis pathway which may be redundant and not essential for activation of the executioner caspases, since activation can occur independently by either caspase-8 or -10 in the extrinsic pathway [28,30,31].

Similar to these findings, our parallel population-based case-control study of NHL (461 cases; 535 controls), which evaluated the same SNPs as the current study, found *CASP3* Ex8 + 567 T >C and *CASP9* Ex5 + 32 G >A to be associated with a significantly decreased risk of NHL, another haematological malignancy [14]. The allele frequencies for our non-Hispanic Caucasian controls are similar to those in the SNP500 database, indicating that our controls are representative and it is highly unlikely that the similar findings found in multiple myeloma and NHL are due to the use of the same controls. Further, the *CASP9* -1263A >G (rs4645978) and -712C >T (rs4645981) polymorphisms significantly decreased and significantly increased risk of lung cancer in Koreans, respectively [9]; however, the *CASP9* -905T >G (rs4645980) and -293del (rs4645982) polymorphisms were not associated with lung cancer risk. The IVS12-19G >A, but not -678del (rs numbers not reported), polymorphism in the *CASP8* gene was also associated with small cell lung cancer in this population [10]. Variants of caspase genes (*CASP8* D302I and *CASP10* V410I) have also been associated with reduced risk of breast cancer [12,13]. Recently, an analysis of Han Chinese case-control studies, totaling 4995 cancer patients and 4972 controls, found that a six nucleotide deletion in the promoter region of *CASP8* was associated with lung, breast, esophageal, gastric, colorectal and cervical cancers [11].

This study adds to the limited data available on genetic susceptibility to multiple myeloma. Our study had a low response rate, which could potentially introduce selection bias. However, a recent study has found that genotyping frequencies for different genes in several pathways are very similar based on participation status [32]. Furthermore, this was a study of predominantly caucasians; however, results adjusted for both race and age were similar to those adjusted for only age. Finally, the moderate sample size may lead to false positive and false negative findings [33].

In conclusion, this population-based case-control study is the first, to our knowledge, to report decreased risks of multiple myeloma associated with variants in caspase genes. The protective associations observed for polymorphisms in two of the four key caspase genes, *CASP3* Ex8 + 567 T >C and *CASP9* Ex5 + 32 G >A, suggest that genetic variation in *CASP* genes may play an important role in the aetiology of multiple myeloma. Further replication studies or pooled analyses are needed to confirm our findings.

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Table 1
Characteristics of multiple myeloma cases and controls

	Cases	%	Controls	%	p-value
Age (years)					
<50	10	7.8	93	18.0	0.02
50–59	26	20.3	94	18.2	
60–69	41	32.0	119	23.1	
70+	51	39.9	210	40.7	
Race					<0.001
White	114	89.1	502	97.3	
Black	14	10.9	14	2.7	0.07
Education					
Graduate degree/professional	11	8.6	73	14.1	
College degree	17	13.3	102	19.8	
Vocational/some college	37	28.9	151	29.3	
High school graduate	44	34.4	130	25.2	11.6
<12 years	19	14.8	60		

Table 2
Odds ratios (OR) and 95% confidence intervals (CI) for the association of *CASP* polymorphisms and risk of multiple myeloma

Genotype	Cases (%)	Controls (%)	OR* (95% CI)	p-value	p-trend
<i>CASP3</i> Ex8-281C >A (rs6948)					
CC	36 (29)	132 (26)			
AC	56 (46)	260 (51)	0.8 (0.5–1.3)	0.36	
AA	31 (25)	120 (23)	1.0 (0.6–1.7)	0.88	
AC or AA	87 (71)	380 (74)	0.9 (0.6–1.3)	0.47	0.84
<i>CASP3</i> Ex8 + 567T >C (rs1049216)					
TT	69 (57)	265 (52)			
CT	51 (42)	208 (41)	1.0 (0.6–1.4)	0.83	
CC†	2 (2)	33 (7)	0.2 (0.0–1.0)	0.05	
CT or CC	53 (43)	241 (48)	0.9 (0.6–1.3)	0.46	0.16
<i>CASP8</i> Ex14-271A > T (rs13113)					
TT	43 (36)	179 (35)			
AT	61 (51)	237 (47)	1.1 (0.7–1.6)	0.79	
AA	16 (13)	91 (18)	0.7 (0.4–1.4)	0.31	
AT or AA	77 (64)	328 (65)	1.0 (0.6–1.5)	0.88	0.45
<i>CASP9</i> Ex5 + 32G > A (rs1052576)					
GG	41 (33)	130 (25)			
AG	65 (52)	252 (49)	0.8 (0.5–1.3)	0.41	
AA	29 (16)	129 (25)	0.5 (0.3–0.9)	0.02	
AG or AA	85 (67)	381 (75)	0.7 (0.5–1.1)	0.12	0.02
<i>CASP10</i> Ex2-171A > G (rs3900115)					
AA	28 (23)	122 (24)			
AG	61 (50)	256 (51)	1.0 (0.6–1.7)	0.90	
GG	32 (26)	125 (25)	1.1 (0.6–2.0)	0.71	
AG or GG	93 (77)	381 (76)	1.1 (0.7–1.7)	0.81	0.71

* Adjusted for age.
† Exact test.