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Insulin-like growth factor-1- and interleukin-6-related gene variation and risk of multiple myeloma

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Abstract

Insulin-like growth factor (IGF)-1 and interleukin (IL)-6 promote the proliferation and survival of multiple myeloma cells. Variation in genes related to IGF-1 and IL-6 signaling may influence susceptibility to multiple myeloma. To assess their etiologic role, we examined the association of 70 tagging single nucleotide polymorphisms (SNP) in seven IGF-1 and three IL-6 pathway genes with multiple myeloma risk in two prospective cohorts, the Nurses' Health Study and Health Professionals Follow-up Study. Among participants who provided DNA specimens, we identified 58 women and 24 men with multiple myeloma and matched two controls per case. We used multivariable logistic regression models to assess the association of the SNPs or tagged haplotypes with multiple myeloma risk. Several SNPs had suggestive associations with multiple myeloma based on large odds ratios (OR), although corresponding omnibus p-values were not more than nominally significant (i.e., at $p<0.05$). These SNPs included rs1801278 in the gene encoding insulin receptor substrate-1 (IRS1; C/T v. C/C genotypes; OR=4.3, 95% confidence interval (CI)=1.5-12.1), and three IL-6 receptor SNPs: rs6684439 (T/T v. C/C: 2.9, 1.2-7.0), rs7529229 (C/C v. T/T; 2.5, 1.1-6.0), and rs8192284 (C/C v. A/A; 2.5, 1.1-6.0). Additional SNPs in genes encoding IGF-1, IGF binding protein-2, IRS2, and gp130 also demonstrated suggestive associations with multiple myeloma risk. We conducted a large number of statistical tests, and the findings may be due to chance. Nonetheless, the data are consistent with the hypothesis that IGF-1- and IL-6-related gene variation influences susceptibility to multiple myeloma and warrant confirmation in larger populations.

Keywords

multiple myeloma; genetic sceptibility; IGF-1; IL-6; epidemiology

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Introduction

Insulin-like growth factor (IGF)-1 and interleukin (IL)-6 have well-documented roles in the pathogenesis of multiple myeloma. IGF-1 has proliferative and antiapoptotic effects on multiple myeloma cells and can modulate their migration (1-3). In addition, the IGF-1 receptor (IGF-1R) is universally expressed on multiple myeloma cells, and higher expression levels are correlated with poorer patient prognosis (4,5). Furthermore, selective inhibition of IGF-1R triggered antiproliferative, proapoptotic events in human myeloma cells (5). IL-6 secretion in the multiple myeloma tumor environment also results in promotion of tumor growth (6-8), and circulating levels of IL-6 have been shown to predict prognosis in multiple myeloma patients (9).

IGF-1 or IL-6 dysregulation may also have a role in the etiology of multiple myeloma, and it is plausible that variation in genes related to IGF-1 or IL-6 signaling influences susceptibility to this malignancy. A population-based case-control study with 150 cases, 112 family controls, and 126 population controls from Los Angeles County reported associations of variant genotypes with risk of multiple myeloma in two polymorphisms in the gene encoding IL-6 (IL6) (10); the single nucleotide polymorphism (SNP) rs1800796 (-572 G/C) was positively associated and the tandem repeat polymorphism -373 A_nT_n inversely associated with risk. Two other SNPs in IL6 (-174 G/C and -597 G/A) were not associated with multiple myeloma in case-control studies (10-13). The studies published to date have examined candidate SNPs and may have overlooked informative genetic variants. To assess whether inherited variation in genes that encode molecules involved in IGF-1 or IL-6 signaling is associated with susceptibility to multiple myeloma, we analyzed common tag SNPs and haplotypes in ten genes in the prospective cohorts of the Nurses' Health Study and Health Professionals Follow-up Study.

Materials and Methods

Study population

The Nurses' Health Study was established in 1976 when 121,700 female registered nurses ages 30 to 55 years, residing in 11 states within the United States (US), returned an initial questionnaire (14). The Health Professionals Follow-up Study was initiated in 1986 among 51,529 US male health professionals ages 40 to 75 years who completed the enrollment questionnaire. Follow-up questionnaires have been collected biennially from members of both cohorts to update information on lifestyle and disease history. We obtained blood samples from 32,826 Nurses' Health Study participants from 1989 to 1990, and from 18,018 Health Professionals Follow-up Study participants from 1993 to 1994. The protocols for collecting and processing the blood samples were similar in both cohorts and have been published in detail elsewhere (15). Briefly, most specimens were received within 26 hours of venipuncture and were immediately centrifuged, separated, and placed in liquid nitrogen freezer storage. From 2001 to 2004, we received buccal cell samples from 33,040 Nurses' Health Study participants who had not provided a blood sample, using a mouthwash protocol that was observed to yield high quality and quantity of DNA (16). Within one week of receipt of the buccal cell specimens, we extracted DNA using Qiagen DNA extraction (Qiagen Inc., Valencia, CA) and stored the DNA in liquid nitrogen freezers. The protocols for these studies were approved by the Human Subjects Research Committees at Brigham and Women's Hospital and Harvard School of Public Health. Informed consent was implied by the participants' completion and return of the enrollment questionnaires and blood specimens. Signed informed consent forms were obtained from women who contributed buccal cell specimens.

Case ascertainment

The same procedures were followed to ascertain cancer cases in both cohorts. Specifically, on the follow-up questionnaires, participants reported new diagnoses of cancer that had occurred in the previous two years. Deaths among non-responding cohort members were identified using the National Death Index, which was previously shown to be highly sensitive and specific in these cohorts (17,18). For each self-report or cause of death indication of multiple myeloma, we obtained written permission to obtain the medical records. A trained physician then reviewed the records to confirm the diagnosis according to established clinical criteria (19), and to confirm the date of diagnosis.

Study design

We included all cohort members with an archived DNA specimen and confirmed diagnoses of multiple myeloma that occurred at least three months after biospecimen collection, and through 2002 in women or 2004 in men. Participants who had reported other cancer diagnoses (other than non-melanoma skin cancer) prior to the multiple myeloma diagnosis date were ineligible. In the Health Professionals Follow-up Study, due to the small number of cases with archived DNA, we also included men with confirmed prevalent diagnoses of multiple myeloma at time of blood collection (i.e., those that occurred after study enrollment but prior to blood collection). We randomly selected two matched controls per case from a “risk set” of study participants who were alive and had no history of cancer (other than non-melanoma skin cancer) as of the given case's diagnosis date. The matching factors included cohort, which corresponded to gender, month and year of birth (± 12 months), and month and year of blood or cheek cell collection (± 2 months). Within the Nurses' Health Study, we also matched on source of DNA (buffy coat v. buccal).

SNP selection

We included SNPs in seven genes that encode molecules related to IGF-1 signaling: IGF-1 (IGF1), IGF binding protein-1, -2, and -3 (IGFBP1, IGFBP2, IGFBP3), IGF-1R (IGF1R), and insulin receptor substrate-1 and -2 (IRS1, IRS2) (20). We also included variants in three IL-6-related genes: IL6, IL-6 receptor (IL6R), and IL6ST (i.e., gp130, a component of the IL-6 receptor complex that is required for signal transduction) (21). We included 29 SNPs across IGF1, IGFBP1, and IGFBP3 that the National Cancer Institute Breast and Prostate Cancer Cohort Consortium (BPC3) (22) identified as tagging SNPs through resequencing and use of public databases (23,24). For IGFBP2, IRS1, IRS2, IL6, IL6R, and IL6ST, we used the International HapMap Project database (http://www.hapmap.org/) and the aggressive three-marker tagging algorithm of Tagger (http://www.broad.harvard.edu/mpg/tagger/server.html) (25) to identify minimal sets of SNPs with minor allele frequencies of 0.05 or greater in Caucasians, that captured common variation in the gene with a minimum R^2 of 0.8. We also included rs1800795 and rs1800796 in IL6, which were implicated based on an association with circulating IL-6 levels (26) or in a previous multiple myeloma study (10), respectively. The IGF1R gene was too large to analyze using a tag SNP approach. Absent *a priori* information on IGF1R polymorphisms in multiple myeloma, we limited the present analysis to one SNP (rs2229765) that has been associated with plasma IGF-1 levels (27).

Genotyping

Samples were genotyped by a blinded technician using the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA), in 384-well format. The 5' nuclease assay (TaqMan®) was used to distinguish the 2 alleles of a gene. PCR amplification was carried out on 5-20ng DNA using 1 X TaqMan® universal PCR master mix (No Amp-erase UNG), 900nM forward and reverse primers, 200nM of the FAM labeled probe and 200nM of the VIC labeled probe in a 5μl reaction volume. Amplification conditions on a AB 9700 dual plate

thermal cycle (Applied Biosystems, Foster City, CA) were as follows: 1 cycle of 95°C for 10min, followed by 50 cycles of 92°C for 15s and 60°C for 1 min. TaqMan® assays were ordered using either Assays-on-Demand or using the ABI Assays-By-Design service. Characteristics of the assays utilized for the SNPs in IGF1, IGFBP1, and IGFBP3 are described on a public web site (28). Information on the remaining assays is available upon request. For quality controls, we included 24 blinded repeat specimens (21 buffy coat, 3 buccal DNA) in the genotyping runs. The concordance of genotypes in the repeat samples was 100% for each assay. We obtained undetermined genotypes in fewer than 5% of study samples for all the SNPs except rs4845623 (in IL6R), for which we obtained missing genotypes for 17.1% of cases and 11.6% of controls. The SNPs rs4601580 and rs4845371 in IL6R, as well as rs4773092 and rs1805097 in IRS2, were identified as tagging SNPs but could not be genotyped using TaqMan due to the nucleotide content of the flanking sequences.

Statistical analysis

We utilized SAS version 9.1 (SAS Institute, Cary, NC) for all statistical analyses. We examined allele frequencies and departure from Hardy-Weinberg equilibrium with Proc ALLELE. We used Proc HAPLOTYPE to infer haplotypes from unphased genotype data and accounted for the uncertainty in the inferred haplotypes by the expectation-substitution approach (29,30). We analyzed the common haplotypes (i.e., those that occurred with 5% frequency or greater) within the haplotype blocks of IGF1, IGFBP1, and IGFBP3 that were identified by the BPC3 (23, 24) using criteria of Gabriel and colleagues (31). For the remaining genes, we analyzed the multi-marker haplotypes that were identified by Tagger during tag SNP selection. Unavailability of genotype data for rs4845371 prevented haplotype-level analysis in IL6R, because each of three identified multi-marker haplotypes included rs4845371. The multi-marker haplotypes in IL6R would have tagged seven SNPs that were not otherwise identified as tag SNPs. Tagger did not identify multi-marker haplotypes in IGFBP2, IRS1, IL6, or IL6ST.

We obtained odds ratios (OR) and 95% confidence intervals (CI) from unconditional logistic regression models to estimate the relative risk of multiple myeloma for a given genotype or haplotype. For analyses of individual SNPs, we assumed co-dominance of the alleles (i.e., we included separate indicator variables for each of the genotypes except the referent homozygous wild type genotype). When fewer than 15 persons carried the homozygous minor variant genotype, we obtained p-values for global association from the trend test, i.e., from logistic regression models in which the genotypes were modeled ordinally as number of copies of the minor allele. Otherwise, we obtained global p-values from the likelihood ratio test with 2 degrees of freedom, comparing models with covariates only to models with covariates and genotype variables. In haplotype analyses, we used an additive model and included rare haplotypes as an aggregated category. We used the likelihood ratio test as a global test of association between the haplotypes in a given haplotype block and risk of multiple myeloma. We adjusted for study population (i.e., gender), age (continuous, in months), and body mass index (BMI; continuous, in kg/m^2) in all the models (32). We conducted sensitivity analyses, separately excluding the prevalent male cases and the female cases and controls with buccal rather than buffy coat DNA, to determine the influence that these participants had on the main analysis.

Genotype data for the 29 SNPs in IGF1, IGFBP1, and IGFBP3 were available for an additional 1,764 Nurses' Health Study and 696 Health Professionals Follow-up Study participants who had been selected as eligible age-matched controls into genetic susceptibility studies of breast or prostate cancer, respectively (22,33,34). Therefore, we performed secondary analyses to explore the influence of sample size on the main analysis, using a combined data set with the original and supplemental controls (n=2,624 total controls).

Results

In the Nurses' Health Study population, we identified 58 women with confirmed incident diagnoses of multiple myeloma (50 buffy coat, 8 buccal DNA). In the Health Professionals Follow-up Study, we identified 24 men with confirmed multiple myeloma diagnoses (15 incident, 9 prevalent at blood draw). Among the nine men with prevalent diagnoses, six (67%) were diagnosed fewer than 36 months prior to blood collection (median interval, 31 months; range, 3 to 63 months). We successfully matched two controls per case, and thus we analyzed 82 cases and 164 controls in the main analysis. The women were ages 45 to 76 years (mean, 62 years), and the men were ages 48 to 81 years (mean, 65 years), at their respective dates of specimen collection. The majority of participants in each cohort (98.8%) reported their ethnicity as Caucasian. With two exceptions, the genotype distributions were in Hardy-Weinberg equilibrium in the controls; the exceptions were rs2229765 in IGF1R (exact p=0.0037) and rs11618950 in IRS2 (exact p=0.0062). We observed allele frequencies similar to those previously reported in the cohorts for the SNPs in IGF1, IGFBP1, and IGFBP3, and for rs1800795 in IL6 and rs8192284 in IL6R (35-37).

Tag SNP associations with multiple myeloma risk

In the main analysis, we did not observe a highly statistically significant association with multiple myeloma for any of the SNPs. For several SNPs, we observed suggestive associations —larger ORs that corresponded to statistically non-significant or nominally significant (i.e., at $p<0.05$) omnibus p-values. For brevity, Table 1 includes only the SNPs that demonstrated relatively strong suggestive associations with multiple myeloma (i.e., ORs of ≥ 2.0 or the equivalent inverse association) in the main or secondary analysis, and that were based on more than five cases with the relevant genotypes. The complete tag SNP results, including minor allele frequencies, are available on-line in Supplementary Table 1.

The SNPs with stronger suggestive associations included two SNPs in IRS1: participants with the C/T genotype for rs1801278 had a more than four-fold higher risk of multiple myeloma compared to those with the C/C genotype (OR=4.3, 95% CI=1.5-12.1), and individuals with the C/A genotype for rs17208470 had a two-fold increase (OR=2.2, 95% CI=1.1-4.5) compared to those with C/C (Table 1). ORs could not be computed for homozygous minor allele carriers of the latter two SNPs due to the rarity of those genotypes. The p-value for global association was not significant for rs1801278 (P_{global} =0.68) or rs17208470 (P_{global} =0.71). We also observed higher risks of multiple myeloma for three SNPs in IL6R: rs6684439 (P_{global}=0.048; T/T v. C/C; OR=2.9, 95% CI=1.2-7.0), rs7529229 (P_{global}=0.08; C/C v. T/T; OR=2.5, 95% CI=1.1-6.0), and rs8192284 (P_{global}=0.038; C/C v. A/A; OR=2.5, 95% CI=1.1-6.0). Persons with heterozygous genotypes in rs6684439, rs7529229, and 8192284 did not have an increased risk of multiple myeloma.

Non-significant associations with multiple myeloma risk were suggested for two SNPs in IGF1 (rs7965399 and rs2373722), for homozygous minor allele carriers in one SNP each in IGFBP2 (rs9341105) and IRS2 (rs913949), and for persons with a heterozygous genotype in rs11744523 in IL6ST (Table 1). We did not observe statistically significant associations with multiple myeloma risk (i.e., at $p<0.05$) for any SNPs in IGF1, IGFBP1, IGFBP2, IGFBP3, IGF1R, IRS2, IL6, or IL6ST in the main analysis. Common haplotypes in IGF1, IGFBP1, IGFBP3, and IRS2 were not significantly associated with risk of multiple myeloma (data not shown). Exclusion of women with buccal DNA, or of men with prevalent diagnoses of multiple myeloma, did not alter the results (data not shown).

Analysis with supplemental controls

The secondary analysis included 2,706 individuals (82 cases, 2624 controls). The mean age of the 1,764 additional female controls was somewhat younger than that of the 116 female primary analysis controls (58 v. 62 years at blood draw, respectively), whereas the mean age of the 696 male supplemental controls was similar to that of the 48 male primary analysis controls (66 v. 65 years at blood draw, respectively). As in the primary analysis case-control sample, a majority (98.8%) of supplemental controls reported their ethnicity as Caucasian. Overall, the secondary analysis yielded similar results to the main analysis for the SNPs included in Table 1, with the following exceptions: in the augmented study sample, homozygous minor allele carriers for two SNPs in IGF1 (rs2195239, $P_{global}<0.0001$, and rs1996656, $P_{global}=0.19$) had increases in risk of multiple myeloma that were not evident in the main analysis (Table 1). In addition, carriers of the minor allele for rs3110697 in IGFBP3 (rs3110697) had a two-fold increased risk of multiple myeloma that was marginally significant in the secondary analysis ($P_{global}=0.083$) but weaker and non-significant in the main analysis ($P_{global}=0.56$). Associations with multiple myeloma were apparent for rs7965399 ($P_{global}=0.0015$) and rs2373722 ($P_{global}<0.0001$) in IGF1, of similar magnitudes to the non-significant associations observed in the main analysis. No other SNP in IGF1, IGFBP1, or IGFBP3 was associated with multiple myeloma risk in the secondary analysis. The secondary analysis results were not as consistent with those from the main analysis for SNPs that did not demonstrate a suggestive association in the main analysis (Supplementary Table 1)—in particular, some of the SNPs with few or no minor allele carriers in the smaller main analysis population (for example, rs35767 and rs1019731 in IGF1, and rs35539615 and rs1908751 in IGFBP1/IGFBP3) yielded contrasting results in the secondary analysis. As in the main analysis, common haplotypes in IGF1, IGFBP1, and IGFBP3 were not associated with multiple myeloma in the secondary analysis (data not shown).

Discussion

We did not observe highly statistically signficant associations with multiple myeloma risk for any of the 70 SNPs in the present analysis. However, we observed strong suggestive associations of several SNPs in IRS1 and IL6R with risk of multiple myeloma. In the secondary analysis, we also observed suggestive associations for SNPs in IGF1 and IGFBP3. Additional SNPs in IGF1, IGFBP2, IRS2, and IL6ST may demonstrate significant associations with multiple myeloma in a larger study population. We did not observe associations for SNPs in IGFBP1, IGF1R, or IL6.

The secondary analysis provided reassurance regarding the impact of sample size on our main analytic findings. Among the SNPs that demonstrated stronger suggestive associations with multiple myeloma in the primary analysis, we observed ORs of a similar magnitude in both the original and secondary analyses when the corresponding genotype frequencies were not too sparse. The emergence of a few novel statistically significant associations in the secondary analysis suggested that we may have been unable to detect some potentially informative markers in the primary analysis due to limited sample size.

Of the SNPs included in the present analysis, only three have been previously examined in relation to risk of multiple myeloma. Consistent with previous reports (10-13), the IL6 SNP rs1800795 (i.e., -174 G/C) was not associated with multiple myeloma risk in the present analysis. We did not observe an association for rs1800796 in IL6, in contrast to Cozen and colleagues (10). The latter study reported a 2.4-fold increase (95% CI=1.2-4.7) in multiple myeloma risk among carriers of the C allele for rs1800796 when 150 cases were compared with 126 population controls (10). The discrepancies beween the two studies may be due in part to differences in the frequency of the C allele in the study populations. Also in contrast to the present findings, Cozen et al. did not observe an association of rs8192284 (i.e., D358A) in

IL6R with multiple myeloma (cases v. population controls, OR=0.9, 95% CI=0.5-1.6) but did report an association of the minor allele with obesity among the population controls (BMI ≥ 30 v. < 30 kg/m^2, OR=5.4, 95% CI=1.7-17.4) (10). The latter finding is noteworthy because obesity has been consistently related to multiple myeloma risk (32,38-40) and to increased secretion of IL-6 (41,42).

Several of the SNPs for which we observed stronger suggestive associations with multiple myeloma have been associated with other IGF-1- or IL-6-related conditions, or have demonstrated functional effects on the signaling pathways. For example, the minor allele of rs1801278 (i.e., G972R) in IRS1 was associated with an increased risk of colorectal cancer (43). Of interest, IRS-1, which is involved in insulin signaling, is also required to activate signaling pathways that mediate both anti-apoptotic and mitogenic effects of IGF-1 (44,45). In IL6R, the minor allele of rs8192284 was significantly associated with higher circulating levels of IL-6 in the Nurses' Health Study population (37), and with levels of IL-6 and the soluble form of the IL-6 receptor in other populations (46,47). In the present analysis, we observed a suggestive inverse association with multiple myeloma risk for rs2373722 in IGF1. In the Nurses' Health Study, rs2373722 in IGF1 was significantly associated with mammographic density (35); the minor (A) allele was associated with lower mammographic density, and thus the direction of association was consistent with the present findings. In addition, in the Multiethnic Cohort, participants with the C/T genotype for rs7965399 in IGF1 had a significant increase in risk of prostate cancer (23).

Strengths of the present analysis include a strong *a priori* hypothesis based on the well-established roles of IGF-1 and IL-6 in multiple myeloma pathogenesis. We used a tag SNP approach to SNP selection, to improve the opportunity to detect as yet unknown susceptibility markers. The excellent concordance of the QC sample genotypes and low percentage of missing genotypes indicate that measurement error did not distort the findings. In addition, we matched cases to controls closely on other risk factors for multiple myeloma (age, gender) and adjusted for these factors and BMI in the analysis, so that the reported 95% CIs account for variability in risk related to those factors. The availability of additional control data for the secondary analyses enabled us to explore the impact of sample size on our results.

Limitations of the study should also be noted. It is possible that we did not include potentially informative susceptibility markers due to inadequate coverage of the genes by the selected tagging SNPs, or due to weak associations of causal and tag SNPs. Also, the inability to genotype two tagging SNPs in each of IRS2 and IL6R precluded the evaluation of markers at the SNP and haplotype level in those genes. For the two SNPs with evidence for departure from Hardy-Weinberg equilibrium, misclassification of genotypes may have biased the effect estimates, although the genotype distributions may have resulted from sampling variability rather than genotyping errors. The high concordance of genotypes that we observed in the quality control samples corroborates the latter explanation. We included both prevalent and incident male cases, which could introduce a survival bias; however, sensitivity analyses that excluded the prevalent cases yielded similar results to the main analyses. We relied upon self-reported ethnicity and did not have genotype data with which to control for potential bias due to population stratification (48,49). In a recent analysis of population structure among Nurses' Health Study participants who are also included in the National Cancer Institute's Cancer Genetic Markers of Susceptibility (CGEMS) Project, fewer than 1% of the individuals who self-reported to be of European ancestry were found to have genetic markers indicative of substantial African or Asian ancestry (50). Furthermore, we are not aware of evidence that the SNPs in the present analysis vary systematically across European populations. Therefore, although we cannot directly demonstrate a lack of this bias, we consider it unlikely that population stratification explains or has distorted the present findings. We conducted a large number of statistical tests relative to the sample size, and any of the findings may simply be

due to chance. We did not perform adjustment for multiple comparisons because most of the suggestive associations were only nominally significant at best and would not have been significant after adjustment. Nonetheless, the strong a priori hypotheses and the reports of associations for some of the SNPs with IGF-1- or IL-6-related cancers, other conditions, and/or relevant functional effects, lend credibility to the present findings.

In conclusion, we report findings that are consistent with the hypothesis that inherited variation in genes that encode molecules important to IGF-1 and IL-6 signaling may influence susceptibility to multiple myeloma. For all but three of the variants that we examined, the present study is the first to explore their association with multiple myeloma. Future studies to examine the interaction of IGF-1- and IL-6-related susceptibility markers with obesity are of interest (32,38-40). In addition, because only preliminary conclusions can be drawn from the present analysis regarding the association of the IGF-1- or IL-6-related genes with risk of multiple myeloma, confirmation of the present findings in both Caucasian and non-Caucasian populations is of paramount interest. The identification of variants in IGF-1 and IL-6 signaling-related genes that are associated with risk of multiple myeloma could provide valuable clues to mechanisms of susceptibility to this malignancy, which may in turn inform the development of effective strategies for prevention.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Association of tagging SNPs in genes related to IGF-1 or IL-6 signaling with risk of multiple myeloma: selected findings*

		Main analysis			Secondary analysis	
SNP	Genotype	Cases (n=82) N (%)†	Controls (n=164) N (%)†	OR (95% CI)‡	Controls (n=2624)§ N (N%)†	OR (95% CI)‡
IGF-1 signaling pathway genes						
IGF1						
rs7965399	TT	70 (88.6)	145 (92.9)	1.0 (ref)**	2328 (92.8)	1.0 (ref)**
	CT	9 (11.4)	11 (7.1)	2.0 (0.8-5.1)	174 (6.9)	1.8 (0.9-3.6)
	CC	0	0	NC††	6 (0.2)	NC††
	P_{global}‖			0.60		0.0015
rs2195239	CC	43 (55.1)	85 (53.5)	1.0 (ref)	1511 (59.5)	1.0 (ref)
	CG	26 (33.3)	63 (39.6)	0.8 (0.4-1.5)	903 (35.6)	1.0 (0.6-1.7)
	GG	9 (11.5)	11 (6.9)	1.6 (0.6-4.5)	125 (4.9)	2.6 (1.2-5.5)
	P_{global}			0.45		<0.0001
rs2373722	GG	71 (91.0)	136 (86.1)	1.0 (ref)	2121 (84.8)	1.0 (ref)
	GA	7 (9.0)	22 (13.9)	0.5 (0.2-1.3)	372 (14.9)	0.5 (0.2-1.1)
	AA	0	0	NC	8 (0.3)	NC
	P_{global}			0.93		<0.0001
rs1996656	AA	51 (63.0)	104 (65.0)	1.0 (ref)	1685 (68.4)	1.0 (ref)
	AG	24 (29.6)	48 (30.0)	1.0 (0.6-1.9)	712 (28.9)	1.3 (0.8-2.1)
	GG	6 (7.4)	8 (5.0)	1.6 (0.5-5.2)	68 (2.8)	3.5 (1.4-8.6)
	P_{global}			0.55		0.19
IGFBP1			(reported in Supplementary Table 1)			
IGFBP2						
rs9341105	TT	43 (55.1)	88 (55.3)	1.0 (ref)	N/A‡‡	
	TC	28 (35.9)	64 (40.3)	0.9 (0.5-1.6)		
	CC	7 (9.0)	7 (4.4)	3.2 (0.9-11.2)		
	P_{global}			0.87		
IGFBP3						
rs3110697	GG	20 (25.0)	45 (27.8)	1.0 (ref)	858 (34.6)	1.0 (ref)

			Main analysis		Secondary analysis	
SNP	Genotype	Cases (n=82) N (%)†	Controls (n=164) N (%)†	OR (95% CI)‡	Controls (n=2624)§ N (N%)†	OR (95% CI)‡
	GA	41 (51.3)	86 (53.1)	1.1 (0.5-2.1)	1177 (47.4)	1.6 (0.9-2.7)
	AA	19 (23.8)	31(19.1)	1.5 (0.7-3.4)	448 (18.0)	2.0 (1.1-3.7)
	P_{global}			0.56		0.083
IGF1R			(reported in Supplementary Table 1)			
IRS1						
rs1801278	CC	69 (86.3)	153 (95.0)	1.0 (ref)	N/A	
(G972R)	CT	11 (13.8)	8 (5.0)	4.3 (1.5-12.1)		
	TT	0	0	NC		
	P_{global}			0.68		
rs17208470	CC	61 (77.2)	138 (85.7)	1.0 (ref)	N/A	
	CA	18 (22.8)	21 (13.0)	2.2 (1.1-4.5)		
	AA	0	2 (1.2)	NC		
	P_{global}			0.71		
IRS2						
rs913949	GG	57 (69.5)	109 (67.3)	1.0 (ref)	N/A	
	GA	19 (23.2)	46 (23.4)	0.8 (0.4-1.5)		
	AA	6 (7.3)	7 (4.3)	2.3 (0.7-8.1)		
	P_{global}			0.57		
IL-6 signaling pathway genes						
IL6			(reported in Supplementary Table 1)			
IL6R						
rs6684439	CC	21 (25.9)	58 (35.8)	1.0 (ref)	N/A	
	CT	42 (51.9)	85 (52.5)	1.4 (0.7-2.8)		
	TT	18 (22.2)	19 (11.7)	2.9 (1.2-7.0)		
	Pglobal			0.048		
rs7529229	TT	21 (25.9)	51 (32.3)	1.0 (ref)	N/A	
	TC	41 (50.6)	88 (55.7)	1.1 (0.6-2.2)		
	CC	19 (23.5)	19 (12.0)	2.5 (1.1-6.0)		

			Main analysis		Secondary analysis	
SNP	Genotype	Cases (n=82) N (%)†	Controls (n=164) N (%)†	OR (95% CI)‡	Controls (n=2624)§ N (N%)†	OR (95% CI)‡
	P_{global}			0.08		
rs8192284	AA	24 (30.4)	54 (34.2)	1.0 (ref)	N/A	
	AC	37 (46.8)	87 (55.1)	0.9 (0.5-1.7)		
	CC	18 (22.8)	17 (10.8)	2.5 (1.1-6.0)		
	P_{global}			0.038		
IL6ST (gp130)						
rs11744523	TT	51 (66.2)	129 (81.1)	1.0 (ref)	N/A	
	TA	26 (33.8)	30 (18.9)	1.7 (0.9-3.3)		
	AA	0	0	NC		
	P_{global}			0.26		

Abbreviations: SNP, single nucleotide polymorphism; IGF, insulin-like growth factor; IL, interleukin; OR, odds ratio; CI, confidence interval; ref, reference category; IGFBP, IGF binding protein; NC, not computed; N/A, not available; IGF1R, IGF-1 receptor; IL6R, IL-6 receptor; IL6ST, IL-6 signal transducer.

* The complete results, including minor allele frequencies among controls, are available on-line in Supplementary Table 1.

† The N's may not sum to the column total due to the omission of undetermined genotypes; the %'s reflect the proportion of successfully genotyped samples and may not sum to 100% due to rounding.

‡ The ORs and 95% CIs are from unconditional logistic regression models controlling for study population (i.e., gender), age (months), and body mass index (kg/m^2).

§ The count includes 164 original matched controls and 2460 supplemental controls.

// The p-values for global association were obtained from the trend test when fewer than fifteen persons in the main analysis carried the homozygous minor variant genotype, and otherwise from the likelihood ratio test with 2 d.f.

** Carriers of the homozygous wild type genotype comprise the reference category.

†† The OR was not computed due to one or more absent genotypes.

‡‡ Genotype data were not available for SNPs in IGFBP2, IGF1R, IRS1, IRS2, IL6, IL6R, or IL6ST in the supplemental controls.