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Brief communication

Could age modify the effect of genetic variants in IL6 and TNF-α genes in multiple myeloma?

Alessandro Martino[a,d,*], Gabriele Buda[b], Valentina Maggini[c], Francesco Lapi[c], Antonella Lupia[a], Domenica Di Bello[a], Enrico Orciuolo[b], Sara Galimberti[b], Roberto Barale[a], Mario Petrini[b], Anna Maria Rossi[a]

[a] Department of Biology, Genetics division, University of Pisa,Italy
[b] Department of Oncology, Transplants and Advanced Technologies, Section of Hematology, Pisa University,Italy
[c] Department of Preclinical and Clinical Pharmacology, Interuniversitary Center of Molecular Medicine and Applied Biophysics (CIMMBA), University of Florence, Florence, Italy
[d] German Cancer Research Center (DKFZ), Genomic Epidemiology Group, Heidelberg, Germany

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ABSTRACT

Cytokines play a central role in multiple myeloma (MM) pathogenesis thus genetic variations within cytokines coding genes could influence MM susceptibility and therapy outcome. We investigated the impact of 8 SNPs in these genes in 202 MM cases and 235 controls also evaluating their impact on therapy outcome in a subset of 91 patients. Despite the overall negative findings, we found a significant age-modified effect of *IL6* and *TNF-α* SNPs, on MM risk and therapy outcome, respectively. Therefore, this observation suggests that genetic variation in inflammation-related genes could be an important mediator of the complex interplay between ageing and cancer.

1. Introduction

Multiple myeloma (MM) is the second most common haematological neoplasm, accounting for 10% of blood cancers and 1% of all cancers. A certain degree of familial aggregation has been observed, suggesting that genetic factors can be involved in the pathogenesis and the evolution of MM [1].

It has been shown that proliferation of normal and malignant plasma cells is under control of a complex network of cytokines, like interleukin (IL)-1β, IL2 and its receptor (IL2R), IL3, IL4, IL6 and IL6R, tumour necrosis factor (TNF)-α, IL10 and IL11 [2]. Therefore, the relationship between cytokine genetic variability and risk of developing MM or therapy outcome has been widely investigated. Nevertheless, results are often controversial and most of the findings failed to be replicated in other studies [3].

To contribute to clarify the role of cytokine genetic variation in the susceptibility to MM, we selected eight missense or functional SNPs in cytokine coding genes (*IL1B* rs16944, *IL1R1* rs2228139, *IL2* rs2069762, *IL2RB* rs228942, *IL6* rs1800797, *IL6R* rs2228145, *TNFα* rs1800629 and *TNFR2* rs1061622) and analyzed the genotype distributions in a case-control study of 202 MM patients and 235 healthy controls. In addition, we evaluated the role of the same variants in relation to therapy response and progression free survival (PFS) after autologous stem cell transplantation (ASCT) in a subgroup of 91 patients that underwent to ASCT after front line treatments.

2. Patients and methods

Between September 1992 and November 2009, 202 MM patients were recruited. Two-hundred and thirty five healthy subjects with a comparable age range (35–87) and gender distribution as MM cases were enrolled. Details are given in supplementary methods.

Complete follow-up and therapy data concerning ASCT were available for 91 out of 202 MM patients. Subjects with complete or partial response were considered Responders (R), while patients with stable or progressive disease were considered Non Responders (NR). Progression Free Survival (PFS) was calculated as the time (months) from the start of treatment (first ASCT) to disease progression or death

* Corresponding author at: German Cancer Research Center (DKFZ) Genomic Epidemiology Group – C055 Im Neuenheimer Feld 580 D-69120 Heidelberg, Germany (DE). Tel.: +49 6221 42 1814; fax: +49 6221 42 1810.
E-mail address: a.martino@dkfz-heidelberg.de (A. Martino).

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Table 1
Demographical and clinical characteristics of the MM patients.

	Cases and controls population		
	Cases	Controls	*p*-value[c]
Age			
Average	61.6 ± 9.9 (35–87)[a]	58.8 ± 10.9 (35–89)[a]	
Median	62 (54–68)[b]	59 (50–67)[b]	**0.00**
Gender (male/female)	108/94	129/106	0.76
Durie-Salmon (I/II/III/n.d.)	34/34/132/2		
ISS (I/II/III/n.d.)	88/32/29/53		
β2-microglobulin *(μg/L)*	2.4 (1.4–4.3)[b]		
Creatinin (mg/dL)	0.9 (0.8–1.1)[b]		
Albumin (g/dL)	4 (3.6–4.3)[b]		
Hemoglobin (mg/dL)	11.6 (10–13.3)[b]		

	Clinical characteristics of 91 subjects receiving ASCT			
	Overall	Clinical characteristics in age strata		
Age at diagnosis				
Average	58.27 ± 8.55 (35–75)[a]			
Median	59 (52–65)[b]	Age < 60 (years)	Age ≥ 60 (years)	*p*-value[c]
Gender (male/female)	48/43	25/22	23/21	0.93
Durie-Salmon (I/II/III)	21/20/50	11/11/25	10/9/25	0.93
ISS (I/II/III)	60/20/11	33/12/2	27/8/9	0.06
β2-microglobulin (μg/L)	2.18 (1.6–4.2)[b]	2.0 (1.4–3.4)[b]	2.4 (1.9–4.9)[b]	**0.03**
Creatinin (mg/dL)	0.9 (0.7–1.0)[b]	0.9 (0.7–1.0)[b]	0.9 (0.7–1.0)[b]	0.28
Albumin (g/dL)	4.1 (3.6–4.3)[b]	4.1 (3.6–4.3)[b]	4.1 (3.7–4.3)[b]	0.97
Hemoglobin (mg/dL)	11.9 (10.6–13.6)[b]	11.9 (10.2–13.7)[b]	11.9 (10.7–13.4)[b]	0.99
1st line therapy R/NR	62/29	29/18	33/11	0.17
Melphalan dosage 100/200 mg/m^2	55/36	11/36	44/0	**0.00**
ASCT R/NR	60/31	29/18	31/13	0.38
ASCT 1/2	35/56	22/25	13/31	0.09
PFS (months)	17 (10–28)[b]	18.0 (10–28)[b]	17 (10.5–28.5)[b]	0.93

ISS: International staging system, R: responders, NR: non responders, ASCT: autologous stem cells transplantation, ASCT 1/2: single or tandem ASCT, PFS: progression free survival. Values in bold show $p < 0.05$.

[a] Mean (range).
[b] Median (25th–75th percentile).
[c] A non-parametrical Kruskal–Wallis test for unpaired samples was used to compare distributions, while a χ^2 test was used to compare proportions.

(Table 1). Sensitivity analysis was also performed calculating the PFS from the date of the second ASCT for patients undergoing tandem transplantation.

Genotyping was carried out using TaqMan assays (Applied Biosystems, Foster City, USA) according to protocol specified from the manufacturer. All genotypes were obtained in duplicate. The Hardy–Weinberg equilibrium (HWE) in controls was tested for each polymorphism by the Chi-square (χ^2) test. Chi-square and Kruskal–Wallis test was used to compare gender and age distribution between cases and controls, respectively.

Unconditional logistic regression was used to assess genotype distributions between cases and controls, as well as between R and NR, considering the homozygotes for the more frequent allele among controls as the reference class. Among cases, PFS was evaluated using the Kaplan–Meier analysis, and log-rank test. Hazard ratio estimates (HR) and 95%CI were calculated using Cox proportional hazard models. Test for interaction was performed through likelihood ratio test (detailed methods are described in supplementary material).

3. Results

All SNPs resulted in HWE and allele frequencies were similar to those already reported in the literature. We observed no differences between distributions of genotypes among cases and controls for each of the studied SNPs (supplementary Table 1). For all the SNPs, no substantial differences between age- and gender-adjusted ORs and unadjusted ORs were observed. Interaction between each SNP and age was also examined, by dividing the subjects in two strata, defined as under and over 60 years (using age median value as cut off). *IL6* rs1800797 (−597A>G) genotypes showed a different distribution between cases and controls, in the stratum over 60 s. In particular, carriers of the A allele resulted significantly less prone to develop MM ($OR_{CARRIERS}$: 0.55, 95%CI: 0.31–0.95, $p = 0.03$) compared to the G/G individuals of the same age stratum (Table 2a).

The median PFS of the 91 MM patients that underwent ASCT was of 17 months (interquartile range 10–28). By examining the relationship between PFS and several clinical parameters in age- and gender-adjusted models (supplementary Table 4), no significant difference was seen, with exception of an increased risk of progression for patients not responding to first line therapy ($HR_{FIRSTLINE\_NR} = 2.44$, 95%CI 1.36–4.39; $p = 0.01$) or to transplantation ($HR_{ASCT\_NR} = 1.74$, 95%CI 1.06–2.87; $p = 0.03$) and for patients receiving low dosage of Melphalan (HR = 1.92, 95%CI 1.12–3.29, $p = 0.02$) (Supplementary Table 4). A multivariate model with all clinical covariates (age, gender, β2-microglobulin, creatinin, albumin, haemoglobin, 1st line regimen, response to 1st line therapy and ASCT, presence/absence of the second ASCT) was also performed (data not shown). No substantial differences between age- and gender-adjusted HRs and multi-adjusted HRs emerged.

We analyzed then the influence of the typed genetic variants on the individual response to therapy in the 91 subjects for which therapy data were available. None of the investigated loci showed association with response to front line treatments, response to ASCT or PFS (Supplementary Tables 2 and 3). However, analyzing interaction with age, we observed that PFS of patients over 60 s was significantly associated with the *TNF-α* rs1800629 (−308G>A) genotype: the carriers of the rs1800629_A allele showed a shorter PFS, with 2.8-fold (HR: 2.79, 95%CI: 1.25–5.67, $p = 0.01$) increased risk of progression respect to patients of the same age stratum with G/G genotype (Table 2b, Fig. 1). A lower Melphalan Dosage (MD, 100 vs. 200 mg/m^2) was administered to all patients over 60 s and to 11 patients of the younger age stratum (most likely because of the presence of severe co-morbidities) therefore we investigate whether the observed interaction between age and genotype could be due to MD. Despite the fact that MD and age strata are often correlated on a clinical basis, the interaction with *TNF-α* rs1800629 genotypes was significant for age strata

Table 2
Age-modified effect of *IL6* and *TNF-α* genetic variants on individual susceptibility and therapy outcome in MM patients.

a. Age-modified effect of *IL6* rs1800797 on the individual susceptibility to MM

IL6 SNP (rs1800797)	Genotypes		OR†	95% C.I.	*P* value
	Cases *N* (%)	Controls *N* (%)			
Total	201	234			
G/G	97 (48.3)	103 (44.0)	1	Ref.	–
G/A	89 (44.3)	109 (46.6)	0.86	0.58–1.28	0.47
A/A	15 (7.4)	22 (9.4)	0.76	0.37–1.57	0.47
G/A+A/A	104 (51.7)	131 (56)	0.85	0.58–1.24	0.39
Age ≤ 60					
G/G	40 (43.0)	65 (48.9)	1	Ref.	–
G/A+A/A	53 (57.0)	68 (51.1)	1.27	0.75–2.17	0.37
Age > 60					
G/G	56 (52.3)	38 (37.6)	1	Ref.	–
G/A+A/A	51 (47.7)	63 (62.4)	0.55	0.31–0.95	**0.03***

b. Age-modified effect of *TNF-α* rs1800629 on PFS.

TNF-α SNP (rs1800629)	Genotypes Cases *N* (%)	HR††	95% C.I.	*P* value
Total	91			
G/G	69 (75.8)	1	Ref.	–
G/A	17 (18.7)	1.59	0.82–3.08	0.17
A/A	5 (5.5)	1.32	0.46–3.75	0.60
G/A+A/A	22 (24.2)	1.51	0.83–2.73	0.17
Age < 60				
G/G	36 (76.6)	1	Ref.	–
G/A+A/A	11 (23.4)	0.78	0.31–1.95	0.60
Age ≥ 60				
G/G	33 (75.0)	1	Ref.	–
G/A+A/A	11 (25.0)	2.79	1.30–5.99	**0.01****

Differences in total numbers are due to failed genotyping. Values in bold show $p < 0.05$.
† Odds ratio (adjusted for age and gender).
†† Hazard ratio (adjusted for age and gender); C.I.: confidence interval.
* Likelihood-ratio test *p*-value = **0.03**.
** Likelihood-ratio test *p*-value = **0.03.**

(Likelihood-ratio test $p = 0.03$), and not MD (Likelihood-ratio test $p = 0.69$). Sensitivity analysis showed consistent results (data not shown).

4. Discussion

Our results, in the overall, did not reveal association between the genetic variants studied and the risk of developing MM or the therapy outcome, confirming part of the findings already reported [3]. Nevertheless, some interesting observations here reported are worth of consideration. As a matter of fact, we observed a statistically significant interaction of age and *IL6* rs1800797 genotype in modifying the risk of developing MM and with the *TNF-α* rs1800629 genotype in modifying PFS. Similar results were reported from Kádár et al. that observed a protective association of *TNF-α* rs1800629_A allele with MM risk, stronger in younger patients [4]. These findings are consistent with the hypothesis that genetic variation in pro-inflammatory cytokines might affect their ability to prevent cancer onset and/or to control disease progression [5]. On the other hand, the "molecular inflammation hypothesis of ageing" suggests that inflammatory processes are of crucial importance in ageing and age-related degenerative diseases, including

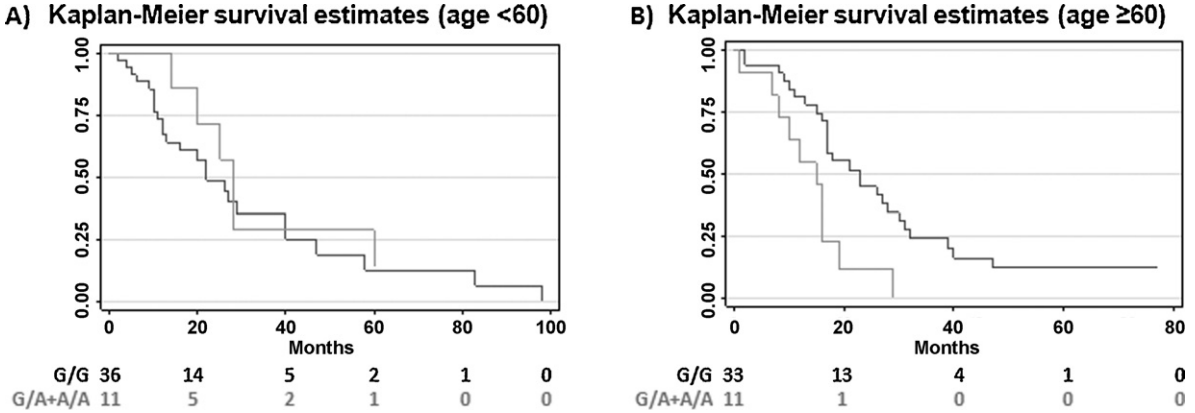

Fig. 1. Age-related effect of *TNF-α* rs1800629 on PFS. Kaplan–Meier survival functions of the homozygotes G/G individuals (black line) and A carriers (grey line) for the *TNF-α* rs1800629 SNP in the two age strata defined as <60 (panel A) and ≥60 (panel B).

cancer [6]. While acute inflammation is a fundamental mechanism of defence, a chronic inflammatory status, though at low to moderate level, might contribute to age-associated morbidity and mortality [7]. Thus, genetic variation in inflammation-related genes could represent an important mediator of the complex interplay between ageing and cancer.

Increased levels of IL6 have been shown in the elderly as well as in cancers patients and genetic variants in *IL6* gene have been associated with both cancer onset and longevity [8,9]. In addition, it has been shown that *IL6* −174G>C genetic polymorphism affect IL6 levels in an age-dependent manner. Carriers of the *IL6* −174_C allele showed a decreased production of IL6 during aging [10]. Lower expression/levels of IL6 has been also associated to *IL6* rs1800797_A allele [11], in nearly complete LD ($r^2 = 0.927$, HapMap CEU population) with the *IL6* −174_C allele. IL6 is one of the most important growth factors for malignant plasma cells and higher IL6 levels have been observed in MM patients [12]. These observation are at the base of the hypothesis that increased IL6 levels can determine higher disease risk. Even if the relationship between gene polymorphisms and serum levels of IL6 is controversial, the lower expression/levels of IL6 associated to the *IL6* rs1800797_A allele would explain the protective association observed for this allele in subjects over 60 s, in which a chronic inflammation status is already established. On the contrary, carrying the *IL6* risk allele would have only a limited impact in subjects under 60 s, who could not be yet (or more recently) entered the "chronic inflammation phase". In the same way, we can argue that the presence of the *TNF-α* rs1800629_A allele, associated to increased level of TNF-α [13], could contribute to accentuate inflammatory status associated to ageing and age-related morbidity and mortality and explain the here reported association of this SNP with a shorter PFS of patients over 60 s.

In conclusion, individual susceptibility to MM, as for other cancer and age-related diseases, could be seen as the resultant of the complex interaction between inflammation mechanisms and ageing. On the basis of our observation, we hypothesize that genetic variability at cytokines *loci*, by altering the normal production of these mediators, can exert important influences on both these processes.

Nevertheless, this study presents some limitations, as i.e. the relatively small sample size, that can affect the robustness of the findings. The suggested hypothesis that genetic variation in inflammation-related genes could interact with ageing modulating the individual risk to develop cancer remains intriguing. Therefore, further studies are needed to clarify the effect of cytokine SNPs and the role of the age in their influence on both risk of developing MM and on treatment outcome.

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Author's contribution

A.M., G.B., V.M. and A.M.R. designed the study, interpreted the results and wrote the original manuscript. G.B., S.G. and E.O. recruited MM patients. A.L., D.D.B. and A.M. performed the sample genotyping. V.M. and F.L. performed the statistical analysis. R.B. and M.P. contributed to the proofreading of the final manuscript. All the authors carefully revised and approved the final version of the manuscript. A.M and G.B. contributed equally to this work.

Conflict of Interest

None of the authors has conflict of interests to declare.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.leukres.2012.02.009.

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