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LETTER TO THE EDITOR

Polymorphisms in xenobiotic transporters *ABCB1*, *ABCG2*, *ABCC2*, *ABCC1*, *ABCC3* and multiple myeloma risk: a case–control study in the context of the International Multiple Myeloma rESEarch consortium

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Multiple myeloma (MM) is a hematological neoplasm that arises from a single clone of malignant plasma cells in the bone marrow. In Europe, 4.6/100 000 males and 3.2/100 000 females every year develop MM, with a median age at diagnosis around 60 years.[1]

The observation of a higher risk to develop MM among first-degree relatives of MM patients in several population-based case–control studies supports the idea that genetic factors are involved in MM pathogenesis.[2] Therefore, several studies focusing on various genes and pathways have tried to identify single-nucleotide polymorphisms (SNPs) associated with the susceptibility to the disease.[3,4]

The detoxification and elimination of xenobiotic compounds is one of the most investigated processes in cancer susceptibility, with several evidences of its association with cancer risk.[5] ATP-binding cassette (ABC) subfamily B, member 1 (*ABCB1* or *MDR1*); subfamily G, member 2 (*ABCG2* or *BCRP*); subfamily C, member 2 (*ABCC2* or *MRP2*); subfamily C, member 1 (*ABCC1* or *MRP1*) and subfamily C, member 3 (*ABCC3* or *MRP3*) are efflux pumps that have a key role in determining the intracellular levels of xenobiotic and toxic compounds, thus protecting cells. ABC transporters are expressed in many tissues, including the blood, and they have been recently showed to be characteristically expressed in hematopoietic stem cells (HSCs).[6] Beside, the exposure to pesticides and toxic compounds, most of which are substrates of ABC transporters, has been shown to increase MM and MGUS risk.[7,8] Therefore, it is a reasonable hypothesis that genetic variation within these genes can affect the exposure of hematopoietic cells to toxic compounds resulting in an increased/decreased risk of MM.

Despite the large number of studies investigating the role of variants in ABC transporters in cancer susceptibility, including hematological malignancies,[9,10] to date only one investigated the role of three variants in *ABCB1* gene and MM susceptibility.[11] Therefore, we performed a two-phase candidate gene association study to comprehensively evaluate the role of genetic variants of ABCs transporter genes in relation to MM risk. We selected 54 SNPs in *ABCB1*, *ABCG2* and *ABCC2* genes using a tagging approach to take into account for all the common genetic variability within these genes and four additional functional polymorphisms in *ABCC1* and *ABCC3* to test their impact on MM susceptibility (a complete list of the selected SNPs is available in Supplementary Table I). In the context of an International Consortium named IMMEnSE (International Multiple Myeloma rESEarch), we collected more of 700 MM cases and 900 controls from Italy, Spain, Poland, Portugal and France. A subset of 633 MM patients and 835 healthy controls with a similar distribution between gender and age (χ^2 $P = 0.753$, Kruskal–Wallis $P = 0.307$) was available for this study and was used for the initial screening of the 58 genetic variants selected. Baseline characteristics of the population are reported in Table 1. The IMMEnSE biobank is set up at the German Cancer Research Center (DKFZ) in Heidelberg, where genotyping has been conducted using both TaqMan (ABI, Applied Biosystems, Foster City, CA, USA) and KASPar (KBioscence, Hoddesdon, UK) technologies and adequate quality control procedures (as described in the Supplementary methods). All the SNPs were in Hardy–Weinberg Equilibrium (HWE) among controls ($P > 0.001$) with the exception of the *ABCC2* SNP rs3740073 ($P < 0.001$) that was therefore excluded from further analysis.

Logistic regression was used to assess the main effects of the genetic polymorphisms on MM risk using a co-dominant and a dominant inheritance model adjusted for age, gender and region of origin. For each SNP, the more common allele in the controls was assigned as the reference category (for detailed material and methods see Supplementary Information). Of the 58 variants screened, 13 SNPs resulted associated with MM risk in the IMMEnSE population at the conventional *P*-value level of $P < 0.05$ (Supplementary Table II). Haplotype analysis (Supplementary Table III) and gene–gene interaction analysis did not add any significant information to the single SNP analysis. With the aim to further investigate the associations found in the first phase, an independent case–control set of German origin was collected through the collaboration with the Heidelberg Myeloma Group.

Using the same methodology described above, we genotyped the 13 SNPs associated in the first phase in 564 MM cases and 1471 healthy controls from Germany (Table 1) with a similar distribution between genders (χ^2 $P = 0.141$), although age was significantly higher for the controls (Kruskal–Wallis $P = 0.0001$). All the SNPs resulted in HWE among controls ($P > 0.001$). The two *ABCB1* SNPs rs2235013 and rs10256836 were replicated in the Heidelberg population (Supplementary Table IV).

We analyzed the results for the whole population of 1197 MM cases and 2306 controls (Supplementary Tables V, VI). Interestingly, the *ABCB1* SNPs rs10264990 and rs17327442 resulted associated with MM risk reaching the *P*-value threshold adjusted for multiple testing ($P < 0.0014$; Table 2). To evaluate heterogeneity of the findings between populations, we performed a meta-analysis across all the different regions in the IMMEnSE and Heidelberg populations. Results showed the absence of statistically significant heterogeneity among different case–control sets and confirmed the associations (Supplementary Figure 1). To further asses the robustness of our findings, we performed 100 000 permutations to compare *P*-values from randomly generated empirical distributions for the SNPs rs10264990 and rs17327442 with the observed ones. We confirmed in this way the statistically significant associations of the T carriers for the rs10264990 with a decreased risk of MM ($P = 0.015$) and of the T/T homozygous for the rs17327442 with an increased risk of MM ($P = 0.009$; Table 2).

At the best of our knowledge, this is the largest study on genetic susceptibility of MM and has a sufficient statistical power (over 0.80 for a co-dominant model) to detect an odds ratio = 1.57 at $\alpha = 0.0014$ (study-wise significance *P*-threshold) for a SNP with a MAF = 0.05 in the overall population. Beside, this is the first comprehensive study that captures all the common genetic

Table 1. Demographical characteristics of cases and controls

Center	*Cases*			*Controls*		
	Gender M/F (Total)	*Mean Age (± S.d.)*	*Median Age (Min-Max)*	*Gender M/F (Total)*	*Mean Age (± S.d.)*	*Median Age (Min-Max)*
First Study Population (IMMEnSE consortium)						
Pisa, Italy[a]	115/108 (223)	62.60 (±9.90)	63 (35-87)	129/105 (234)	58.77 (±10.89)	58.5 (35-89)
Lodz, Poland[b]	50/47 (97)	61.80 (±10.55)	62 (39-86)	66/80 (146)	69.53 (±6.71)	69 (55-98)
Salamanca, Spain[c]	66/58 (124)	62.70 (±11.60)	62 (31-93)	137/125 (262)	65.56 (±12.85)	66 (24-92)
Granada, Spain[d]	23/36 (59)	63.25 (±10.30)	63 (39-86)			
Lyon, France[e]	43/32 (74)	55.60(±8.97)	57 (34-75)	47/47 (94)	33.31 (±14.80)	30 (18-60)
Braga, Portugal[f]	26/29 (55)	66.78 (±10.49)	68 (43-86)	54/45 (99)	60.76 (±7.72)	58 (51-85)
Total	323/310 (633)	62.10 (±10.64)	62 (31-93)	433/402 (835)	60.15 (±15.18)	62 (18-98)
Replication Study population (Heidelberg Myeloma Group)						
Heidelberg, Germany[g]	323/241 (564)	54.58 (±7.82)	56 (25-66)	789/682 (1471)	56.31 (±9.89)	59 (18-68)
Overall population						
Total	646/551 (1197)	58.43 (±10.10)	59 (25-93)	1222/1084 (2306)	56.78 (±12.73)	59 (18-98)

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Table 2. Significant associations of *ABCB1* SNPs rs10264990 and rs17327442 with MM risk in the overall population

SNP (rs)	*Cases* N *(%)*	*Controls* N *(%)*	*OR*[a]	*95%CI*	P-*value*	P-*trend*	P-*perm*
ABCB1 rs10264990							
C/C	549 (47.1)	958 (41.7)	1	Ref.	–	**0.023**	
C/T	475 (40.7)	1059 (46.0)	0.77	0.66 - 0.90	**0.001**		
T/T	142 (12.2)	283 (12.3)	0.85	0.68 - 1.07	*0.171*		*0.869*[*]
C/T + T/T	617 (52.9)	1342 (58.3)	0.79	0.68 - 0.91	**0.001**		**0.015**[**]
ABCB1 rs17327442							
A/A	824 (69.7)	1600 (69.9)	1	Ref.	–	*0.191*	
A/T	309 (26.1)	643 (28.1)	0.92	0.78 - 1.08	*0.309*		
T/T	50 (4.2)	46 (2.0)	1.99	1.32 - 3.02	**0.001**		**0.009**[*]
A/T + T/T	359 (30.3)	689 (30.1)	0.99	0.85 - 1.16	*0.928*		*1*[**]

Genotype distribution among MM cases and controls in the overall population of the ABCB1 SNPs rs10264990 and rs17327442. [a]Odds Ratios (OR) are adjusted for age, gender and region of origin. Differences in sample numbers are due to failures in genotyping. Results in bold show $P<0.05$. [*]*P*-value obtained after 100 000 permutations following a co-dominant model. [**]*P*-value obtained after 100 000 permutations following a dominant model.

variation within *ABCB1*, *ABCG2* and *ABCC2* genes and investigates variants in *ABCC1* and *ABCC3* in relation to MM risk. Our results confirm the findings of the only study already published in the literature showing no associations of the same three *ABCB1* variants previously tested (or their tag SNP) with MM risk.[11]

Our results suggest a possible low-penetrance role for the two genetic variants rs10264990 and rs17327442 within *ABCB1* gene in modulating individual susceptibility to MM can be hypothesized.

The SNP rs17327442 has been already found associated with genetic susceptibility to Crohn's disease,[12] whereas the SNP rs10264990 has not been associated with any disease risk. Both these SNPs are located in intronic regions of the *ABCB1* gene and no functional data have been reported to date. To explain our observation, we investigated the putative function of the two SNPs with the FastSNP tool.[13] Although the *ABCB1* SNP rs10264990 has no known or predicted function, the genetic *ABCB1* variant rs17327442 is predicted to be an 'intronic enhancer'. In particular, the rs17327442_T allele is predicted to introduce a binding site for the transcription factors T-cell acute lymphocytic leukemia 1 (*TAL1*). This latter finding is particularly intriguing. It has been shown that *TAL1* is a crucial factor for HSC differentiation and its deregulation can lead to oncogenesis in T cells by altering several downstream genes. Several ABC family members are regulated by *TAL1*, and in particular *ABCG2*, *ABCE1* and *ABCB10* are activated in erythroid cell lines after *TAL1* knocking down, while *ABCC1* expression is repressed.[14,15] Although *ABCB1* seems not to be one of the targets for *TAL1* regulation, one can hypothesize that the introduction of a binding site for the transcription factor by the rs17327442_T allele could determine its interaction with *TAL1*. This could therefore result in an aberrant regulation of *ABCB1* expression during normal HSC development. Owing to the broad spectrum of substances transported by this pump, including regulators and mediators of B-cell growth and survival, an alteration of its normal expression could enhance the exposure of HSCs to mutagens or proliferating signals that could therefore justify the increased risk to develop MM associated with the rs17327442 T/T genotype.

In conclusion, our results suggest that genetic variants within *ABCB1* gene can have a role on the risk to develop MM. Nevertheless the association of the *ABCB1* SNP rs17327442 T/T homozygotes with a twofold higher risk to develop MM has to be replicated on independent populations to clarify its real impact.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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