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ORIGINAL ARTICLE

Polymorphisms in regulators of xenobiotic transport and metabolism genes *PXR* and *CAR* do not affect multiple myeloma risk: a case–control study in the context of the IMMEnSE consortium

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The exposure to pesticides and toxic compounds in xenobiotic transport and metabolism genes has been shown to affect risk of developing multiple myeloma (MM). Therefore, we hypothesized that genetic variations in xenobiotic transport and metabolism regulator genes *PXR* (*NR1I2*) and *CAR* (*NR1I3*) could determine a difference in MM susceptibility. Ten tagging single-nucleotide polymorphisms (SNPs) for *PXR* and seven for the *CAR* genes were selected and genotyped in 627 MM cases and 883 controls collected in the context of the International Multiple Myeloma rESEarch (IMMEnSE) consortium. None of the 17 SNPs investigated showed significant association with MM risk either alone or when combined in haplotypes. Significant SNP–SNP interactions were not found, neither with 58 previously genotyped polymorphisms in ABC transporters. We can therefore exclude that common genetic variants in the xenobiotic transport and metabolism regulator genes *PXR* and *CAR* affect MM risk.
Journal of Human Genetics (2013) **58,** 155–159; doi:10.1038/jhg.2012.149; published online 10 January 2013

INTRODUCTION

Multiple myeloma (MM) is a hematological neoplasm with a world-wide incidence of 1.5/100 000 new cases/year. The disease is slightly more frequent in males than females, and the mean age at diagnosis is around 60 years.[1] It has been shown that MM could evolve from an asymptomatic premalignant stage termed monoclonal gammopathy of undetermined significance (MGUS). The rate of progression of this condition to MM is around 1.5% per year, and about 15–17% of MGUS subjects develop MM.[2] Among other proposed risk factors, pesticides and toxic compounds have been shown to affect the risk of developing MM and MGUS.[3,4] In particular, higher incidence rates of both MM and MGUS have been shown among farmers and agricultural workers.[3,5–7]

In addition, converging evidences of MM in monozygotic twins and familial aggregation of MM strongly suggest that MM etiology

has a robust genetic component.[8] Several case–control studies investigating the role of single-nucleotide polymorphisms (SNPs) in MM risk have been conducted, and associations of SNPs in genes involved in xenobiotic metabolism with MM risk have been reported.[9,10] Recently, we found a strong association of SNPs in the key xenobiotic transporter *ABCB1* gene with the risk of MM in the largest candidate-gene study on MM risk conducted to date.[11] Both evidences that the exposure to toxic compounds, and in particular pesticides, can increase the risk of MM (and its premalignant condition MGUS) and that several SNPs within genes involved in xenobiotic transport and metabolism are associated with MM risk suggest that the xenobiotic metabolism process could have an important role in MM susceptibility and that genetic variation in key genes within this pathway can further affect the individual risk to develop MM. Although the role of genetic variation in xenobiotic

metabolism enzymes and transporters has been already extensively studied, no study focused on the role of regulators of xenobiotic transport and metabolism.

The pregnane X receptor (PXR or NR1I2) and the constitutive androstane receptor (CAR or NR1I3) are the most known and well-characterized regulators of xenobiotic transport and metabolism. These proteins belong to the nuclear receptor subfamily and are activated by numerous xenobiotics, drugs, bile acids and hormones. It has been shown that several environmental, occupational and natural products, among which pesticides (some of which specifically linked with MM risk[3,5–7]), can activate (or inactivate) both PXR and CAR.[12] The activation of PXR and CAR leads to the induction of several detoxification enzymes (that is, phase I cytochrome P450 enzymes, phase II enzymes, such as uridine diphospho-glucorunosyltransferase, glutathione-S-transferases and sulfutransferases, and the phase III transporters, such as ABCB1 and ABCG2).[12] In addition, *PXR* and *CAR* have shown to be highly polymorphic genes with several SNPs identified within their coding or regulatory sequences. Indeed, genetic variants within *PXR* and *CAR* genes have been related to differential gene expression of some of the detoxifying enzymes activated by these receptors.[12–14] For this reason, it has been hypothesized that part of the individual variability in xenobiotic metabolism efficiency could be due to the genetic variations within these regulator genes.[15]

Therefore, to address the hypothesis that genetic variants in *PXR* and *CAR* genes could affect the risk of MM, we selected 17 SNPs in the *PXR* and *CAR* genes and genotyped them in 627 MM cases and 883 controls.

MATERIALS AND METHODS

Study population
The study population consisted of 627 MM cases and 883 controls (Table 1). This study way carried out in the context of the International Multiple Myeloma rESEarch (IMMEnSE) consortium, a recently created working group that aims to study genetics and pharmacogenetics of MM.[10,16,17] Briefly, cases were defined by a confirmed diagnosis of MM, according to the International Myeloma Working Group criteria.[18] Incident cases of MM diagnosed between 1990–2010 were recruited, whereas subjects with uncertain diagnosis or MGUS were excluded. Different region-specific subpopulations of controls were selected among the general population, as well as among hospitalized subjects with different diagnoses, excluding cancer.

For each subject, informed consent to collect fresh blood and perform DNA extraction for research purpose was requested and collected individually by each center. The study has been approved by the competent ethical committees.

SNP selection and genotyping
We used a tagging SNP approach for the selection of the genetic variants within the *PXR* and the *CAR* genes. The entire set of common genetic variants (including 5 kb upstream of the first exon and 5 kb downstream of the last exon of each gene), with minor allele frequency (MAF) $\geqslant 5\%$ in Caucasians from the International HapMap Project (release no. 26; http://www.hapmap. org), was included for both genes. Tagging SNPs were selected using the Tagger algorithm available through Haploview (http://www.broad.mit.edu/mpg/haploview/; http://www.broad.mit.edu/mpg/tagger/), using pairwise SNP selection with a minimum r^2 threshold of 0.8. This selection resulted in 10 tagging SNPs for *PXR* and 7 for *CAR*. Genotyping was carried out at the German Cancer Research Center (DKFZ) in Heidelberg, where the IMMEnSE bio-bank is set up, on genomic DNA extracted from peripheral blood, using both TaqMan (Applied Biosystems, Foster City, CA, USA) and KASPar (KBioscence, Hoddesdon, UK) technologies. The order of DNAs from cases and controls was randomized on plates to ensure that an equal number of cases and controls were analyzed simultaneously. For quality-control purpose, duplicates of 10% of the samples were interspersed throughout the plates. PCR plates for both TaqMan and KASPar assays were read on an ABI PRISM 7900HT instrument (Applied Biosystems). The SDS Software, version 2.4 (Applied Biosystems) was used to determine the genotypes. Subjects whose samples had a call rate $<75\%$ were dropped from any analysis. Once subjects with low call rate were removed, all SNPs had a call rate $>95\%$.

Statistical analysis
The Hardy–Weinberg equilibrium was tested in controls for each polymorphism. The χ^2- and Kruskal–Wallis tests were used to compare gender and age distributions between MM patients and controls. Unconditional 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. For each SNP, the more common allele in the controls was assigned as the reference category. All analyses were adjusted for age (continuous), gender and region of origin, and were conducted with STATA software (StataCorp, College Station, TX, USA).

Because of the large number of SNPs analyzed, a conservative threshold for statistical significance was applied, based on a revised version of the Bonferroni method.[19]

Haplotype blocks were identified from the genotyping data of the controls using SNPtool (http://www.dkfz.de/de/molgen_epidemiology/tools/SNPtool.html)[20] and the Haploview v4.2 software. A MAF >0.05, Hardy–Weinberg equilibrium $P>0.001$ and a call rate $>75\%$ were used as cut-off values. Individual haplotypes were then statistically inferred using the PHASE v.2.1.1 algorithm, based on a Bayesian approach (http://www.stat.Washington.edu/stephens/).[21,22] Unconditional logistic regression was used to estimate risk. The most frequent haplotype was set as reference, whereas haplotypes with a frequency $<3\%$ were declared as rare and combined in a single category.

Table 1 Demographical characteristics of IMMEnSE cases and controls used in this study

Region[a]	Cases			Controls			
	Gender M/F (Tot)	Mean Age (± S.d.)	Median Age (Range)	Gender M/F (Tot)	Mean Age (± S.d.)	Median Age (Range)	Control type
IT	114/107 (221)	62.7 (±9.8)	63 (35–87)	124/104 (228)	58.9 (±10.9)	59 (35–89)	General population
PL	49/48 (97)	62.1 (±10.4)	63 (39–86)	67/78 (145)	69.5 (±6.7)	69 (55–98)	Blood donors
ES	84/89 (173)	62.8 (±11.4)	62 (31–93)	168/151 (319)	65.2 (±12.6)	66 (24–92)	Hospitalized subjects
FR	44/33 (77)	55.9 (±9.0)	57 (34–75)	46/46 (92)	33.0 (±14.8)	30 (18–60)	Blood donors
PT	29/30 (59)	67.2 (±10.3)	69 (43–86)	54/45 (99)	60.7 (±7.7)	58 (51–85)	Blood donors
Total	320/307 (627)	62.2 (±10.6)	62 (31–93)	459/424 (883)	60.4 (±15.0)	62 (18–98)	

Abbreviations: ES, Spain; FR, France; IMMEnSE, International Multiple Myeloma rESEarch; IT, Italy; PL, Poland; PT, Portugal.
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Table 2 Genotype distribution of the typed SNPs in *PXR* and *CAR* genes among MM cases and controls

SNP (rs)	Cases (%)	Controls (%)	OR[a]	95% CI	P-value	P-trend
PXR rs10511395						
C/C	429 (69.5)	623 (70.7)	1.00	Ref		0.423
A/C	160 (25.9)	228 (25.9)	1.02	0.81–1.30	0.851	
A/A	28 (4.6)	30 (3.4)	1.38	0.81–2.35	0.232	
A/C + A/A	188 (30.5)	258 (29.3)	1.06	0.85–1.33	0.588	
PXR rs1054190						
C/C	452 (74.0)	656 (74.8)	1.00	Ref		0.451
C/T	137 (22.4)	200 (22.8)	1.00	0.78–1.28	0.993	
T/T	22 (3.6)	21 (2.4)	1.56	0.84–2.88	0.155	
C/T + T/T	159 (26.0)	221 (25.2)	1.05	0.83–1.34	0.680	
PXR rs11917714						
C/C	412 (65.9)	591 (67.2)	1.00	Ref		0.819
C/T	190 (30.4)	250 (28.5)	1.07	0.85–1.35	0.535	
T/T	23 (3.7)	38 (4.3)	0.84	0.49–1.44	0.536	
C/T + T/T	213 (34.1)	288 (32.8)	1.04	0.84–1.30	0.698	
PXR rs12488820						
C/C	223 (36.3)	296 (33.7)	1.00	Ref		0.126
C/T	289 (47.0)	407 (46.4)	0.93	0.74–1.18	0.574	
T/T	103 (16.7)	175 (19.9)	0.79	0.58–1.06	0.119	
C/T + T/T	392 (63.7)	562 (66.3)	0.89	0.72–1.11	0.299	
PXR rs13071341						
G/G	430 (69.6)	593 (67.4)	1.00	Ref		0.807
A/G	166 (26.9)	269 (30.6)	0.85	0.67–1.07	0.158	
A/A	22 (3.5)	18 (2.0)	1.70	0.90–3.22	0.102	
A/G + A/A	188 (30.4)	287 (32.6)	0.90	0.72–1.12	0.355	
PXR rs3732359						
A/A	352 (58.7)	516 (39.4)	1.00	Ref		0.981
A/G	209 (34.8)	291 (33.5)	1.04	0.83–1.30	0.720	
G/G	39 (6.5)	62 (7.1)	0.90	0.59–1.37	0.619	
A/G + G/G	248 (41.3)	353 (40.6)	1.02	0.82–1.26	0.881	
PXR rs13059232						
C/C	255 (41.2)	383 (43.6)	1.00	Ref		0.815
C/T	299 (48.3)	390 (44.4)	1.16	0.93–1.44	0.192	
T/T	65 (10.5)	106 (12.0)	0.94	0.66–1.33	0.711	
C/T + T/T	364 (58.8)	496 (56.4)	1.11	0.90–1.37	0.326	
PXR rs3732357						
A/A	300 (48.7)	437 (49.7)	1.00	Ref		0.258
A/G	240 (39.0)	361 (41.0)	0.94	0.75–1.17	0.589	
G/G	76 (12.3)	82 (9.3)	1.31	0.92–1.85	0.130	
A/G + G/G	316 (51.3)	443 (50.3)	1.01	0.82–1.24	0.934	
PXR rs1357459						
T/T	328 (53.6)	463 (52.9)	1.00	Ref		0.424
C/T	249 (40.7)	345 (39.4)	1.02	0.82–1.27	0.850	
C/C	35 (5.7)	67 (7.7)	0.75	0.49–1.17	0.206	
C/T + C/C	274 (46.4)	412 (47.1)	0.98	0.79–1.20	0.838	
CAR rs3003596						
A/A	218 (35.4)	335 (38.1)	1.00	Ref		0.571
A/G	296 (48.0)	393 (44.7)	1.16	0.93–1.46	0.191	
G/G	102 (16.6)	151 (17.2)	1.04	0.77–1.41	0.799	
A/G + G/G	398 (64.6)	544 (61.9)	1.13	0.91–1.40	0.264	

Table 2 (Continued)

SNP (rs)	Cases (%)	Controls (%)	OR[a]	95% CI	P-value	P-trend
CAR rs3813627						
G/G	264 (42.7)	371 (42.0)	1.00	Ref		0.642
G/T	276 (44.7)	392 (44.4)	0.98	0.79–1.23	0.882	
T/T	78 (12.6)	120 (13.6)	0.91	0.66–1.26	0.581	
G/T + T/T	354 (57.3)	512 (58.0)	0.97	0.78–1.19	0.750	
CAR rs11265571						
A/A	441 (73.1)	635 (73.5)	1.00	Ref		0.911
A/T	147 (24.4)	207 (24.0)	1.01	0.79–1.29	0.911	
T/T	15 (2.5)	22 (2.5)	0.97	0.49–1.89	0.921	
A/T + T/T	162 (26.9)	229 (26.5)	1.01	0.80–1.28	0.937	
CAR rs2307418						
T/T	404 (64.2)	575 (65.7)	1.00	Ref		0.836
G/T	193 (31.1)	268 (30.6)	1.02	0.81–1.27	0.879	
G/G	23 (3.7)	32 (3.7)	1.05	0.60–1.83	0.863	
G/T + G/G	216 (34.8)	300 (34.3)	1.02	0.82–1.27	0.851	
CAR rs2502805						
C/C	348 (56.6)	508 (57.7)	1.00	Ref		0.527
C/T	220 (35.8)	313 (35.6)	1.05	0.84–1.30	0.693	
T/T	47 (7.6)	59 (6.7)	1.16	0.77–1.74	0.484	
C/T + T/T	267 (43.4)	372 (42.3)	1.06	0.86–1.31	0.564	
CAR rs4073054						
A/A	245 (39.8)	346 (39.4)	1.00	Ref		0.770
A/C	291 (47.2)	412 (46.9)	0.98	0.78–1.22	0.855	
C/C	80 (13.0)	120 (13.7)	0.94	0.68–1.31	0.720	
A/C + C/C	371 (60.2)	532 (60.6)	0.97	0.78–1.20	0.785	
CAR rs4233368						
C/C	360 (57.6)	524 (59.8)	1.00	Ref		0.391
A/C	225 (36.0)	302 (34.4)	1.09	0.88–1.36	0.439	
A/A	40 (6.4)	51 (5.8)	1.15	0.74–1.78	0.538	
A/C + A/A	265 (42.4)	353 (40.2)	1.10	0.89–1.35	0.377	

Abbreviations: CI, confidence interval; MM, multiple myeloma; OR, odds ratio; SNP, single-nucleotide polymorphism.
[a]ORs are adjusted for age, gender and region of recruitment. Differences in sample numbers are due to failures in genotyping.

SNP–SNP interactions were tested using the nonparametric Multifactor Dimensionality Reduction approach. Detailed information is described elsewhere,[23] and the Multifactor Dimensionality Reduction software is available from http://www.epistasis.org.

RESULTS AND DISCUSSION

MM cases and controls had similar gender and age distributions ($\chi^2 = 0.717$, Kruskal–Wallis = 0.39). All the SNPs were in Hardy–Weinberg equilibrium among controls ($P > 0.02$), with the exception of the *PXR* rs2461818 ($P = 0.0003$), which was therefore excluded from further analysis.

To correct for multiple testing, we calculated M_{eff} values for each candidate gene separately (PXR = 8, CAR = 3) and for the whole study (by adding the individual M_{eff} values). The study-wise M_{eff} was 11. We therefore used a study-wise statistical significance P-value of $0.05/11 = 0.0045$. None of the SNPs resulted associated with MM risk, with either a co-dominant or dominant inheritance model (Table 2).

Table 3 Haplotype blocks in *PXR* and *CAR* genes and their distribution among MM cases and controls in the IMMEnSE population

Gene	SNPs in haplotype block	Haplotypes	Cases N (%)	Controls N (%)	OR[a]	CI	P-value
PXR Block 1	rs12488820, rs13071341	CG	741 (59.1)	1004 (56.9)	1.00	Ref	
		TG	301 (24.0)	456 (25.8)	0.90	0.75–1.07	0.225
		TA	207 (16.5)	306 (17.3)	0.92	0.75–1.12	0.396
		CA	5 (0.4)	—			
PXR Block 2	rs11917714, rs3732359,	CACC	720 (57.4)	1050 (59.5)	1.00	Ref	
	rs10511395, rs1054190	TGCC	235 (18.7)	330 (18.7)	1.02	0.84–1.24	0.822
		CAAT	181 (14.4)	241 (13.6)	1.10	0.89–1.36	0.385
		CGCC	66 (5.3)	93 (5.3)	1.02	0.73–1.42	0.914
		Others	52 (4.2)	52 (2.9)	1.43	0.96–2.13	0.076
CAR Block 1	rs4233368, rs4073054,	CCTACA	427 (34.0)	630 (35.7)	1.00	Ref	
	rs2307418, rs3003596,	AATGTA	286 (22.8)	388 (22.0)	1.09	0.90–1.33	0.378
	rs2502805 rs11265571	CAGACA	235 (18.7)	331 (18.7)	1.05	0.85–1.30	0.628
		CATGCT	165 (13.2)	250 (14.2)	0.97	0.77–1.23	0.830
		CATACA	56 (4.5)	82 (4.6)	1.00	0.70–1.43	0.990
		Others	85 (6.8)	85 (4.8)	**1.51**	**1.09–2.10**	***0.014***

Abbreviations: IMMEnSE, International Multiple Myeloma rESEarch; MM, multiple myeloma; OR, odds ratio; SNP, single-nucleotide polymorphism.
Haplotype distribution among MM cases and controls for *PXR* and *CAR* genes in the IMMEnSE population.
[a]ORs are adjusted for age, sex and region of origin. Results in bold show $P<0.05$.

We reconstructed haplotypes within *PXR* and *CAR* genes based on the linkage disequilibrium blocks observed in the controls. Two haplotype blocks were identified in the *PXR* and one in the *CAR* gene. None of the individual haplotypes showed association with MM risk (Table 3). The statistically significant association for the *others* group (that is, rare haplotypes lumped together) within the *CAR* haplotype block is most likely because of the heterogeneity of the group. Further analyses within this group are prevented by the small numbers of each individual haplotype.

Finally, we evaluated SNP–SNP interactions using the nonparametric Multifactor Dimensionality Reduction approach. We investigated the interaction between *PXR* and *CAR* genotypes. In addition, we verified the presence of inter-gene interactions between the *PXR* and *CAR* genotypes and 58 previously genotyped polymorphisms in ABC transporters *ABCB1*, *ABCG2*, *ABCC2*, *ABCC1* and *ABCC3*.[11] We performed a stratified analysis of *PXR* and *CAR* genotypes among groups defined according to *ABCB1* genotypes that we previously showed to be strongly associated with MM risk.[11] Nevertheless, no evidence of associations with MM risk emerged in any case (Supplementary Table I).

At the best of our knowledge, this is the first investigation of the *PXR* and *CAR* genetic variation in relation to MM risk. Despite the strong rationale and the existing evidences suggesting a role of genetic variants in xenobiotic transport and metabolism genes in influencing MM risk, our results show no effect of SNPs in *PXR* and *CAR* genes in modifying individual susceptibility to the disease. In this study, we analyzed also the haplotype structure within the two genes and the possible SNP–SNP interactions, including the analysis of previously obtained genotypes for SNPs within ABC transporters. Moreover, our study had a sufficient statistical power to evidence the effect of common low-penetrance genetic variants, such as those expected to affect cancer susceptibility. Indeed, we achieved a statistical power of 80% to discover a minimum odds ratio (OR) of 1.34 with the smallest MAF observed among the selected tagging SNPs (0.14, rs1054190) and $\alpha=0.05$. The minimum OR detectable with the same power decreased to 1.24 with the highest MAF observed (0.43, rs12488820). Thus, we had sufficient power to detect risks of the magnitude commonly observed with genetic polymorphisms.

In the light of our thorough investigation showing overall negative results, we are keen to exclude a role of common genetic variations in the xenobiotic transporter and metabolism regulator genes *PXR* and *CAR* in MM etiology. Although the tag SNP approach accounts for >95% of the genetic variations with a MAF ⩾0.05 in both genes, including functional variants, we cannot exclude the presence of functional SNPs with a lower frequency that could still affect MM risk. Further studies in this sense are needed to identify and establish genetic risk factors for MM.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on Journal of Human Genetics website (http://www.nature.com/jhg)