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CD38 gene polymorphisms and genetic predisposition to multiple myeloma

Polimorfizm genu CD38 a predyspozycja genetyczna do szpiczaka mnogiego

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ABSTRACT

Single nucleotide polymorphisms (SNPs) of adhesion and signaling genes may influence the etiopathogenesis of multiple myeloma (MM). *CD38* molecule and its ligand CD31 are expressed and interact in malignant plasma cells and MM microenvironment. In this study we evaluated allele frequencies and distribution of two potentially functional *CD38* SNPs, intronic rs6449182 (184C>G) and missense rs1800561 (418C>T, $Arg^{140}Trp$) in 175 Caucasian patients with MM and 207 healthy blood donors. The carriers of variant G allele of the rs6449182 SNPs were found to have significantly elevated risk of MM as compared to non-carriers; odds ratio = 5.69 (95% confidence interval = 3.7–8.7), $p < 0.0001$. In contrast, rs1800561 SNP minor T allele was detected at very low and comparable frequencies in patients and controls groups. In conclusion, our data suggest that inherited genetic variation in *CD38* gene may impact on the risk of MM development.

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Introduction

Multiple myeloma (MM) represents a B cell malignancy characterized by clonal plasma cells proliferation in the bone marrow, monoclonal protein in the blood and associated organ dysfunction [1]. While the etiology of MM is largely unknown, there is strong evidence for an inherited genetic susceptibility to this tumor. Presence of the genetic background is supported by the reports on MM cases in

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monozygotic twins as well as the two- to four-fold increased MM risk in first-degree relatives of patients with MM [2–4]. This heritable risk is likely a consequence of the co-inheritance of low-risk genetic variants, mainly single-nucleotide polymorphisms (SNPs). SNPs are a significant source of genetic variation in humans, and are thought to be responsible, at least partially, for the individual differences in genetic susceptibility to complex diseases including MM [5].

Interestingly, findings on familial aggregation of cases of MM, chronic lymphocytic leukemia (CLL), non-Hodgkin lymphoma and Hodgkin lymphoma indicate that these different B-cell malignancies share partially common genetic background [3, 6–8]. Our recent study showed that two potentially functional SNPs of *CD38* gene contribute to the increased risk of CLL [9, 10]. Human *CD38* is a type II transmembrane glycoprotein expressed in immature hematopoietic cells, down-regulated by mature cells, and re-expressed at high levels by activated B cells, T cells, natural killer cells, and dendritic cells [11, 12]. *CD38* displays two, probably independent, functions including a transmembrane signaling receptor and an ectoenzyme [13]. Acting as a receptor *CD38* is involved in cell-cell interactions. Engagement by its ligand CD31 (also known as the platelet endothelial cellular adhesion molecule-1, PECAM-1), followed by an increase in intracellular Ca^{2+}, induces activation and differentiation signals in B, T and natural killer (NK) cells [14–16]. Furthermore, heterophilic (CD31/*CD38*) and homophilic (CD31/CD31) interactions of these adhesion molecules play a key role in lymphocyte adhesion and extravasation [17]. As an ectoenzyme *CD38* exerts both cyclase and hydrolase activities, synthesizing molecules involved in the regulation of cytoplasmic calcium levels [18]. The extracellular domain of *CD38* contains an enzymatic site that can synthesize cyclic ADP ribose (cADPR) from nicotine adenine dinucleotide (NAD+) and is capable to hydrolase cADPR and adenosine diphosphate ribose (ADPR) [18]. cADPR functions as a universal second messenger involved in a distinct pathway of intracellular Ca^{2+} mobilization, resulting in a variety of physiological effects such as activation, proliferation, differentiation and migration [18]. Importantly, the *CD38* gene, located in the short arm of chromosome 4 (4p15), is highly polymorphic [19].

Interestingly, *CD38* is also expressed on MM plasma cells and *CD38*/CD31 interactions are important for MM evolution [20]. However, little is known on the biological and clinical significance of *CD38* SNPs in the context of MM development. In this case-control study we compared the frequencies of two potentially functional *CD38* SNPs, rs6449182 (184C>G) and rs1800561 (418C>T) in MM patients and unaffected controls of Polish Caucasian origin.

Patients and methods

Study subjects

The study included 175 patients diagnosed with MM at the Department of Hematology, Medical University of Lodz or referred to this center for consultation between February 1993 and December 2006. Peripheral blood samples were collected at the time of diagnosis in 5 ml serum tubes and stored at −20 °C temperature until analyzed. Control individuals were 207 volunteer Caucasian blood donors at the local blood bank, residents of the Lodz city region. Fresh peripheral blood samples (2 ml) were obtained from controls between 1999 and 2008. Ethnicity and cancer-free status were self-reported at the time of blood collection.

The study has been reviewed and approved by the Bioethical Committee of the Medical University of Lodz (approval no. RNN/200/11/KE). All patients and controls provided informed consent.

DNA extraction and genotyping

Blood DNA obtained from peripheral blood leukocyte fraction was purified on a QIAamp spin column (Qiagen, Hilden, Germany). The protocol for DNA isolation from body fluids provided by the manufacturer was used with modification as follows: 5 μg of RNA poly (A) (Pharmacia Biotech, Uppsala, Sweden) was added to 1 ml of serum to serve as a carrier to improve the recovery of small amounts of DNA. Lysis was ensured by adding 20 μl of Qiagen Proteinase K solution and 1 ml of buffer AL (QIAamp® DNA mini kit). After 10-min incubation at 56 °C 1 ml of ethanol was added. The mixture was loaded on the QIAamp spin column and centrifuged at 20 000 × *g* for 1 min. The column was washed twice by adding 500 μl of buffer AW (QIAamp® DNA mini kit) and centrifuged at 20 000 g for 1 min. Finally, DNA was incubated for 5 min at room temperature with 50 μl of AE buffer and eluted by centrifugation.

The investigated polymorphisms were analyzed using polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP) method as describe previously [9]. Briefly, for rs6449182 (184C>G) SNP detection assay forward primer 5′-CCGGGTGGTGCTGAGTAGGGAGTC-3′ and reverse primer 5′-CTACGCAGCAGAGCCACCGAGCAG-3′ (IDT, Illinois) were used. The PCR reactions were done in a 20 μl PCR tube containing 50 ng of genomic DNA, 1.5 pmol/l of each primer, 1.5 mmol/l of MgCl2, 250 nmol/l of dNTPs, and 2 units of Taq polymerase (Promega) with amplification conditions as follows: initial denaturation at 95 °C for 5 min, followed by 35 cycles of 95 °C for 30 s, 63 °C for 30 s, and 72 °C for 30 s followed by an extension step of 72 °C for 10 min in a Biometra T Personal thermocycler (Biometra). The 128-bp amplicon was digested with 1 unit of enzyme *PvuII* (Promega) analyzed on 6% paa gel overnight. The presence of the rs6449182 C allele resulted in the digestion of the amplicon to 63 bp and 65 bp products.

The PCR-RFLP assay to detect rs1800561 (418C>T) SNP included forward primer 5′-GACATGCTACTAAATTGATCT-CAG-3′ and reverse primer 5′-CTACGCAGCAGAGCCACCGAG-CAG-3′ (IDT, Illinois). The PCR mixes consisted of 50 ng of genomic DNA, 5 pmol/l of each primer, 1.6 mmol/l of $MgCl_2$, 200 nmol/l of dNTPs, and 1 unit of Taq polymerase (Promega) in a 20 μl PCR tube. The amplification reactions were performed with the following cycling variables: initial denaturation at 95 °C for 5 min, followed by 30 cycles of 94 °C for 30 s, 50 °C for 30 s, and 72 °C for 30 s, with a final extension step at 72 °C for 10 min. The amplified DNA product was

Table I – Baseline characteristics at diagnosis of 175 Polish multiple myeloma patients included to the study

Variable	*n* (%)
Age (years)	
median	61
range	39–87
Sex	
male	93 (53)
female	81 (46)
Myeloma serotype	
IgG	91 (52.0)
IgA	48 (27.4)
IgD	1 (0.6)
Light chain disease	35 (20.0)
Hemoglobin (g/dL)	
median	9.2
range	5.6–15.4
Durie–Salmon stage	
I	9 (5)
II	29 (17)
III	81 (46)
data not available[a]	56 (32)
Renal insufficiency	
present	31 (18)
absent	87 (50)
data not available[a]	57 (32)

[a] For patients ($n = 57$) referred to the Department of Hematology of Medical University of Lodz for ambulatory consultation some data from the time of diagnosis were not available.

digested overnight with 1 unit of enzyme *AciI* (New England BioLabs). Digests were detected by electrophoresis on 6% paa gel as a combination of uncut 248-bp band (rs1800561T allele) and 146-bp and 102-bp bounds for digested fragments (rs1800561C allele).

Statistical analysis

The statistical analysis was performed using IBM SPSS Statistics 20 (IBM Corporation, Armonk, NY) statistical package. The permutation test was applied to assess accordance with the Hardy–Weinberg equilibrium within each case and control group. Unconditional logistic regression was used to estimate the odds ratios (ORs) with 95% confidence intervals (95%CI) for associations of case-control status with the *CD38* genotypes. Baseline laboratory and clinical parameters at diagnosis among carriers of different *CD38* genotypes were compared by the chi^2 or the Kruskal–Wallis tests as appropriate. For all calculations $p < 0.05$ was considered significant.

Table II – Allele frequencies of *CD38* gene SNP rs6449182 and rs1800561 in Polish Caucasian multiple myeloma patients and controls. *P*-value for deviation from Hardy–Weinberg equilibrium was derived from the permutation test

CD38 alleles	MM cases (%) *n* = 175	*p*	Controls (%) *n* = 207	*p*
rs6449128 SNP				
C	206 (58.9)	0.01	348 (84.1)	0.8
G	144 (41.1)		66 (15.9)	
rs1800561 SNP				
C	347 (99.1)	0.99	414(100.0)	0.99
T	3 (0.9)		0	

Results

Among the 175 MM patients included to the study there were 93 (53%) males and 81 (46%) females of a median age 61 years. Details of the baseline clinical characteristics at diagnosis are listed in Table I. For a proportion of patients ($n = 57$) who were referred to the Department of Hematology of Medical University of Lodz for an ambulatory consultation some information on laboratory results at diagnosis was missing (Table I).

Genotyping was successful in all individuals investigated within the study. Allele frequencies of the *CD38* SNPs rs6449182 and rs1800561 in patient and control populations are summarized in Table II. Both tested SNPs were in accordance with Hardy–Weinberg equilibrium regarding control group (Table II). Consistent with low rs1800561 T allele frequency observed previously among Caucasian populations, we detected only 3 heterozygous individuals (rs1800561 CT genotype) in the patient group, while only wild-type homozygous subjects (rs1800561 CC genotype) were identified in the MM patient group. Therefore, further analyses were restricted to the rs6449182 polymorphism. The allele frequency and distribution of rs6449182 SNP differed significantly between patients and controls. We observed higher frequencies of the variant allele rs6449182 G in patients with MM as compared to healthy controls, (0.411 *versus* 0.159, $p < 0.001$) (Table II). Moreover, an association between MM and heterozygous rs6449182 GC genotype was observed; OR = 5.1 (95% CI = 3.3–7.9). Interestingly, the MM risk was further elevated with rs6449182 GG homozygous genotype (OR = 12.7; 95% CI = 6.9–23.4) suggesting an allele-dose effect. The associations between *CD38* SNPs genotypes and MM risk are presented in Table III.

Subsequently, we analyzed potential relation between clinical and laboratory variables at diagnosis of MM and

Table III – Associations between *CD38* gene SNP rs6449182 genotypes and risk of multiple myeloma

rs6449182 SNP genotypes	Cases	Controls	OR	95% CI	*p*
CC	51	145	1.00		
GC	104	58	5.10	(3.28–7.92)	<0.0001
GG	20	4	14.22	(5.68–35.61)	<0.0001
CC	51	145	1.00		
GC + GG	124	62	5.69	(3.71–8.73)	<0.0001

CD38 alleles. Median age at diagnosis was comparable in carriers of rs6449182 CC, CG and GG genotypes and reached 64, 60 and 62 years respectively, $p = 0.32$. Similarly, patients' median age at MM diagnosis did not differ between the wild-type rs1800561 CC homozygotes (61 years) and carriers of heterozygous rs1800561 CT genotype (68 years), $p = 0.23$. Furthermore, we did not detect any significant differences among different *CD38* genotypes regarding such parameters as sex ($p = 0.55$ for rs6449182 SNP analysis and $p = 0.74$ for rs1800561 CT analysis), Durie–Salmon clinical stage ($p = 0.33$ for rs6449182 SNP analysis and $p = 0.78$ for rs1800561 CT analysis), baseline hemoglobin concentration ($p = 0.40$ for rs6449182 SNP analysis and $p = 0.69$ for rs1800561 CT analysis) and presence of renal insufficiency at diagnosis of MM ($p = 0.13$ for rs6449182 SNP analysis and $p = 0.09$ for rs1800561 CT analysis) (data not shown).

Discussion

In this study we hypothesized on the potential role of *CD38* SNPs as determinants of MM predisposition based on the data on *CD38* and CD31 expression in MM and microenvironment cells and their interactions. We found a significant association between genotypes containing common variant *CD38* rs6449182 SNP G allele and the risk of MM development.

To our knowledge, no studies testing the relation of *CD38* gene SNPs to MM have been reported to date. However, rs6449182 *CD38* gene polymorphism was linked to susceptibility to CLL as well as some clinical phenotypes in different diseases. Aydin et al., showed significant association between rs6449182G allele and advanced clinical stage and elevated risk of Richter's transformation in patients with CLL [21]. Subsequently, our group found that rs6449182 contributes to CLL predisposition and affects *CD38* expression [9]. In accordance with our finding Abramenko et al., described significant association between rs6449182 GG genotype and CLL risk in Ukrainian Caucasians [22]. Finally, the metaanalysis of the three studies confirmed the influence of this SNP on CLL predisposition [23]. Earlier, it had been reported that rs6449182 SNP influenced the maintenance of peak bone mineral density in women as well as clinical characteristics of systemic lupus erythematosus [24, 25]. The biological mechanisms underlying these associations are unknown. However, they can be related to altered *CD38* protein expression as shown in our previous study [9].

Despite strong epidemiological data supporting the inherited background of MM, to date no loci have been unambiguously established as risk factors for MM development. The most common approach to identify such loci was to screen potentially functional or haplotype-tagging SNPs in candidate genes involved in different pathways regulating malignant plasma cell proliferation or survival. Interleukin 6 (IL-6) is a main cytokine driving myeloma growth and differentiation [26]. In some trials IL-6 SNPs rs1800796 as well as IL-6 receptor rs6684439 and rs7529229 SNPs were found to significantly alter risk to develop MM [27, 28]. Furthermore, tumor necrosis factor (TNF) haplotypes involving rs1800629 and rs909253 SNPs were associated with MM risk in some studies, but not in others [29, 30]. Another source of potential susceptibility alleles to tumorigenesis are functional variants in genes involved in such processes as DNA repair, cell cycle control and apoptosis. The example of such findings is influence of rs963248 SNP of XRCC4 and rs1051685 SNP of XRCC5 DNA repair genes on the risk to develop MM [31].

A limitation of our study, as well as majority of MM susceptibility studies reported to date, is relatively small sample size due in part to the low incidence of the disease. In a consequence the statistical power is too low to detect variants associated with low OR. This is particularly important considering the fact that the genetic risk to develop MM is likely influenced by many low-risk alleles. Even if metaanalyses can overcome the size limitations that occur in single studies, there are so far limited data on particular predisposition loci. To overcome these obstacles large groups of patients from different research groups can be simultaneously studied in a consortium. Recently, at least two international consortia with objective to investigate MM genetic background have been founded, namely IMMEnSE (International Multiple Myeloma rESEarch) including 12 research groups across Europe and the MAGIC (MyelomA Genetics International Consortium), including 16 research groups in Europe, Asia, Australasia, the Middle East and the Americas [32, 33]. Furthermore, hypotheses on candidate-genes in MM are problematic as there is little understanding of the disease etiology. An unbiased approach to detect the cancer associations is genome wide association studies (GWAS). Recently, a metaanalysis of two first GWAS in MM was published [34]. The studies investigated the total of 1675 MM cases and 5903 control subjects, and identified risk loci at 3p22.1 (rs1052501) and 7p15.3 (rs4487645) and a promising association at 2p23.3 (rs6746082). These novel predisposition variants, if confirmed by subsequent association studies, may lead to important etiological insights in MM.

In conclusion, this study found that inherited polymorphism rs6449182 of *CD38* gene is associated with a predisposition to MM. Our finding may have implications for understanding the role of *CD38* molecule in the pathogenesis of MM.

Authors' contributions/Wkład autorów

ZS-R – study design, data collection and interpretation, manuscript preparation, literature search, JS – data collection, OG-I – data collection, manuscript preparation, TR – data interpretation, manuscript preparation, KJ – study design, statistical analysis, manuscript preparation, literature search.

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Conflict of interest/Konflikt interesu

None declared.

Ethics/Etyka

The work described in this article have been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform Requirements for manuscripts submitted to Biomedical journals.

The own research were conducted according to the Good Clinical Practice guidelines and accepted by local Bioethics Committee, all patients agreed in writing to participation and these researches.

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