

Polymorphisms within genes encoding Ikaros family proteins IKZF1 and IKZF3 in multiple myeloma patients treated with thalidomide

Piotr Łacina^{1,B–F}, Diana Porzuczek^{2,B,C,E,F}, Katarzyna Bogunia-Kubik^{1,C,E,F}, Grzegorz Mazur^{3,C,E,F}, Aleksandra Butrym^{4,5,A–F}

¹ Laboratory of Clinical Immunogenetics and Pharmacogenetics, Hirsfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, Poland

² Lower Silesian Oncology, Pulmonology and Hematology Center, Wrocław, Poland

³ Department of Emergency Medical Service, Wrocław Medical University, Poland

⁴ Department of Cancer Prevention and Therapy, Wrocław Medical University, Poland

⁵ Oncological Clinical Trials Support Center at Dr Alfred Sokolowski Specialist Hospital in Walbrzych, Poland

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;

D – writing the article; E – critical revision of the article; F – final approval of the article

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online)

Dent Med Probl. 2024;61(6):885–892

Address for correspondence

Aleksandra Butrym

E-mail: aleksandra.butrym@gmail.com

Funding sources

Scientific submission SUB. A360.21.083, Wrocław Medical University, Poland.

Conflict of interest

None declared

Acknowledgements

None declared

Received on July 20, 2023

Reviewed on December 30, 2023

Accepted on February 7, 2024

Published online on December 20, 2024

Cite as

Łacina P, Porzuczek D, Bogunia-Kubik K, Mazur G, Butrym A. Polymorphisms within genes encoding Ikaros family proteins IKZF1 and IKZF3 in multiple myeloma patients treated with thalidomide. *Dent Med Probl.* 2024;61(6):885–892. doi:10.17219/dmp/183776

DOI

10.17219/dmp/183776

Copyright

Copyright by Author(s)

This is an article distributed under the terms of the

Creative Commons Attribution 3.0 Unported License (CC BY 3.0)

(<https://creativecommons.org/licenses/by/3.0/>).

Abstract

Background. Multiple myeloma (MM) is a hematological malignancy characterized by the presence of abnormal plasma cells. It is associated with anemia, bone lesions and renal dysfunction. Immunomodulatory drugs (IMiDs) are commonly used in MM treatment. Recent studies indicate that their therapeutic effect is caused by binding to cereblon (CRBN), which in turn causes the degradation of 2 important immune cell regulatory factors, IKZF1 and IKZF3. These are necessary for the anti-myeloma effect of IMiDs. Their expression level has been shown to affect MM survival and response to treatment. Potentially important single-nucleotide polymorphisms (SNPs) in the genes coding for IKZF1 and IKZF3 have been identified, but they have not been analyzed in MM patients before.

Objectives. The study was designed to establish the relationship between 4 SNPs in the genes coding for IKZF1 (rs61731359, rs4132601 and rs10272724) and IKZF3 (rs907091), and MM survival, response to treatment and other parameters.

Material and methods. The study involved 222 MM patients, as well as 100 control individuals. The *IKZF1* and *IKZF3* genotypes were determined by the LightSNiP assay. Genotyping was performed in the real-time polymerase chain reaction (PCR) LightCycler 480 device.

Results. No difference was observed between the patients and the controls for any of the SNPs, but the *IKZF1* and *IKZF3* variants were associated with various clinical parameters. Allele *IKZF1* rs4132601 G was more common in the patients with worse response to first-line therapy ($p = 0.040$), particularly in the patients treated with thalidomide ($p = 0.017$). The patients tended to have worse overall survival. *IKZF3* rs907091 CC was detected more commonly in the patients in stage I than in those in stages II and III, according to the International Staging System (ISS) criteria ($p = 0.015$). This genotype was also associated with a higher albumin level ($p = 0.033$), and was less common in the patients with the albumin level below 3.5 g/dL ($p = 0.030$).

Conclusions. Our results suggest that *IKZF1* rs4132601 and *IKZF3* rs907091 may affect response to treatment and progression in patients with MM.

Keywords: single nucleotide polymorphism, multiple myeloma, Ikaros transcription factor

Introduction

Multiple myeloma (MM) is an incurable hematological malignancy, which is characterized by the monoclonal growth of abnormal cells derived from transformed plasma cells. Multiple myeloma is a progressive disease that evolves from monoclonal gammopathy of undetermined significance (MGUS) and smoldering multiple myeloma (SMM) into symptomatic MM.¹ It is estimated that 35,730 new MM cases will be diagnosed in the United States in 2023 and 12,590 patients will die due to the disease.² In the Polish population, MM accounts for 18% of all hematological malignancies, and over 2,000 new cases are reported annually.³ The disease is diagnosed mostly in elderly people, and the median age at diagnosis is 69 years.² Multiple myeloma is associated with poor prognosis; however, new drugs have greatly improved 5-year overall survival and patients' quality of life (QoL).^{4,5}

Multiple myeloma typically leads to and is associated with anemia, lytic bone lesions, hypercalcemia, and renal dysfunction.⁶ It is believed that both environmental and genetic factors are responsible for the development of MM.¹ Drugs currently available for MM treatment include proteasome inhibitors, monoclonal antibodies, 3-phosphoinositide kinase inhibitors, and immunomodulatory drugs (IMiDs). Immunomodulatory drugs are a group that comprises thalidomide and its derivatives – lenalidomide and pomalidomide. They exert their therapeutic effect by binding to cereblon (CRBN), a component of the E3 ubiquitin ligase complex.^{7,8} By binding to CRBN, IMiDs change the substrate specificity of the E3 ubiquitin ligase complex, and cause it to ubiquitinate and target for proteasomal degradation two B cell transcription factors – Ikaros family zinc finger protein 1 (IKZF1, Ikaros) and Ikaros family zinc finger protein 3 (IKZF3, Aiolos). Ikaros family proteins are known to be the major regulators of immune cell development, particularly with regard to CD4⁺ T helper cells, as well as B cells during their early and late development.^{9,10} IKZF1 acts as a tumor suppressor in normal cells, but in MM cells, it becomes a transcriptional activator of oncogenes and promotes cancer cell survival.¹¹ The knockdown and overexpression of IKZF1 and IKZF3 reduces the sensitivity of MM cells to IMiDs.^{12–14} Both IKZF1 and IKZF3 are needed for IMiDs to work, and the inactivation of either of them leads to MM cell cycle arrest.¹⁵ The degradation of IKZF1 and IKZF3 may lead to MM cell apoptosis through the downregulation of interferon regulatory factor 4 (IRF4) and c-MYC. It has also been shown that IKZF1 and IKZF3 repress interferon-stimulated genes (ISGs), including *CD38*, the expression of which is increased upon IMiD treatment.

Earlier studies established that the IKZF1 expression levels affected response to therapy and survival in patients treated with IMiDs.^{16–18} However, as of yet, no studies have investigated whether genetic variability

within the genes coding for IKZF1 and IKZF3 may affect survival or response to treatment in MM patients. Our previous studies showed that the single-nucleotide polymorphisms (SNPs) located in the genes associated with IMiD metabolism might correlate with response to treatment and survival in MM patients.^{19–21} In the present study, we aimed to analyze selected SNPs in the *IKZF1* and *IKZF3* genes in the context of MM. Three SNPs were in *IKZF1* (rs61731359, a missense Asn>Asp substitution; rs4132601, a 3' untranslated region (3'UTR) variant and a potential miRNA binding site; and rs10272724, located near *IKZF1* and affecting survival in acute lymphoblastic leukemia) and one in *IKZF3* (rs907091, a 3'UTR variant and a potential miRNA binding site).^{22–25} The 4 SNPs were analyzed in relation to MM susceptibility, response to treatment, survival, and association with diagnostic and prognostic markers, such as albumin, beta-2-microglobulin (β 2-M), C-reactive protein (CRP), or calcium (Ca).

Material and methods

Patients and controls

The study included 222 patients diagnosed with MM, 111 women and 111 men, aged 37–88 years (median age: 64 years). The only inclusion criterion for patients to enter the study was a diagnosis of MM. The exclusion criterion was a diagnosis of any other malignancy.

According to the International Staging System (ISS) criteria, 54 patients were in stage I, 67 in stage II and 81 in stage III.²⁶ Detailed information on patients is presented in Table 1. In addition, 100 healthy blood donors (50 women and 50 men) served as a control group. All patients and control individuals were from the Lower Silesia region in western Poland, and were treated between 2015 and 2020. The study was performed in accordance with the Declaration of Helsinki, and received the approval of the Ethics Committee at Wrocław Medical University, Poland (no. KB-297/2016).

Sample size

The sample size analysis was conducted based on one of the primary objectives of the study, assuming a lower occurrence of the *IKZF1* rs4132601 *G* allele in patients with better response (complete or very good partial remission) to first-line therapy (effect size: 0.30). The minimum required sample size to detect this difference, assuming $\alpha = 5\%$, a power of 90% and a confidence level of 95%, was 184 patients in total. Additionally, a 10% risk of incomplete data was considered. The final minimum required sample size was 203 participants. The sample size analysis was performed using the G*Power software.²⁷

Table 1. Patients' characteristics

Data		Number of patients
Age [years]	≤60	70
	>60	137
Sex	M	111
	F	111
ISS	I	54
	II	67
	III	81
Hypercalcemia	yes	38
	no	154
Anemia	yes	69
	no	126
β2-M [μg/mL]	≤3	74
	>3	126
CRP [mg/L]	≤5	75
	>5	64
Albumin [mg/dL]	≤3.5	71
	>3.5	86

ISS – International Staging System; β2-M – beta-2-microglobulin; CRP – C-reactive protein; M – male; F – female.

Immunomodulating therapy

Regarding first-line therapy, 127 patients were treated with the thalidomide-containing regimens (thalidomide 100 mg/day), including 89 patients treated with cyclophosphamide, thalidomide and dexamethasone (CTD) up to 6 cycles, and the subsequent continuous thalidomide monotherapy. Of the rest of the patients, 76 were not treated with thalidomide, and 19 patients lacked data on thalidomide treatment. Response to first-line therapy was as follows: complete remission (CR) in 37 patients; very good partial remission (VGPR) in 29 patients; partial remission (PR) in 77 patients; minor remission (MR) in 11 patients; stable disease (SD) in 19 patients; and progressive disease (PD) in 12 patients. For the patients treated with the thalidomide-containing regimens, the response was as follows: CR in 24 patients; VGPR in 18 patients; PR in 49 patients; MR in 8 patients; SD in 15 patients; and PD in 8 patients. Response criteria are well described in a previous study.³

Genotyping

DNA was extracted from the samples of peripheral blood taken on ethylenediaminetetraacetic acid (EDTA), using Maxwell® 16 Blood DNA Purification Kit (Promega Corp., Madison, USA) or QIAamp DNA Blood Midi Kit (Qiagen, Hilden, Germany), following the manufacturers' recommendations. The *IKZF1* and *IKZF3* genotypes were determined by the LightSNiP assay (TIB Molbiol, Berlin, Germany), which uses specific probes allowing

to distinguish genotypes based on melting curves (melting curve genotyping). Genotyping was performed in the real-time polymerase chain reaction (PCR) LightCycler 480 device (Roche Diagnostics, Rotkreuz, Switzerland) on a 96-well plate. A negative control with no DNA was included. Conditions for PCR were as follows: 95°C for 10 min; 45 cycles of 95°C for 10 s, 60°C for 10 s and 72°C for 15 s. This was then followed by one cycle of 95°C for 30 s and 40°C for 2 min, and gradual melting from 75°C to 40°C. The genotypes were distinguished based on the melting temperatures of the samples and the shape of their melting curves.

Statistical analysis

The null hypothesis that there is no difference in the presence of the *IKZF1/IKZF3* alleles between patients and controls was tested with Fisher's exact test or the χ^2 test, using the website <http://vassarstats.net>. The genotypes were tested for deviation from the Hardy–Weinberg equilibrium using the χ^2 test. Survival was assessed with the Wilcoxon test and the Kaplan–Meier survival curves. Other associations were tested either with Fisher's exact test or the Mann–Whitney *U* test. All of these tests, except for Fisher's exact test and the χ^2 test, were performed with the Real Statistics Resource Pack for Microsoft Excel (<https://real-statistics.com/free-download/real-statistics-resource-pack>). A *p*-value <0.05 was considered statistically significant.

Results

Distribution of the *IKZF1* and *IKZF3* alleles and genotypes among patients with multiple myeloma and control individuals

All patients and control individuals (control group) were genotyped for rs4132601, rs10272724 and rs907091, while 203 patients and all control individuals were genotyped for rs61731359. Details about the genotype and allele frequencies are presented in Table 2. Genotype frequencies for all 4 SNPs were in accordance with the Hardy–Weinberg equilibrium in both patients and controls. We detected no statistically significant differences between the patients and the controls in any of the 4 studied SNPs, in any of the 3 investigated models (dominant, recessive and additive) (Table 3). Regarding rs61731359A>G, only allele A was detected, as all the subjects carried genotype AA. Two *IKZF1* SNPs, rs4132601 and rs10272724, were in very high linkage disequilibrium ($r^2 = 0.96$, $D' = 0.98$), prompting us to select only one of them for further analysis. Due to the reasons listed above, rs61731359 and rs10272724 were not included in further analysis.

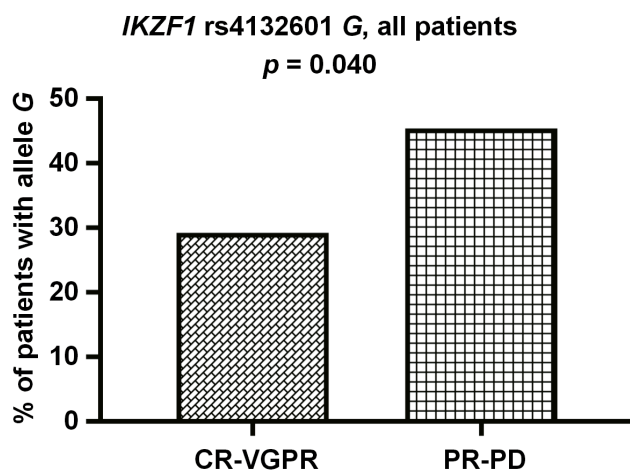
Table 2. *IKZF1* and *IKZF3* genotypes and alleles

Genotypes and alleles		MM patients	Controls N = 100
<i>IKZF1</i> rs61731359 A>G N = 203 (MM patients)	AA	203 (100.0%)	100 (100.0%)
	AG	0 (0.0%)	0 (0.0%)
	GG	0 (0.0%)	0 (0.0%)
	A	1.000	1.000
	G	0.000	0.000
<i>IKZF1</i> rs4132601 T>G N = 222 (MM patients)	TT	132 (59.5%)	61 (61.0%)
	TG	77 (34.7%)	34 (34.0%)
	GG	13 (5.9%)	5 (5.0%)
	T	0.768	0.780
	G	0.232	0.220
<i>IKZF1</i> rs10272724 T>C N = 222 (MM patients)	TT	133 (59.9%)	60 (60.0%)
	TC	76 (32.2%)	35 (35.0%)
	CC	13 (5.9%)	5 (5.0%)
	C	0.770	0.775
	T	0.230	0.225
<i>IKZF3</i> rs907091 C>T N = 222 (MM patients)	CC	42 (18.9%)	17 (17.0%)
	CT	116 (52.3%)	53 (53.0%)
	TT	64 (28.8%)	30 (30.0%)
	C	0.451	0.439
	T	0.549	0.561

MM – multiple myeloma.

Association of the *IKZF1* and *IKZF3* polymorphisms with response to immunomodulating therapy

Allele *IKZF1* rs4132601 G was less common among the patients with better response (CR-VGPR) to first-line therapy (19/66 vs. 54/119; $p = 0.040$). This association could

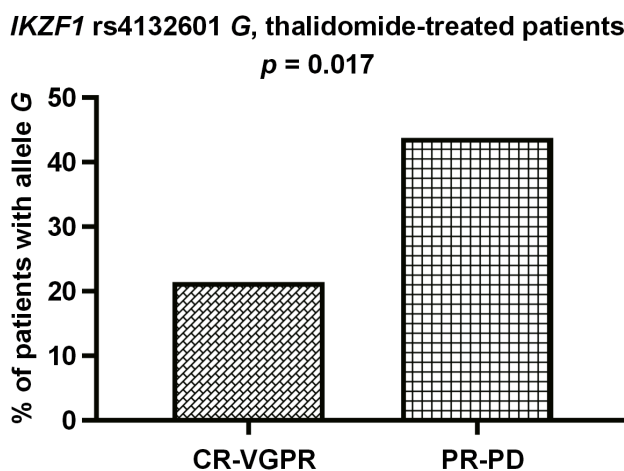
**Table 3.** Case–control comparison between the multiple myeloma (MM) patients and the controls in 3 genetic models for *IKZF1* and *IKZF3* single-nucleotide polymorphisms (SNPs)

Model		p-value
Dominant	rs61731359 A>G	1.000
	rs4132601 T>G	0.807
	rs10272724 T>C	1.000
	rs907091 C>T	0.757
Recessive	rs61731359 A>G	1.000
	rs4132601 T>G	0.802
	rs10272724 T>C	0.802
	rs907091 C>T	1.000
Additive	rs61731359 A>G	1.000
	rs4132601 T>G	0.937
	rs10272724 T>C	0.951
	rs907091 C>T	0.932

also be observed in the subgroup of patients treated with thalidomide (9/42 vs. 35/80; $p = 0.017$) (Fig. 1). Additionally, we observed a trend for worse response to treatment in the patients with allele *IKZF3* rs907091 C, although this was not statistically significant ($p = 0.086$) (Fig. 2).

Association of the *IKZF1* and *IKZF3* polymorphisms with survival and disease progression

Overall and disease-free survival analyses were conducted for the *IKZF1* and *IKZF3* SNPs. While no statistically significant associations were observed for any of the analyzed SNPs, we noticed that the patients with allele *IKZF1* rs4132601 G tended to have shorter overall survival than the patients without it ($p = 0.104$) (Fig. 3).

**Fig. 1.** Distribution of the *IKZF1* rs4132601 G allele in the patients after first-line therapy showing complete or very good partial remission (CR-VGPR), and in the patients showing only partial remission, minor remission, no remission, or progression of the disease (PR-PD)

The left panel illustrates the association for all patients, regardless of the type of treatment, while the right panel includes only the patients undergoing treatment that included thalidomide. In both groups, allele G was less commonly detected.

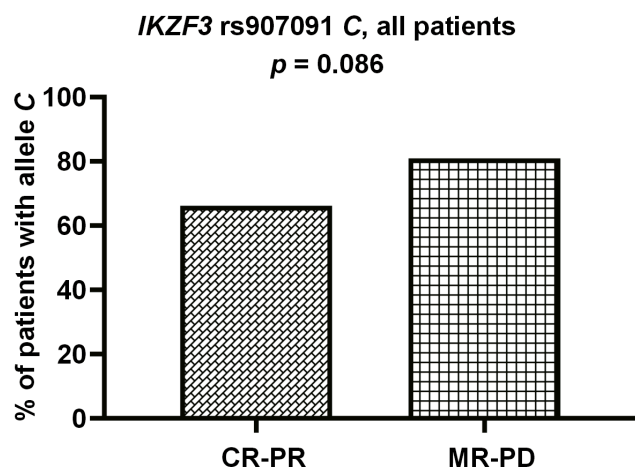


Fig. 2. Distribution of the *IKZF3* rs907091 C allele in the patients after first-line therapy showing complete, very good or partial remission (CR-PR), and in the patients showing only minor remission, no remission or progression of the disease (MR-PD)

Regarding *IKZF3*, we found genotype rs907091 CC to be more common in the patients in stage I than in those in stages II and III, according to the ISS criteria (16/54 vs. 21/148; $p = 0.015$) (Fig. 4).

Associations of the *IKZF1* and *IKZF3* polymorphisms with other prognostic factors

We observed that the median albumin blood concentration was slightly higher in the patients with genotype *IKZF3* rs907091 CC than in the patients without this genotype (3.80 g/dL vs. 3.55 g/dL; $p = 0.033$). Furthermore,

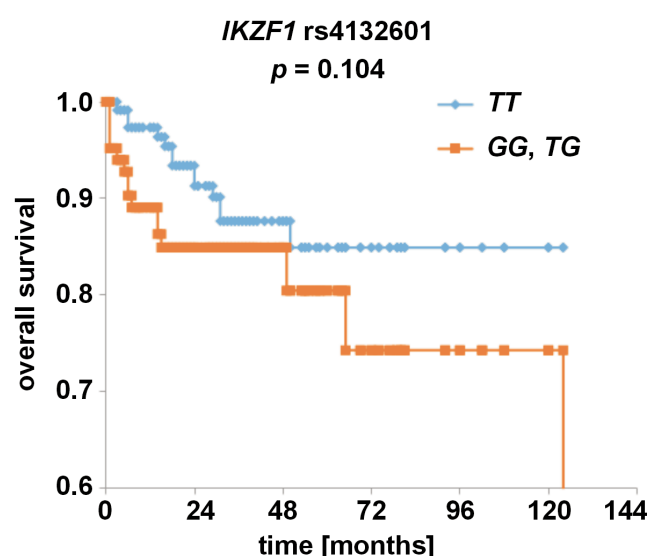


Fig. 3. Kaplan–Meier curves for *IKZF1* rs4132601, visualizing overall survival. The red line shows overall survival for the patients with allele G (genotypes GG and TG), while the blue line represents the patients lacking allele G (genotype TT).

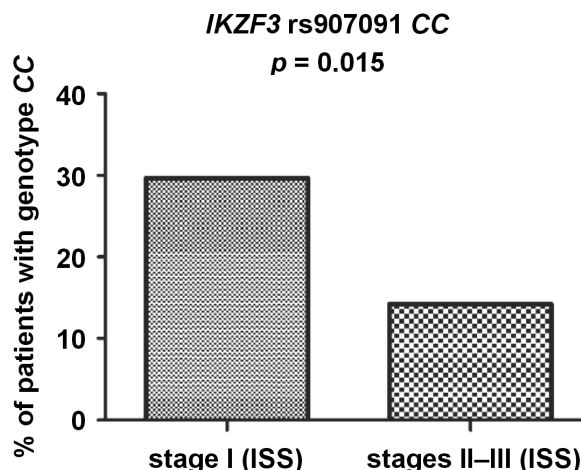


Fig. 4. Distribution of the *IKZF3* rs907091 CC genotype in the patients characterized by being in either stage I, or stage II–III of the disease, according to the International Staging System (ISS) criteria

The CC genotype was more prevalent among the patients in the initial stage.

we noticed that CC homozygosity was less common in the patients with the albumin levels below 3.5 g/dL than in those with the albumin levels above 3.5 g/dL (9/71 vs. 24/86; $p = 0.030$) (Fig. 5), with the albumin concentration below 3.5 g/dL being associated with worse prognosis.²⁸ Additionally, the patients with genotype *IKZF1* rs4132601 GG tended to have lower albumin concentration than the patients without this genotype (3.35 g/dL vs. 3.65 g/dL; $p = 0.057$).

Regarding other clinical parameter – hemoglobin – allele *IKZF3* rs907091 C was more commonly detected in the patients with abnormal hemoglobin blood levels (<10 g/dL) than in the patients with normal levels (55/69 vs. 81/126; $p = 0.034$) (Fig. 6). We also observed that the patients with allele *IKZF1* rs4132601 G tended to have higher Ca blood levels (9.53 mg/dL vs. 9.25 mg/dL; $p = 0.082$) and slightly higher age on diagnosis (65.0 years vs. 63.5 years; $p = 0.053$) than the patients without this allele, although this was not statistically significant. No associations regarding the levels of β 2-M, creatinine and CRP, or the presence of light chains were detected.

Discussion

Immunomodulatory drugs are a major group of drugs used to treat MM. However, their mechanism of action has not been fully elucidated. It has been established that the molecular target of IMiDs is CRBN. While a ubiquitin ligase-independent pathway for IMiD action has been described, the anti-myeloma effect of IMiDs is most often ascribed to the degradation of *IKZF1* and *IKZF3* by the E3 ubiquitin ligase complex upon IMiD binding to CRBN.^{12,13,25} In the present study, we investigated the *IKZF1* and *IKZF3* genetic variants and analyzed their influence on survival, response to treatment and diagnostic

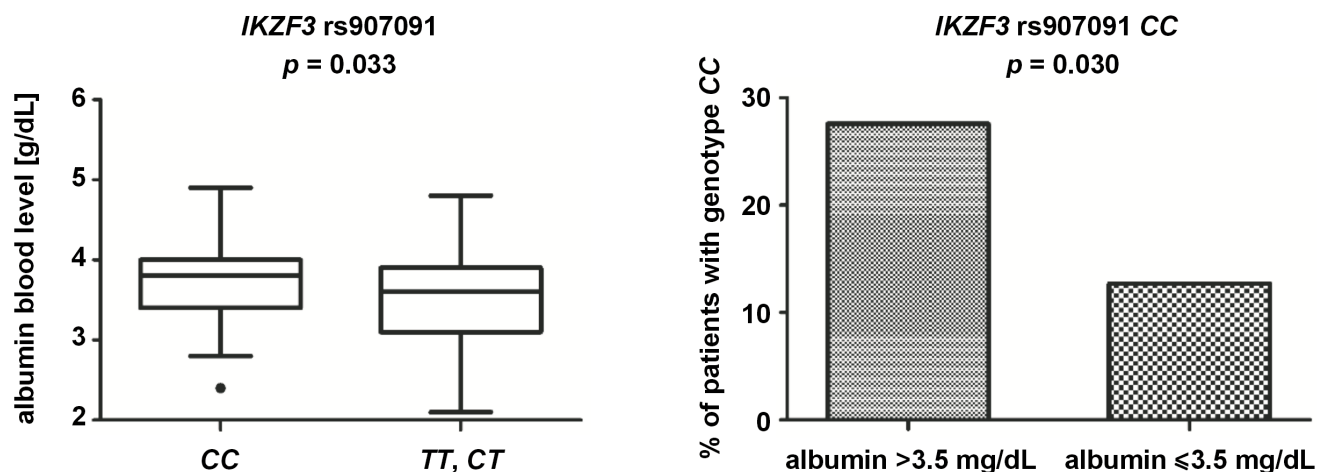


Fig. 5. Albumin blood levels in the patients carrying the *IKZF3* rs907091 CC genotype

The left panel shows differences in the albumin blood concentration between the patients with genotype CC and those without it (genotypes TT and CT). The CC genotype was associated with higher albumin levels. The right panel shows the distribution of the CC genotype in the patients with the albumin levels above and below 3.5 mg/dL, with the levels below the 3.5 mg/dL cut-off point being associated with worse prognosis. The patients with albumin lower than 3.5 mg/dL less commonly presented with the CC genotype.

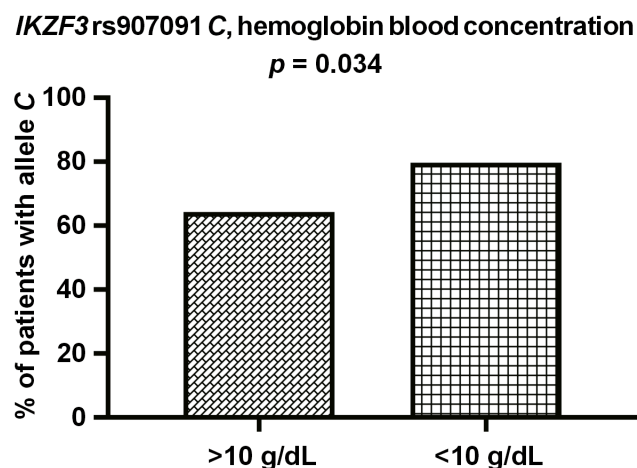


Fig. 6. Hemoglobin blood levels in the patients carrying the *IKZF3* rs907091 C allele

The graphs show differences in the distribution of allele C in the patients with higher and lower hemoglobin blood concentration. Allele C was more common in the patients with lower hemoglobin blood concentration.

markers in MM patients. Previous studies found differences in survival and response to treatment between patients with different *IKZF1* expression levels,^{16–18} which may be caused by the interference of the genetic background. To the best of our knowledge, there has been no study analyzing the influence of SNPs in the genes coding for *IKZF1* or *IKZF3* on MM. Our study showed that allele rs4132601 G was associated with worse response to treatment, especially in patients treated with thalidomide, and might be more common in patients with shorter overall survival, although the latter association was not statistically significant. Furthermore, the G allele tended to be more common in patients with higher Ca blood levels, while the GG genotype tended to be more frequent in patients with lower albumin blood levels, which are associ-

ated with worse prognosis.²⁵ However, it was not associated with susceptibility to MM.

IKZF1 rs4132601 was first identified as a risk variant in childhood acute lymphoblastic leukemia (ALL) in a study by Papaemmanuil et al,²⁹ and has mostly been studied in the context of this disease. The researchers found the G allele to be associated with a higher risk for ALL and a lower *IKZF1* mRNA expression level in a dose-dependent fashion.²⁹ This association with the ALL risk was confirmed in various European, North African, Middle Eastern and Southeast Asian populations, although it was not detected in studies on ALL patients from Taiwan, Latvia and Iran.^{24,30–39} The rs4132601 SNP was also analyzed in Polish diffuse large B cell lymphoma (DLBCL) patients.⁴⁰ While the SNP was not associated with DLBCL susceptibility, allele G was found to correlate with longer progression-free and overall survival,⁴⁰ contrary to our results in MM. This may be attributed to differences between the 2 diseases and the fact that DLBCL is not treated with IMiDs; therefore, the role of *IKZF1* in this disease may be different from its role in MM. It is worth noting that our study showed that rs4132601 is in strong linkage disequilibrium with another *IKZF1* SNP, rs10272724. It is thus possible that the effects ascribed to rs4132601 may, in fact, be due to a genetic variation in rs10272724.

Another SNP covered in our present study was rs907091, located in the gene coding for *IKZF3*, also known as Aiolos. Both *IKZF1* and *IKZF3* are needed for IMiDs to work, and the inactivation of either of them can lead to MM cell cycle arrest.¹⁵ We found rs907091 CC to be more common in MM patients with albumin levels above 3.5 g/dL (a marker of better prognosis) and to correlate with higher albumin levels in general. The rs907091 CC was also more commonly detected in patients in stage I of MM than in those in more advanced stages II and III, according to the ISS criteria. These findings may suggest

that rs907091 CC is associated with better prognosis and slower progression of MM. On the other hand, we also observed that allele rs907091 C was more often detected in patients with abnormally low (<10 g/dL) hemoglobin levels. *IKZF3* rs907091 was first described in a haplotype-based analysis that found it to be associated with gene expression changes.⁴¹ *IKZF3* rs907091 is located in a predicted miR-326 recognition element (binding site) and miR-326 is expected to bind to rs907091 T (a variant absent in rs907091 CC homozygotes). Accordingly, patients with rs907091 T are reported to have lower *IKZF3* expression.⁴² The rs907091 T was also been described to be associated with a higher risk for systemic lupus erythematosus (SLE) and multiple sclerosis (MS), with the CC genotype playing a protective role.^{42–45} The protective effect of the CC genotype in these studies seems to correspond well with our results with regard to MM, although it should be noted that SLE and MS differ significantly from MM. No studies on *IKZF3* rs907091 have been conducted in the context of MM or other hematologic malignancies.

Our study also included rs61731359, a functionally relevant *IKZF1* SNP causing a missense Asn>Asp amino acid change.²² To the best of our knowledge, this variant has not been analyzed before in any group of patients. It did not have a polymorphic character in our group of MM patients and control individuals, as only one allele (A) was detected. This absence of allele G may have been due to the relatively small number of patients and controls investigated. Further studies on rs61731359 in MM and other diseases may be needed to elucidate its role.

Limitations

The relatively small number of patients and controls is a major limitation of our study. Another limitation that could have influenced our results is the non-homogeneous therapy used for MM treatment. Further studies on larger cohorts of patients are needed to confirm our results.

Conclusions

In conclusion, our present study shows that rs4132601 in the gene coding for *IKZF1* and rs907091 in the gene coding for *IKZF3* may be associated with response to treatment, survival, and the diagnostic and prognostic parameters in MM patients, such as the stage of the disease and albumin blood concentration.

Ethics approval and consent to participate

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Board of Wrocław Medical University, Poland (approval No. KB-297/2016).

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

ORCID iDs

Piotr Łacina  <https://orcid.org/0000-0002-9659-9403>

Diana Porzuczek  <https://orcid.org/0000-0002-2195-9312>

Katarzyna Bogunia-Kubik  <https://orcid.org/0000-0001-9744-0376>

Grzegorz Mazur  <https://orcid.org/0000-0001-6610-2008>

Aleksandra Butrym  <https://orcid.org/0000-0002-8199-2018>

References

1. Fonseca R, Blood E, Rue M, et al. Clinical and biologic implications of recurrent genomic aberrations in myeloma. *Blood*. 2003;101(11):4569–4575. doi:10.1182/blood-2002-10-3017
2. GBD 2015 Disease and Injury Incidence and Prevalence Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990–2015: A systematic analysis for the Global Burden of Disease Study 2015. *Lancet*. 2016;388(10053):1545–1602. doi:10.1016/S0140-6736(16)31678-6
3. Dmoszyńska A, Usnarska-Zubkiewicz L, Walewski J, et al. Recommendations of Polish Myeloma Group concerning diagnosis and therapy of multiple myeloma and other plasmacytic dyscrasias for 2017 [in Polish]. *Acta Haematol Pol*. 2017;48(2):55–103. doi:10.1016/j.achaem.2017.05.003
4. Kobayashi H, Abe Y, Miura D, et al. Clinical features and predictive factors of early mortality in patients with MM outside of clinical trials. *Blood*. 2018;132(Suppl 1):5593. doi:10.1182/blood-2018-99-113750
5. Kyle RA, Therneau TM, Rajkumar SV, et al. A long-term study of prognosis in monoclonal gammopathy of undetermined significance. *N Engl J Med*. 2002;346(8):564–569. doi:10.1056/NEJ-Moa01133202
6. Chen M, Zhao Y, Xu C, Wang X, Zhang X, Mao B. Immunomodulatory drugs and the risk of serious infection in multiple myeloma: Systematic review and meta-analysis of randomized and observational studies. *Ann Hematol*. 2018;97(6):925–944. doi:10.1007/s00277-018-3284-y
7. Ito T, Ando H, Suzuki T, et al. Identification of a primary target of thalidomide teratogenicity. *Science*. 2010;327(5971):1345–1350. doi:10.1126/science.1177319
8. Chang XB, Stewart AK. What is the functional role of the thalidomide binding protein cereblon? *Int J Biochem Mol Biol*. 2011;2(3):287–294. PMID:22003441. PMCID:PMC3193296.
9. Powell MD, Read KA, Sreekumar BK, Oestreich KJ. Ikaros zinc finger transcription factors: Regulators of cytokine signaling pathways and CD4⁺ T helper cell differentiation. *Front Immunology*. 2019;10:1299. doi:10.3389/fimmu.2019.01299
10. Sellars M, Kastner P, Chan S. Ikaros in B cell development and function. *World J Biol Chem*. 2011;2(6):132–139. doi:10.4331/wjbc.v2.i6.132
11. Osada N, Kikuchi J, Iha H, et al. c-FOS is an integral component of the IKZF1 transactivator complex and mediates lenalidomide resistance in multiple myeloma. *Clin Transl Med*. 2023;13(8):e1364. doi:10.1002/ctm2.1364
12. Lu G, Middleton RE, Sun H, et al. The myeloma drug lenalidomide promotes the cereblon-dependent destruction of Ikaros proteins. *Science*. 2014;343(6168):305–309. doi:10.1126/science.1244917

13. Krönke J, Udeshi ND, Narla A, et al. Lenalidomide causes selective degradation of IKZF1 and IKZF3 in multiple myeloma cells. *Science*. 2014;343(6168):301–305. doi:10.1126/science.1244851
14. Abe Y, Ishida T. Immunomodulatory drugs in the treatment of multiple myeloma. *Jpn J Clin Oncol*. 2019;49(8):695–702. doi:10.1093/jjco/hyz083
15. Fedele PL, Willis SN, Liao Y, et al. IMiDs prime myeloma cells for daratumumab-mediated cytotoxicity through loss of Ikaros and Aiolos. *Blood*. 2018;132(20):2166–2178. doi:10.1182/blood-2018-05-850727
16. Krönke J, Kuchenbauer F, Kull M, et al. IKZF1 expression is a prognostic marker in newly diagnosed standard-risk multiple myeloma treated with lenalidomide and intensive chemotherapy: A study of the German Myeloma Study Group (DSMM). *Leukemia*. 2017;31(6):1363–1367. doi:10.1038/leu.2016.384
17. Bolomsky A, Hübl W, Spada S, et al. IKAROS expression in distinct bone marrow cell populations as a candidate biomarker for outcome with lenalidomide-dexamethasone therapy in multiple myeloma. *Am J Hematol*. 2017;92(3):269–278. doi:10.1002/ajh.24634
18. Tachita T, Kinoshita S, Ri M, et al. Expression, mutation, and methylation of cereblon-pathway genes at pre- and post-lenalidomide treatment in multiple myeloma. *Cancer Sci*. 2020;111(4):1333–1343. doi:10.1111/cas.14352
19. Butrym A, Rybka J, Łacina P, et al. Polymorphisms within beta-catenin encoding gene affect multiple myeloma development and treatment. *Leuk Res*. 2015;39(12):1462–1466. doi:10.1016/j.leukres.2015.10.007
20. Butrym A, Łacina P, Rybka J, et al. Cereblon and IRF4 variants affect risk and response to treatment in multiple myeloma. *Arch Immunol Ther Exp (Warsz)*. 2016;64(Suppl 1):151–156. doi:10.1007/s00005-016-0442-6
21. Łacina P, Butrym A, Mazur G, Bogunia-Kubik K. BSG and MCT1 genetic variants influence survival in multiple myeloma patients. *Genes (Basel)*. 2018;9(5):226. doi:10.3390/genes9050226
22. Yang L, Luo Y, Wei J. Integrative genomic analyses on Ikaros and its expression related to solid cancer prognosis. *Oncol Rep*. 2010;24(2):571–577. doi:10.3892/or_00000894
23. Xu Z, Taylor JA. SNPinfo: Integrating GWAS and candidate gene information into functional SNP selection for genetic association studies. *Nucleic Acids Res*. 2009;37(Web Server issue):W600–W605. doi:10.1093/nar/gkp290
24. Bahari G, Hashemi M, Naderi M, Taheri M. *IKZF1* gene polymorphisms increased the risk of childhood acute lymphoblastic leukemia in an Iranian population. *Tumour Biol*. 2016;37(7):9579–9586. doi:10.1007/s13277-016-4853-0
25. Eichner R, Heider M, Fernández-Sáiz V, et al. Immunomodulatory drugs disrupt the cereblon–CD147–MCT1 axis to exert antitumor activity and teratogenicity. *Nat Med*. 2016;22(7):735–743. doi:10.1038/nm.4128
26. Greipp PR, San Miguel J, Durie BG et al. International staging system for multiple myeloma. *J Clin Oncol*. 2005;23(15):3412–3420. doi:10.1200/JCO.2005.04.242
27. Faul F, Erdfelder E, Buchner A, Lang AG. Statistical power analyses using G*Power 3.1: Tests for correlation and regression analyses. *Behav Res Methods*. 2009;41(4):1149–1160. doi:10.3758/BRM.41.4.1149
28. Kim JE, Yoo C, Lee DH, Kim SW, Lee JS, Suh C. Serum albumin level is a significant prognostic factor reflecting disease severity in symptomatic multiple myeloma. *Ann Hematol*. 2010;89(4):391–397. doi:10.1007/s00277-009-0841-4
29. Papaemmanuil E, Hosking FJ, Vijayakrishnan J, et al. Loci on 7p12.2, 10q21.2 and 14q11.2 are associated with risk of childhood acute lymphoblastic leukemia. *Nat Genet*. 2009;41(9):1006–1010. doi:10.1038/ng.430
30. Lautner-Csorba O, Gézi A, Semsei AF, et al. Candidate gene association study in pediatric acute lymphoblastic leukemia evaluated by Bayesian network based Bayesian multilevel analysis of relevance. *BMC Med Genomics*. 2012;5:42. doi:10.1186/1755-8794-5-42
31. Tayel SI, El-Hefnway SM, Abo El-Fotoh WM, El-Zayat RS. The genetic variants of *IKZF1* gene linked with the growing risk of childhood acute lymphoblastic leukaemia. *Curr Mol Med*. 2019;19(1):32–39. doi:10.2174/1566524019666190219123900
32. Al-Absi B, Razif MF, Noor SM, et al. Contributions of *IKZF1*, *DDC*, *CDKN2A*, *CEBPE*, and *LMO1* gene polymorphisms to acute lymphoblastic leukemia in a Yemeni population. *Genet Test Mol Biomarkers*. 2017;21(10):592–599. doi:10.1089/gtmb.2017.0084
33. Mullighan CG, Su X, Zhang J, et al.; Children's Oncology Group. Deletion of *IKZF1* and prognosis in acute lymphoblastic leukemia. *N Engl J Med*. 2009;360(5):470–480. doi:10.1056/NEJMoa0808253
34. Vijayakrishnan J, Sherborne AL, Sawangpanich R, Hongeng S, Houlston RS, Pakakasama S. Variation at 7p12.2 and 10q21.2 influences childhood acute lymphoblastic leukemia risk in the Thai population and may contribute to racial differences in leukemia incidence. *Leuk Lymphoma*. 2010;51(10):1870–1874. doi:10.3109/10428194.2010.511356
35. Mosaad YM, El-Ziny MA, Darwish A, et al. *IKZF1* rs4132601 and rs11978267 gene polymorphisms and acute lymphoblastic leukemia: Relation to disease susceptibility and outcome. *J Pediatr Hematol Oncol*. 2020;42(7):420–428. doi:10.1097/MPH.0000000000001874
36. Al-Zayan NR, Ashour MJ, Abuwarda HN, Sharif FA. *ARID5B*, *IKZF1*, *GATA3*, *CEBPE*, and *CDKN2A* germline polymorphisms and predisposition to childhood acute lymphoblastic leukemia. *Pediatr Hematol Oncol*. 2024;14(2):103–113. doi:10.1080/08880018.2023.2234946
37. Lin CY, Li MJ, Chang JG, et al. High-resolution melting analyses for genetic variants in *ARID5B* and *IKZF1* with childhood acute lymphoblastic leukemia susceptibility loci in Taiwan. *Blood Cells Mol Dis*. 2014;52(2–3):140–145. doi:10.1016/j.bcmd.2013.10.003
38. Kreile M, Piekuse L, Rots D, Dobelev Z, Kovalova Z, Lace B. Analysis of possible genetic risk factors contributing to development of childhood acute lymphoblastic leukaemia in the Latvian population. *Arch Med Sci*. 2016;12(3):479–485. doi:10.5114/aoms.2016.59920
39. Bardsiri MS, Zehtab S, Karami N, Farsinejad A, Ehsan M, Fatemi A. Association of *IKZF1* and *CDKN2A* gene polymorphisms with childhood acute lymphoblastic leukemia: A high-resolution melting analysis. *BMC Med Genomics*. 2022;15(1):171. doi:10.1186/s12920-022-01325-6
40. Bielska M, Borowiec M, Jesionek-Kupnicka D, et al. Polymorphism in *IKZF1* gene affects clinical outcome in diffuse large B-cell lymphoma. *Int J Hematol*. 2017;106(6):794–800. doi:10.1007/s12185-017-2315-0
41. Verlaan DJ, Berlivet S, Hunninghake GM, et al. Allele-specific chromatin remodeling in the *ZBP2/GSDMB/ORMDL3* locus associated with the risk of asthma and autoimmune disease. *Am J Hum Genet*. 2009;85(3):377–393. doi:10.1016/j.ajhg.2009.08.007
42. Richardson K, Lai CQ, Parnell LD, Lee YC, Ordoñas JM. A genome-wide survey for SNPs altering microRNA seed sites identifies functional candidates in GWAS. *BMC Genomics*. 2011;12:504. doi:10.1186/1471-2164-12-504
43. Cai X, Qiao Y, Diao C, et al. Association between polymorphisms of the *IKZF3* gene and systemic lupus erythematosus in a Chinese Han population. *PloS One*. 2014;9(10):e108661. doi:10.1371/journal.pone.0108661
44. Farh KKH, Marson A, Zhu J, et al. Genetic and epigenetic fine mapping of causal autoimmune disease variants. *Nature*. 2015;518(7539):337–343. doi:10.1038/nature13835
45. Keshari PK, Harbo HF, Myhr KM, Aarseth JH, Bos SD, Berge T. Allelic imbalance of multiple sclerosis susceptibility genes *IKZF3* and *IQGAP1* in human peripheral blood. *BMC Genet*. 2016;17:59. doi:10.1186/s12863-016-0367-4