 DOI: https://doi.org/10.5114/ceji.2021.104425

Shared epitope and polymorphism of *MICA* and *NKG2D* encoding genes in Greek and Polish patients with rheumatoid arthritis

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Abstract

The present study aimed to analyse and compare the distribution of MICA (rs1051792) and NKG2D/KLRK1 (rs1154831, rs1049174, rs2255336) polymorphisms in 61 Greek and 100 Polish patients with rheumatoid arthritis in relation to the presence of the HLA-DRB1 shared epitope and clinical parameters. Genotyping of selected polymorphism was performed using real-time PCR. HLA-DRB1 shared epitope alleles segregated differently in Greek and Polish patients but in both populations were detected in over 60% of cases. The rs1051792-A variant was more common among SE-positive Polish patients ($p = 0.003$) while the rs1049174-G allele was more frequently observed in Greeks than in Poles ($p < 0.001$). Moreover, among Greek patients, the rs1051792-GG homozygotes more frequently presented with anti-CCP antibodies and rheumatoid factor (RF), while carriers of the rs1049174-G variant and rs1154831-CC homozygotes were characterized by lower disease activity scores ($p < 0.05$ in all cases). These results imply that, in addition to the HLA-DRB1 SE alleles, MICA and NKG2D polymorphisms may also play a role in rheumatoid arthritis.

Key words: *HLA-DRB1 shared epitope, NKG2D, MICA, polymorphism, rheumatoid arthritis.*

(Cent Eur J Immunol 2021; 46 (1): 92-98)

Introduction

Rheumatoid arthritis (RA) is described as a common autoimmune disease, characterized by chronic, symmetric joint inflammation. It causes bone and cartilage destruction leading to functional disability and is considered as an individual, social and economic problem [1, 2]. RA occurs in 1% of the population worldwide with female predominance [3]. The risk of RA can increase up to 60% in people with certain genetic factors [4].

One of the most significant genetic links to the disease is the HLA-DRB1 shared epitope and the major hypothesis describing it was enunciated in the late 1980s. The shared epitope (SE) is a specific five amino acid sequence in positions 70 to 74 within the antigen-binding groove of HLA-DR-β chains [5].

The shared epitope is responsible for antigen presentation, T-cell repertoire and self-peptide selection and may have a role in autoreactive adaptive immune response stimulation. HLA-DRB1 is recognized as being associated with RA in those patients who are positive for the rheumatoid factor (RF) or anti-cyclic citrullinated peptide (anti-CCP) [6]. It was found that 70% of anti-CCP positive patients possessed one of the SE variants [7]. It seems that anti-CCP positive and anti-CCP negative disease types are different in terms of the genetic background of RA development [8].

Furthermore, the strength of association is different for separate allelic variants. In North Europeans, a pow-

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Submitted: 11.12.2019; Accepted: 26.05.2020

erful risk variant is HLA-DRB1*04:01, especially within the HLA-DRB1*04:04/04:01 heterozygous genotype. In contrast, DRB1*13:01 seems to play a protective role in anti-CCP positive disorder [9, 10]. Among Greek RA patients, DRB1*04:05, DRB1*01:01 and DRB1*10:01 belong to the most frequent SE alleles [11]. This is not surprising, as in general there are differences in HLA-DRB1 allele distribution between the Polish and southern European populations [12].

Despite that, environmental factors such as smoking, which has been identified as the main inductor of citrullination, may be harmful in combination with HLA-DRB1 molecules and promote the autoimmune reaction [13].

Variability within the *KLRK1* gene coding for an activator of the immune system — Natural Killer Group 2, Member D (NKG2D), a receptor belonging to the C-type lectin-like family of transmembrane proteins – constitutes another RA related genetic factor. The NKG2D receptor is expressed on the natural killer (NK) cell surface as well as CD8+T cells and γδ+ T cells [14]. The T lymphocytes and NK cells, both of which express NKG2D, are recognized as being an important part of RA pathogenesis [15]. This may suggest potential involvement of NKG2D in RA. Increased numbers of unique CD4+ cells lack the CD28 molecule and express the NKG2D receptor. It was also observed in peripheral blood and synovial tissue of patients suffering from this disease [15, 16].

NKG2D is associated with multiple ligands, but among the first described were MHC class I-chain related proteins, MICA and MICB, with the ability to activate NK cells [17]. Exposure to cellular stress, infection, inflammation and DNA damage upregulate MIC expression [18]. Previously investigated single nucleotide polymorphisms in *MICA* and *NKG2D* regions suggested an association with susceptibility to RA in German and Indian patients [19, 20]. Nonetheless, to the best of our knowledge, there has been no study examining *MICA* and *NKG2D* variants in both Polish and Greek populations.

Our recent findings regarding the effect of *MICA* [21] and *NKG2D* polymorphism on RA treatment outcome [22] have prompted us to analyse and compare the genetic variability of Greek and Polish patients with respect to the presence of the HLA-DRB1 SE as well as *MICA* and *NKG2D* variants.

Material and methods

Patients and controls

61 Greek and 100 Polish RA patients, diagnosed according to the 2010 American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) criteria, were investigated. Collected data of the patients are summarized in Table 1. The study was approved by the Wrocław Medical University Ethics Committee and Evangelismos Hospital.

Genotyping

The single nucleotide polymorphisms (SNPs) in the genes coding for MICA and its receptor, NKG2D, were selected based on available literature analysis as well as search results from the NCBI Database of Short Genetic Variations (dbSNP). Information regarding predicted functional consequences of SNPs was obtained using the SNPinfo Web Server [23]. The following SNPs were selected: *MICA* rs1051792 (G>A; missense variant in exon 3 – Val129Met), *KLRK1* rs1154831 (C>A; intronic variant; potential transcription factor binding site), *KLRC4-KLRK1* rs1049174 (C>G; 3′ untranslated region (UTR); potential miRNA binding site) and *KLRC4-KLRK1* rs2255336 (A>G; nonsynonymous polymorphism in exon 4 – Thr72Ala).

Genotyping for *MICA* rs1051792, *KLRK1* rs1154831 and *KLRC4-KLRK1* rs1049174 was performed using a LightSNiP assay (TIB MOLBIOL, Germany). The *KLRC4-KLRK1* rs2255336 was studied employing a TaqMan SNP genotyping assay (Thermo Fisher Scientific, Waltham, MA, USA). The LightCycler 480 Real-Time PCR system (Roche Diagnostics, Rotkreuz, Switzerland) was used in this analysis. HLA-DRB1 alleles were genotyped by polymerase chain reaction (PCR) followed by hybridization with sequence-specific oligonucleotide probes (PCR-SSOP), PCR with sequence specific primers (PCR-SSP) or sequence-based typing (SBT). Genotyping results were correlated with clinical parameters.

Statistical analysis

Potential associations between examined SNPs and clinical parameters of RA patients were analysed by Fisher's exact test for parametric values. The same test was also used to compare genotype variation distribution within patients. *P*-values less than 0.05 were considered statistically significant. All statistical calculations were performed using the GraphPad7 Prism software.

Table 1. Characteristics of Polish and Greek rheumatoid arthritis (RA) patients

Variables	RA patients (*N*)	
	Poland (*n* = 100)	Greece (*n* = 61)
Females/males (% females)	92/8 (92.0)	37/24 (60.7)
Age (years) [mean ±SD]	49.6 (±11.7)	65.8 (±10.7)
Disease onset (years) [mean ±SD]	37.9 (±11.9)	52.6 (±16.2)
DAS28 level at baseline [mean ±SD]	6.50 (±0.65)	3.71 (±1.62)
CRP level at baseline [mean ±SD]	23.1 (±29.6)	2.04 (±2.78)
anti-CCP positive [%]	90 (90.0%)	46 (75.4%)
RF-positive [%]	54 (54.0%)	47 (77.0%)

DAS28 – disease activity score, CRP – C-reactive protein, RF – rheumatoid factor, anti-CCP – anti-cyclic citrullinated peptide autoantibodies, SD – standard deviation

Table 2. HLA-DRB1 shared epitope frequency in rheumatoid arthritis (RA) patients

Variables	Poland (n = 100)	Greece (n = 61)
HLA-DRB1	***n* (%)**	***n* (%)**
SE positive	72 (72.0)	38 (62.3)
*01:01	29 (35.8)	13 (29.5)
*01:02	2 (2.47)	2 (4.54)
*04:01	27 (33.3)	3 (6.82)
*04:04	10 (12.3)	5 (11.4)
*04:05	0 (0.0)	11 (25.0)
*04:08	7 (8.64)	1 (2.27)
*10:01	6 (7.41)	9 (20.5)

*The most frequent HLA-DRB1 SE alleles are marked in bold. Note that the frequencies of HLA-DRB1*04:01, *04:05, *10:01 significantly differ between the two groups of patients ($p < 0.001$, $p < 0.001$, and $p = 0.044$, respectively).*

Results and discussion

HLA-DRB1 SE has been of particular interest for many years, especially in regard to its association with RA [10, 11].

Table 2 depicts SE risk alleles in both Greek and Polish RA patients. As expected, the majority of patients, 62.3% of Greek and 72.0% of Polish RA cases of the present study, were characterized by the presence of HLA-DRB1 SE alleles. Also in line with the results of the previous studies, involving both studied populations of RA patients, DRB1*01:01, *04:05 and *10:01 were predominantly represented among Greek patients [24-26] while DRB1*01:01 and *04:01 alleles were predominantly represented among Polish patients [10]. The latter DRB1*04:01 allele was associated with RA susceptibility in various populations as shown, for example, also for UK patients [27]. According to another study in UK patients, HLA-DRB1*04:04 is more frequently observed than HLA-DRB1*01:01, especially in anti-CCP-positive and RF-positive RA patients [28]. On the other hand, in Korean RA patients, HLA-DRB1*04:05 correlated with the disease [29], whereas other HLA-DRB1 alleles, such as *04:05, *04:01, *09:01, *01:01, *14:01, *16:02, *04:03, and *14:05, were significantly associated with RA in the Japanese [30]. These results underline the differences in HLA-DRB1 SE segregation between various populations and confirm the predominant representation of specific HLA-DRB1 SE alleles associated with the RA risk among Greek and Polish patients.

For the past few years, non-classical HLA polymorphism, including MICA and NKG2D, has been investigated in Polish patients with RA [21, 22, 31]. As those publications were focused only on one population, there is still a lack of knowledge about the effect of these genetic factors in Greeks [11, 26]. Therefore, in the present study, *MICA* and *NKG2D* polymorphisms were also analysed in a group of Greek patients.

Table 3. Distribution of *MICA* and *NKG2D* alleles, genotypes in rheumatoid arthritis (RA) patients

Variables	Poland *n* (%)	Greece *n* (%)
MICA rs1051792		
GG	37 (37.0)	26 (44.1)
GA	45 (45.0)	24 (40.7)
AA	18 (18.0)	9 (15.2)
G	119 (59.5)	76 (64.4)
A	81 (40.5)	42 (35.6)
NKG2D rs1154831		
CC	71 (71.0)	37 (60.7)
CA	27 (27.0)	20 (32.8)
AA	2 (2.00)	4 (6.60)
C	169 (84.5)	94 (77.0)
A	31 (15.5)	28 (23.0)
NKG2D rs1049174		
CC	42 (42.0)[a]	10 (16.4)
CG	49 (49.0)	31 (50.8)
GG	9 (9.00)	20 (32.8)[b]
C	133 (66.5)	51 (41.8)
G	67 (34.5)	71 (58.2)
NKG2D rs2255336		
AA	2 (2.00)	4 (6.56)
AG	35 (35.0)	18 (29.5)
GG	63 (63.0)	40 (65.6)
A	39 (19.5)	24 (19.7)
G	161 (80.5)	98 (80.3)

[a] *CC vs. G+, $p < 0.001$,* [b] *GG vs. C+, $p < 0.001$*

Firstly, we compared the distributions of *MICA* alleles and their association with HLA-DRB1 alleles in both groups of patients. As shown in Table 3, no significant differences were observed in genotype distribution between Polish and Greek patients within *MICA* rs1051792. In both groups the *AA* homozygous *MICA* genotype was the rarest.

No significant differences were observed in the presence of the SE among Greek patients with various *MICA* rs1051792 alleles or genotypes. However, the Polish *A* allele carriers were more likely to possess one of the HLA-DRB1 SE alleles ($p = 0.003$), particularly DRB1*01:01 and/or DRB*01:02 ($p = 0.001$) (Fig. 1).

Secondly, *MICA* rs1051792 polymorphism was investigated for a potential association with clinical parameters in both Polish and Greek RA patients. A subsequent anal-

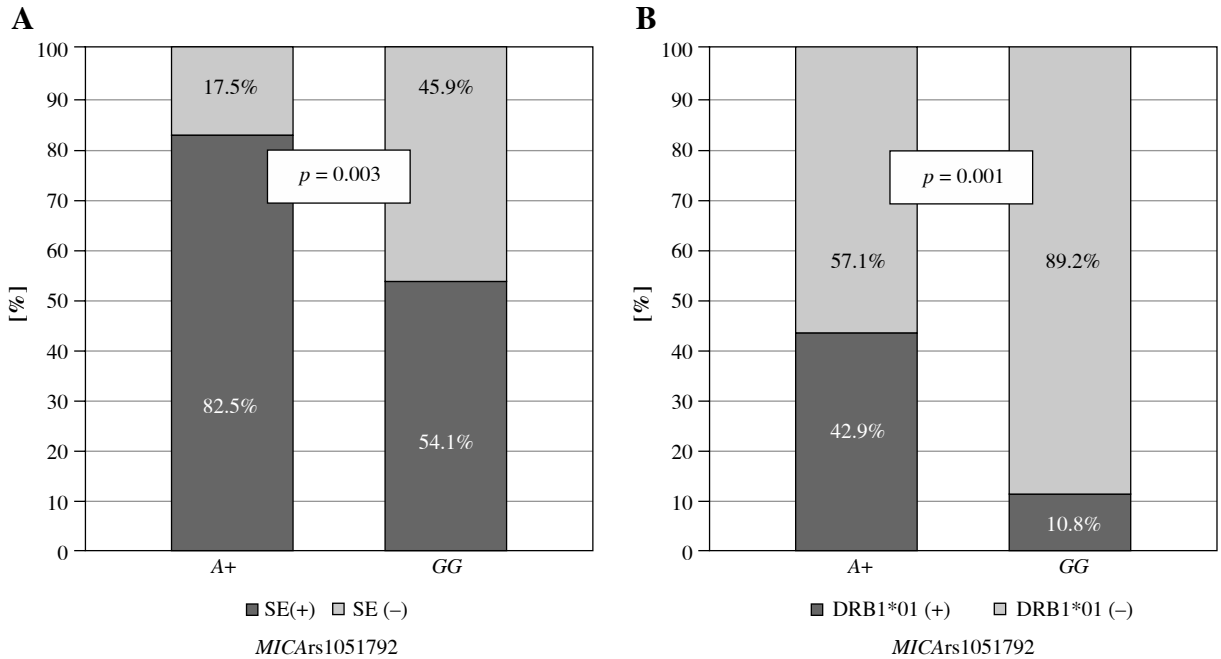

Fig. 1. Relationship between *MICA* rs1051792 genotype and presence of HLA-DRB1 SE alleles in Polish RA patients. *MICA A* allele carriers were more likely to possess one of the HLA-DRB1 SE alleles, (**A**) especially HLA-DRB1*01:01 and/or DRB1*01:02 (**B**)

ysis revealed that the *GG* homozygotes are more frequent among Greek patients with anti-CCP antibodies and RF as compared to those carrying the *A* variant ($p = 0.038$ and $p = 0.028$, respectively) (Fig. 2). It has been also documented by Alexiou *et al*. that a higher level of anti-CCP antibodies may have prognostic significance in Greek patients with extra-articular manifestation [32] as well as radiographic joint damage [33].

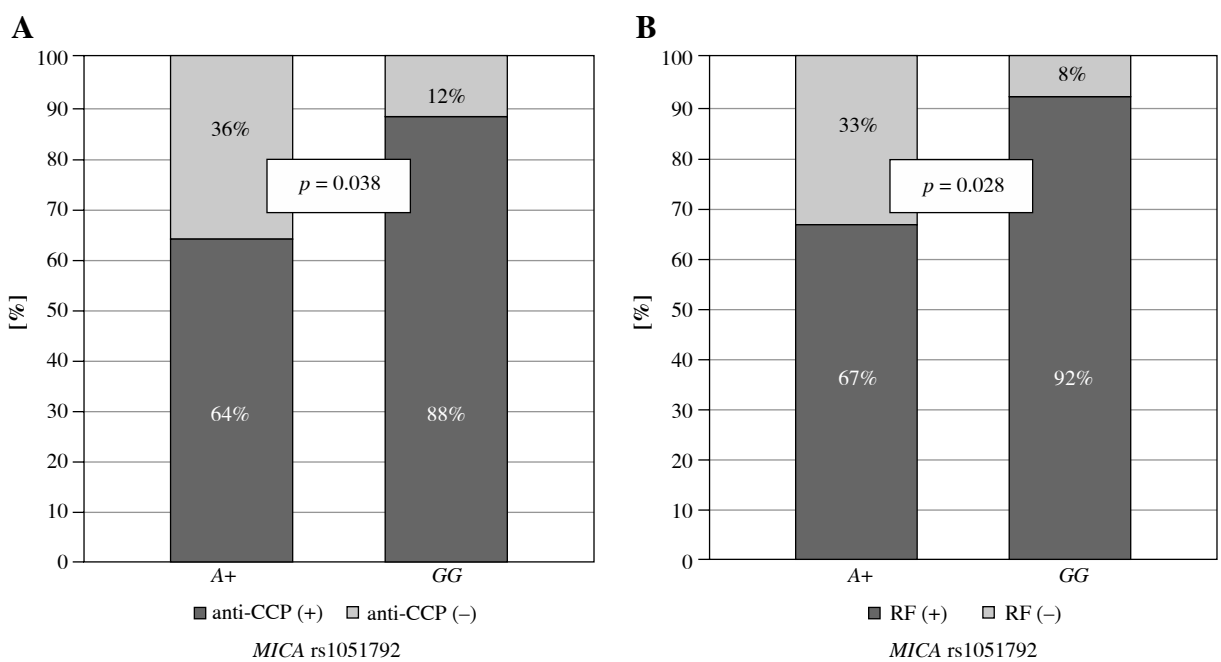

Fig. 2. Relationship between *MICA* rs1051792 genotype and clinical parameters in Greek RA patients. *MICA GG* homozygous patients more frequently presented with anti-CCP antibodies (**A**) and RF (**B**) as compared to those carrying the A variant

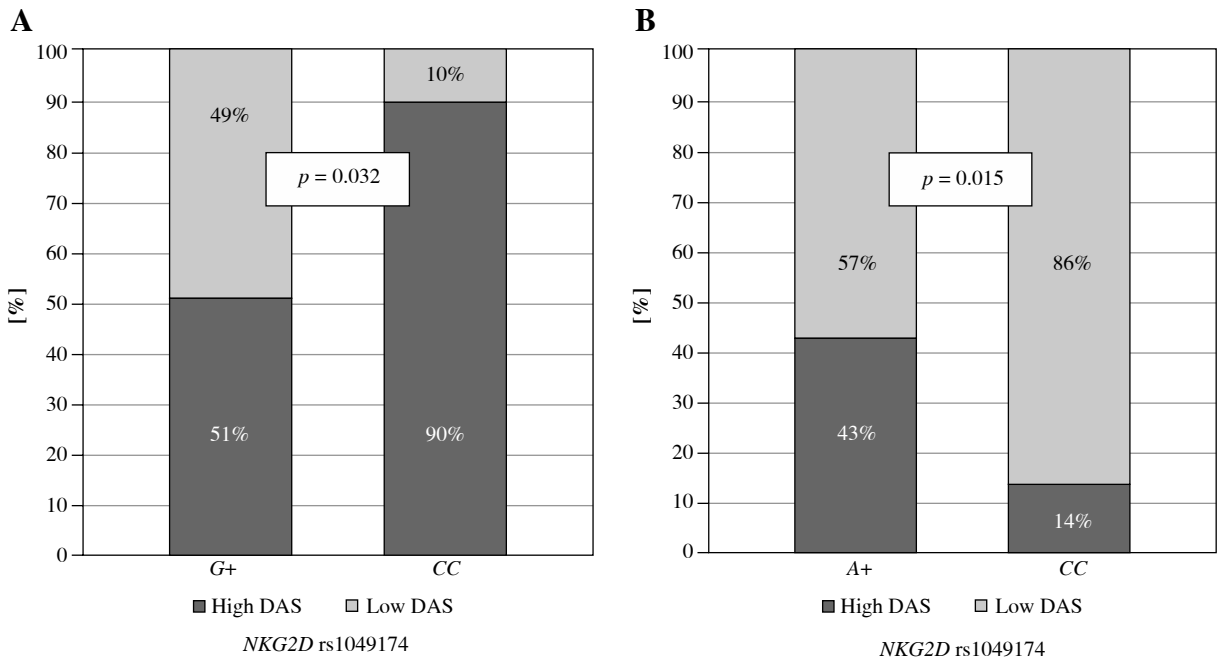

Fig. 3. Relationship between disease activity score and *NKG2D* genotypes in Greek RA patients. Lower disease activity score characterised patients carrying the *NKG2D* rs1049174 *G* variant (**A**) or being *NKG2D* rs1154831 *CC* homozygotes (**B**)

On the other hand, these relationships between anti-CCP and RF positivity and *MICA GG* homozygosity detected in Greeks were not seen in the Polish group of RA patients. Note, however, the results of our previous study by Iwaszko *et al*. documenting that *MICA* rs1051792 polymorphism can affect the efficiency of anti-tumour necrosis factor (TNF) therapy in Polish patients [21]. Thus this *MICA* polymorphism seems to play a significant role also in Polish RA patients. In a German and French cohort, the *MICA* rs1051792 *G* allele was also associated with disease development risk [19]. Interestingly, the correlation between *GG* homozygotes and RF presence was shown in Tunisians [34], which is in line with our results for the Greek cohort.

In the third part of the study, we focused on analysis of the *NKG2D* genetic variants. The mechanism and functions of MICA-128Met/Val (*G/A*) dimorphism in disease pathogenesis remain partly obscure [35]. However, it has been documented that the protein encoded by the MICA-129-Met (*A*) allele is characterized by stronger interaction with NKG2D than the alternative Val (*G*) isoform. Therefore, downregulation of NKG2D by the Met (*A*) variant is more efficient.

The role of *NKG2D* genetic variants, including those selected in the present study (rs1049174, rs2255336), has been previously investigated by Mariaselvam *et al*. in regard to RA susceptibility in an Indian population. However, this study did not reveal any statistically significant correlation between those single nucleotide polymorphisms and clinical parameters of the disease [20].

In this present study, the *NKG2D* rs1049174 *G* variant and *GG* homozygosity were more frequent among the Greeks than the Polish patients ($p < 0.001$) (Table 3). However, no significant differences were observed in genotype distribution between Polish and Greek patients within *NKG2D* rs1154831 and *NKG2D* rs2255336.

Moreover, the *NKG2D* rs1049174 *G* variant was found to be associated with lower disease activity score in Greek RA patients ($p = 0.032$, Fig. 3A). In addition, *NKG2D* rs1154831 *CC* homozygotes were also characterized by a lower disease activity score ($p = 0.015$, Fig. 3B). This is a novel observation not previously described. This finding is supported by a Korean study conducted by Park *et al.*, where presence of *NKG2D* rs2255336 wild type homozygosity, but not *NKG2D* rs1049174, resulted in an increased risk of RA [36].

Summarizing the above, HLA-DRB1 SE alleles are well-documented risk factors for RA development, but the association of particular alleles varies between populations. The vast majority of Polish and Greek patients with RA are positive for SE alleles, but DRB1*01:01, *04:05 and *10:01 predominate among Greek while DRB1*01:01 and *04:01 predominate among Polish patients. Differences between the two populations are also seen with respect to the other genetic loci. In Polish patients the presence of DRB1 SE is associated with the *MICA* rs1051792 *A* allele. Among Greek patients, the *NKG2D* rs1051792-*GG* homozygotes more frequently presented with anti-CCP antibodies and RF, while carriers of the *NKG2D* rs1049174-*G*

variant and rs1154831-*CC* homozygotes were characterized by lower disease activity scores ($p < 0.05$ in all cases).

In conclusion, the results of the present study show that HLA-DRB1 SE alleles, as well as *MICA* and *NKG2D* genetic variants, are associated with RA development and their frequencies differ between the Greek and Polish populations of RA patients.

Acknowledgments

This study was supported by the National Science Centre Poland (grant number 2016/21/B/NZ5/01901).

The authors declare no conflict of interest.

References

1. Knevel R, Huizinga TWJ, Kurreeman F (2017): Genomic influences on susceptibility and severity of rheumatoid arthritis. Rheum Dis Clin North Am 43: 347-361.
2. Smolen JS, Aletaha D, McInnes IB (2016): Rheumatoid arthritis. Lancet 388: 2023-2038.
3. Alam J, Jantan I, Bukhari SNA (2017): Rheumatoid arthritis: Recent advances on its etiology, role of cytokines and pharmacotherapy. Biomed Pharmacother 92: 615-633.
4. MacGregor AJ, Snieder H, Rigby AS et al. (2000): Characterizing the quantitative genetic contribution to rheumatoid arthritis using data from twins. Arthritis Rheum 43: 30-37.
5. Gregersen PK, Silver J, Winchester RJ (1987): The shared epitope hypothesis. an approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis. Arthritis Rheum 30: 1205-1213.
6. McInnes IB, Schett G (2011): The Pathogenesis of rheumatoid arthritis. N Engl J Med 365: 2205-2219.
7. Bodis G, Toth V, Schwarting A (2018): Role of human leukocyte antigens (HLA) in autoimmune diseases. In: Methods Mol Biol 1802: 11-29.
8. Bax M, Van Heemst J, Huizinga TWJ, Toes REM (2011): Genetics of rheumatoid arthritis: What have we learned? Immunogenetics 63: 459-466.
9. Silman AJ, Pearson JE (2002): Epidemiology and genetics of rheumatoid arthritis. Arthritis Res 4: 265-272.
10. Pazdur J, Płoski R, Bogunia-Kubik K, et al. (1998): Can HLA-DRB1 typing have prognostic value in patients with undifferentiated chronic arthritis? Tissue Antigens 51: 678-680.
11. Ioannidis JPA, Tarassi K, Papadopoulos IA, et al. (2002): Shared epitopes and rheumatoid arthritis: Disease associations in Greece and meta-analysis of Mediterranean European populations. Semin Arthritis Rheum 31: 361-370.
12. Jungerman M, Sanchez-Mazas A, Fichna P, et al. (1997): HLA class II DRB1, DQA1 and DQBI polymorphisms in the Polish population from Wielkopolska. Tissue Antigens 49: 624-628.
13. Klareskog L, Stolt P, Lundberg K, et al. (2006): A new model for an etiology of rheumatoid arthritis: Smoking may trigger HLA-DR (shared epitope)-restricted immune reactions to autoantigens modified by citrullination. Arthritis Rheum 54: 38-46.
14. Babic M, Romagnani C (2018): The role of natural killer group 2, member D in chronic inflammation and autoimmunity. Front Immunol 9: 1219.
15. Ahern DJ, Brennan FM (2011): The role of natural killer cells in the pathogenesis of rheumatoid arthritis: Major contributors or essential homeostatic modulators? Immunol Lett 136: 115-121.
16. Groh V, Bruhl A, El-Gabalawy H, et al. (2003): Stimulation of T cell autoreactivity by anomalous expression of NKG2D and its MIC ligands in rheumatoid arthritis. Proc Natl Acad Sci U S A 100: 9452-9457.
17. Burgess SJ, Maasho K, Masilamani M, et al. (2008): The NKG2D receptor: immunobiology and clinical implications. Immunol Res 40: 18-34.
18. Choy MK, Phipps ME (2010): MICA polymorphism: biology and importance in immunity and disease. Trends Mol Med 16: 97-106.
19. Kirsten H, Petit-Teixeira E, Scholz M, et al. (2009): Association of MICA with rheumatoid arthritis independent of known HLA-DRB1 risk alleles in a family-based and a case control study. Arthritis Res Ther 11: 60.
20. Mariaselvam CM, Tamouza R, Krishnamoorthy R, et al. (2017): Association of NKG2D gene variants with susceptibility and severity of rheumatoid arthritis. Clin Exp Immunol 187: 369-375.
21. Iwaszko M, Świerkot J, Dratwa M, et al. (2020): Association of MICA-129Met/Val polymorphism with clinical outcome of anti-TNF therapy and MICA serum levels in patients with rheumatoid arthritis. Pharmacogenomics J 20: 760-769.
22. Iwaszko M, Świerkot J, Kolossa K, et al. (2018): Influence of NKG2D genetic variants on response to anti-TNF agents in patients with rheumatoid arthritis. Genes (Basel) 9: 1-15.
23. Xu Z, Taylor JA (2009): SNPinfo: integrating GWAS and candidate gene information into functional SNP selection for genetic association studies. Nucleic Acids Res 37: W600-W605.
24. Stavropoulos C, Spyropoulou M (1997): HLA-DRB1 alleles in Greek rheumatoid arthritis patients and their association with clinical characteristics. Eur J Immunogenet 24: 265-274.
25. Carthy D, Ollier W, Papasteriades C, et al. (1993): A shared HLA-DRB1 sequence confers RA susceptibility in Greeks. Eur J Immunogenet 20: 391-398.
26. Drosos AA, Lanchbury JS, Panayi GS, Moutsopoulos HM (1992): Rheumatoid arthritis in greek and british patients. a comparative clinical, radiologic, and serologic study. Arthritis Rheum 35: 745-748.
27. Boki KA, Panayi GS, Vaughan RW, et al. (1992): HLA Class ii sequence polymorphisms and susceptibility to rheumatoid arthritis in greeks. the hla-drβ shared epitope hypothesis accounts for the disease in only a minority of greek patients. Arthritis Rheum 35: 749-755.
28. Mackie SL, Taylor JC, Martin SG, et al. (2012): A spectrum of susceptibility to rheumatoid arthritis within HLA-DRB1: stratification by autoantibody status in a large UK population. Genes Immun 2: 120-128.
29. Mok JW, Lee YJ, Kim JY, et al. (2003): Association of MICA polymorphism with rheumatoid arthritis patients in Koreans. Hum Immunol 64: 1190-1194.
30. Kochi Y, Yamada R, Kobayashi K, et al. (2004): Analysis of single-nucleotide polymorphisms in Japanese rheumatoid arthritis patients shows additional susceptibility markers besides the classic shared epitope susceptibility sequences. Arthritis Rheum 50: 63-71.

31. Iwaszko M, Świerkot J, Kolossa K, et al. (2015): Polymorphisms within the human leucocyte antigen-E gene and their associations with susceptibility to rheumatoid arthritis as well as clinical outcome of anti-tumour necrosis factor therapy. Clin Exp Immunol 182: 270-277.
32. Alexiou I, Germenis A, Koutroumpas A, et al. (2008): Anti-cyclic citrullinated peptide-2 (CCP2) autoantibodies and extra-articular manifestations in Greek patients with rheumatoid arthritis. Clin Rheumatol 27: 511-513.
33. Alexiou I, Germenis A, Ziogas A, et al. (2007): Diagnostic value of anti-cyclic citrullinated peptide antibodies in Greek patients with rheumatoid arthritis. BMC Musculoskelet Disord 8: 1-7.
34. Achour Y, Kammoun A, Ben Hamad M, et al. (2014): Association study of MICA gene polymorphisms with rheumatoid arthritis susceptibility in south Tunisian population. Int J Immunogenet 41: 486-492.
35. Isernhagen A, Malzahn D, Bickeböller H, Dressel R (2016): Impact of the MICA-129Met/Val dimorphism on NKG2D-mediated biological functions and disease risks. Front Immunol 7: 588.
36. Park KS, Park JH, Song YW (2008): Inhibitory NKG2A and activating NKG2D and NKG2C natural killer cell receptor genes: susceptibility for rheumatoid arthritis. Tissue Antigens 72: 342-346.