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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

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Summary

Involvement of the non-classical human leucocyte antigen-E (HLA-E) in both innate and acquired immune response suggests its possible role in development of autoimmune pathologies. This study was undertaken to investigate relationships between the HLA-E gene single nucleotide polymorphisms (SNPs) and a risk of rheumatoid arthritis (RA), as well as to evaluate a potential of these polymorphisms to modulate clinical outcome of anti-tumour necrosis factor (TNF) treatment in female patients. A total of 223 female patients with RA receiving anti-TNF biological therapy and 134 female healthy subjects were enrolled into the study. Genotypings for two SNPs within the HLA-E gene (rs1264457 HLA-E*01:01/01:03; rs1059510 HLA-E*01:03:01/01:03:02) were performed using a polymerase chain reaction (PCR) amplification employing LightSNiP assays. Clinical response was evaluated according to the European League Against Rheumatism (EULAR) criteria at 12 and 24 weeks after initiation of the therapy. The frequency of the HLA-E*01:01/01:01 genotype was decreased significantly in RA patients in comparison to controls ($P = 0{\cdot}031$). The presence of the HLA-E*01:01/01:01 genotype in patients correlated with better EULAR response after 12 weeks of anti-TNF treatment, while 01:03 allele carriers were generally unresponsive to the treatment ($P = 0{\cdot}014$). The HLA-E*01:03/01:03 genotype was also over-represented among non-responding patients in comparison to HLA-E*01:01/01:01 homozygotes ($P = 0{\cdot}021$). With respect to the HLA-E rs1059510 variation, a better response after 12 weeks was observed more frequently in patients carrying the HLA-E*01:03:01/01:03:01 genotype than other genotypes ($P = 0{\cdot}009$). The results derived from this study imply that HLA-E polymorphisms may influence RA susceptibility and affect clinical outcome of anti-TNF therapy in female RA patients.

Keywords: anti-TNF therapy, HLA-E polymorphism, rheumatoid arthritis, TNF inhibitors

Introduction

Rheumatoid arthritis (RA) constitutes one of the most common autoimmune inflammatory diseases, affecting approximately 1% of the population worldwide, and is characterized by synovial inflammation and joint destruction leading to significant disability and early mortality. The aetiology of RA is multifarious and has not yet been elucidated fully, although genetic and environmental factors have been implicated in the disease development. The genetic contribution to RA susceptibility has been estimated at approximately 50% [1]. The introduction of anti-tumour necrosis factor (anti-TNF) biological drugs in clinical management has revolutionized the treatment of RA; however, up to 30% of patients do not respond to the therapy [2,3]. Such heterogeneity in clinical outcomes among patients may reflect different genetic backgrounds of anti-TNF-treated patients. In this context, genetic variants affecting the anti-TNF therapy could serve as biomarkers to predict response to treatment and to optimize the use of anti-TNF agents [4].

It has been suggested that natural killer (NK) and T cells are involved in the initiation and maintenance of RA [5,6]. Non-classical human leucocyte antigen-E (HLA-E) acts as a ligand for the CD94/NKG2 receptors, including inhibitory CD94/NKG2A and activatory CD94/NKG2C molecules [7–9], expressed on both NK and $CD8^+$ T lymphocytes [10,11]. The interaction between these receptors and HLA-E induces a cascade of competing activating and inhibitory signals, resulting in a modulation of NK and T lymphocytes function [12]. Disruption of these signals can lead to an inadequate immune response of NK and T cells and promote autoimmune reactions. Additionally, HLA-E can be also recognized by T cells in a T cell receptor (TCR)-dependent manner [13–15]. Involvement of the HLA-E in both innate and acquired immune responses suggests its possible role in development of autoimmune pathologies.

The HLA-E molecule is one of the least polymorphic members of the HLA I family and only two functional alleles, HLA-E*01:01 and HLA-E*01:03, have been reported in human populations at high frequencies [16]. These alleles differ at only one amino acid position (non-synonymous mutation) located in codon 107 of the α heavy chain domain [17]. This substitution was implicated in functional differences resulting from their distinct biological and biophysical properties. Proteins encoded by the both alleles have the same intracellular expression levels, but HLA-E*01:01-encoded molecules exhibit lower cell surface expression in comparison to HLA-E*01:03-encoded proteins. The HLA-E*01:03 variant also displays higher peptide-binding affinity and thermal stability than the HLA-E*01:01 [17]. In addition, the silent polymorphism in codon 77 differentiates the HLA-E*01:03 allele further into HLA-E*01:03:01 and HLA-E*01:03:02 variants. The HLA-E polymorphism was implicated in susceptibility to inflammatory autoimmune diseases, including Behçet's disease (BD) [18], type I diabetes mellitus (T1D) [19], pemphigus vulgaris (PV) [20] and multiple sclerosis (MS) [21]. To date, there are no published studies addressing the possible association of the genetic variants in the HLA-E gene with RA.

Female sex constitutes a risk factor for RA. In comparison to men, women are two to three times more prone to develop RA, exhibit a more aggressive disease course and display worse responses to treatments with biological agents [22]. The higher prevalence of RA among women may reflect gender differences in the innate and adaptive immune systems [23,24]. The exact mechanisms underlying this sexual dimorphism in the immune response remain obscure; however, sex hormones, environmental factors and microchimerism have been implicated in this phenomenon [25,26]. Genetic background may also contribute to this immunological disparity. Genetic differences between men and women have been implicated in distinct gender-specific patterns of susceptibility to many diseases, including autoimmune disorders [27,28]. Furthermore, some studies revealed female-specific associations between polymorphisms within genes related to the immune system and RA, suggesting the existence of genetic predisposition factors specific for women [29–35]. In the context of these reports, the present study focused on the female patient population with RA.

This study was undertaken to investigate relationships between HLA-E gene polymorphisms and the risk of RA development, as well as to evaluate the potential of the HLA-E polymorphisms to act as predictive markers of response to anti-TNF treatment in female patients with rheumatoid arthritis.

Materials and methods

Patients and controls

The study group comprised 223 female patients of Caucasian origin diagnosed with active, adult-onset RA and enrolled into treatment with anti-TNF agents. The following inclusion criteria were applied to select patients for the current study: RA was confirmed according to the 2010 American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) classification criteria; resistance to treatment with at least two disease-modifying anti-rheumatic drugs (DMARDs); presence of active disease [defined as a Disease Activity Score in 28 joints (DAS28) $\geq 5{\cdot}1$) prior to initiation of anti-TNF therapy; ongoing treatment with one of the four anti-TNF biological agents (adalimumab, etanercept, infliximab, certolizumab) at the time of the study; age more than 18 years; complete medical history and physical examination; and consent to participate in the study. Exclusion criteria comprised: clinically significant impairment of hepatic and renal function; co-existence of other systemic diseases of connective tissue besides RA; infections with hepatotrophic viruses; infections resistant to therapy; ongoing history of cancer or uncontrolled diabetes; alcohol abuse; pregnancy or breastfeeding; insufficient clinical records; and unwillingness or inability to co-operate. Baseline characteristics of the patients are summarized in Table 1. The control group consisted of 134 unrelated healthy female blood donors (age range = 23–55; mean age = $47{\cdot}1 \pm 8{\cdot}7$) of the same ethnic origin as the patients, with no personal history of autoimmune diseases. Controls were recruited from the Blood Bank of Wroclaw. The study was approved by the Wroclaw Medical University Ethics Committee and written informed consent was obtained from all participants.

The patients were administered recommended doses of TNF-inhibitors: 3 mg/kg of body weight of infliximab given as intravenous infusions at weeks 0, 2 and 6, and every 8 weeks thereafter, subcutaneous injections of adalimumab at 40 mg every other week, subcutaneous injections of etanercept at 50 mg every week and subcutaneous injections of certolizumab pegol 400 mg at weeks 0, 2 and 4 and 200 mg every 2 weeks thereafter. In most cases DMARDs, glucocorticoids and/or non-steroidal anti-inflammatory

Table 1. Patient characteristics.

RA patients	$n = 223$
Clinical characteristics	
Age (years) [mean (±s.d.)]	52·2 (±12·3)
Disease duration (years) [mean (±s.d.)]	13·2 (±8·1)
Disease onset (years) [mean (±s.d.)]	39·1 (±12·1)
DAS28 at baseline [mean (±s.d.)]	6·5 (±0·6)
CRP at baseline [mean (±s.d.)]	22·0 (±33·7)
RF-positive (%)	64·8%
Anti-CCP$^+$ (%)	94·8%
Anti-TNF drugs	
Etanercept	54%
Adalimumab	33%
Infliximab	7%
Certolizumab pegol	6%
Glucocorticosteroids	91%
Methotrexate	92%

DAS28 = disease activity score 28; CRP = C-reactive protein; RF = rheumatoid factor; anti-CCP = anti-cyclic citrullinated peptide antibodies; s.d. = standard deviation; TNF = tumour necrosis factor.

drugs were maintained at stable levels during follow-up. In total, 92% of patients were treated with methotrexate (mean dose: 22 mg per week) and 91% of patients were treated with glucocorticoids (mean dose of prednisone: 6·6 mg per day).

Clinical features of the patients were evaluated systematically at baseline as well as at 12 and 24 weeks after initiation of the therapy with TNF inhibitors. Disease activity of RA patients was assessed using the DAS28 score based on four components, including the number of swollen and tender joints, C-reactive protein (CRP) level, erythrocyte sedimentation rate (ESR) and patient's global assessment of general health expressed on the visual analogue scale (VAS, mm). Clinical response was evaluated according to the EULAR response criteria based on combination of an individual amount of change in a DAS28 score in relation to a DAS28 score attained at a given end-point [36,37]. The level of disease activity was interpreted as low (DAS28 $\leq$ 3·2), moderate (3·2 $<$ DAS28 $\leq$ 5·1) or high (DAS28 $>$ 5·1). The patients were categorized as good, moderate or non-responders. A good response was defined as improvement in a DAS28 score (ΔDAS28) $>$ 1·2 from baseline in combination with a DAS28 $\leq$ 3·2 at end-point. Moderate response was defined as ΔDAS28 $>$ 1·2 and DAS28 at end-point $>$ 3·2 or 0·6 $<$ ΔDAS28 $\leq$ 1·2 and DAS28 at end-point $\leq$ 5·1. Lack of response was defined as ΔDAS28 $\leq$ 0·6 or 0·6 $<$ ΔDAS28 $\leq$ 1·2 and DAS28 at end-point $>$ 5·1.

HLA-E genotyping

DNA was extracted from peripheral blood taken on ethylenediamine tetraacetic acid (EDTA) using the Maxwell 16 Blood DNA Purification Kit (Promega Corp., Madison, WI, USA) following the recommendations of the manufacturer.

Determinations of the HLA-E*01:01/01:03, rs1264457 and HLA-E*01:03:01/01:03:02 and rs1059510 variants were carried out by the LightSNiP typing assay (TIB-MolBiol, Berlin, Germany) on a LightCycler 480 real-time polymerase chain reaction (PCR) system (Roche Applied Science, Mannheim, Germany). LightSNiP assay constitutes a powerful tool for single nucleotide polymorphism (SNP) typing that relies on real-time PCR reaction combined with analysis of the melting curve of a SNP-specific probe. A probe's melting temperature decreases with each mismatch to a studied region, allowing identification of polymorphic variants. The reagents used for each reaction included 1·6 µl $MgCl_2$, 14·4 µl H_2O, 1 µl of LightSNiP reagent mix (containing premixed primers and probes specific to each variant, designed and manufactured by TIB Molbiol) and 2 µl of FastStart DNA Master HybProbe (Roche Diagnostics), which were set up in a final volume of 20 µl containing 1 µl of DNA solution. The cycling conditions were as follows: initial denaturation at 95°C for 10 min, followed by 45 cycles of denaturation at 95°C for 10 s, annealing at 60°C for 10 s and extension at 72°C for 15 s. Fluorescence was assessed at the end of each annealing phase. The melting curve analysis of the amplification products was performed at 95°C for 30 s, 40°C for 2 min and 75°C with a ramp of 1·5°C/s and continuous acquisition of data.

Statistical analysis

The Hardy–Weinberg equilibrium was evaluated for genotype frequencies of two SNPs within the HLA-E gene in the patient and control groups using the Population Genetics package for R (cran: genetics, version 1.3.8.1). Patient characteristics comprising the main clinical and demographic features were described using mean and standard deviation for continuous variables and frequencies and proportions for categorical ones. The frequencies of the alleles and genotypes in the patients and healthy controls were compared using Fisher's exact test. The efficacy of the anti-TNF treatment was evaluated in accordance to EULAR criteria. To assess the effect of studied SNPs on clinical outcome, Fisher's exact test was employed to compare patients with a good response to TNF inhibitors to those with a poor response to the treatment. Response to anti-TNF treatment was assessed at two different time-points: at the 12th and 24th weeks after initiation of the therapy. The absolute values of the DAS28 and CRP levels and their changes between baseline and the 12th and 24th weeks in relation to studied SNPs were analysed using the Wilcoxon–Mann–Whitney test. A P-value $<$ 0·05 was considered statistically significant. All statistical calculations were performed with R software (version 3.0.2; x86_64-pc-linux-gnu) [38]. Power analyses were performed retrospectively using Quanto software (version 1.2.4) assuming the additive model, minor allele frequency (MAF) of 35% and the RA population risk of 1%. These simulations indicated that the power of the

presented calculations ranged between 86 and 88% at the 0·05 significance level [39].

Results

Distribution of the HLA-E genotypes and alleles in RA patients and healthy control group

The allele and genotype frequencies of the HLA-E rs1264457 and rs1059510 polymorphisms observed in patients and controls are presented in Table 2. The distributions of the HLA-E rs1264457 and rs1059510 genotypes were in accordance with the Hardy–Weinberg equilibrium in both the female RA patients and the control group. The distributions of the HLA-E rs1264457 alleles and genotypes differed between patients and healthy individuals. The frequency of the HLA-E*01:01/01:01 genotype was decreased significantly in RA patients in comparison to the control group [odds ratio (OR) = 0·61, confidence interval (CI) = 0·38–0·98, $P = 0·031$]. This result was accompanied by a significant increase in the frequency of the HLA-E*01:03 allele in patients compared to controls (OR = 1·43, CI = 1·03–1·98, $P = 0·027$). The HLA-E*01:03/01:03 genotype also indicated a trend to appear more frequently among patients than healthy controls when compared to the HLA-E*01:01/01:01 homozygotes, although it did not reach the level of statistical significance (OR = 1·96, CI = 0.95–4·17, $P = 0·069$). No differences were detected regarding the frequencies of HLA-E rs1059510 alleles or genotypes between patients and controls.

Relationships between HLA-E gene polymorphisms and EULAR responses at 12 and 24 weeks of anti-TNF treatment

Significant differences were detected for the HLA-E rs1264457 polymorphism in relation to anti-TNF treatment outcome in female RA patients (Table 3). Twelve weeks after initiation of treatment the good/moderate response was displayed more frequently by patients with the HLA-E*01:01/01:01 genotype, while HLA-E*01:03 allele carriers were generally unresponsive to treatment (OR = 8·26, CI = 1·23–352·79, $P = 0·014$). Also the HLA-E*01:03/01:03 genotype was over-represented among the non-responder patient group in comparison with the HLA-E*01:01/01:01 homozygous patients (OR = 10·08, CI = 1·07–492·92, $P = 0·021$). With respect to the HLA-E rs1059510 variation, unresponsiveness to treatment after 12 weeks was observed more frequently in patients carrying the HLA-E*01:03:02/01:03:02 when compared with the HLA-E*01:03:01/01:03:01 genotype carriers (OR = 7·06, CI = 0·75–90·37, $P = 0·047$). Conversely, the HLA-E*01:03:01/01:03:01 genotype was associated with a better EULAR response (OR = 6·41, CI = 1·43–59·40, $P = 0·009$). The analyses performed for both HLA-E rs1264457 and HLA-E rs1059510 failed to demonstrate any significant association with EULAR response measured after 24 weeks of anti-TNF therapy.

Distribution of the HLA-E alleles and genotypes in relation to selected clinical features of female RA patients

The frequency of the HLA-E genotypes and alleles in female RA patients were analysed in relation to selected clinical and laboratory parameters (Table 4). A significant association was observed between the HLA-E rs1059510 polymorphism and a CRP level 24 weeks after the implementation of the drug regimen. The patients homozygous for the HLA-E*01:03:02 allele demonstrated a lesser average decrease of CRP level after 24 weeks of anti-TNF treatment than carriers of the HLA-E*01:03:01 allele or HLA-E*01:03:01/01:03:01 genotype ($P = 0·009$ and $P = 0·020$, respectively). This outcome was accompanied by higher absolute CRP values after 24 weeks of therapy in patients with the HLA-E*01:03:02/01:03:02 homozygous genotype in comparison to HLA-E*01:03:01 allele carriers ($P = 0·013$) as well as HLA-E*01:03:01 homozygotes

Table 2. Distribution of human leucocyte antigen (HLA)-E alleles and genotypes in female rheumatoid arthritis (RA) patients and healthy control group.

	Patients [*n* (%)]	Controls [*n* (%)]	OR (95% CI)	*P*
HLA-E rs1264457	*N* = 220	*N* = 134		
01 : 01	252 (57·3%)	176 (65·7%)	**0·70 (0·50–0·97)**	**0·027**
01 : 03	188 (42·7%)	92 (34·3%)	**1·43 (1·03–1·98)**	**0·027**
01 : 01|01 : 01	70 (31·8%)	58 (43·3%)	**0·61 (0·38–0·98)**	**0·031***
01 : 01|01 : 03	112 (50·9%)	60 (44·8%)	1·28 (0·81–2·02)	0·275
01 : 03|01 : 03	38 (17·3%)	16 (11·9%)	1·54 (0·79–3·10)	0·223
HLA-E rs1059510	*N* = 209	*N* = 132		
01 : 03 : 01	281 (67·2%)	188 (71·2%)	1·21 (0·85–1·71)	0·309
01 : 03 : 02	137 (32·8%)	76 (28·8%)	1·21 (0·85–1·71)	0·309
01 : 03 : 01|01 : 03 : 01	93 (44·5%)	69 (52·3%)	0·73 (0·46–1·16)	0·182
01 : 03 : 01|01 : 03 : 02	95 (45·5%)	50 (37·9%)	1·37 (0·86–2·19)	0·179
01 : 03 : 02|01 : 03 : 02	21 (10%)	13 (9·8%)	1·02 (0·47–2·31)	1·000

*01:01|01:01 *versus* 01:01|01:03 + 01:03|01:03. OR = odds ratio; 95% CI = 95% confidence interval; *n* = number of individuals studied for a given single nucleotide polymorphism (SNP); *n*, frequency of an allele/number of individuals with a genotype.

Table 3. European League Against Rheumatism (EULAR) responses at 12 and 24 weeks of anti-tumour necrosis factor (TNF) treatment in relation to human leucocyte antigen (HLA)-E polymorphisms in female rheumatoid arthritis (RA) patients.

	EULAR 12 weeks		EULAR 24 weeks	
	Non-responders [n (%)]	Responders [n (%)]	Non-responders [n (%)]	Responders [n (%)]
HLA-E rs1264457				
01 : 01	**13 (38·2%)***	**234 (58·8%)**	11 (50%)	231 (58·3%)
01 : 03	**21 (61·8%)**	**164 (41·2%)**	11 (50%)	165 (41·7%)
01 : 01|01 : 01	**1 (5·9%)**	**68 (34·2%)**†	3 (27·3%)	66 (33·3%)
01 : 01|01 : 03	11 (64·7%)	98 (49·2%)	5 (45·5%)	99 (50·0%)
01 : 03|01 : 03	**5 (29·4%)**‡	**33 (16·6%)**	3 (27·3%)	33 (16·7%)
HLA-E rs1059150				
01 : 03 : 01	**16 (47·1%)**§	**257 (68·4%)**	13 (59·1%)	252 (67·4)
01 : 03 : 02	**18 (52·9%)**	**119 (31·6%)**	9 (40·9%)	122 (32·6%)
01 : 03 : 01|01 : 03 : 01	**2 (11·8%)**	**87 (46·3%)****	4 (36·4%)	84 (44·9%)
01 : 03 : 01|01 : 03 : 02	12 (70·6%)	83 (44·1%)	5 (45·5%)	84 (44·9%)
01 : 03 : 02|01 : 03 : 02	**3 (17·6%)**††	**18 (9·6%)**	2 (18·2%)	19 (10·2%)

*01:01 *versus* 01:03, $P = 0{\cdot}029$, odds ratio (OR) = 0·44, 95% confidence interval (CI) = 0·20–0·95. †01:01\|01:01 *versus* 01:01\|01:03 + 01:03\|01:03, $P = 0{\cdot}014$, OR = 8·26, 95% CI = 1·23–352·79. ‡01:03\|01:03 *versus* 01:01\|01:01, $P = 0{\cdot}021$, OR = 10·08, 95% CI = 1·07–492·92. §01:03:01 *versus* 01:03:02, $P = 0{\cdot}021$, OR = 0·41, 95% CI = 0·19–0·89. **01:03:01\|01:03:01 *versus* 01:03:01\|01:03:02 + 01:03:02\|01:03:02, $P = 0{\cdot}009$, OR = 6·41, 95% CI = 1·43–59·40. ††01:03:02\|01:03:02 *versus* 01:03:01\|01:03:01, $P = 0{\cdot}047$, OR = 7·06, 95% CI = 0·75–90·37.

($P = 0{\cdot}022$). Taking into account the HLA-E rs1264457 variant and the CRP level, no significant effects were observed. No associations of HLA-E polymorphisms with the presence of anti-cyclic citrullinated peptide (anti-CCP) antibodies were found, nor any association of studied SNPs with presence of rheumatoid factor (RF). Furthermore, the comparison of baseline DAS28 values or change in DAS28 values (ΔDAS28) between the different genotypes did not expose significant differences.

Discussion

The present study provides evidence of potential engagement of the HLA-E in pathogenesis of rheumatoid arthritis in female patients. The HLA-E*01:01/01:01 homozygous individuals were more frequent among healthy controls than in the female RA patient group. Furthermore, the HLA-E*01:03 allele prevailed over the HLA-E*01:01 allele among patients. The clinical outcome of anti-TNF treatment in RA patients was also associated with the HLA-E polymorphism. Patients bearing the HLA-E*01:01/01:01 genotype achieved significantly better EULAR responses than patients with HLA-E*01:03/01:03 or HLA-E*01:01/01:03 genotypes. These results imply that presence of the HLA-E*01:01/01:01 genotype may be associated with reduced risk of RA and may increase the probability of good response to anti-TNF agents. Conversely, the HLA-E*01:03 variant may contribute to a lower chance of EULAR good response and correlate with inefficiency to TNF blockade therapy. The cohort comprising only male patients was also analysed with regard to the HLA-E polymorphisms (data not shown). There were no significant

Table 4. Human leucocyte antigen (HLA)-E gene polymorphisms in relation to selected clinical features in female rheumatoid arthritis (RA) patients.

	HLA-E rs1264457			HLA-E rs1059510		
	01 : 01 01 : 01	01 : 01 01 : 03	01 : 03 01 : 03	01 : 03 : 01 01 : 03 : 01	01 : 03 : 01 01 : 03 : 02	01 : 03 : 02 01 : 03 : 02
DAS28 at baseline	6·50 (±0·63)	6·51 (±0·62)	6·52 (±0·56)	6·53 (±0·62)	6·48 (±0·61)	6·55 (±0·60)
DAS28 at week 24	3·26 (±1·08)	3·39 (±1·13)	3·44 (±1·18)	3·24 (±1·06)	3·43 (±1·18)	3·61 (±1·11)
Δ DAS28	−3·24 (±1·16)	−3·10 (±1·14)	−3·14 (±1·25)	−3·27 (±1·14)	−3·05 (±1·22)	−2·94 (±1·11)
CRP at baseline	20·3 (±18·7)	25·1 (±43·7)	16·8 (±18·9)	17·4 (±16·7)	27·4 (±45·2)	14·9 (±19·5)
CRP at week 24	6·3 (±7·3)	8·2 (±15·0)	7·6 (±7·6)	6·5 (±9·1)	7·4 (±13·6)	**8·9 (±7·8)***
Δ CRP	−13·9 (±17·8)	−17·0 (±40·6)	−9·1 (±21·4)	−10·8 (±16·7)	−20·2 (±43·0)	**−6·0 (±22·2)**†
RF^+	45 (33%)	66 (49%)	25 (18%)	61 (47%)	55 (42%)	15 (11%)
Anti-CCP^+	53 (30%)	96 (54%)	29 (16%)	73 (43%)	79 (47%)	16 (10%)

*01:03:02\|01:03:02 *versus* 01:03:01\| 01:03:01 + 01:03:01\| 01:03:02, $P = 0{\cdot}013$; 01:03:02\|01:03:02 *versus* 01:03:01\| 01:03:01, $P = 0{\cdot}022$. †01:03:02\|01:03:02 *versus* 01:03:01\| 01:03:01 + 01:03:01\| 01:03:02, $P = 0{\cdot}009$; 01:03:02\|01:03:02 *versus* 01:03:01\| 01:03:01, $P = 0{\cdot}020$. DAS28 and CRP values represent mean ± standard deviation, RF^+ and anti-CCP^+ values represent frequencies of RF positive and anti-CCP positive patients. DAS28 = disease activity score 28; CRP = C-reactive protein; anti-CCP = antibodies to cyclic citrullinated peptide.

differences in the frequencies of the HLA-E genotypes and alleles among these patients in relation to male healthy subjects. Also, no significant associations were observed in this group between HLA-E variants and response to anti-TNF treatment, anti-CCP antibodies presence, RF status or levels of CRP and DAS28.

The role of the HLA-E in the development of autoimmunity is supported by several studies. The HLA-E*01:03/01:03 genotype has been associated with increased risk of Behçet's disease (BD) [18]. Moreover, this genotype was found to be a risk factor for pemphigus vulgaris (PV) development [20]. Additionally, the frequency of the HLA-E*01:03/*01:03 genotype was elevated in patients with multiple sclerosis (MS) [21] and HLA-E*01:01/*01:03 heterozygosity has been associated with severe and early-onset manifestations of type I diabetes mellitus (T1D) [19]. The results mentioned suggest the deleterious effect of the HLA-E*01:03 variant in the context of autoimmunity development, which is in agreement with the results derived from the present report.

The experiments with murine model of RA (collagen-induced arthritis; CIA) and MS (experimental allergic encephalomyelitis; EAE) revealed that NK cells are involved in the regulation of autoreactive $CD4^+$ T cells via the Qa (the equivalent of HLA-E in rodents)-1-NKG2A inhibitory pathway [40–42]. Interruption of the interaction between Qa-1 and CD94/NKG2A, through anti-NKG2A antibody administration or genetic disruption, resulted in up-regulated activity of NK cells against pathogenic $CD4^+$ T cells and was associated with inhibition of the disease development. The results of these studies imply that a presence of the Qa-1 molecule on self-reactive $CD4^+$ T cells confers protection from lysis mediated by $NKG2A^+$ NK cells. It can be hypothesized that the HLA-E polymorphism, resulting in different HLA-E surface expression on the potentially pathogenic autoreactive T cells, may determine their susceptibility to lysis mediated by $NKG2A^+$ NK cells. In this scenario, highly expressed HLA-E*01:03 molecules may be responsible for increased transmission of inhibitory signals via the CD94/NKG2A receptors, leading to diminished ability of NK cells to regulate autoreactive T cells. Consistently, lower cell surface expression of HLA-E*01:01, compared to HLA-E*01:03, may decrease binding between the HLA-E and CD94/NKG2A receptor and, in consequence, fail to provide sufficient inhibitory signal to NK cells and enhance lysis of pathogenic T cells. Augmentation of NK-dependent lytic activity may confer protection from RA development.

In addition, some T cells express CD94/NKG2A receptors and exhibit an ability to interact with HLA-E molecules. Engagement of the CD94/NKG2A receptors expressed on $CD8^+$ regulatory T cells (T_{regs}) Qa-1 by ligands on activated, autoreactive $CD4^+$ T cells dampened the suppressor functions of these cells in a study concerning the EAE [43]. Genetic disruption of the Qa-1–CD94/NKG2A pathway caused an impairment of CD94/NKG2A-mediated inhibition and augmented $CD8^+$ T cell-mediated suppression of proinflammatory $CD4^+$ T cells. In this context, high expressing HLA-E*01:03 variant may be related to increased HLA-E-restricted inhibition of the $CD8^+$ T_{reg} cells and may dampen their suppressive activity.

The HLA-E molecule may also be recognized by a subset of HLA-restricted regulatory $CD8^+$ T cells in a TCR-dependent manner and this signalling pathway has been implicated in the pathogenesis of autoimmune diseases [13]. Experiments conducted on a murine EAE model indicate that engagement of the T cell receptor on regulatory $CD8^+$ T cells by Qa-1–peptide complexes triggers suppressive activity of these cells towards potentially autoreactive $CD4^+$ T clones, and disruption of this interaction was associated with enhanced susceptibility to the development of EAE [43]. Furthermore, the essential role of the HLA-E-restricted $CD8^+$ T cell-mediated pathway in maintenance of peripheral self-tolerance was also confirmed by *in-vivo* studies concerning patients with type 1 diabetes (T1D) [44]. In the context of this recognition pathway, it was proposed that the HLA-E*01:01 and HLA-E*01:03 alleles may differ in their capacity to interact with TCR receptors on T regulatory cells and the HLA-E*01:03 variant may be responsible for less effective TCR recognition [20].

With respect to the HLA-E rs1059510 polymorphism, in this study the HLA-E*01:01:03 variant correlated with improved outcome of anti-TNF therapy. This silent polymorphism comprises a C → T substitution and is located in codon 77 of the HLA-E gene. The exact mechanism underlying the association of the HLA-E polymorphism with different patterns of anti-TNF treatment response remains elusive. According to *in-silico* analysis using a candidate SNP approach (SNPinfo Web Server) [45], variation at this site was predicted to be 'possibly damaging', suggesting potential functional consequences of this polymorphism. None the less, the synonymous substitutions do not trigger amino acid change in the protein; they may influence RNA processing and post-transcriptional regulation, translational machinery stability and co-translational folding [46]. Such possible consequences of silent SNPs may lead to differences in protein conformations, functions and expression levels. However, these considerations remain theoretical, and functional studies are indispensable to validate and clarify the potential role of this SNP in the RA pathogenesis.

Deliberating the results of this study, existing limitations must be taken into account. The study was based on a sample with limited size and such a single case–control study may not thoroughly highlight existing relations between HLA-E polymorphisms and RA. The study assumed a significance level of 0·05, which might be considered liberal, and lacked a replication sample. Therefore, further studies in the Caucasian population as well as populations with different genetic backgrounds are prerequisite to confirm

the implication of HLA-E variations in the pathogenesis of RA.

To our knowledge, this is the first study investigating the relationships between the HLA-E polymorphism and predisposition to RA as well as the clinical outcome of anti-TNF therapy in female patients. The results derived from this study indicate possible involvement of the HLA-E in the genetic background of RA in the female population of Caucasian origin. The obtained data imply a potential of the HLA-E polymorphisms to influence RA susceptibility and to modulate the clinical outcome of anti-TNF treatment. Nevertheless, further genetic studies are indispensable to confirm the role of these variations in pathogenesis of RA in female patients.

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Disclosure

None of the authors has any conflicts of interest to declare.

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