Interleukin-21 gene polymorphism rs2221903 is associated with disease activity in patients with rheumatoid arthritis

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Abstract

Introduction: Interleukin-21 (IL-21) is a cytokine which plays a significant role in the pathogenesis and disease activity of rheumatoid arthritis (RA). Genetic polymorphisms in the *IL-21* gene may alter the synthesis of IL-21. The aim of this study was to examine IL-21 and IL-21R polymorphisms in patients with RA.

Material and methods: We examined 422 patients with RA and 338 healthy controls. Single nucleotide polymorphisms (SNPs) within the *IL-21* (rs6822844 G>T, rs6840978 C>T, rs2221903 T>C) and *IL-21R* (rs2285452 G>A) genes were genotyped using TaqMan genotyping assays.

Results: There were no statistically significant differences in the distribution of studied genotypes and alleles between RA patients and the control group. To examine whether IL-21 polymorphisms affect disease activity in RA patients, we compared the distribution of IL-21 genotypes between patients with DAS28 ≤ 2.5 (patients with remission of disease symptoms) and patients with DAS28 > 2.5 (patients with active RA). Among patients with DAS28 > 2.5, increased prevalence of rs2221903 CT and CC genotypes was observed (OR = 1.54; 95% CI: 1.04–2.28; p = 0.035).

Conclusions: The results of this study suggest that IL-21 and IL-21R gene polymorphisms are not risk loci for RA susceptibility, whereas the *IL-21* rs2221903 polymorphism is associated with disease activity.

Key words: rheumatoid arthritis, interleukin-21, polymorphism.

Introduction

Rheumatoid arthritis is a multifactorial disease leading to joint destruction and numerous extra-articular manifestations. In the pathogenesis of rheumatoid arthritis (RA), proinflammatory cytokines play a significant role, inducing inflammatory responses as well as the release of other mediators of inflammation. Interleukin-21 (IL-21), an immunomodulatory type 1 cytokine, is produced by CD4+ T cells including T follicular helper cells, Th17 cells and natural killer (NK) T cells and has pleiotropic effects on both innate and adaptive immune responses [1]. Interleukin-21 increases the proliferation of activated CD4+ and CD8+ T cells and

inhibits the differentiation of inducible regulatory T cells [2, 3]. Moreover, IL-21 can directly act on B cells, leading to activation of the immune response. Thus, the effect of IL-21 on B cells may contribute to the development of autoimmune diseases [4, 5]. Numerous studies suggest that IL-21 is a cytokine playing an important role in RA pathogenesis and in the development and maintenance of inflammatory status in joints and tissues in RA patients [6, 7]. Previous studies have revealed increased levels of IL-21 in RA patients, which correlated with disease activity parameters [8].

It has been shown that the expression of IL-21 and IL-21R may be modulated by the genetic polymorphisms in genes coding IL-21 and IL-21R [9, 10]. Genetic polymorphisms have been studied in various diseases, as factors associated with increased disease risk [11–14]. The aim of this study was to examine IL-21 and IL-21R polymorphisms in patients with RA compared with control subjects.

Material and methods

Subjects

We examined 422 patients (340 female, 82 male, mean age: 57.5 ±12.5 years) with rheumatoid arthritis diagnosed according to the criteria of the American College of Rheumatology/European League against Rheumatism [15]. Consenting RA patients treated between 2010 and 2013 in the Department of Rheumatology, County Hospital in Szczecin, Poland were enrolled in the study. All subjects were Caucasian, from the Pomeranian region of Poland. The patients were treated with low doses of methotrexate and glucocorticosteroids.

Disease activity was determined on the basis of the DAS28 score. Those patients with DAS28 ≤ 2.5 were classified as subjects in remission of disease symptoms, while those with DAS28 > 2.5 were classified as subjects with an active form of RA [16, 17].

The control group was selected randomly from the population of the Pomeranian region of Poland and consisted of 338 healthy Caucasian subjects (261 female, 77 male) without autoimmunological diseases (mean age: 60.6 ±15.4 years). The study was approved by the ethics committee in Pomeranian Medical University, Szczecin, Poland, and written informed consent was obtained from all subjects.

Genotyping

DNA was extracted from 200 µl whole blood samples using a GeneMATRIX Quick Blood DNA Purification Kit (EURx, Poland). SNPs within *IL-21* (rs6822844 G>T, rs6840978 C>T, rs2221903 T>C) and *IL-21R* (rs2285452 G>A) genes were genotyped using pre-validated TaqMan genotyping assays (Life Technologies, USA). Fluorescence data were captured using a 7500 Fast Real-Time PCR System (Applied Biosystems, USA).

Statistical analysis

Chi-square (χ^2) and Fisher's exact tests were used to compare genotype and allele frequencies between the study groups. $P < 0.05$ was considered statistically significant. The age at onset was compared between genotypes using the Kruskal-Wallis test.

Results

Clinical and demographic data of patients and the control group are shown in Table I. The distributions of studied genotypes were in Hardy-Weinberg equilibrium and are shown in Table II. As shown in Table II, there were no statistically significant differences in the distribution of studied genotypes and alleles between RA patients and the control group.

To examine whether IL-21 polymorphisms affect disease activity in RA patients, we compared the distribution of IL-21 genotypes between patients with DAS28 ≤ 2.5 (patients with remission of disease symptoms) and patients with DAS28 > 2.5 (patients with active RA). Among patients with DAS28 > 2.5, the prevalence of rs2221903 CT and CC genotypes was revealed (OR = 1.54; 95% CI: 1.04–2.28; $p = 0.035$) (Table III).

Additionally, we examined the associations between the studied polymorphisms and clinical pa-

Table I. Clinical and demographic parameters of patients with rheumatoid arthritis (RA) and control group

Parameter	RA	Control group
N	422	338
Sex (F/M)	340/82	261/77
Age [years] Mean ± SD	57.47 ±12.45	60.62 ±15.35
Disease duration Mean ± SD	10.07 ±8.32	–
Age at disease onset Mean ± SD	47.40 ±13.22	–
Rheumatoid factor (positive)	75.36%	–
Erosive RA	80.09%	–
Extra-articular manifestations	17.06%	–
DAS28	3.45 ±2.39	–

N – number of patients, F – number of females, M – number of males.

Table II. Distribution of IL-21 and IL-21R genotypes in RA patients and control group

Variable	RA patients		Control group		*P*-value[a]		*P*-value[b]	OR (95% CI)
	n	%	*n*	%				
IL-21 rs2221903 genotype:								
TT	174	41.23	151	44.67		CC + CT vs. TT	0.38	1.15 (0.86–1.54)
CT	193	45.74	148	43.79	0.60	CC vs. CT + TT	0.58	1.15 (0.74–1.78)
CC	55	13.03	39	11.54		CC vs. TT	0.41	1.22 (0.77–1.95)
						CT vs. TT	0.81	1.13 (0.83–1.54)
						CC vs. CT	0.44	1.08 (0.68–1.72)
IL-21 rs2221903 allele:								
T	541	64.10	450	66.57				
C	303	35.90	226	33.43		C vs. T	0.30	1.12 (0.90–1.38)
IL-21 rs6822844 genotype:								
GG	313	74.17	255	75.45		TT + GT vs. GG	0.74	1.07 (0.77–1.49)
GT	103	24.41	79	23.37	0.90	TT vs. GT + GG	1.00	1.20 (0.34–4.30)
TT	6	1.42	4	1.18		TT vs. GG	1.00	1.22 (0.34–4.38)
						GT vs. GG	0.73	1.06 (0.76–1.49)
						TT vs. GT	1.00	1.15 (0.31–4.22)
IL-21 rs6822844 allele:								
G	729	86.37	589	87.13				
T	115	13.63	87	12.87		T vs. G	0.70	1.07 (0.79–1.44)
IL-21 rs6840978 genotype:								
CC	287	68.01	227	67.16		TT + CT vs. CC	0.82	0.96 (0.71–1.31)
CT	123	29.15	104	30.77	0.73	TT vs. CT + CC	0.64	1.38 (0.54–3.56)
TT	12	2.84	7	2.07		TT vs. CC	0.64	1.36 (0.53–3.50)
						CT vs. CC	0.69	0.94 (0.68–1.28)
						TT vs. CT	0.48	1.45 (0.55–3.82)
IL-21 rs6840978 allele:								
C	697	82.58	558	82.54				
T	147	17.42	118	17.46		T vs. C	1.00	1.00 (0.76–1.30)
IL-21R rs2285452 genotype:								
GG	239	56.63	207	61.24		AA + GA vs. GG	0.21	1.21 (0.90–1.62)
GA	165	39.10	110	32.55	0.12	AA vs. GA + GG	0.25	0.67 (0.35–1.28)
AA	18	4.27	21	6.21		AA vs. GG	0.41	0.74 (0.39–1.43)
						GA vs. GG	0.10	1.30 (0.96–1.76)
						AA vs. GA	0.12	0.57 (0.29–1.12)
IL-21R rs2285452 allele:								
G	643	76.18	524	77.51				
A	201	23.82	152	22.49		A vs. G	0.58	1.08 (0.85–1.37)

[a]χ^2 test, [b]Fisher exact test. IL-21 rs2221903, HWE: examined group p = 0.92, control group p = 0.81; IL-21 rs6822844, HWE: examined group p = 0.54, control group p = 0.63; IL-21 rs6840978, HWE: examined group p = 0.87, control group p = 0.26; IL-21R rs2285452, HWE: examined group p = 0.14, control group p = 0.21.

Table III. Comparison between patients with active RA (patients with DAS28 > 2.5) and patients in disease remission (patients with DAS28 ≤ 2.5)

Variable	Patients with DAS28 ≤ 2.5		Patients with DAS28 > 2.5		*P*-value[a]		*P*-value[b]	OR (95% CI)
	N = 173		*N* = 249					
	n	%	*n*	%				
IL-21 rs2221903 genotype:								
TT	82	47.4	92	36.9	0.10	CC + CT vs. TT	0.035*	1.54 (1.04–2.28)
CT	71	41.0	122	49.0		CC vs. CT + TT	0.56	0.80 (0.44–1.44)
CC	20	11.6	35	14.1		CC vs. TT	0.21	1.56 (0.84–2.91)
						CT vs. TT	0.06	0.65 (0.43–0.99)
						CC vs. CT	1.00	0.98 (0.53–1.83)
IL-21 rs2221903 allele:								
T	235	67.90	306	61.4				
C	111	32.10	192	38.6		C vs. T	0.057	1.33 (0.99–1.77)
IL-21 rs6822844 genotype:								
GG	127	73.4	186	74.7	0.86	TT + GT vs. GG	0.82	1.07 (0.69–1.66)
GT	44	25.4	59	23.7		TT vs. GT + GG	1.00	0.72 (0.13–3.96)
TT	2	1.2	4	1.6		TT vs. GG	1.00	0.73 (0.13–4.06)
						GT vs. GG	0.73	1.09 (0.70–1.71)
						TT vs. GT	1.00	0.67 (0.12–3.83)
IL-21 rs6822844 allele:								
G	298	86.1	431	86.5				
T	48	13.9	67	13.5		T vs. G	0.92	1.04 (0.70–1.54)
IL-21 rs6840978 genotype:								
CC	114	65.9	173	69.5	0.41	TT + CT vs. CC	0.46	1.18 (0.78–1.78)
CT	52	30.1	71	28.5		TT vs. CT + CC	0.24	2.06 (0.64–6.59)
TT	7	4.0	5	2.0		TT vs. CC	0.24	2.12 (0.66–6.86)
						CT vs. CC	0.66	1.11 (0.72–1.71)
						TT vs. CT	0.36	1.91 (0.57–6.36)
IL-21 rs6840978 allele:								
C	280	80.9	417	83.7				
T	66	19.1	81	16.3		T vs. C	0.31	1.21 (0.85–1.74)
IL-21R rs2285452 genotype:								
GG	88	50.9	151	60.6	0.11	AA + GA vs. GG	0.06	1.49 (1.01–2.20)
GA	78	45.1	87	34.9		AA vs. GA + GG	1.00	0.91 (0.35–2.40)
AA	7	4.0	11	4.5		AA vs. GG	1.00	1.09 (0.41–2.92)
						GA vs. GG	0.04*	1.54 (1.03–2.30)
						AA vs. GA	0.62	0.71 (0.26–1.92)
IL-21R rs2285452 allele:								
G	254	73.4	389	78.1				
A	92	26.6	109	21.9		A vs. G	0.12	1.29 (0.94–1.78)

*[a]χ^2 test, [b]Fisher's exact test, *p < 0.05.*

Table IV. Analysis of clinical parameters in relation to IL-21 and IL-21R genotypes

Genotype	*N*	Age at onset [years]		Rheumatoid factor positive		Erosive RA		Extra-articular manifestations	
		Mean ± SD	*P*-value[a]	%	*P*-value[b]	%	*P*-value[b]	%	*P*-value[b]
IL-21 rs2221903:									
TT	174	47.04 ±13.57		75.45		81.50		18.97	0.68
CT	193	47.49 ±13.21	0.86	74.60	0.89	78.24	0.61	15.54	
CC	55	48.22 ±12.28		77.78		83.33		16.36	
IL-21 rs6822844:									
GG	313	47.54 ±13.36		75.66		79.42		17.89	0.34
GT	103	46.46 ±12.84	0.16	74.00	0.85	82.52	0.78	13.59	
TT	6	56.17 ±9.77		83.33		83.33		33.33	
IL-21 rs6840978:									
CC	287	47.43 ±13.39		75.54		79.30		16.38	0.85
CT	123	46.71 ±12.55	0.20	74.38	0.86	82.93	0.63	18.70	
TT	12	53.75 ±15.12		81.82		75.00		16.67	
IL-21R rs2285452:									
GG	239	47.18 ±12.56		77.49		80.17		17.15	0.79
GA	165	47.58 ±14.30	0.88	71.60	0.33	78.79	0.29	17.58	
AA	18	48.67 ±11.81		82.35		94.44		11.11	

[a]*Kruskal-Wallis test,* [b]χ^2 *test.*

rameters of RA. There were no significant associations between the studied genotypes and age of disease diagnosis, rheumatoid factor, extra-articular manifestations and joint erosions (Table IV).

Discussion

In this study we examined the genetic polymorphisms in genes coding IL-21 and IL-21R in patients with RA. Our results showed no significant differences in the distribution of the studied genotypes between RA patients and controls, suggesting that IL-21 SNPs are not the genetic loci predisposing to RA development. We also compared the distribution of studied genotypes between patients with disease remission (DAS28 ≤ 2.5) and patients with the active form of disease (DAS28 > 2.5). Our results indicated an increased frequency of rs2221903 CT and CC genotypes in patients with the active form of RA. Previous studies have revealed that IL-21 is a cytokine which plays a significant role in the pathogenesis of RA.

Li *et al.* have shown that IL-21 induces T-cell activation and proinflammatory cytokine secretion in RA. These authors also reported that IL-21R was overexpressed in the inflamed synovial membranes and in peripheral blood or synovial fluid leukocytes of RA patients [18]. In another study, Xing *et al*. found that IL-21 can promote the proliferation of synovial tissue and proinflammatory cytokine production in RA patients [19]. Numerous other studies have revealed the important role of IL-21 in RA activity. Rasmussen *et al.*, Sglunda *et al.* and Liu *et al.* observed that IL-21 serum concentrations were significantly higher in RA patients than in healthy controls and correlated with DAS28 values [8, 20, 21].

IL-21 supported B cell activation, proliferation and antibody secretion via the IL-21R pathway. Kwok *et al.* reported that IL-21 was up-regulated in the synovium, synovial fluid, and serum of patients with RA and in the synovium and serum of mice with collagen-induced arthritis (CIA), an animal model of RA [22]. IL-21 induced RANKL expression in mixed joint cells and CD4+ T cells from mice with CIA and in CD4+ T cells and fibroblast-like synoviocytes from patients with RA. Moreover, IL-21 enhanced in vitro osteoclastogenesis without the presence of RANKL-producing cells and by inducing RANKL expression in CD4+ T cells and fibroblast-like synoviocytes. Sakuraba *et al.* found that IL-21 receptor knockout mice were resistant to the development of CIA, and IL-21 receptor expression on B cells, but not on T cells, was essential for the development of CIA [23].

Maiti *et al.* evaluated associations between rs6822844 and celiac disease, rheumatoid arthritis, type 1 diabetes mellitus, primary Sjögren's syndrome, and systemic lupus erythematosus.

These authors observed an association between rs6822844 and multiple autoimmune diseases [24].

So far the association between *IL-21* gene polymorphisms and IL-21 serum levels has not been widely investigated. Li *et al.* found that *IL-21* rs2221903 was by interaction with *IL-21R* rs3093301 associated with serum IL-21 levels in patients with chronic hepatitis B virus infection [9]. Lan *et al.* found that *IL-21* rs2055979 correlated with IL-21 serum levels in patients with systemic lupus erythematosus [10]. Unfortunately the association between *IL-21* gene polymorphisms and the expression or the function of the IL-21 gene was not investigated. Our study is also limited by the lack of measurement of serum IL-21 levels and lymphocyte IL-21 mRNA expression levels in patients with different IL-21 genotypes.

The above studies have indicated that IL-21 is a cytokine which plays an important role in RA pathogenesis, especially in RA activity. The results of our study suggest that *IL-21* gene polymorphisms are not genetic risk loci for RA susceptibility, whereas the *IL-21* rs2221903 polymorphism is associated with RA activity. Probably the differences in IL-21 synthesis associated with this polymorphism or linkage with other gene polymorphisms may influence the disease activity in RA patients. However, this hypothesis requires further investigation.

Conflict of interest

The authors declare no conflict of interest.

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