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GENETIC ASPECTS OF RHEUMATOID ARTHRITIS

Abstract of Doctoral Thesis
Speciality – Rheumathology

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The doctoral thesis is available at the library of Riga Stradins University and on the RSU website – www.rsu.lv

Secretary of the Promotion Council:

Dr. med., professor **Inara Logina**

CONTENT

ABBREVIATIONS AND KEYWORDS

A	– adenine
ACPA	– anti-citrullinated peptide antibodies
AntiCCP	– antibodies against cyclic citrullinated peptide
bp	– base pair(s)
C	– cytosin
CRP	– C-reactive protein
DAS28	– disease activitys score 28
DMARD	– disease modifying anti-rheumatic drugs
DNS	– deoxyribonucleic acid
ESR	– erythrocyte sedimentation rate
FD	– fandkcional deficit
G	– guanin
GWAS	– genome-wide association study
HLA	– human leukocyte antigen
IL	– interleukin
KLF12	– Kruppel-like factor 12
PCR	– polymerase chain reaction
PTPN22	– protein tyrosine phosphatase non-receptor22
RA	– rheumatoid arthritis
RF	– rheumatoid factor
RTG	– rentgenological
SE	– shared epitope
SJC	– swollen joints counts
SNP	– single nucleotide polymorphism
T	– tymidin
TJC	– tender joints count
TNF	– tumor necrosis factor
USA	– United States of America
VAS	– visual analogue scale

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory destructive joints disease with essential personal, social and economic impact. Locally inflammatory cells invade acellular synovium, promoting hyperplasia and pannus tissue formation, that lead to cartilage destruction, adjacent bone erosions and loss of function in the affected joint. Inflammation characterized by a progressive course with remissions and exacerbations, which ultimately leads to severe joint deformation, contracture and ankylosis formation. Progressive joint damage, internal organ involvement, persistent disease activity cause long-term disability. In 10 years after disease onset only less than half of patients continue to work or normally function in everyday life. In long term, systemic inflammation affects various organs, significantly increasing the risk of atherosclerosis and lymphoma. Along with the inevitable long-standing antirheumatic drugs, adverse reactions and psychological burden associated with early disability and social instability, untreated RA causes reduction of life expectancy on average by 10 years.

There are great differences in the clinical picture of RA, as well as in the disease course. In the long-term the disease may take place only with joint involvement or may be associated with extraarticular manifestations, clinical features may overlap with other rheumatic diseases, so-called overlap syndromes, or with other autoimmune diseases such as Sjögren's syndrome and Hashimoto thyroiditis. Course of the disease is highly variable ranging from mild cases without erosions, sometimes spontaneously remitting disease to severe, rapidly progressive and destructive RA. Genetic risk factor analysis, antibodies responses and clinical studies suggest, however, that clinical RA as a whole, is composed from pathogenetically distinct subgroups, and thatthese subgroups have to be adjusted to different treatment strategies. RA is regarded

as an autoimmune disease, meaning the breakdown of the immunological tolerance against self in a given moment of the patient's life. The cause of this destructive process is still unknown. Presence of antibodies and slowly increasing C reactive protein level for several years before clinical symptoms suggest that inflammation may have started long before the patient's visit to the physician. Differences between ethnic groups in susceptibility to RA, disease heterogeneity and variations in clinical, radiological and laboratory findings suggest, that several factors influence the occurrence and progression of RA, where the genetic component has a large impact. Genetic markers have many important advantages over conventional biological markers: genotypes are stable, detectable before or at the onset of the disease and does not change during treatment.

There is evidence that early and aggressive treatment of RA is more effective, provides a favorable clinical, radiological and functional outcome and increases the rate of disease remission. It is well known that the erosive changes develop quite early. It is very important to identify the RA within a few weeks or months after its onset, because with immediate use of disease-modifying antirheumatic drugs (DMARDs) better results are achieved than if DMARD use is delayed. The combination of biological drugs and methotrexate therapy or the combination of traditional DMARDs more effectively slows the progression of radiological changes compared with methotrexate monotherapy. Because of the correlation between radiological damage and disability it is hypothesized that radiological damage prevention reduces the amount of disability over the years. The patient is more likely to achieve remission with early treatment. One can be certain that the diagnosis of rheumatoid arthritis has to be made as soon as possible in order to start DMARD treatment immediately. Biological medications are very effective in the treatment of severe RA, but their wide use in many countries is limited because of the high cost, as well as due unknown remote side effects.

The clinical and serological characteristics are not the only characteristics to predict prognosis for the particular patient. Additional knowledge about the genetics of RA can help to identify patients with a prognostically severe and aggressive course of the disease and to promote targeted therapy with potential clinical and economic benefits. Great progress in genetic research of RA has been achieved over the past ten years. In addition to the HLA loci, more than 30 genetic markers are strongly associated with susceptibility to RA. Although it is a remarkable achievement, a large proportion of genetic risk factors remains to be clarified. Some of these missing factors are rare genetic variations with allele frequencies less than five or even one per cent and its identification requires extensive investigations of the genome. Moreover, with few exceptions, identified genetic factors role is unknown. Thus, new markers identification needs new, based on hypotheses, studies to determine the role of these markers in disease pathogenesis.

Genetic markers for RA were widely studied in different populations, but have not so far been investigated in the Latvian population. Given the fact that genetic polymorphisms may prevail the particular population, data obtained in this study shows the influence of genetic factors on manifestations of RA in Latvia. Focus on RA research was motivated by the fact that RA is the most common form of inflammatory arthritis. According to statistics in Latvia more than 10 thousand patients have RA and about 500 people become sick each year. Summarizing of obtained results and data analysis can supplement existing information about etiology and pathogenesis of RA, factors that affect disease activity, severity and prognosis, as determined by the study up to date.

Aim of the research

To investigate the association of genetic factors to susceptibility to rheumatoid artritis and its manifestations in Latvian population.

Objectives of the research

1. To create well characterized group of RA patients.
2. To clarify the involvement of RA susceptibility genetic markers.
3. To investigate the relation of genetic markers on age of RA onset.
4. To verify the influence of genetic markers on presence of antibodies.
5. To investigate the genetic markers correlation with rheumatoid process activity.
6. To explore the genetic markers relation to the severity of RA.

Research hypothesis

Genetic polymorphisms for PTPN22 gene (rs2476601), KLF12 gene (rs1324913), TNFA gene promoter -308 position (rs1800629), IL6 gene promoter position -174 (rs1800795), IL18 gene promoter -607, -656 position (rs1946519, rs1946518), IL10 gene promoter -592, -819, -1082 position (rs1800872, rs1800871, rs1800896) relate to the risk of developing and phenotypic expression of the rheumatoid arthritis in Latvian population.

Scientific and practical novelty

Doctoral thesis summarizes the data about RA patients in Latvia. Be defined stages of the disease, activity and severity of disease according to current evaluation criteria. Be determined nine polymorphisms - PTPN22 gene (rs2476601), KLF12 gene (rs1324913), TNFA gene promoter -308 position (rs1800629), IL6 gene promoter position -174 (rs1800795), IL18 gene promoter -607, -656 position (rs1946519, rs1946518) , IL10 gene promoter -592, -819, -1082 position (rs1800872, rs1800871, rs1800896), and data was analyzed in respect on disease susceptibility, activity and severity.

The study obtained scientifically reliable results about the influence of the genetic markers on RA susceptibility and phenotypic expression of the disease. Statistical significance demonstrated between PTPN22 1858C/T,

KLF12C/A, IL6 and IL18 polymorphisms susceptibility to RA in Latvian population. KLF12C/A polymorphism, recently detected polymorphism, in contrast to other studies in the world, shows an influence on the susceptibility to disease in Latvian population. KLF12C/A and IL6 polymorphisms have an influence on the presence of antibodies. PTPN22 1858T allele bearing genotypes and KLF12 A allele homozygous genotype are possible predictors of erosive disease. TNFA genotypes may influence on disease pathogenesis depending on age of the disease onset and IL10 promoter polymorphisms have an influence on disease activity. Based on the selected polymorphisms, it is possible that RA in women represent a separate disease subgroup.

Additional knowledge about the genetics of RA can help in early diagnosis of RA, disease manifestations prognosis and treatment planning with potential clinical and economic benefits, as well as provide insight into the mediators of tissue damage, which may represent potentially new therapeutic targets for RA.

Structure of the research

This doctoral thesis is originally written in Latvian. It consists of the following parts: abbreviations and keywords, introduction, literature review, patients, materials and methods, results, discussion, conclusions, practical recommendations, references list. The work consists of 153 pages in total, including 55 tables, 34 pictures and one attachment. The reference list consists of 253 references.

On the basis of doctoral thesis were published and accepted for publication five scientific articles, six abstracts and three poster presentations.

Approbation of the doctoral thesis occured on the 30 August 2011 on Riga Stradins internal medicine department session, Museum of Medical History, Antonijas street 1, Riga.

1. MATERIALS AND METHODS

A total of 105 patients with established RA according to the revised criteria of the American College of Rheumatology for RA [1], and 242 healthy subjects were consecutively recruited into the study. Each participant that participated in the study was given a reference number. All met and signed an information consent form of biological material (blood) DNA research.

All patients were treated in Riga's Eastern Clinical University Hospital Rheumatology Department on an outpatient or inpatient basis for the period from 10th January to 30th October 2008. The following data were obtained from the RA patients: age, gender, age of disease onset, biometrical characteristics (weight, height, abdominal circumference),duration of morning stiffness, number of tender (TEN) and swollen (SW) joints, visual analogue scale (VAS) of pain and disease activity, physician assessment of disease activity; laboratory parameters (rheumatoid factor (RF) and anticycliccitrullinated peptides (antiCCP) antibodies, C reactive protein (CRP) and erythrocyte sedimentation rate (ESR)); rentgenological (RTG) stage; functional disability level; medication.

A total of 242 healthy subjects (without autoimune connective tissue systemic diseases) with similar age and sex distribution were available for the study from the Genome Database of Latvian Population with a median age of 53 years, range 17-84 years, female 195 (81.3%) and male 45 (18.8%)).

The following characteristics were used for assessment of RA activity: the tender and swollen joint count, patient pain and disease activity score, using visual analogue scale (VAS), physician global assessment of disease activity, (VAS), functional disability assessment, acute-phase parameters (ESR and CRP), calculated DAS28. The degree of functional disability evaluated using the 1991 American College of Rheumatology revised criteria for the

classification of functional disability in patient with RA. Simple x-rays of the hands and feet were analyzed using the Steinbrocker method for the presence of erosions.

PTPN22 (rs2476601) and KLF12 (rs1324913) genotyping: genomic DNA was extracted using standard phenol – chlorophorm extraction protocol. Extracted DNA was then dissolved in H2O. Genotyping of the PTPN22 (rs2476601) and KLF12 (rs1324913) was performed by a Taqman Pre-Designed SNP Genotyping Assays (Applied Biosystems, Foster City, California, USA) [2] on ABI 7500 Real-Time PCR system (Applied Biosystems) according to the supplier's recommendations.

Cytokines SNPs (TNFA gene promoter region -308 position (rs1800629), IL6 -174 (rs1800795), IL18 -607, -656 (rs1946519, rs1946518), IL10 -592, -819, -1082 positions (rs1800872, rs1800871, rs1800896)) DNA sequencing analysis: DNA promoter region harbouring SNPs, was PCR amplified using specific primers. Amplicons were then purified using Sap-ExoI protocol. Sequences were obtained by direct sequencing of amplified products, using forward PCR primer, on 3100 ABI prism DNA sequencer (Applied Biosystems). Praimer used in the study shown in the table 1.1. Following PCR mix and conditions were used. PCR mix for 1 sample contained 15mkl of 2x PCR Master Mix (Fermentas Life Sciences, Lithuania), forward and reverse primers (1mM) and 28 ng of dried genomic DNA. The cycling conditions of PCR were as follows: 5 minutes of initial denaturation at 95°C, following 32 cycles of 15 seconds at 95°C, 30 seconds at 56°C, 30 seconds at 72°C, and final extension - 10 minutes at 72°C.

Table 1.1.

Praimers used in the study

Praimer name	Primer sequence	PCR produkta lenght (bp)
TNFA-308F	5'-ACAGGCCTCAGGACTCAACA-3'	
TNFA-308R	5'-GCACCTTCTGTCTCGGTTTC-3'	364bp
TNFA-308SEQ	5'-AACACAGCTTTTCCCTCCAA-3'	
IL6-174F	5'-TCGTGCATGACTTCAGCTTT-3'	
IL6-174R	5'-GCCTCAGACATCTCCAGTCC-3'	328bp
IL18-607F	5'- ATTCAGGACTTCCCCTTCCT-3'	
IL18-607R	5'-CACTCTGCTCTTCAAACGTTACAT-3'	402bp
IL18-656F	5'- ATTCAGGACTTCCCCTTCCT-3'	
IL10-PĶR-F	5'-TTCCCCAGGTAGAGCAACAC-3'	
IL10-PĶR-R	5'-ATCCTCAAAGTTCCCAAGCA-3'	685bp
IL10-819, 592SEQ	5'-TCTAAGGCCAATTTAATCCAAGG-3'	
IL10-1082SEQ	5'-GATGGGGTGGAAGAAGTTGA-3'	

F – forward primer, R – reverse primer, SEQ – sequences primer

Hypothesis testing in accordance with the objectives set and data type were used in the following statistical methods: Chi-square test, Fishe's exact test, Kolmogorov-Smirnov test, Kruskal Wallis test, single factor dispersion analysis ANOVA (*Analysis Of Variance*), Spearman's rank correlation coefficient, logistic regresion analyzis. Potential associations between alternative alleles and the phenotypes were estimated in various genetic models (e.g. allelic, dominant and recessive models, respectively). Genetic association with the defined phenotype was evaluated for allelic, dominant and recessive genetic models. P value less than 0.050 was considered statistically significant. If p was greater than 0.050, but at the same time less than 0.100, the value was considered as statistical differences tendency. Odds ratio was calculated with 95% confidence interval. Statistical analysis was performed using statistical software SPSS (*Statistical package for the social sciences*, SPSS Inc, Chicago, IL), version 13.0, 2004.

2. RESULTS

2.1. Patients and control group characteristics

This study analyzed data from 105 RA patients. Demographic characteristics are summarized in the table 2.1.

Table 2.1.

Demographic characteristics of the patients with rheumatoid arthritis

Characteristics	Patients n=105	Controls n=242
Age (years)	58 (26-83)	53 (17–84)
Female (%)	89 (84.8)	195 (81.3)
Male ge(%)	16 (15.2)	45 (18.8)
Smokers/Ex-smokers	32 (30.5)	72 (34.8)
Non-smokers	73 (69.5)	135 (65.2)
Age of disease onset	52 (16–80)	
Disease duration (years)	4.7 (0.1-49.4)	
RA onset before 50 y.o.	47 (44.8)	
after 50 y.o.	58 (55.2)	

Value given as mediana (range) or as number (%)

Investigated 89 (84.8%) women and 16 (15.2%) men with a mean age 58.71 ± 11.18 years, mean age at the time of disease onset 50.52 ± 13.38. Before the age 50 RA was started to 47 (44.8%) patients and after 50 years old to 58 (55.2%) patients. Smokers or former smokers represented 30.5%. Clinical and laboratory characteristics were shown in table 2.2.

Table 2.2.

Clinical and laboratory characteristics of the patients with rheumatoid arthritis

Raksturojums	Value	Number
SJC (28)	6 (0 - 20)	105
TJC (28)	8 (0 - 26)	105
VAS of pain (mm)	49 (1 - 97)	105
VAS disease activity (mm)	50 (2 - 93)	105
Physician assessment of disease activity (mm)	34 (4 - 89)	105
ESR (mm/h)	26 (2 - 76)	105
CRP (mg/l)	6.90 (0.00 - 113.4)	104
DAS28 (ESR)	5.04 (0.93 - 7.77)	105
DAS28 (CRP)	4.5 (1.84 - 7.6)	104
Rheumatoid factor+	79 (76.7)	103
Rheumatoid factor -	24 (23.3)	103
AntiCCP antibodies +	58 (79.45)	73
AntiCCP antibodies -	15 (20.55)	73

Value given as mediana (range) or as number (%)
SJC – swollen joints count, TJC – tender joints count, VAS – visual analogue scale, ESR – erythrocyte sedimentation rate, CRP – C reactive protein, DAS28 – disease activity score, AntiCCP – antibodies against anti cyclic citrullinated peptide

Patients with active, erosive RA with pronounced functional instability were recruited into the study (Figures 2.1-2.3.).

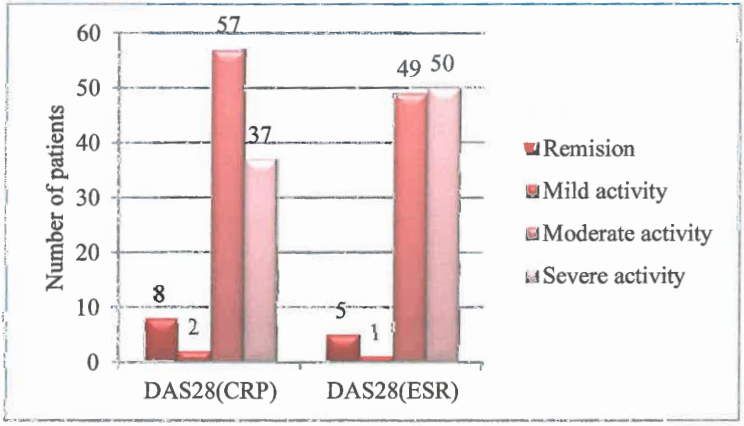

Figure 2.1. **Patients distribution depending on DAS28**
CRP– C reactive protein, ESR– erythrocyte sedimentation rate, DAS28– disease activity score

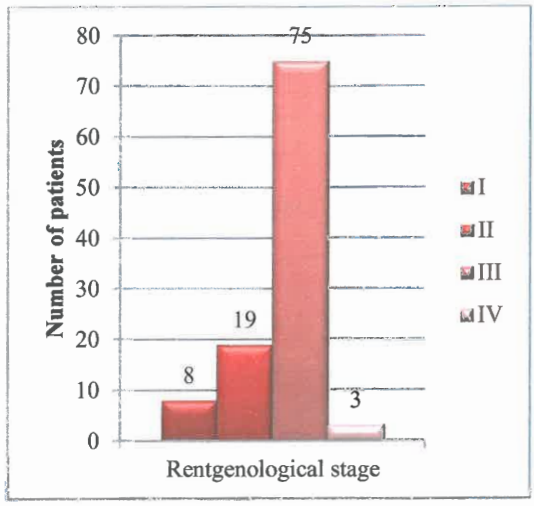

Figure 2.2. **Patients distribution depending on rentgenological stages**

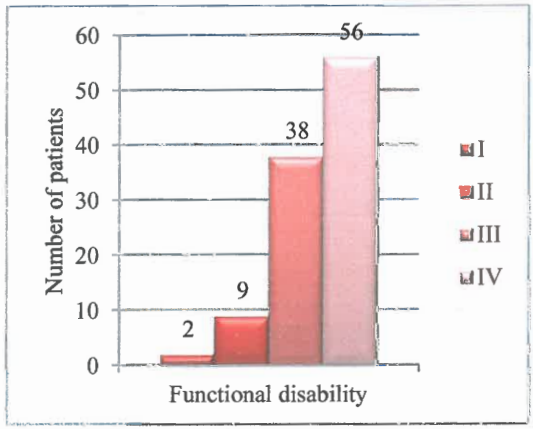

Figure 2.3. **Patients distribution depending on functional disability**

To find the correlation between the clinical characteristics of patients with RA, a correlation analysis was done. A positive correlation was observed between disease activity variables (DAS28 CRP, DAS28 ESR) and radiological stages, the greater the disease activity, the higher the RTG stage (more severe

RA) ($p = 0.005$; $p = 0.009$, respectively); between the radiological stages and functional disability levels, the greater the RTG stage, the stronger is functional disability ($p < 0.001$); between disease duration and radiological stages, and the disease duration and functional disability levels, the higher the disease duration, the greater the RTG stage ($p < 0.001$) and functional disability ($p < 0.001$).

2.2. Alleles and genotypes distribution between patients with rheumatoid arthritis

This study used polymorphisms markers shown in the table 2.3.

Table 2.3.

SNP markers used in the study

SNP gene	Common allele	Rare allele	MAF controls	MAF patients	HVV controls p
rs2476601 **PTPN22**	C	**T**	0.10	0.24	0.68
rs1324913 **KLF12**	C	**A**	0.32	0.35	0.04
rs1800629 **TNF-308**	G	**A**	0.10	0.11	0.06
rs1800795 **IL6-174**	G	**C**	0.50	0.60	0.30
rs 1946519 **IL18-607**	C	**A**	0.36	0.37	0.48
rs 1946518 **IL18-656**	G	**T**	0.36	0.37	0.54
rs1800872 **IL10-592**	C	**A**	0.26	0.27	0.27
rs1800871 **IL10-819**	C	**T**	0.26	0.27	0.25
rs1800896 **IL10-1082**	A	**G**	0.44	0.40	0.71

MAF – minor allele frequency, HWE – Hardy-Weinberg equilibrium

There were statistically significant differences in the distribution of the PTPN22 rs2476601 alleles and genotypes between patients with RA and the control group (sk. 2.4. tab.). T allele was found in 23.6% patients with RA and 10.1% in the control group, C allele was found in 75.5% patients with RA and 89.9% in the control group (p=0.000006).

Table 2.4.

PTPN22 C1858T (rs2476601) alleles and genotypes distribution between patients with RA

Genetic model	Patients n=94	Control n=238	p	OR [95% TI]
Alleles T/C	46/142	48/428	0.000006	1.96 [1.53-2.53]
TT/CT/CC	5/36/53	3/42/193	0.00001	
Dominant (TT+CT/CC)	41/53	45/193	0.000007	2.21 [1.60-3.06]
Recesive (TT/CT+CC)	5/89	3/235	0.044	2.28 [1.29-4.00]

Value given as number (%)

Homozygous TT genotype and T allele bearing genotypes were more frequent in patients with RA (Figure 2.4.).

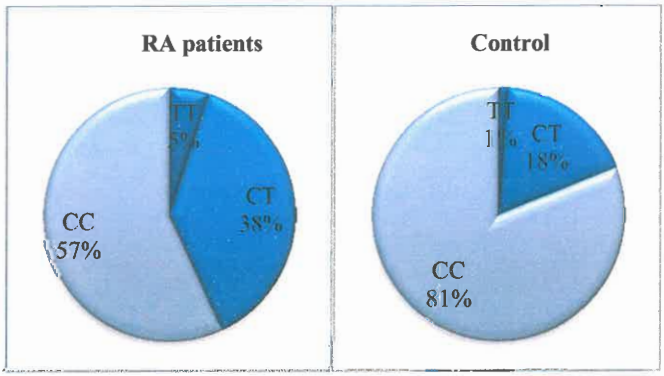

Figure 2.4. **PTPN22 C1858T genotypes distribution between patients with RA** TT – homozigotic TT genotype, CT – heterozygotic CT genotype, CC – homozigotic CC genotype, n – genotypes count

There were significant differences in the distribution of the KLF12 genotypes between patients with RA and the control group (Table 2.5.). AA genotype was more frequent in patients with RA (Figure 2.5.).

Table 2.5.

KLF12 C/A (rs1324913) alleles and genotypes distribution between patients with RA

Genetic model	Patients n=94	Control n=242	p	OR [95% TI]
Alleles A/C	65/123	156/328	0.584	1.08 [0.84-1.39]
AA/CA/CC	15/35/44	18/120/104	0.024	
Dominant AA+CA/CC	50/44	138/104	0.542	0.90 [0.64-1.26]
Recesive AA/AC+CC	15/79	18/224	0.024	1.74 [1.15-2.65]

Value given as number (%)

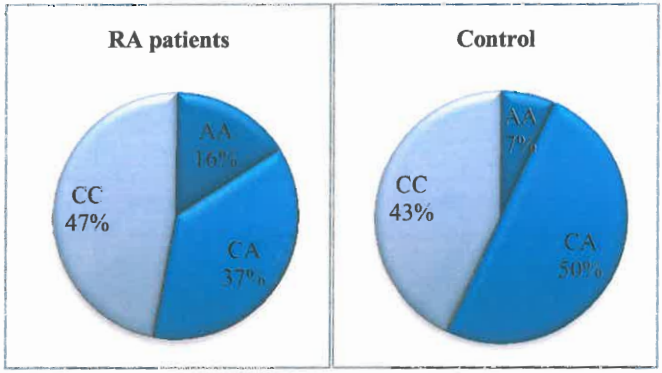

Figure 2.5. **KLF12 C/A genotypes distribution between patients with RA** AA – homozigotc AA genotype, CA – heterozygotic CA genotype, CC – homozigotic CC genotype, n – genotypes count

There were statistically significant differences in the distribution of the IL6 -174G/C alleles and genotypes berween patients with RA and the control group (sk. 2.6. tab.).

Table 2.6.

IL6-174 (rs1800795) alleles and genotypes distribution between patients with RA

Genetic modelis	Patients n=105	Control n=240	p	OR [95% TI]
Alleles C/G	126/84	238/242	0.013	1.34 [1.07-1.69]
CC/GC/GG	38/50/17	63/112/65	0.047	
Dominant CC+GC/GG	88/17	175/65	0.029	1.61 [1.02-2.55]
Recesive CC/GC+GG	38/67	63/ 177	0.072	1.37 [0.99-1.89]

Value given as number (%)
*p=0.015, compared CC/GG frequences between patients with RA and control group OD=1.81 95%CI=1.11-2.97 (Fisher's exact test)

C allele was found in 126 (60.0%) patients with RA and in 238 (49.6%) control group and G allele was found in 84 (40.0%) patients with RA and in 242 (50.4%) control group. Homozigotic CC genotype and C allele bearing genotypes were more frequent in RA patient group, p=0.047 (Chi square test) and p=0.029 (Fisher's exact test), respective (Figure 2.6.).

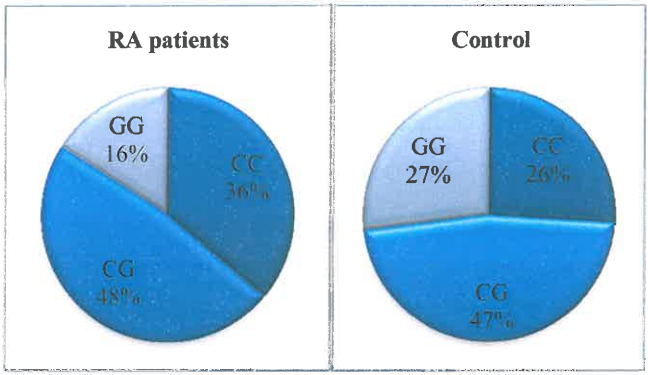

Figure 2.6. **IL6-174 G/C genotypes distribution between patients with RA**
CC – homozygotic CC genotype, CG – heterozygotic CG genotype, GG – homozygotic GG genotype, n – genotypes count.

There were no statistically significant differences in the distribution of the IL18-607 alleles (p=0,863) and genotypes (p=0,102) in patients with RA. But there were statistically significant differences in the IL18-607 heterozygotic genotype distribution, compared with homozygotic between patients with RA and the control group (OR=1.39, 95% TI=1.01-1.93, p=0.046) (Figure 2.7.).

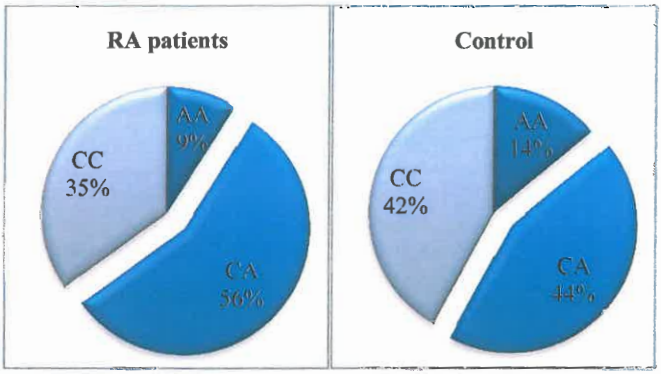

Figure 2.7. **IL18-607 C/A genotypes distribution between patients with RA**
AA – homozygotic AA genotype, CA – heterozygotic CA genotype, CC – homozygotic CC genotype, n – genotypes count.

Similar genotypes distribution was found for IL18-656 C/T polymorphism, heterozygotic GT genotype was more frequent in patients with RA than in the control group, compared with homozygotic GG and TT genotypes, (OR=1.38, 95%TI=1.00-1.90, p=0.060).

2.3. Genotypes distribution in women's group with rheumatoid arthritis

Analysing genotypes distribution between patients with RA and the control group based on gender (Table 2.7.), for PTPN22 C1858T, KLF12 C/A

polymorphisms were found statistically significant differences in genotypes distribution between women with RA and the control group (p=0.00002, p=0.040).

Table 2.7.

Genotypes distribution between women and men with RA and the control group

SNP	Gender	Genotype	Patients	Control	p
PTPN22	W	TT/CT/CC	4/32/44	1/37/156	0.00002
	M	TT/CT/CC	1/4/9	2/5/35	0.300
KLF12	W	AA/CA/CC	14/30/36	16/97/82	0.040
	M	AA/CA/CC	1/5/8	2/21/22	0.746

Value given as number (%), W – women, M – men

Statistically differences tendency was found in IL18-607 C/A, -656 G/T genotypes distribution, respective p=0.084, p=0.097. Significant difference appeared, compared heterozygotic genotypes with homozygotic, in case of IL18-607 CA p=0.041. Because number of the men's group was not large enough, genotypes distribution between men's and control groups was not analysed.

2.4. Genotypes association with age of onset of rheumatoid arthritis

There was a tendency for association in the distributions of the KLF12 genotypes between RA patients with disease onset before age 50 (p=0,081). AA genotype (recesive model of the KLF12 polymorphism) was more frequent in patients with RA onset before age 50 (OR=2.13, 95%TI 1.05-4.29, p=0.067) (Figure 2.8.).

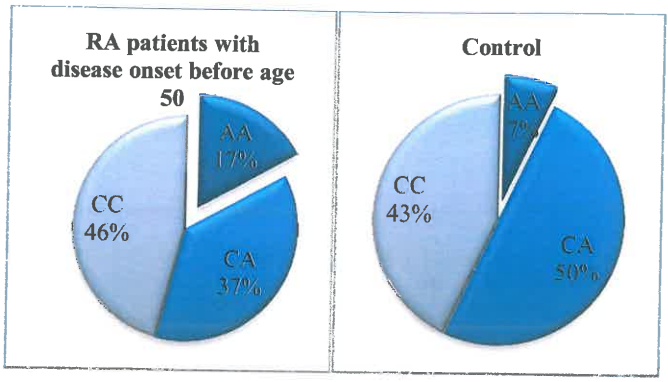

Figure 2.8. **KLF12 genotypes distribution between RA patients with age of disease onset before age 50** AA – homozygotic AA genotype, CA – heterozygotic CA genotype, CC – homozygotic CC genotype, n – genotypes count

There was a tendency for association in the distributions of the IL6-174 genotypes between RA patients with disease onset before age 50 (p=0.075). CC genotype (recesive IL6-174 polimorfisma modelis) was more frequent in patients with RA onset before age 50, OR=1.82, 95%TI 1.08-3.06, p=0.034.

Heterozygotics CA and GT genotypes were more frequent in patients with RA onset after age 50 (OR=1.61, 95% TI=1.01-2.58, p=0.056 and OR=1.59, 95% TI=0.99-2.54, p= 0.057, respective).

Analysing genotype associations in the RA patients group with disease onset before or after age 50, showed a tendency for association in the distributions of the TNFA-308 genotypes between RA patients and age of disease onset before or after age 50 was observed (Figure 2.9.). A allele bearing genotypes were more prevalent in patients with disease onset before age 50 (OR=1.60 95%TI 1.06–2.42, p=0.056).

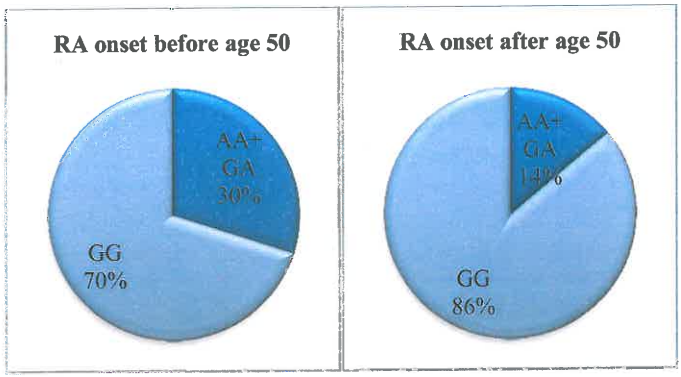

Figure 2.9. **TNFA-308 G/A genotypes association with age of disease onset** AA – homozygotic AA genotype, GA – heterozygotic GA genotype, GG – homozygotic GG genotype, n – genotypes count

2.5. Genotypes associations with RF, antiCCP antibodies

There was a significant difference in the distribution of the KLF12 genotypes between RF-positive RA patients and the control group (p=0.006). Susceptible AA genotype was strongly associated with RF-positive disease compared with CA+CC genotypes (OR=2.22, 95% TI=1.40-3.51, p=0.006) (Figure 2.10.).

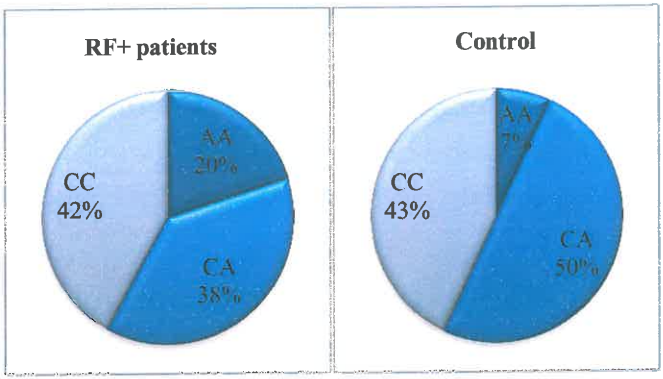

Figure 2.10. **KLF12 C/A genotypes distribution between RF+ RA patients and control** AA – homozygotic AA genotype, CA – heterozygotic CA genotype, CC – homozygotic CC genotype, n – genotypes count.

Moreover, within the RA patient group there were significant differences in the genotypes distribution found between RF-positive and RF-negative patients (p=0.05), where homozygous AA genotype was found only between RF-positive, not RF-negative patients (OR=1.41, 95% TI=1.23-1.63, p=0.018) (Figure 2.11.).

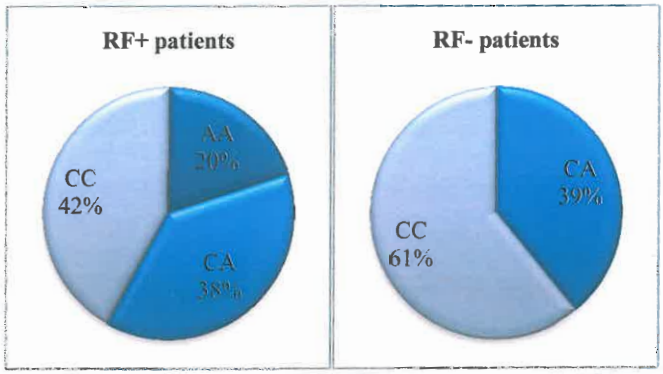

Figure 2.11. **KLF12 C/A genotypes asociations with positive/negative RF patients** AA – homozygotic AA genotype, CA – heterozygotic CA genotype, CC – homozygotic CC genotype, n – genotypes count.

Compared homozygotics AA and CC genotypes, were found significant diferences (OR=1.48, 95% TI 1.20-1.83, p=0.013).

There was a tendency for association in IL6 -174 genotypes distribution between RF+ RA patients and controls (p=0.055). C allele bearing genotypes (dominant model) were more frequent in the RF+ RA patient group, n=68 (86%), compared with controls, n=175 (73%) (OR=1.93, 95%TI 1.08–3.46, p=0.022).

Also a tendency for association was found IL6-174 genotypes distribution in the antiCCP+ RA patients group compared with control (p=0.051). C allele bearing genotypes were more frequent in the antiCCP+ RA patients group, n=51 (88%), compared with controls, n=175 (73%) (OR=2.32, 95%TI 1.10–4.89, p=0.017).

2.6. Asociations of genotypes with activity of rheumathoid arthritis

To find out immunosuppressive therapy differences in patients with distinct disease activities, two immunosuppressive treatment groups were developed:

group A: no treatment, monotherapy, two and three medications;

group B: no treatment, glicocorticosteroids in mono or combined treatment and other immunosuppressive medications. Despite the fact that either the combined treatment (group A) or glicocorticosteroids (group B) have an influence on disease activity, the Chi square test showed, that there were no differences between immunosuppressive treatment groups and disease activities. Therefore further analyses on these groups of patients were not divided based on medications received.

In the study compared RA aktivities parameters SNP genotypes distributions. There were significant differences between IL10-592 C/A genotypes distribution and RA activity: TJC28 ($p=0.009$), VAS physician assessment of disease activity ($p=0.025$), ESR ($p=0.049$), DAS28 ESR ($p=0.018$) (Figure 3.14.), as well as asociation between IL10-592 C/A genotypes distribution and DAS28 CRO ($p=0.063$). AA genotype was found with more active disease.

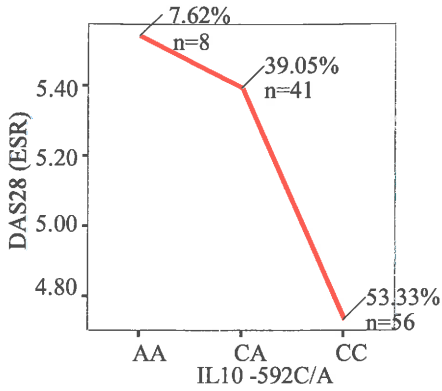

Figure 2.12. **Association IL10-592 C/A genotypes with DAS28 (ESR)**
DAS28 (ESR) – disease activity score, was found using ESR values, ESR – erythrocyte sedimentation rate (mm/h), n – genotypes count

Similar significant differences were found for IL10-819 C/T polimorfism, where TT genotype was found with more active disease. There was no associations found with ESR values, patient VAS of pain and disease activity values.

2.7. Association genotypes with severity of rheumatoid arthritis

Patients with erosive arthritis (III and IV RTG stages) joined in one group and other patients (I and II RTG stages) in another group. There were statistiski significant diferences PTPN22 genotypes distribution in patients with RA depending from RTG stages (Table 2.8.). T allele bearing genotypes frequently were found in patients with RA with III–IV RTG stages. The significant differences remained after adjusting for age gender and smoking status (OR=1.11, 95% TI=1.02-1,22, p=0.017 and OR=1.28, 95% TI=1.06-1.20, p=0.00006) (Figure 2.13.).

Table 2.8.

Association of PTPN22 C1858T between RA patients and controls in dependence of RTG stages

Genetic model	Patients	Controls	p [a]	p [b]	OR [95% TI]
		I-II RTG stage			
	n=25	n=238			
(TT/CT/CC)	0/10/15	3/42/193	0.026	0.017	1.11 [1.02-1.22]
Dominant (TT+ CT/CC)	10/15	45/193	0.020	0.016	3.00 [1.23-7.31]
Recessive (TT/CT +CC)	0/25	3/235	1.000	0.999	--
		III-IV RTG stage			
	n=69	n=238			
(TT/CT/CC)	5/26/38	3/42/193	0.00002	0.00006	1.28 [1.06-1.20]
Dominant (TT+ CT/CC)	31/38	45/193	0.00003	0.00008	3.35 [1.84-6.10]
Recessive (TT/CT +CC)	5/64	3/235	0.016	0.023	5.8 [1.28-26.07]

p[a] value were obtained from Chi square or Fisher's exact tests
p[b] values were obtained from logistic regression adjusted for age, gender and smoking status. Odds ratio (OR) corresponds to Exp (B) value obtained from logistic regression.

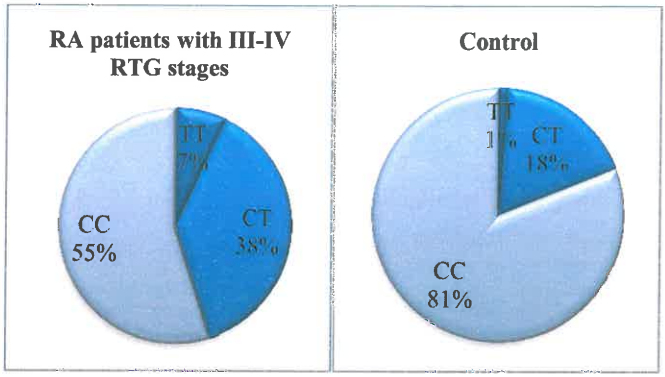

Figure 2.13. **Associations of PTPN22 C1858T between RA patients with III–IV RTG stages and controls** TT – homozygotic TT genotype, CT – heterozygotic CT genotype, CC – homozygotic CC genotype, n – genotypes count

There were no differences found between KLF12 genotypes distribution between RA patients in dependence of RTG stages. Homozygotic AA genotype associated with erosive disease compared with CA+CC (III–IV RTG stage), OR=2,93, 95%TI 1,29–6,66, p=0,010. Significant differences preserved after adjusting for age gender and smoking status, OR=2,93, 95% TI=1,29-6,66, p=0,01.

Analyzing genotypes associations with RTG stages in the RA patients group, found no significant differences between genotypes distributions for all studied polimorphisms.

Because of the duration of RA influence the development of erosion, the the study also analyzed the genotypes association with RTG stages depending on the disease duration under five years, five and more years. Differences were not found between the genotypes distribution for all polymorphisms studied.

3. DISCUSSION

The aim of the study was to investigate the influence of genetic factors on manifestations of rheumatoid arthritis in Latvian population. This study selected cytokine genes and the importance of their role in the pathogenesis of RA is described in several previous studies. PTPN22 (rs2476601) is a well known risk factor for RA in different populations. Demonstrating the association of this polymorphism with RA, we prove that the RA in the Latvian population is genetically similar to RA in other Caucasian European populations. KLF12 is a recently described gene with controversial association with RA, but with polymorphisms, which may explain the mechanism of influence parvovirus B19 on development of RA, showing the relationship of genetic and environmental. Considering that now in the treatment of RA puts great emphasis on new biological drugs, of which TNFα and IL-6 inhibitors, according to literature data were chosen the most important single nucleotide candidate polymorphisms in TNFA gene promoter -308 position (rs1800629) and IL6 gene promoter -174 position (rs1800795). IL-18 is a cytokine with many biological functions, may represent potential therapeutic targets, so we chose two SNPs IL18 gene promoter -607 and -656 positions (rs1946519, rs1946518). IL-10 is antiinflammatory cytokine, in order to clarify the genetic factor association with RA studied SNP of the IL10 gene promoter -592, -819, -1082 positions (rs1800872, rs1800871, rs1800896).

In accordance with the objectives of the study, demographic, clinical and laboratory test (including genetic) data from105 adult RA patients have been obtained, systematized and statistically processed. This is the first time genotyping was performed in Latvia for this population of patients and the information received was attributed to the phenotypic manifestations of the disease. Compared in the study obtained demographic and clinical charac-

teristics with in literature reported, significant differences have not been observed, which shows known populations similarity of studied methods and provides the opportunity to compare the study findings with other authors.

Patients' demographic characteristics were analyzed in the total study group and compared in subgroups. Analyzing gender, women dominated (84.8%) with a female and male ratio 6:1 in the general population. These observations were partly supported by previous publications, reported RA incidences positive correlations with age, as well as with female gender [3]. RA is one of many autoimmune diseases that are predominant in women with female-to-male ratio 2:1 to 3:1. Sex hormones can have significant effects on the cells known to participate in RA. However, the specific mechanisms responsible for increased susceptibility to RA in women are uncertain. *Padyukov L.* et al analyzed genetic associations with RA [4] showed association of IL10-1087 genotypes in woman with RA compared with men and control. We found association in genotypes distribution in women with RA for PTPN22 1858C/T, KLF12 C/A and IL18-607 C/A, -656 G/T polymorphisms. Based on the studied polymorphisms, women's RA seems to represent a separate disease subgroups. More studies are needed and possibly performed on a larger patient group.

AntiCCP antibodies were determined for 70% of patients, the antiCCP antibody positivity determined in 80% of cases (58 patient). Recently, an in-depth studied the HLA-DRB1 SE alleles and ACPA role in predisposition to RA and it was observed that the SE alleles induce ACPA risk and that these antibodies explain the SE alleles association with RA. In addition, these observations are the first evidence that etiopathology of ACPA-positive and ACPA-negative RA is different [5]. In recently GWAS *Padyukov L.* et al [6] analysed ACPA-positive (n=1147) and ACPA-negative (n=774) patients with RA, showed differences in risk allele distribution, which actually maintains the hypothesis of different ethiopathogenesis for ACPA positive and ACPA negative

RA. In study analyzed polymorphisms associations with susceptibility to antiCCP positive and antiCCP negative RA,but other phenotypic characteristics analyzed in the total RA patient group, without dividing on antiCCP existence due relatively small groups of patients.

Disease activity was assessed by DAS28 using CRP or ESR characteristics. Patients mainly with active severe RA were observed in the study. Analyzing the possible effects of drug therapy on disease activity, differences were not found in RA activity between immunosuppressive treatment groups. Therefore, further analysis of these patient groups were not be divided based on treatment received and the observed differences between the subgroups studied (analysed genotypes associations with disease activity variables) were attributed to the effects of studied polymorphisms.

According to correlation analysis, the greater the duration of the illness, the greater the rentgenological stage and functional disability level. Because disease duration affects rentgenological stage, the study analyzed genotypes associations with rentgenological stages, depending on the disease duration, and there were no differences found in the distribution of genotypes for all studied polymorphisms. Therefore, further analysis of these patient groups was not divided based on disease duration and the observed differences between the subgroups studied (analysed genotypes associations with radiological stages) were attributed to the effects of studied polymorphisms.

An association between **PTPN22** and RA has presently been demonstrated in several populations [7-10]. Because the PTPN22 T allele is very rare in Japan [11], it is the gene where susceptibility to RA is specific for particular ethnic or racial groups. In the present study, we replicated the previously reported association between the PTPN22 C1858T polymorphism and susceptibility to RA in Latvian population. PTPN22 1858T allele may predispose individuals to the development of RA. In our study the 1858 T allele frequency was 23.6% in individuals with RA and 10.1% in control. These allele

frequencies in our study were higher than those reported in some Caucasian populations, such as Spanish [12,13], German [14], English [7] and were lower than the Swedish population [15]. Therefore, our results were consistent with the previously reported increasing south to north gradient in the frequency of the 1858 T variant in white European populations [15,16].

Statistically significant differences in PTPN22 C1858T genotypes distribution was observed between RA patients with disease onset before and after 50 years of age and the control group. Because statistically significant differences were not found in the PTPN22 C1858T genotypes distribution in the patient with RA depending on the age of onset disease before or after 50 years, these data attributed to the association of the A allele and the risk of developing RA.

The association of RA susceptibility with the PTPN22 1858 T allele was confirmed in multiple studies. Some authors showed association with antibody status (RF, antiCCP) and sex. *Begovich A.* et al [12] observed an increased frequency of the 1858 T allele between 475RA patients (13.8%) compared with 475 healthy controls (8.8%) in a North American population and replicated their findings using a different cohort of individuals. They also reported an association between the PTPN22 C1858T polymorphismu and RF-positive disease. *Orozco G.* et al [13] studied PTPN22 rs2476601 genotypes distribution in Spanish population, between 826 RA patients and 1036 control, and reported T allele frequency 10.4% in cases, compared with 7.4% in control. They found no association with RF-positivity, but observed a higher frequency of the T-bearing genotypes in male patients and in patients without extraarticular disease, although this skewing did not reach statistical significance after correction for multiple tests. *Johansson M.* et al [17] found that there was an association between PTPN22 1858T and anti-CCP antibodies and that the combination gives a specificity of 100% for diagnosing RA. *Pierer M.* at al [14] found, that the frequency of the PTPN22 C1858T polymorphism

in the German population was higher in male RA patients compared with female RA patients, indicating that this genetic contribution to pathogenesis might be more prominent in men. In contrast of the study by *Pierer M.* at al [14] we found an association in the distribution of PTPN22 rs2476601 T-bearing genotypes in female patients. One possible explanation of these findings is that male and female RA are partially diverging disease entities, which has been suggested previously based on clinical observations [18].

Stratifying the patients according to RF and antiCCP status, we found associations of PTPN22 rs2476601 T with disease in RF-positive and RF-negative groups, so also antiCCP-positive and antiCCP-negative groups. *Simkins H.* et al [19] described the similar pucture, studying the PTPN22 C1858T polymorphism in patients with RA (n = 869) in New Zealand, and explained it with clinical, genetic and environmental heterogeneity between populations. On the other hand, in the New Zealand study, antibody status was not specified in the control group and comparisons were made with the entire control group (n = 563). Regarding to antibodies status, in the study no differences were found between genotypes distribution and RF or antiCCP antibody status in the patient group, therefore these data attributed to the effects of the 1858T allele on the susceptibility to RA in our study.

Little is known about the effect of the 1858T allele on disease severity and activity. [20]. Most studies investigating the effect of 1858T on clinical characteristics have found no association of 1858T with disease activity or severity [14,21]. We found no associations PTPN22 rs2476601 T with disease activity parameters, but strong associations were found between distributions of PTPN22 1858T bearing genotypes in patients with erosive RA compared with the control group, where differences were preserved after adjusting for age, gender and smoking status. Interestingly, *Marinou I.* et al [22] found, that RA patients (n=964) with PTPN22 1858T allele have greater radiographic destruction than patients without 1858T allele. On the other hand, *Wesoly J.* et

al [23] examined rate of joint destruction, using mean baseline and yearly Sharp-van der Heijde scores [24] of radiographs of the hands and feet of RA patients with different PTPN22 genotypes, and found no difference between risk allele carriers and noncarriers. It is possible that 1858T allele might be a predictor of the erosive disease, but not have an influence on disease progression.

In contrast study by Julia A. et al [25], reported protective effect from **KLF12** C/A polymorphism (OR for GWAS and replication study were 0.73 and 0.77, respectively), we found an association between the KLF12 C/A polymorphism and susceptibility to RA. The AA genotype was more frequent in RA patients than in matched healthy controls, suggesting that this genotype may predispose individuals to the development of RA. The similar distribution of genotypes was found between women.

RF is present in the majority of patients with RA, and its role in the etiology remains unclear. Determination of any genetic differences between RF-positive and RF-negative patients may shed light on the role of RF in disease pathogenesis. In our study RF-positive individuals homozygous for KLF12 A allele was found only in RF positive patients (p=0,018). This data suggested that there might be an association between the KLF12 AA genotype and RF production.

Associations were found between distributions of KLF12 genotypes in patients with erosive RA compared with control. It is possible that KLF12 AA genotype might be used as a predictor of the erosive disease.

On the other hand, recently *Eyre S.* et al [26] in a large British cohort did not find any relationships of KLF12 gene polymorphisms (rs1887346, rs9565072) with RA, explained it either by linkage with other polymorphisms within the gene, or with other genes, or with the variation of genetic susceptibility between ethnic groups.

The **IL6 -174** G/C polymorphism is associated with susceptibility to systemic-onset juvenile idiopathic arthritis [27,28], although other data appear to rule out any important role of this polymorphism in susceptibility to RA. *Pascual M.* et al [29] found no difference in the distribution of IL6-174 genotypes or alleles frequencies between RA patients (n=163) and controls (n=157), however, observed a significant difference in the mean age at disease onset between IL6 genotypes. IL6-174 polymorphism has not been studied a lot in respect to RA severity and activity. *Pawlik A.* et al [30], concluded that distribution of IL6 genotypes in 98 RA patients did not differ from that in control subjects, but demonstrated that active RA with higher DAS28, ESR, number of swollen and tender joints was more frequently diagnosed in patients with a GG genotype, compared with homozygous CC and GC. *Marinou I.* et al [22] reported that IL6-174G allele was associated with more severe disease, but only in antiCCP-positive patients.

The **TNFA** -308G/A promoter polymorphism has been studied in relation to the susceptibility and/or severity to RA, but the results have been controversial. In some studies the susceptibility to RA was associated with the A allele [31], in others with the G [32], but a majority of previous studies failed to demonstrate any association between the TNFA-308 promoter polymorphism with the susceptibility to RA [33]. In agreement with a majority of previous studies, were found no association between the TNFA-308 promoter polymorphism and the susceptibility to RA in our study.

Cuenca J. et al [34] concluded that there is a gradient in the distribution of the TNFA-308A allele according to ethnicity. The presence of the TNFA -308A allele has also been linked to increased severity in a variety of autoimmune and inflammatory disorders, including RA [35]. *Khanna D.* et al [36] showed an association between AA/GA genotypes and progression of radiographic damage in patients with early seropositive RA. *Cvetkovic J.* et al [32] demonstrated that RA patients GA heterozygous for the TNFA-308

genotype had a more severe course of disease. On the other hand, *Barton A.* et al [37] found that the TNFA-308G allele showed a trend toward a worse radiological outcome at 5 years, as measured by the presence/absence of erosions, in patients with inflammatory arthritis. *Nemec P.* et al [33] found a positive association of the GG genotype with more severe disease. Moreover, they found that the G allele of the TNFA-308 is associated with the worse functional ability of RA patients. *Pawlik A.* et al [38] did not find any association between the TNFA-308 genotypes and disease activity and severity parameters. Our findings are consistent with the report from Pawlik et al. We did not find any association of the TNFA-308 polymorphism with disease activity parameters and erosion existence.

The present data appear to rule out an important role of -308 TNFA polymorphisms in the susceptibility to RA. However, it may contribute to the pathogenesis of the disease by influencing the age at disease onset. Interestingly, this depends on the existence of A allele in our study. AA and GA genotypes were found more frequently in patients with disease onset before 50 years old ($p = 0.056$).

Reports evaluating the role of **IL18** promoter polymorphisms in RA patients are various. Different SNPs were studied with different results. *Sivalingam S.* et al [39] found that the controls had a significantly higher frequency of AA genotype at position IL18-607 when compared to RA patients and concluded that the AA genotype at position IL18 -607 is associated with a protective effect against development of RA in Chinese individuals. *Rueda B.* et al [40] observed no statistically significant differences for -607C/A and -137C/G IL18 promoter polymorphisms between RA patients and controls. RA patients were stratified according to sex, age at the onset of the disease rheumatoid factor status, and extraarticular manifestations and no association was found with these polymorphisms. Therefore it concluded that studied polymorphisms within the IL18 promoter region do not play a major role in RA

predisposition. *Gracie J.* et al [41] found that the -607C/-137C haplotype was more prevalent in Caucasian RA patients than in the control and concluded that SNP of both positions contribute to the genetic background of RA pathogenesis. *Pawlik A.* et al [42], studied seven IL18 gene SNPs and found no significant differences in the distributions of the genotypes except for rs360722 and haplotypes between RA patients and a control group. Age at RA diagnosis was lower in carriers of the -607 CC and rs187238 GG genotypes. Erosive disease was diagnosed more frequently in patients with the -607 CC and AC genotypes than in AA homozygotes. We found statistically significant difference IL18 -607 heterozygous genotype distribution compared with homozygous between RA patients and the control (p=0,046), as well as a tendency for association in heterozygous genotype distribution compared with homozygous between patients with RA onset after 50 years and control group (p=0,056). Not found significant associations of genotypes with erosive RA, antibody status, disease activity.

The possible explanation for discordant results could be the linkage with other polymorphisms with in the gene or within other genes. Another explanation might be the variation of genetic susceptibility between ethnic groups [43]. Allelic heterogeneity exists between ethnic groups, and different variations within the same gene should contribute to disease risk [44].

It is well known that gene promoter polymorphisms can affect the level of protein production. The gene sequence can be associated with cytokine phenotype. So, homozygotes for the one allele are the highest producers of the cytokine, homozygotes for another allele are the lowest producers of the cytokine and heterozygotes respective are intermediate. But on the other hand, we can assume that homozygots for the any allele are the lowest producers of the cytokine and heterozygots are the highest producers of the cytokine. *Khripko O.* et al [45] studied distribution of IL18 allele variants at positions -607C/A and -137G/C in healthy donors from Siberia and the influence of these

allele variants on the level of IL-18 production by their peripheral blood mononuclear cells. Authors found that lipopolysaccharide stimulated production of IL-18 from healthy donors is significantly greater in those carrying IL18-607 CA genotype than that from donors with the CC genotype.

IL-10 is a cytokine with different anti-inflammatory effects and plays a role in the pathogenesis of RA by inhibiting a systemic inflammatory reaction. The **IL10** promoter polymorphisms have been studied in relation to the susceptibility and/or severity to RA. *Hee C.* et al [46] observed a significant difference in allele frequencies (IL10-824 CT,TT, -597 CA,AA) between RA patients (n=84) and healthy volunteers (n=95) and showed that the -1087A/-824T/-597A (ATA) haplotype, which comprises all mutant alleles, was associated with lower IL-10 production when compared with the other haplotypes. *Padykov L.* et al [4] showed an association of IL10-1087 genotypes in woman with RA. *Marinou I.* et al. [22] concluded that IL-10 -592C was associated with more severe radiographic damage only in antiCCP and RF-negative patients. *Moreno O.* et al [47] also concluded that IL-10 promoter polymorphisms were not important for development or severity of rheumatoid arthritis in Colombian population. *Pawlik A.* et al [48] found no correlation between IL-10 polymorphism and disease activity parameters such as ESR, CRP, number of swollen and tender joints too.

But we found significant diferences between IL10-592, -819 polymorphisms genotypes distributions and disease activity parameters. IL10 -592 AA, CA and IL10 -819 TT, CT were found with more active disease compared with CC genotypes in both positions. Therefore, IL10 -592, -819 polymorphisms with rare alleles are associated with active disease.

Considering all of the above, it is evident that one polymorphism affects development of various symptoms of the disease. At the same time different genetic factors participate in the formation of the particular phenotype. PTPN22 1858C/T, KLF12C/A, IL6 and IL18 polymorphisms are associated

with susceptibility to RA in Latvian population. KLF12C/A and IL6 polymorphisms may affect antibodies status. PTPN22 1858T allele bearing genotypes and KLF12 A allele homozygous genotype are possibly the forerunners of erosive disease. TNFA genotypes may influence disease pathogenesis, depending on the age of disease onset and IL10 promoter polymorphisms may influence disease activity (Table 3.1.).

Table 3.1.

Summary of associations observed in the study

SNP	Susceptibility to RA	Age of disease onset	Antibody status	Disease activity	Disease severity
PTPN221858C/T	+				+
KLF12C/A	+	+	+		+
TNF-308G/A		+			
IL6-174G/C	+	+	+		
IL18-607C/A	+	+			
IL18-656G/T	+				
IL10-592C/A				+	
IL10-819C/T				+	
IL10-1082A/G					

Our increased understanding of the molecular pathogenesis of RA, have seen the development of various biological drugs that act against specific components of the immune system. A wide range of biological therapy is available today for a patient with RA. Clinical studies and life experiences show significant heterogeneity of treatment efficacy between patients, although there are serious side effects from these drugs. Although the response to these drugs is largely beneficial, a substantial part of patients do not give a predictable response. To maximize the benefit / risk ratio and to minimize joint damage, is to define prognostic treatment predictors and, ideally, the medication side effects predictors. The high cost of medications requires a personalized approach to each patient's treatment. This is the so-called

"personalized medicine", which allows an optimal match treatment to the patient, reduces the maximum period of disease activity, protects patients from possible side effects of treatment failure and provides parallel reduction of the treatment cost. RA is multigenetic disease with a wide range of genetic polymorphisms that influence disease susceptibility and expression. Identification of genetic polymorphisms maymost importantly help with an early diagnosis of RA in cases with early undifferentiated arthritis or with atypical course. It is not so easy to determine when the RA actually starts clinically, but it is important to recognize RA in preclinical stage to prevent progression to blooming, classified disease which can become persistent and destructive. Secondly, familiar biomarkers can help in prognostic algorithms development, which promotes therapeutic purposefulness especially in prognostically worse disease, resulting in earlier disease control and reduced joint damage. Thirdly, can provide insight into the mediator of tissue damage, which may represent potential therapeutic targets in RA.

To summarize, existing knowledge about the importance of genetic markers of in RA, it is clear that the individual marker has little effect and it explains only a small portion of RA inheritance. Therefore, current determination of the individual markers have a small importance in actual clinical practice. However, knowledge about the association between genetic factors and disease, and particularly the understanding of biological processes, increases the comprehension of disease etiology and pathogenesis and provides a new way for therapy. In addition, improving the understanding of the interaction between genetic and environmental factors is an important factor for future discoveries.

4. CONCLUSIONS

1. This doctoral thesis summarized data about RA patients in Latvia. Information was collected about stages, activity and severity of RA in accordance with the current assessment criteria. This study established the phenotype and genotype database for further research.
2. Clarified RA susceptibility genetic markers in the Latvian population: PTPN22 1858T allele is a strong risk factor for developing RA; KLF12 C/A polymorphism in the homozygous have an influence to the risk of developing RA; IL6 -174G/C and IL18 -607C/A, -656G/T polymorphisms also associated with RA susceptibility in Latvian population; women's RA represents a separate disease subgroup.
3. Observed genetic markers association with age of RA onset. KLF12 AA genotype and IL6 -174 CC genotypes associated with the disease onset before 50 years, IL18 -607 heterozygous genotype is associated with disease onset after 50 years, for TNFA-308 polymorphism A allele bearing genotypes are more frequently in patients with disease onset before 50 years.
4. Clarified genetic markers association with the presence of antibodies. With RF positive RA linked KLF12 AA genotype and IL6-174 C allele-bearing genotypes With antiCCP positive RA related IL6-174 C allele-bearing genotypes.
5. There is genetic markers correlation with rheumatoid process activity: active disease was observed with rare allele bearing IL10 -592 C/A, -819 C/T genotypes, analysing TJC 28, assessment of disease activity, ESR, DAS28 ESR.
6. Observed genetic markers association with RA severity: PTPN22 1858T allele bearing genotypes and KLF12 AA genotype are associated with erosive RA.

5. PRACTICAL RECOMENDATIONS

For practicing physicians and based on the study findings, it may be recommended:

1. continue to monitor and investigate RA patients using actual disease assessment criteria for disease activity, severity, prognosis, to more accurately assess the disease expression and course;
2. to identify genetic polymorphisms in early, undifferentiated arthritis, arthritis with atypical course in order to recognize the RA in earlier stage and in time initiate necessary treatment;
3. to identify genetic polymorphisms in patients with RA for disease course prediction and treatment strategy selection.

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PUBLICATIONS RELATED TO THE SUBJECT OF THE THESIS

Scientific publications

1. *Mihailova A*, Mikazane H., Klovins J., Nikitina-Zake L. Promoter polymorphisms of the TNFA (-308) and Interleikin-6 (-174) genes in Latvian patients with rheumatoid arthritis. RSU Collection of Scientific Papers, 2010, 146–152.
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1. *Mihailova A*, Kakurina N., Mikazane H., Klovins J., Nikitina-Zake L. Influence of interleukin 10 gene polymorphisms on disease activity in Latvian patients with rheumatoid arthritis. Tēzes; RSU Zinātniskā konference, Aprīlis, 2011, Rīga; 122.lpp.
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Presentations

1. Cytokine gene polymorphisms in Latvian patients with rheumatoid arthritis. Poster II–29. RSU Scientific conference; Riga, March 19, 2010.

2. Genetic polymorphisms in Latvian rheumatoid arthritis. Poster THU0029. Annual European congress of Rheumathology EULAR2010; Roma, Italy, June 17, 2010.
3. Influence of interleukin 10 gene polymorphisms on disease activity in Latvian patients with rheumatoid arthritis. Stenda referāts II–66. RSU Scientific conference; Riga, April 15, 2011.

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