The glucocorticoid receptor polymorphisms in systemic autoimmune diseases

PhD thesis

Anna Bazsó

Doctoral School of Molecular Medicine
Semmelweis University

Supervisor: Emese Kiss MD, D.Sc.

Official reviewers: Csaba Horváth MD, D.Sc.,
Judit Pulai MD, Ph.D.

Head of the Final Examination Committee: Attila Szabó MD, D.Sc.

Members of the Final Examination Committee:
Zsolt Nagy MD, Ph.D,
László Sallai MD, Ph.D.

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INTRODUCTION

Until now, glucocorticoids (GCs) due to their anti-inflammatory and immunsuppressive effects are one of the most effective agents in the therapy of several autoimmune disorders including systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA). Both endogenous and exogenous glucocorticoids contribute to the down-regulation of disease activity and the outcome of organ damages in SLE and RA via the intracellular glucocorticoid receptor (GR). Highly studied polymorphisms of the *GR* gene are associated with altered sensitivity to glucocorticoids. The N363S and BclI polymorphisms have been associated with increased, while the ER22/23EK and A3669G polymorphisms have been associated with decreased glucocorticoid sensitivity.

The glucocorticoid receptor sensitivity can show a high degree of individual differences. Furthermore, SNPs may alter the sensitivity for endogenous glucocorticoids, therefore may have significance in the pathomechanism of SLE and RA.

Systemic lupus erythematosus (SLE) is a chronic, autoimmune disease which can damage many organs. Despite of the survival of SLE patients has improved over the last 50 years, owing to the innovative therapies, the most appropriate scheme is absent. However, the first therapeutic option is still the glucocorticoids, treat-to-target therapy could improve the disease outcome supported by clinical trials. Lupus activity can be reduced by glucocorticoids dramatically otherwise the side effects of long-term used glucocorticoids worsening quality of life. Moreover, specific biomarkers that could predict the efficacy and side effects of glucocorticoids are still absent.

Although the exact etiology is unknown, rheumatoid arthritis (RA) as a chronic, inflammatory disorder is mediated by several pathways. Dysregulation of the hypothalamic-pitutiary-adrenal (HPA) axis, the relatively low cortisol level, high concentrations of proinflammatory cytokins– mainly tumor necrosis alpha (TNF-α), interleukin-6 (IL-6), interleukin-17 (IL-17)- might contribute to the pathogenesis of RA. Glucocorticoid receptors (GR) are important elements in

determining the sensitivity and effects of GC at cellular level, and probably play a role both in the pathomechanism and the response to therapy of rheumatoid arthritis. Despite of the benefit in the early and prolonged therapy, the advers effects of the glucocorticoids are a potential risk in the managment of RA, also. Moreover, observations suggest that a great proportion of RA population fails to respond to exogeneous GC administration. Studies highly suggest to determine the individual glucocorticoid sensitivity apart from the glucocorticoid dose. GCs manifest their effect by binding to membrane and intracellular receptors. The glucocorticoid receptor alpha (GRα) is known to be the active isoform. The precise function glucocorticoid receptor beta (GRß) is still not detailed clearly, it seems to exert negative dominant effect on GRα and it also may have independent transcriptional activity. Associations with autoimmune and inflammatory diseases have been reported. Therefore, GRß could contribute to the GC resistence in RA.

AIMS

1) To study the role of GR single nucleotid polymorphisms (SNPs) in the pathogenesis and clinical course of systemic lupus erythematosus (SLE).

2) To study also the role of the three GR polymorphisms (BclI, N363S, 9β) in the pathogenesis of rheumatoid arthritis (RA).

3) To analyze the association of the clinical, immunserological parameters of SLE and the GR genetic variants as BclI, N363S és az A3669G.

4) To study the association of the clinical, immunserological parameters of RA and the BclI, N363S és az A3669G polymorphisms.

5) To investigate if an exist their possible interaction of anti-TNFα therapy in RA.

MATRIALS AND METHOD

1. Polymorphisms of human glucocorticoid receptor gene in systemic lupus erythematosus.

1.1.Patients and clinical data

GR gene polymorphisms were analysed in 104 patients diagnosed and regularly followed-up tracked with SLE at the National Institute of Rheumatology and Physiotherapy. The control group contained 160 healthy individuals from the Hungarian population. In the SLE group, patients who have been presented with glucocorticoid therapy were selected. In the SLE group the female to male ratio was 89%, while in the control group was 69.37%. The patients average age was 47.9 ± 13.1 year, while in the control population this ratio was 52.73 ± 14.7 year. The average age at the diagnosis of SLE was 31,1 ± 13,2 year.

All members of the SLE and healthy control groups were Caucasians origin. The research was approved by the local Ethical Committee of Semmelweis University (SE TUKEB 12/2013). Agreed written

consent was obtained from all patients. SLE was classified as per the more recent 2012 SLICC-ACR (Systemic Lupus Collaborating Clinics revised and validated the American College of Rheumatology) criteria. Particular manifestations of SLE -if have been ever presented from the onset of the disease- were selected and defined as in the classification criteria. Regarding neuropsychiatric manifestation we used the ACR Ad Hoc Committee on neuropsychiatric lupus nomenclature as providing a definition of 19 manifestations.

2. Glucocorticoid receptor polymorphisms in rheumatoid arthritis

2.1.Patients and clinical data

One-hundred and fourty-six Caucasian patients who met the classification criteria of the American College of Rheumatism (ACR) 2010 were included in the study. The

control group consisted of 160 healthy individuals from the Hungarian population, without personal history of RA. The percentage of females was 90.41% and 69.38% in the RA population and in controls, respectively. The patients' age was 58.28±12.04 years, while in the control population 52.7±14.7 years. The age at the appearance of RA was 50.44±14.62. Additionally, all patients were either rheumatoid factor (RF) positive and anti-cyclic citrullined peptide (anti-CCP) positive and/or had joint erosions. Radiographic data and RF were avaiable in 100%, anti-CCP were avaible 95.21%, and anti-DNA data were avaiable in 93.81% of patients (n=146). Patients were selected into two groups according to therapy: those treated with conventional synthetic disease modifying anti-rheumatic drugs (csDMARDs) (n=81, 55.48%) and receiving anti-TNFα therapy (n=65, 44.52%). Anti-TNFα therapy was initiated after treatment failure with at least 2 csDMARDs and still having active RA, as indicated by the disease activity score (DAS28> 3,2). The control group included 160 healthy, unrelated Hungarian volunteers (49 men, 111 woman, age 52.7 (14.7) years) without family history of autoimmune

diseases. The research was approved by the local Ethical Committee of Semmelweis University (SE TUKEB 12/2013). Written informed consent was obtained from all patients.

Clinical data of the 146 RA patients containing detailed disease parameters, such as tender and swollen joint counts, visual analoge score (VAS), disease activity score (DAS28-ESR) and Health Assessment Questionnaire (HAQ) were documented at the time of the study. At the time of this cross-sectional study every patient reached the therapeutic target (remission or LDA (low disease activity)) as defined by European League Against Rheumatism (EULAR) committee. According to clinical charts, 34 % and 71% of patients ever received systemic or local GC treatment in short time respectively, however 52% and 0% received systemic or local GC at the time of the study. Patients received <7.5 mg equivalent dose of prednisolon (PED) GC at the time of the study. The cumulated GC doses of patients were averaged from the onset of disease to the time of the study and the doses were converted to hydrocortizone to compare.

3. Immunserological analysis

In the SLE study, the following immune serology parameters were tested in all patients: Anti-dsDNA antibody, anti-ribosomal-P-protein antibody, anti-chromatin antibody, anti-C1q antibody, anti-SSA, anti-SSB were tested by ELISA (ORGENTEC Diagnostika GmbH, Mainz, Germany). Anti-Sm antibody, anti-Cardiolipin antibody IgG, anti-Beta2-GPI antibody IgM and IgG were tested by ELISA (INOVA Diagnostics, San Diego, CA, USA). Serum complement C3, C4 were tested by nephelometry (Siemens Healthcare Diagnostic Products GmbH, Marburg, Germany). Lupus anticoagulant was tested according to international recommendation.

In the RA study, apart from the basic inflammatory labor parameters (erythocyte sedimentation rate [ESR], C-reactive protein [CRP]), autoantibodies were also measured. In addition to rheuma factor (RF) and anti-cyclic citrullinated peptide (anti-CCP), the anti-double stranded deoxyribonucleic acid (anti-dsDNA) and anti-

Cardiolipin immunoglobulin M and G (a-CL IgM and IgG) antibodies were also measured in all patients with RA. Anti-dsDNA was tested with ELISA (ORGENTEC Diagnostika GmbH, Mainz, Germany). Anti-CCP, anti-Cardiolipin IgM and IgG antibodies were tested with ELISA (INOVA Diagnostics, San Diego, CA, USA), RF was tested by nephelometry (Siemens Healthcare Diagnostic Products GmbH, Marburg, Germany).

4. Disease-specific clinical data

The disease-specific clinical data were tested by the medical professional concepts. These tests were based on echocardiography, kidney biopsy, histopathology, X-Ray and MR examinations.

5. DNA extraction and genotyping of GR gene polymorphisms

Genotyping of the BclI, N363S and A3669G polymorphisms, was performed in peripheral blood DNA isolated with commercially available DNA Isolation kit (QIAamp DNA Blood Mini Kit (QiampDNA Blood Kit, Qiagen, USA). Genotypes for the BclI and the N363S variants were determined by allele-specific polymerase chain reaction (PCR) as earlier reported. Genotypes for the A3669G polymorphism were analysed using a primer-probe set purchased as predesigned Taqman allelic discrimination assay according to the manufacturer's instructions (Applied Biosystems, Applied Biosystems Group 850 Lincoln Center Drive Foster City, CA) on a 7500 Fast Real Time PCR System (Applied Biosystems).Genotypes of the GR gene: BclI, N363S and A366G were compared between SLE patients to a control group consisting of 160 healthy individuals.

6. Statistical analysis

For statistical analysis Statistica software (7.0 version, Statsoft Inc) was used. The Hardy-Weinberg balance was tested and did not show alteration for any

polymorphisms. The differences between allele frequencies and prevalence of various symptoms were evaluated by chi-square or Fischer exact tests. The demographic data were analysed by student's T test. Significant results were defined if p value was less than 0.05.

RESULTS

1. **Polymorphisms of human glucocorticoid receptor gene in systemic lupus erythematosus.**

1.1. Demographic findings and immunserological parameters

The immunserological parameters of patients showed high degrees of diversity. The anti-Sm (referring for kidney and neuro-psychiatric manifestation) and anti-SSA antibody (showing subacut cutan erythematosus) positivities were 25.96% and 39.42%, respectively. The frequency of anticardiolipin, anti-β-2-glikoprotein I

antibodies and lupus anticoagulant were as follows: 31.73%, 22.12% and 21.15%.

1.2. The allele frequency of BclI, N363Sand A3669G of the *GR* gene polymorphisms in patients with systemic lupus erythematosus and the control population

The occurrence of BclI polymorphism in patients with SLE was significant lower than control population (0.26 vs. 0.35; p=0.025). The frequency of N363S and A3669G polymorphisms did not alter in the patients and healthy controls (N363S: 0.03 vs. 0.03, p=0.873; A3669G: 0.16 vs. 0.22, p=0.179).

1.3. The association between BclI, N363S and A3669G *GR* gene polymorphisms and clinical parameters of patients with systemic lupus erythematosus

1.3.1. Associations between of BclI polymorphism and the clinical symptoms

There was a significant association between of the psychiatrics symptoms and the carrier status of BclI polymorphism (p=0.02), while a tendency (p=0.06) with central nervous system symptoms. Patients with BclI polymorphisms suffered from neuropshichiatric symptoms more often than patients without BclI polymorphism. No statistically significant differences were found between the BclI polymorphism and other clinical parameters of SLE.

1.3.2. Associations between the N363S polymorphism and clinical symptoms

No statistically significant association were detected between the N363S polymorphism carrier status and SLE symptoms.

1.3.3. Association between the A3669G polymorphism and the clinical symptoms

Impact of A3669G polymorphism contributed a strong association with the psychiatric symptoms.

Contrary to the association between BclI polymorphisms and psychiatric symptoms, these symptoms occurred less frequent in patients who carried the A3669G SNP compared to non carriers. No other significant association between this SNP and clinical parameters were detected.

2. Glucocorticoid receptor polymorphisms in rheumatoid arthritis

2.1. Demographic and disease specific characteristics

In this study the mean age was similar in the RA and control group. Not surprisingly, the percentage of females in the RA group was significantly higher than in the control group (90.41% and 69.38%, respectively, $p<0.00001$).

Anti-TNFα was administered in 44.52% of RA cases. The age at the onset of disease in anti-TNFα-treated patients was significantly lower compared to the patients without anti-TNFα treatment. Both RF and anti-CCP were present in 95.20% of the RA patients.

Neither aDNA, nor aCCP or RF levels differed significantly between the anti-TNFα-treated and non-treated group. However, when grouping patients according to their antibody levels, significantly more patients treated with anti-TNFα had higher aCCP and RF levels than those not treated (p=0.003 and p=0.014, respectively).

Characteristics of the study and control population are presented.

2.2. The allele frequencies of the GR gene SNPs

The BclI allele frequency was significantly lower in RA patients compared to controls (p=0.0104). The higher proportion of BclI in the control population suggests that the increased sensitivity to endogenous glucocorticoids associated with this polymorphism results in a lower risk of developing RA. There were no significant associations regarding N363S or 9β allele frequencies and development of RA.

2.3. Associations between genotypes and clinical and immunoserological parameters

There was no difference in age between carriers and non-carriers of the BclI and N363S polymorphisms. 9ß carriers were older, but not significantly, at the onset of the disease than controls (53.00 vs 48.85, respectively, p=0.095). There was no significant correlation between the GR SNP carrier status and the RA disease activity measures. However, the tender joint counts tended to be lower in homozygous than in heterozygous BclI carriers (2.69±3.07 vs. 6.18±6.16; respectively, p=0.053). Interestingly, patients with homozygous BclI allele had significantly higher level of aDNA than heterozygous BclI carriers (20.15± 26.65 IU vs 7.63± 7.73 IU**, p=0.005**). This difference was maintained when considering only patients without anti-TNFα therapy (22.75±33.40 IU vs 7.20±7.85 IU, p=0.0345), while anti-TNFα-treated patients showed only tendency (16.00±11.98 vs 8.083±7.73, p=0.069). The anti-CCP level was significantly lower in case of heterozygous 9β

patients treated with anti-TNFα contrary to the non-carriers (388.7± 485.1 U vs 708.8±668.1 U, **p=0.0467).** Since the duration of the average glucocorticoid therapy in RA patients was quite short, we did not find any unequivocal correlation between the duration of GC therapy or the average GC dosis and the GR polymorphisms. Similarly, there was no association between the body mass index (BMI) or the BMI change during the GC therapy and the SNPs (not shown).

CONCLUSION

1) We have found that the prevalence of BclI polymorphism was lower in the SLE group as compared to healthy controls. These findings are in concordance with the fact that BclI polymorphism increases glucocorticoid sensitivity and may have a preventive role in the development of SLE.
2) Presently, our findings show that the more frequent appearance of the minor allele of BclI polymorphism is associated with a lower risk of

developing RA by increasing GC sensitivity. In conrast to previous studies, we did not find associations between other investigated GR polymorphisms (N363S, 9β) and the susceptibility to RA.

3) One interesting association was found, however, between carrier status of BclI and neuro-psychiatric symptoms. These symptoms developed more frequently in the BclI positive SLE group and, in addition, these symptoms were less prevalent in the A3669G carriers. These findings strongly agree with an earlier study of va*n Oosten* and collages. The BclI polymorphism increases while the A3669G polymorphism decreases the sensitivity of glucocorticoid receptors and may have influence on the development of psychiatric syndromes.

4) The age at the onset of disease of RA patients without anti-TNFα was significantly higher compared to the patients with anti-TNFα treatment. It suggests that patients with higher

disease activity, anti-CCP positivity and poor prognostic factors predispose to initiate an anti-TNFα treatment earlier in life. However, the age of 9ß carrier RA patients was higher but not significantly, than controls, which is controversary knowing its effect causing relative GC resistance. Otherwise, carrying the 9ß SNP may result in the need of anti-TNFα treatment, which may contribute to the significantly lower anti-CCP levels in these patients compared to those not treated with anti-TNFα. However, these results could only partially be explained by the minor effects of these SNPs, on the other hand, individual and enviromental factors of our studied population may also contribute to the differences to previous studies

5) Homozygous BclI patients in the RA population and also in the subgroup of patients not treated with anti-TNFα showed a significantly higher aDNA level compared to heterozygous carriers. Homozygous BclI patients treated with anti-TNFα showed only tendency. Knowing the fact

that anti-TNFα could induce antinuclear antibodies (ANA) including anti-DNA antibodies (19), moreover drug-induced *lupus* is developed by anti-TNFα therapy *in a smaller proportion* of patients (20), our results were somewhat unexpected. In the study population, the anti-DNA level did not differ significantly between patients with or without anti-TNFα therapy in the analyzed gene polymorphims, There was no correlation between the aDNA and aCCP levels in the two (anti-TNFα treated and not-treated) populations, either. This finding potentially excludes the role of polyclonal B-cell activity at the background. According to the GC sensitizing effect of BclI results indicated lower tender joint count in homozygous carriers, altough this association did not reach satistical significance. However, this tendency was consequent regardless of anti-TNFα therapy. We did not find any correlation between the other documented clinical immunoserological and laboratory parameters and also the csDMARD therapies

Publications

Publications related to the dissertations:

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Appendix

Original article. Related to the dissertations, accepted in Clinical and Experimental Rheumatology, publication is currently proceeding.

F1. Bazsó A, Kövesdi A, Rásonyi R, Nagy E, Patócs A, Poór G, Kiss E. Glucocorticoid receptor polymorphisms in rheumatoid arthritis. A single centre results. Clin and Exp Rheumatol. Accepted in 02/09/2019.