**in vivo** *38*: 774-784 (2024)
doi:10.21873/invivo.13501

# Association Between *AIRE* Polymorphisms rs870881(C>T), rs1003854(T>C) and Rheumatoid Arthritis Risk: A Hungarian Case-control Study

BALINT BERCZI[1], NÓRA NUSSER[2], IVAN PETER[2], BALAZS NEMETH[1,2] and ZOLTAN GYONGYI[1]

[1]*Department of Public Health Medicine, Medical School, University of Pécs, Pécs, Hungary;*
[2]*Harkány Thermal Rehabilitation Centre, Harkány, Hungary*

**Abstract.** *Background/Aim: Autoimmune regulator (AIRE) is a transcription factor that plays pivotal role in controlling autoimmunity. In the thymus, it supports the presentation of peripheral tissue antigens to developing T cells, where recognition of these self-antigens negatively selects the autoimmune naïve T-cells by central tolerance. Studies demonstrated that single-nucleotide polymorphisms (SNPs) in AIRE alter transcription and propagate clonal survival of autoimmune T cells, therefore increase susceptibility to autoimmune diseases. This study intended to identify SNPs in exon and intron sequences that determine AIRE transcription, where their genotypes are associated with rheumatoid arthritis (RA) risk and clinical parameters. Patients and Methods: After a thorough in silico research, we enrolled 100 patients with RA and 100 healthy controls to analyze the association of SNP rs870881(C>T) and rs1003854(T>C) in AIRE coding sequence with RA risk by using five different genetic models and selected clinical parameters. Multiplex quantitative polymerase chain reaction was used to determine allelic discrimination of SNPs. RA risk was assessed by odds ratios (ORs) and confidence intervals (CIs). Results: In a recessive model of rs878081, minor allele TT homozygotes were associated with RA (p=0.032, OR=5.44, 95%CI=1.16-25.52); in a recessive model of rs1003854, minor allele CC homozygotes were associated with RA (p=0.047, OR=4.84, 95%CI=1.02-23.02). Higher C-reactive protein (CRP) levels in patients with RA were significantly associated with minor allele homozygotes in recessive and codominant genetic models (p=0.029 and p=0.043, respectively) of rs1003854. Conclusion: Genotypes for minor alleles of rs878081 and rs1003854 might be involved in RA pathogenesis and risk prediction.*

*Correspondence to:* Balázs Németh, MD, Department of Public Health Medicine, Medical School, University of Pécs, Szigeti str. 12, H-7624 Pécs, Hungary. E-mail: balazs.nemeth@aok.pte.hu

*Key Words:* Autoimmune regulator, single-nucleotide polymorphism, allelic discrimination, rheumatoid arthritis, C-reactive protein.



Rheumatoid arthritis (RA) is a common disease of immune dysfunction characterized by chronic synovitis; the resultant polyarticular arthritis with extra-articular symptoms causes functional impairment. The disease prevalence is 0.5-1% among adults, and the annual incidence in developed countries is 5-50 per 100,000 adults (1).

The immunopathology of RA has not been completely clarified; however, many studies have reported that some autoimmune T cells clonally expand and survive negative selection of the adaptive immune system. These autoimmune T cells then infiltrate the synovium and cause RA (2, 3).

Despite the significant association between major histocompatibility complex (MHC) *HLA-DRB1* and RA by numerous genome-wide association studies (4-7), the two latest transethnic, high-throughput genome-wide association studies have associated 51 novel risk loci with RA using a combined total of 33,954 cases and 90,649 controls (8, 9). One of these risk loci is the coding sequence for *AIRE*, which is indispensable for the control of autoimmunity.

*AIRE* is located in the 21q22.3 chromosomal region and encodes the 545 amino acid transcription factor autoimmune regulator (AIRE), which is located in the nucleus of medullary thymic epithelial cells (mTECs) (10). During postnatal development of the immune system, AIRE binds to the DNA of mTECs to ensure the promiscuous, ectopic expression of peripheral tissue self-antigens in the thymic environment, which is the "educational" site of the incoming naïve T cell precursors, thymocytes (2). Self-antigens are presented on MHC molecules of mTECs to the T cell

receptors on thymocytes (2). If self-antigens are recognized by the T-cell receptor (TCR) of thymocytes, these naïve T-cells become autoreactive and negative selection clonally deletes them by apoptosis to ensure a normal T cell population without autoreactivity (2, 10). This negative selection is the last step in the process of immunological central tolerance (2). AIRE consists of several structural domains: the caspase-recruitment domain controls interactions with binding partners; the SAND domain has DNA-binding and transcription properties and controls peripheral tissue antigen production; the PHD fingers read the histone code; and the LXXLL sequences interact with the ubiquitous transcription activators CREB-binding protein (CBP) and signal transducers and activators of transcription (STAT) protein (10-13). Certain functional mutations in the *AIRE* protein-coding sequence result in the loss of negative selection, causing development of the systemic autoimmune disease autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), also known as autoimmune polyendocrine syndrome type 1 (APS-1), which causes multiorgan devastation (10, 14).

Quantitatively inappropriate presentation of self-antigens may decrease the effectiveness of central and peripheral tolerance, causing the survival and maturation of autoreactive thymocytes which leads to various autoimmune diseases, including RA. This effect has been confirmed for the glucose-6-phosphate isomerase self-antigen in the K/BxN mouse model of RA (15). Numerous studies describe the fundamental contribution to RA development of defective selection of autoimmune T cells and the resultant synovial infiltration of $CD4^+$ type 1 T helper (Th) 1, Th2, Th17, and regulatory T cells (3, 16-18). We endorse the rising scientific attention on thymic T cell selection and the necessity to analyze the role of gene variants of its key player, AIRE. The role of allelic polymorphisms in *AIRE* transcription was first described in an *in vivo* study that detected two distinct *AIRE* single-nucleotide polymorphisms (SNPs), *AIRE*-230Y and *AIRE*-655G. The haplotype *AIRE*-230T transcriptionally modified *AIRE* gene expression, which elevated the risk of autoimmunity through the alteration of negative selection (19). Although *AIRE* SNPs attract scientific attention, only few case-control studies have analyzed the association between allelic polymorphisms of *AIRE* and autoimmune diseases, such as vitiligo (20), alopecia areata (21), Addison's disease (22), and systemic sclerosis (23). The majority of these molecular epidemiological studies analyzed the association of *AIRE* SNPs with RA. Similarly, we previously conducted a meta-analysis to verify the association of *AIRE* allelic polymorphisms with RA in Asian populations (24). So far, the association in European populations is poorly understood. To date, one study has analyzed *AIRE* and RA in the Spanish population (25). According to our knowledge, our report is the first European study to examine the association of genotypes of *AIRE* allelic polymorphisms and RA risk using five different genetic models (allelic, dominant, recessive, codominant heterozygous, codominant homozygous, and overdominant) to determine risk genotypes.

## Patients and Methods

*Preliminary in silico research*. We performed preliminary *in silico* research to identify SNPs that could be relevant to RA risk. We used the Ensembl automatic annotation of the human genome sequence (GRCh38.p13) and the UCSC Genome Browser (GRCh38 assembly) as a human genome database (26, 27). Our inclusion criteria for SNPs to be tested were global minor allele frequency (MAF) of 0.01-0.5 and presence of the variant in at least one of the following databases: 1000 Genomes Project Phase III (including the HapMap project), NCBI dbSNP, NHLBI GO Exome Sequencing Project (human only), Exome Aggregation Consortium (human only), Genome Aggregation Database (human only), and NHLBI Trans-Omics for Precision Medicine (human only) (28-32).

Hits were excluded as they had a low probability of detection in our study population. Therefore, we established a criterion to exclude a genetic variant if it existed in the 1000 Genomes Project database with a global MAF of 0.01-0.5 but the MAF was less than 0.01 in the EUR or CEU subpopulations.

*Study subjects*. Patients with RA and healthy control subjects were consecutively recruited by voluntary participation from individuals receiving outpatient or inpatient care at the Harkány Thermal Rehabilitation Centre, Hungary. We collected blood samples from the recruited subjects from May 2018 to September 2022; the subjects gave informed consent and received a study information sheet.

Blood samples were obtained and collected in citrate tubes (one 4 ml tube per subject). Whole blood was stored at −75˚C until analysis. Our inclusion criteria for the patients with RA were that the subjects aged more than 18 years, had signed a consent statement, and had been clinically diagnosed with RA, based on the American College of Rheumatology classification (33). Our inclusion criteria for the healthy control subjects were that they aged more than 18 years, had signed a consent statement, and did not have RA, based on the American College of Rheumatology classification and had blood values of C-reactive protein (CRP) ≤20 mg/dl and rheumatoid factor (Rf) ≤25 IU/ml (34). Ultimately, we analyzed data from 100 patients with RA and 100 healthy control subjects.

*Laboratory assessment*. The blood samples were analyzed for complete blood count, cholesterol profile, blood sugar profile, erythrocyte sedimentation rate (ESR), CRP, sodium, potassium, urea, creatinine, glutamate-oxaloacetate transaminase, glutamate-pyruvate transaminase, gamma-glutamyl transferase, alkaline phosphatase, lactate dehydrogenase, creatine kinase, protein albumin, magnesium, phosphorus, and serum bilirubin. We measured rheumatoid factor (Rf), anti-cyclic citrullinated peptide (aCCP), and antinuclear antibodies to confirm seropositivity in patients with RA and seronegativity in control subjects (35, 36). Finally, we calculated the functional status of patients with RA by the Functional Independence Measure and disease activity score with 28-joint counts (DAS28) (37). ESR was quantified using the Westergren method (38). CRP and Rf were quantified at the

Table I. *Demographic and clinical characteristics of study populations.*

| Characteristics | Patients with RA (N=100) | Control subjects (N=100) |
|---|---|---|
| Age (years), mean±SD | 65.38±9.84* | 81.17±4.56 |
| Sex | | |
| Female, no. (%) | 85 (85%) | 68 (68%) |
| Male, no. (%) | 15 (15%) | 32 (32%) |
| ESR (mm/h), mean±SD (normal range=3-13 mm/h) | 40.24±23.90* | 15.43±9.97 |
| CRP (mg/dl), mean±SD (normal range=0-10 mg/dl) | 28.80±29.21* | 6.19±2.53 |
| Rf (IgG) (IU/ml), mean±SD (normal range=0-15 IU/ml) | 166.15±156.39* | 11.79±7.61 |
| aCCP (U/ml), mean±SD (normal range: <20.0 U/ml; weak positivity: ≥20.0-39.0 U/ml; moderate positivity: ≥39.0-59.0 U/ml; strong positivity: ≥59.0 U/ml) | 561.10±583.07* | 4.566±2.65 |
| Seropositive, no. (%) | 66 (66%)* | 0 (0%) |
| Seronegative, no. (%) | 14 (14%)* | 100 (100%) |
| DAS28, mean±SD (disease remission: <2.6; low: ≥2.6-3.1; moderate: ≥3.1-5.1; high: ≥5.1) | 4.93±1.46 | – |

RA: Rheumatoid arthritis; SD: standard deviation; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; Rf: rheumatoid factor; IgG: immunoglobulin G; aCCP: anti-cyclic citrullinated peptide; DAS28: disease activity score with 28-joint counts; *$p$<0.001, compared with control subjects.

Harkány Thermal Rehabilitation Centre using Konelab Arena 20XT Clinical Chemistry Analyzer (Thermo Fisher Scientific, Waltham, MA, USA) in accordance with the manufacturer's recommendations. Levels of aCCP were analyzed using an Immunoscan CCPlus enzyme-linked immunosorbent assay kit (Svar Life Sciences, Malmö, Sweden) at the Department of Immunology and Biotechnology, Medical School, University of Pécs, Hungary, in accordance with the manufacturer's protocols.

*Genomic DNA extraction from blood*. Genomic DNA was isolated using 1 ml DNAzol™ BD reagent (Thermo Fisher Scientific) mixed with 0.5 ml whole blood in accordance with the manufacturer's instructions. Genomic DNA was precipitated with 0.4 ml isopropanol for 5 min at room temperature and then centrifuged. After the removal of the supernatant, the pellet was washed with 0.5 ml DNAzol™ BD reagent and then washed with 1 ml 75% ethanol. The genomic DNA was dried, dissolved in 200 μL Tris EDTA buffer (10mM Tris, pH 8.0, and 1mM EDTA), and quantified with a NanoDrop One (Thermo Fisher Scientific). For genotyping by quantitative polymerase chain reaction, 20 ng genomic DNA was used per reaction.

*Genotyping AIRE SNPs rs878081 (C>T) and rs1003854 (T>C)*. We used the predesigned Minor Groove Binder (MGB) TaqMan probe assays (Thermo Fisher Scientific) to detect SNPs in *AIRE*. The presence of the MGB at the 3' ends of the probe increases the melting temperature, stabilizes the probe–target hybrids, and enables more accurate discrimination between highly homologous allele sequences. After genomic DNA purification from blood samples, multiplex quantitative polymerase chain reaction was performed. FAM and VIC fluorescence intensity signals were detected using the Rotor-Gene 3000 thermal cycler (Corbett Life Science, Qiagen, Crawley, UK). Samples with signals in either the VIC or FAM channels were considered homozygous for the SNP site of interest, whereas samples with signals in both VIC and FAM channels were considered heterozygous for the SNP site of interest.

*Statistical analysis*. Our statistical analyses were performed using SPSS software, version 28.0 (IBM Corp. Released 2021, IBM SPSS Statistics for Windows, Version 28.0; IBM Corp, Armonk, NY, USA). Chi-square tests were used to determine the contingency of genotype distributions with Hardy–Weinberg equilibrium. We also used chi-square tests to check significant differences in the genotype frequencies of control subjects. Clinical continuous variables are represented as mean±standard deviation (SD).

The Shapiro-Wilk test was used to determine normality, and Levene's test was used to analyze the equality of variances. To compare groups for continuous variables, we used independent samples *t*-tests and analysis of variance. To compare medians of non-normally distributed variables, we used Mann-Whitney *U* and Kruskal-Wallis *H* tests with two-tailed significance. Correlation was tested using bivariate correlation with Pearson correlation coefficient and two-tailed significance. Binary logistic regression was used to calculate odds ratios (ORs) with a 95% confidence interval (CI). Statistical significance was determined when *p*-values were less than 0.05. The power was calculated and indicated if it exceeded 0.8 (39).

## Results

*Demographic and clinical characteristics of the study subjects*. The demographic and clinical data of the study subjects are presented in Table I. The mean±SD age of RA patients was 65.38±9.84 years; 85% were female. According to the literature, the mean age of the disease onset is 65 years (1). To minimize the probability of latent cases among controls, we included participants of age 75 or older. Patients and control individuals were all born in Hungary. The RA group demonstrated significantly higher ESR, CRP, Rf immunoglobulin G (IgG) antibody, aCCP levels (all $p$<0.001) and had 60% seropositivity compared with 100

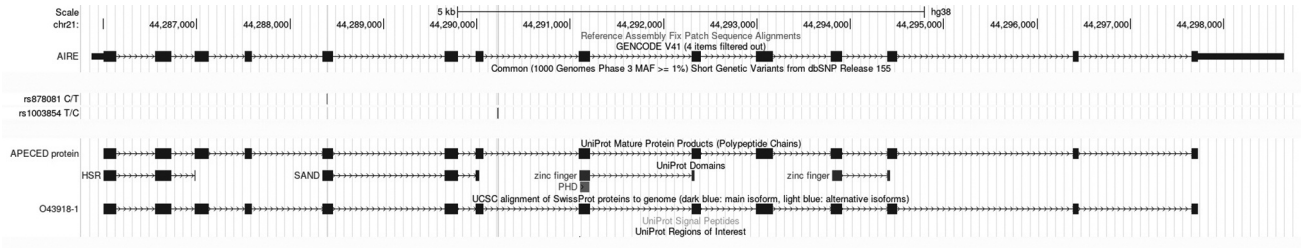


Figure 1. *Domain-coding sequences of AIRE, and the positions of rs878081 and rs1003854 (image generated in UCSC Genome Browser).*

control subjects in whom the aforementioned clinical parameters were in the normal range and whose seronegativity was 100% (34, 40-43). We also calculated DAS28 for patients with RA, where the mean±SD of DAS28 suggested moderate disease activity (44).

*Results of preliminary in silico research*. Using the Ensembl automatic annotation of the human genome sequence and the UCSC Genome Browser as a human genome database, we set up our parameters in the *AIRE* (HGNC Symbol;Acc:HGNC:360) gene sequence (also known as *APECED*, *APS1*, or *PGA1*), which is located in the 21q22.3 chromosomal region between 44,285,838-44,298,648 on the forward strand (26, 27). Without using any restrictions, we identified 23,023 variations (point mutations, indels, and SNPs). To find detectable SNPs, we adjusted the MAF to 0.01-0.5 with at least one representation in each database. We had 68 hits; however, some SNPs had MAFs less than 0.01 in the European (EUR) subpopulation of Utah residents with Northern and Western European ancestry (CEU). We excluded these SNPs by adjusting the MAF to 0.01-0.5 in EUR, because of the low probability of SNP detection in this population. This allowed us to exclude 24 SNPs, and we subsequently shortlisted 44 SNPs in our *in silico* setting. Each of the shortlisted SNPs is found in the 1000 Genomes Project Phase III data set with MAFs 0.01-0.5 and is present in EUR and CEU subpopulations.

Among the 14 exon regions of *AIRE*, exon 5 codes for the SAND domain, which is responsible for transcriptional regulation and DNA binding in mTECs during negative selection. Therefore, we analyzed synonymous polymorphisms in the SAND domain-coding sequence that had MAFs of 0.01-0.5 that might alter messenger RNA (mRNA) splicing, mRNA stability, mRNA structure, protein translation, or cotranslational protein folding (10, 11, 26, 45). In the coding sequence of exon 5, we found one synonymous polymorphism, rs878081, and analyzed its strongly linked intron variant rs1003854, for which the pairwise $r^2$ value for linkage disequilibrium was 0.912. Domain-coding structural sequences of *AIRE* and the positions of rs878081 and rs1003854 are illustrated in Figure 1.

*Genotype frequencies and Hardy–Weinberg equilibrium*. Genotype frequencies in control groups for rs878081 and rs1003854 were in accordance with Hardy–Weinberg equilibrium. No statistically significant difference was found between the expected and observed genotype frequencies in rs878081 ($p$=0.316) and rs1003854 ($p$=0.214); therefore, our sample collection avoided selection bias, nonrandom sampling, and accumulation of relatives.

*Association between rs878081 (C>T) allelic polymorphism and the risk of rheumatoid arthritis*. Allelic polymorphism in rs878081 was analyzed under allelic, dominant, recessive, codominant heterozygous, codominant homozygous, and overdominant genetic models (46). Under the recessive genetic model, we found statistically significant association with RA, where the genotype frequency of TT homozygotes was significantly higher in patients with RA than in healthy control subjects, supporting RA risk (OR=5.44, 95%CI=1.16-25.52, $p$=0.032, power >0.8). The genotype frequency of TT homozygotes was also higher among patients with RA in a codominant homozygous setting than among control subjects; however, the association was barely insignificant (OR=4.68, 95% CI=0.98-22.27, $p$=0.052, power >0.8) but suggested the same direction as the recessive model. No other significant associations were found in the allelic, dominant, codominant heterozygous, or overdominant genetic models. Allele and genotype frequencies of rs878081 in patients with RA and control subjects and risk calculations are presented in Table II. The results of allelic discrimination tests for rs878081 in patients with RA and control subjects are illustrated in Figure 2.

*Association between rs1003854 (T>C) allelic polymorphism and the risk of rheumatoid arthritis*. Allelic polymorphism of rs1003854 was analyzed under allelic, dominant, recessive, codominant heterozygous, codominant homozygous, and overdominant genetic models. The genotype frequency in patients with RA was statistically significantly higher in TT homozygotes than in control subjects, supporting RA risk (OR=4.84, 95%CI=1.02-23.02, $p$=0.047, power >0.8). No other statistically significant association was found under the

Table II. *Distribution of allele and genotype frequencies of AIRE rs878081 in patients with rheumatoid arthritis and control subjects.*

| *AIRE* SNP rs878081 (C>T) | Patients with RA N=100 (%) | Control subjects N=100 (%) | OR (95%CI) | *p*-Value[a] |
|---|---|---|---|---|
| Allele | | | | |
| T | 46 (23.0) | 42 (21.0) | 1.12 (0.70-1.80) | 0.629 |
| C | 154 (77.0) | 158 (79.0) | | |
| Genotype | | | | |
| TT | 10 (10.0) | 2 (2.0) | 4.68 (0.98-22.27) | 0.052† |
| CT | 26 (26.0) | 38 (38.0) | 0.64 (0.34-1.18) | 0.154 |
| CC | 64 (64.0) | 60 (60.0) | | |
| Dominant | | | | |
| CT+TT | 36 (36.0) | 40 (40.0) | 0.84 (0.47-1.49) | 0.629 |
| CC | 64 (64.0) | 60 (60.0) | | |
| Recessive | | | | |
| TT | 10 (10.0) | 2 (2.0) | 5.44 (1.16-25.52) | 0.032*† |
| CC+CT | 90 (90.0) | 98 (98.0) | | |
| Overdominant | | | | |
| CT | 26 (26.0%) | 38 (38.0%) | 0.57 (0.31-1.04) | 0.070 |
| CC+TT | 74 (74.0%) | 62 (62.0%) | | |

SNP: Single-nucleotide polymorphism; OR: odds ratio; CI: confidence interval. abased on logistic regression; *$p<0.05$, †power >0.8.

allelic, dominant, codominant heterozygous, codominant homozygous, or overdominant genetic models. Allele and genotype frequencies of rs1003854 in patients with RA and control subjects and risk calculations are presented in Table III. The results of allelic discrimination tests for rs1003854 in patients with RA and control subjects are illustrated in Figure 3.

*Comparison of clinical parameters under the genetic models of rs878081 and rs1003854 in patients with rheumatoid arthritis and control subjects*. Our study aimed to compare ESR, CRP, Rf, aCCP, and DAS28 levels between the genotype subgroups of genetic models of rs878081 and rs1003854 in patients with RA and control subjects. The first stage was designed to find significant differences by comparing group means, and the second stage was designed to reveal correlations between clinical parameters and genotypes.

In patients with RA, ESRs were significantly higher in the CC subgroup compared to TT homozygotes under the codominant homozygous model of rs1003854 ($p$=0.048). The CC subgroup demonstrated significantly higher CRP levels compared to TT homozygotes in the codominant homozygous model and the TT+TC subgroup in the recessive model ($p$=0.043 and $p$=0.029, respectively). Furthermore, the CC homozygote patients with RA were significantly correlated with CRP under the codominant homozygous and recessive models ($p$=0.006 and $p$=0.002, respectively; r=0.350 and r=0.338, respectively). No other significant differences were found in other clinical variables and genetic models of rs878081 and rs1003854. These results are illustrated in Table IV.

## Discussion

RA is a complex, multifactorial disorder in which genetic and environmental causes contribute to disease initiation (1-3). *In vitro* and *in vivo* studies have demonstrated that presentation of peripheral tissue self-antigens is quantitatively determined by *AIRE* transcription, and *AIRE* SNPs may cause clonal survival (19, 47). Autoreactive $CD4^+$ T cells are recruited to the synovium where they initiate an inflammatory environment by the production of interferon-gamma, tumor necrosis factor-alpha, interleukins (ILs), proinflammatory cytokines, and chemokines, causing tissue destruction (48-50). The inflammatory environment activates fibroblast-like synoviocytes (FLSs) to produce chemokines in order to attract more autoreactive CD4+ T cells, creating a vicious circle (50, 51). This was demonstrated by an *in vitro* study by Bergström *et al*. in which IL-1β and tumor necrosis factor-stimulated FLSs from patients with RA and in which there was 26-fold statistically significantly higher CXCL10 and CCL8 production compared with cells with AIRE expression silenced by sense siRNA (51). Nonstimulated FLSs did not produce chemokines (51). Based on our current understanding, *AIRE* SNPs are important in two ways: they allow the escape of autoreactive $CD4^+$ T cells during central tolerance, and they cause FLSs to produce chemokines to attract increasingly more self-reactive $CD4^+$ cells, thereby maintaining the hyperplastic inflammatory environment (19,

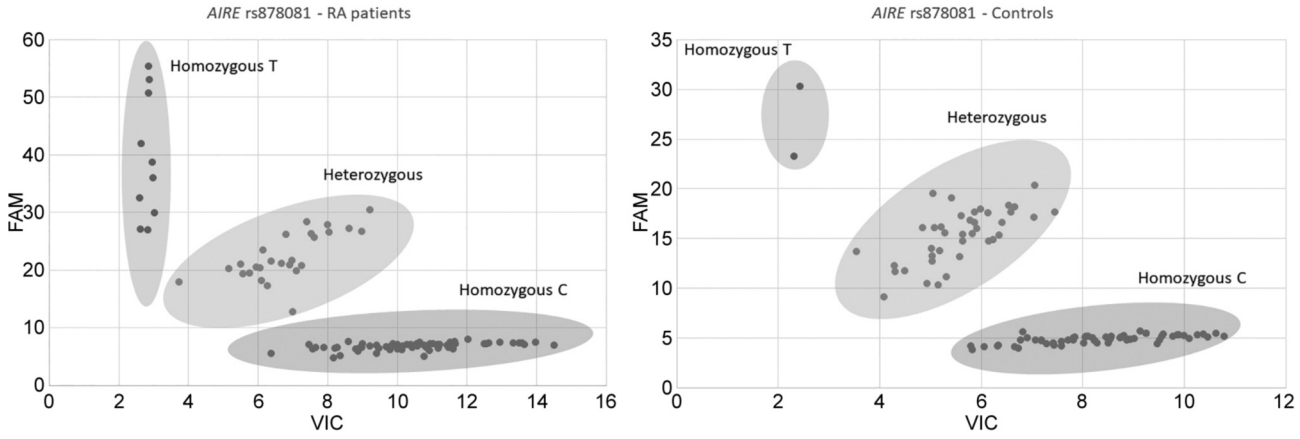


Figure 2. *Result of allelic discrimination tests for rs878081 in patients with rheumatoid arthritis and control subjects.*

Table III. *Distribution of allele and genotype frequencies of AIRE rs1003854 in patients with rheumatoid arthritis and control subjects.*

| *AIRE* SNP rs1003854 (T>C) | Patients with RA N=100 (%) | Control subjects N=100 (%) | OR (95%CI) | *p*-Value[a] |
|---|---|---|---|---|
| Allele | | | | |
| C | 47 (23.5) | 45 (22.5) | 1.05 (0.66-1.68) | 0.812 |
| T | 153 (76.5) | 155 (77.5) | | |
| Genotype | | | | |
| CC | 9 (9.0) | 2 (2.0) | 4.13 (0.85-19.96) | 0.077[†] |
| TC | 29 (29.0) | 41 (41.0) | 0.65 (0.35-1.18) | 0.157 |
| TT | 62 (62.0) | 57 (57.0) | | |
| Dominant | | | | |
| TC+CC | 38 (38.0) | 43 (43.0) | 0.81 (0.46-1.43) | 0.471 |
| TT | 62 (62.0) | 57 (57.0) | | |
| Recessive | | | | |
| CC | 9 (9.0) | 2 (2.0) | 4.84 (1.02-23.02) | 0.047*[†] |
| TT+TC | 91 (91.0) | 98 (98.0) | | |
| Overdominant | | | | |
| TC | 29 (29.0) | 41 (41.0) | 0.58 (0.32-1.05) | 0.076 |
| TT+CC | 71 (71.0) | 59 (59.0) | | |

SNP: Single-nucleotide polymorphism; OR: odds ratio; CI: confidence interval; [a]based on logistic regression; *$p<0.05$, [†]power >0.8.

51). Hence, synonymous SNPs and their strongly related intron variants can be associated with disease activity. In Asian populations, a strong association was found between rs2075776, rs760426, and RA in different genetic models (24, 52-55). Furthermore, Terao *et al*. demonstrated genotype-specific *AIRE* expression in lymphoblastoid cells using the Gene Expression Omnibus database and raised the idea that different genotypes may alter disease activity (56). Allelic polymorphisms of rs878081 have been analyzed in Chinese, Egyptian, and Spanish populations (25, 56, 57). Yang *et al*. measured *AIRE* expression in blood lymphocytes and found significant differences between rs878081 TT and CC homozygotes in Asian populations (56).

Our investigation is the first attempt to analyze different genotypes and RA risk among Hungarian-born, European individuals in allelic, dominant, recessive, codominant heterozygous, codominant homozygous, and overdominant genetic models combined with clinical parameters, such as ESR and CRP. After our preliminary *in silico* selection of SNPs, our results showed in a recessive model (TT *vs*. CC+CT) that rs878081 TT is significantly associated with RA risk ($p$=0.032). The codominant homozygous model (TT *vs*. CC) provided the same result; however, only borderline significance was detected ($p$=0.052). These results made us consider that two copies of the minor alleles may be necessary for a quantitative association with RA risk, as the

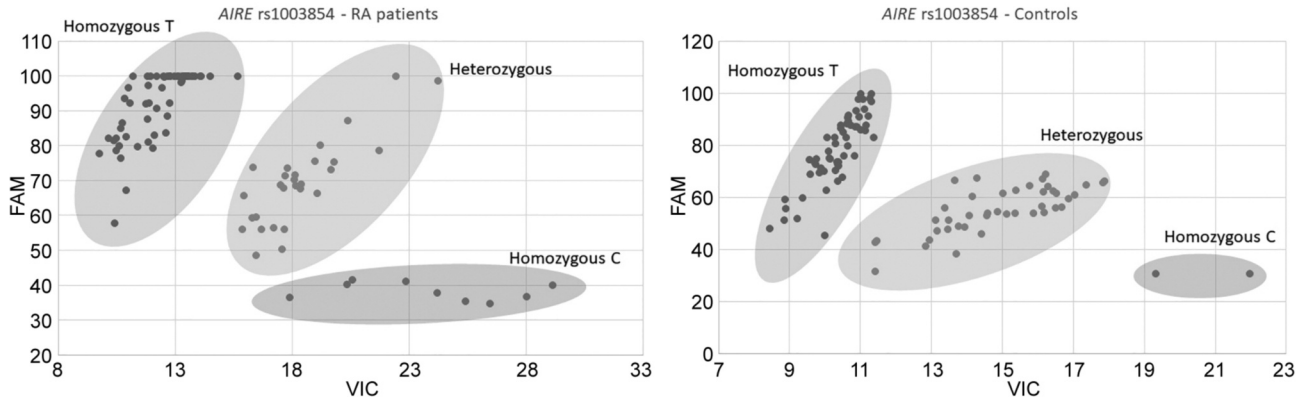


Figure 3. *Result of allelic discrimination tests for rs1003854 in patients with rheumatoid arthritis and control subjects.*

Table IV. *C-reactive protein levels among the genotypic subgroups of genetic models of rs1003854 within patients with rheumatoid arthritis and control subjects.*

| *AIRE* SNP rs1003854 (T>C) | Patients with RA | | | | Control subjects | | | |
|---|---|---|---|---|---|---|---|---|
| | CRP | *p*-Value[a] | Pearson correlation | *p*-Value[b] | CRP | *p*-Value[c] | Pearson correlation | *p*-Value[d] |
| Genotype | | | | | | | | |
| CC | 58.41±47.30 | 0.043* | 0.350 | 0.006** | 6.00±0.00 | 0.486 | 0.002 | 0.990 |
| TC | 22.04±23.03 | 0.094 | –0.086 | 0.469 | 6.49±3.14 | 0.486 | 0.099 | 0.337 |
| TT | 27.21±25.75 | | | | 5.98±2.06 | | | |
| Dominant | | | | | | | | |
| TC+CC | 32.47±35.36 | 0.687 | 0.092 | 0.416 | 6.47±3.06 | 0.430 | 0.096 | 0.347 |
| TT | 26.87±25.55 | | | | 5.98±2.06 | | | |
| Recessive | | | | | | | | |
| CC | 58.41±47.30 | 0.029* | 0.338 | 0.002** | 6.00±0.00 | 0.571 | –0.011 | 0.915 |
| TT+TC | 25.56±24.95 | | | | 6.19±2.56 | | | |
| Overdominant | | | | | | | | |
| TC | 22.09±23.57 | 0.050 | –0.132 | 0.239 | 6.49±3.14 | 0.527 | 0.100 | 0.329 |
| TT+CC | 31.01±30.69 | | | | 5.98±2.02 | | | |

SNP: Single-nucleotide polymorphism; RA: rheumatoid arthritis; CRP: C-reactive protein; [a]genotype subgroup comparison of CRP levels in patients with RA in each genetic model; [b]Pearson correlation of CRP with genotype subgroups of patients with RA in each genetic model; [c]genotype subgroup comparison of CRP levels in control subjects in each genetic model; [d]Pearson correlation of CRP with genotype subgroups of control subjects in each genetic model; *$p$<0.05, **$p$<0.01; Clinical parameters are reported as mean±SD.

SNP is synonymous and resides in exon 5. Exon 5 is in the SAND coding sequence of *AIRE* that is responsible for DNA-binding and cooperation with transcriptional factors, so allelic polymorphisms in this region may functionally determine AIRE production and affect central tolerance (10-13).

Our study analyzed intron variant rs1003854, where significant correlations were found between clinical parameters and genotypes in recessive (CC *vs*. TT+TC) and homozygous codominant (CC *vs*. TT) genetic models. The allelic polymorphism rs1003854 resides in intron 7 and may modify the transcription of *AIRE* by altering alternative-splicing mechanisms with the process of intron-mediated enhancement (58). Interestingly, after we determined RA risk for SNP rs1003854, we concluded that only the recessive and codominant genetic models demonstrated significantly higher levels of CRP ($p$=0.029 and $p$=0.043, respectively) and positive, significant correlation with CRP ($p$=0.002 and $p$=0.006, respectively) when patients with RA were homozygous for the minor C allele. This finding supports the notion that CRP levels vary in genetic models of *AIRE* rs1003854 among patients with RA, reflecting a possible association between *AIRE* genotypes and RA disease activity, where chemokines (such as CXCL10, CCL8, and CCL5) are essential and are strongly correlated with CRP levels (59-61).

To conclude, rs878081(C>T) and rs1003854(T>C) SNPs together with ESR and CRP levels would be useful as potential biomarkers to determine RA susceptibility, follow-up, and prognosis. Before future clinical application, further studies are necessary. To continue contributing to this research area, we intend to carry out studies on larger cohorts of patients with RA and control subjects and to consider multiple loci as well as possible molecular partners of AIRE.

## Conclusion

In conclusion, our data demonstrate the effects of gene polymorphisms in rs878081 and rs1003854 on the risk of RA in the European population, where two copies of the minor alleles and the related homozygous genotypes provided susceptibility. Analyses of the clinical parameters ESR and CRP supported our assumption that the homozygous subgroup for the minor allele of patients with RA may have higher ESR and CRP levels, which might reflect disease activity. Therefore, these clinical parameters may be suitable to detect at-risk populations in the future. Based on medical history, a previous family history of RA may be an indication for a risk genotype test, where the detection would prospectively allow a follow-up of the healthy individual and possible preventive physiotherapy and drug therapy.

## Conflicts of Interest

The Authors declare no conflicts of interest in relation to this study.

## Authors' Contributions

Bálint Bérczi: conceptualization, methodology, formal analysis, investigation, writing – original draft preparation, writing – review & editing, funding acquisition. Nóra Nusser: methodology, resources, writing—original draft preparation, writing—review & editing. Iván Péter: resources, writing—original draft preparation, writing—review & editing. Balázs Németh: resources, writing—original draft preparation, writing—review & editing. Zoltán Gyöngyi: conceptualization, investigation, methodology, data curation, writing—original draft preparation, writing—review & editing, supervision, funding acquisition. All Authors have read and agreed to the published version of the manuscript.

## Acknowledgements

The Authors thank all the patients and control subjects for participation in the study. The Authors would like to also acknowledge the conscientious laboratory assistance by the team of the Harkány Thermal Rehabilitation Centre, Hungary. This research was funded by the ÁOK János Szolcsányi Research Fund supported by the Medical School, University of Pécs, Hungary. The study was conducted in accordance with the guidelines of the Declaration of Helsinki, and the protocol was approved by the Secretary of the Medical Research Council, Scientific and Research Ethics Committee of Hungary (11871-7/2018/EÜIG of 24 April 2018, and 57142-5/2022/EÜIG of 8 November 2022). Informed consent was obtained from all subjects involved in the study.

## References

1 Scott DL, Wolfe F, Huizinga TW: Rheumatoid arthritis. Lancet 376(9746): 1094-1108, 2010. DOI: 10.1016/S0140-6736(10)60826-4

2 Starr TK, Jameson SC, Hogquist KA: Positive and negative selection of T cells. Annu Rev Immunol 21(1): 139-176, 2003. DOI: 10.1146/annurev.immunol.21.120601.141107

3 Mellado M, Martínez-Muñoz L, Cascio G, Lucas P, Pablos JL, Rodríguez-Frade JM: T cell migration in rheumatoid arthritis. Front Immunol 6: 384, 2015. DOI: 10.3389/fimmu.2015.00384

4 McInnes IB, Schett G: The pathogenesis of rheumatoid arthritis. N Engl J Med 365(23): 2205-2219, 2011. DOI: 10.1056/NEJMra1004965

5 Raychaudhuri S, Sandor C, Stahl EA, Freudenberg J, Lee HS, Jia X, Alfredsson L, Padyukov L, Klareskog L, Worthington J, Siminovitch KA, Bae SC, Plenge RM, Gregersen PK, de Bakker PI: Five amino acids in three HLA proteins explain most of the association between MHC and seropositive rheumatoid arthritis. Nat Genet 44(3): 291-296, 2012. DOI: 10.1038/ng.1076

6 MacKay K, Eyre S, Myerscough A, Milicic A, Barton A, Laval S, Barrett J, Lee D, White S, John S, Brown MA, Bell J, Silman A, Ollier W, Wordsworth P, Worthington J: Whole-genome linkage analysis of rheumatoid arthritis susceptibility loci in 252 affected sibling pairs in the United Kingdom. Arthritis Rheum 46(3): 632-639, 2002. DOI: 10.1002/art.10147

7 Kurkó J, Besenyei T, Laki J, Glant TT, Mikecz K, Szekanecz Z: Genetics of rheumatoid arthritis - a comprehensive review. Clin Rev Allergy Immunol 45(2): 170-179, 2013. DOI: 10.1007/s12016-012-8346-7

8 Okada Y, Terao C, Ikari K, Kochi Y, Ohmura K, Suzuki A, Kawaguchi T, Stahl EA, Kurreeman FA, Nishida N, Ohmiya H, Myouzen K, Takahashi M, Sawada T, Nishioka Y, Yukioka M, Matsubara T, Wakitani S, Teshima R, Tohma S, Takasugi K, Shimada K, Murasawa A, Honjo S, Matsuo K, Tanaka H, Tajima K, Suzuki T, Iwamoto T, Kawamura Y, Tanii H, Okazaki Y, Sasaki T, Gregersen PK, Padyukov L, Worthington J, Siminovitch KA, Lathrop M, Taniguchi A, Takahashi A, Tokunaga K, Kubo M, Nakamura Y, Kamatani N, Mimori T, Plenge RM, Yamanaka H, Momohara S, Yamada R, Matsuda F, Yamamoto K: Meta-analysis identifies nine new loci associated with rheumatoid arthritis in the Japanese population. Nat Genet 44(5): 511-516, 2012. DOI: 10.1038/ng.2231

9 Okada Y, Wu D, Trynka G, Raj T, Terao C, Ikari K, Kochi Y, Ohmura K, Suzuki A, Yoshida S, Graham RR, Manoharan A, Ortmann W, Bhangale T, Denny JC, Carroll RJ, Eyler AE, Greenberg JD, Kremer JM, Pappas DA, Jiang L, Yin J, Ye L, Su DF, Yang J, Xie G, Keystone E, Westra HJ, Esko T, Metspalu A, Zhou X, Gupta N, Mirel D, Stahl EA, Diogo D, Cui J, Liao K, Guo MH, Myouzen K, Kawaguchi T, Coenen MJ, van Riel PL, van de Laar MA, Guchelaar HJ, Huizinga TW, Dieudé P, Mariette X, Bridges SL Jr, Zhernakova A, Toes RE, Tak PP, Miceli-Richard C, Bang SY, Lee HS, Martin J, Gonzalez-Gay MA, Rodriguez-Rodriguez L, Rantapää-Dahlqvist S, Arlestig L, Choi HK, Kamatani Y, Galan P, Lathrop M, RACI consortium, GARNET consortium, Eyre S, Bowes J, Barton A, de Vries N, Moreland LW, Criswell LA, Karlson EW, Taniguchi A, Yamada

R, Kubo M, Liu JS, Bae SC, Worthington J, Padyukov L, Klareskog L, Gregersen PK, Raychaudhuri S, Stranger BE, De Jager PL, Franke L, Visscher PM, Brown MA, Yamanaka H, Mimori T, Takahashi A, Xu H, Behrens TW, Siminovitch KA, Momohara S, Matsuda F, Yamamoto K, Plenge RM: Genetics of rheumatoid arthritis contributes to biology and drug discovery. Nature 506(7488): 376-381, 2014. DOI: 10.1038/nature12873

10 Mathis D, Benoist C: Aire. Annu Rev Immunol 27(1): 287-312, 2009. DOI: 10.1146/annurev.immunol.25.022106.141532

11 Bottomley MJ, Collard MW, Huggenvik JI, Liu Z, Gibson TJ, Sattler M: The SAND domain structure defines a novel DNA-binding fold in transcriptional regulation. Nat Struct Biol 8(7): 626-633, 2001. DOI: 10.1038/89675

12 Ragvin A, Valvatne H, Erdal S, Årskog V, Tufteland KR, Breen K, Øyan AM, Eberharter A, Gibson TJ, Becker PB, Aasland R: Nucleosome binding by the bromodomain and PHD finger of the transcriptional cofactor p300. J Mol Biol 337(4): 773-788, 2004. DOI: 10.1016/j.jmb.2004.01.051

13 Plevin MJ, Mills MM, Ikura M: The LxxLL motif: a multifunctional binding sequence in transcriptional regulation. Trends Biochem Sci 30(2): 66-69, 2005. DOI: 10.1016/j.tibs.2004.12.001

14 Akirav EM, Ruddle NH, Herold KC: The role of AIRE in human autoimmune disease. Endocrinol 7(1): 25-33, 2011. DOI: 10.1038/nrendo.2010.200

15 Perera J, Liu X, Zhou Y, Joseph NE, Meng L, Turner JR, Huang H: Insufficient autoantigen presentation and failure of tolerance in a mouse model of rheumatoid arthritis. Arthritis Rheum 65(11): 2847-2856, 2013. DOI: 10.1002/art.38085

16 Walser-Kuntz DR, Weyand CM, Weaver AJ, O'Fallon WM, Goronzy JJ: Mechanisms underlying the formation of the T cell receptor repertoire in rheumatoid arthritis. Immunity 2(6): 597-605, 1995. DOI: 10.1016/1074-7613(95)90004-7

17 Kouskoff V, Korganow AS, Duchatelle V, Degott C, Benoist C, Mathis D: Organ-specific disease provoked by systemic autoimmunity. Cell 87(5): 811-822, 1996. DOI: 10.1016/s0092-8674(00)81989-3

18 Goronzy JJ, Weyand CM: Thymic function and peripheral T-cell homeostasis in rheumatoid arthritis. Trends Immunol 22(5): 251-255, 2001. DOI: 10.1016/s1471-4906(00)01841-x

19 Lovewell TR, McDonagh AJ, Messenger AG, Azzouz M, Tazi-Ahnini R: The AIRE -230Y polymorphism affects AIRE transcriptional activity: Potential influence on AIRE function in the thymus. PLoS One 10(5): e0127476, 2015. DOI: 10.1371/journal.pone.0127476

20 Tazi-Ahnini R, McDonagh AJ, Wengraf DA, Lovewell TR, Vasilopoulos Y, Messenger AG, Cork MJ, Gawkrodger DJ: The autoimmune regulator gene (*AIRE*) is strongly associated with vitiligo. Br J Dermatol 159(3): 591-6, 2008. DOI: 10.1111/j.1365-2133.2008.08718.x

21 Wengraf DA, McDonagh AJ, Lovewell TR, Vasilopoulos Y, Macdonald-Hull SP, Cork M.J, Messenger AG, Tazi-Ahnini R: Genetic analysis of autoimmune regulator haplotypes in alopecia areata. Tissue Antigens 71(3): 206-212, 2008. DOI: 10.1111/j.1399-0039.2007.00992.x

22 Eriksson D, Bianchi M, Landegren N, Dalin F, Skov J, Hultin-Rosenberg L, Mathioudaki A, Nordin J, Hallgren Å, Andersson G, Tandre K, Rantapää Dahlqvist S, Söderkvist P, Rönnblom L, Hulting AL, Wahlberg J, Dahlqvist P, Ekwall O, Meadows JRS, Lindblad-Toh K, Bensing S, Rosengren Pielberg G, Kämpe O: Common genetic variation in the autoimmune regulator (AIRE) locus is associated with autoimmune Addison's disease in Sweden. Sci Rep 8(1): 8395, 2018. DOI: 10.1038/s41598-018-26842-2

23 Ferrera F, Rizzi M, Sprecacenere B, Balestra P, Sessarego M, Di Carlo A, Filaci G, Gabrielli A, Ravazzolo R, Indiveri F: AIRE gene polymorphisms in systemic sclerosis associated with autoimmune thyroiditis. Clin Immunol 122(1): 13-17, 2007. DOI: 10.1016/j.clim.2006.09.013

24 Bérczi B, Gerencsér G, Farkas N, Hegyi P, Veres G, Bajor J, Czopf L, Alizadeh H, Rakonczay Z, Vigh É, Erőss B, Szemes K, Gyöngyi Z: Association between AIRE gene polymorphism and rheumatoid arthritis: a systematic review and meta-analysis of case-control studies. Sci Rep 7(1): 14096, 2017. DOI: 10.1038/s41598-017-14375-z

25 García-Lozano JR, Torres-Agrela B, Montes-Cano MA, Ortiz-Fernández L, Conde-Jaldón M, Teruel M, García A, Núñez-Roldán A, Martín J, González-Escribano MF: Association of the AIRE gene with susceptibility to rheumatoid arthritis in a European population: a case control study. Arthritis Res Ther 15(1): R11, 2013. DOI: 10.1186/ar4141

26 Cunningham F, Allen JE, Allen J, Alvarez-Jarreta J, Amode MR, Armean IM, Austine-Orimoloye O, Azov AG, Barnes I, Bennett R, Berry A, Bhai J, Bignell A, Billis K, Boddu S, Brooks L, Charkhchi M, Cummins C, Da Rin Fioretto L, Davidson C, Dodiya K, Donaldson S, El Houdaigui B, El Naboulsi T, Fatima R, Giron CG, Genez T, Martinez JG, Guijarro-Clarke C, Gymer A, Hardy M, Hollis Z, Hourlier T, Hunt T, Juettemann T, Kaikala V, Kay M, Lavidas I, Le T, Lemos D, Marugán JC, Mohanan S, Mushtaq A, Naven M, Ogeh DN, Parker A, Parton A, Perry M, Piližota I, Prosovetskaia I, Sakthivel MP, Salam AIA, Schmitt BM, Schuilenburg H, Sheppard D, Pérez-Silva JG, Stark W, Steed E, Sutinen K, Sukumaran R, Sumathipala D, Suner MM, Szpak M, Thormann A, Tricomi FF, Urbina-Gómez D, Veidenberg A, Walsh TA, Walts B, Willhoft N, Winterbottom A, Wass E, Chakiachvili M, Flint B, Frankish A, Giorgetti S, Haggerty L, Hunt SE, IIsley GR, Loveland JE, Martin FJ, Moore B, Mudge JM, Muffato M, Perry E, Ruffier M, Tate J, Thybert D, Trevanion SJ, Dyer S, Harrison PW, Howe KL, Yates AD, Zerbino DR, Flicek P: Ensembl 2022. Nucleic Acids Res 50(D1): D988-D995, 2022. DOI: 10.1093/nar/gkab1049

27 Kent WJ, Sugnet CW, Furey TS, Roskin KM, Pringle TH, Zahler AM, Haussler D: The human genome browser at UCSC. Genome Res 12(6): 996-1006, 2002. DOI: 10.1101/gr.229102

28 Cavalli-Sforza LL, Bodmer WF: The genetics of human populations. 1st ed. San Francisco, CA, USA, Dover Publications, pp. 41-118, 1971.

29 Fairley S, Lowy-Gallego E, Perry E, Flicek P: The International Genome Sample Resource (IGSR) collection of open human genomic variation resources. Nucleic Acids Res 48(D1): D941-D947, 2020. DOI: 10.1093/nar/gkz836

30 Sherry ST, Ward M, Sirotkin K: dbSNP-database for single nucleotide polymorphisms and other classes of minor genetic variation. Genome Res 9: 677-679, 1999. DOI: 10.1101/gr.9.8.677

31 Karczewski KJ, Francioli LC, Tiao G, Cummings BB, Alföldi J, Wang Q, Collins RL, Laricchia KM, Ganna A, Birnbaum DP, Gauthier LD, Brand H, Solomonson M, Watts NA, Rhodes D, Singer-Berk M, England EM, Seaby EG, Kosmicki JA, Walters RK, Tashman K, Farjoun Y, Banks E, Poterba T, Wang A, Seed C, Whiffin N, Chong JX, Samocha KE, Pierce-Hoffman E, Zappala Z, O'Donnell-Luria AH, Minikel EV, Weisburd B, Lek

M, Ware JS, Vittal C, Armean IM, Bergelson L, Cibulskis K, Connolly KM, Covarrubias M, Donnelly S, Ferriera S, Gabriel S, Gentry J, Gupta N, Jeandet T, Kaplan D, Llanwarne C, Munshi R, Novod S, Petrillo N, Roazen D, Ruano-Rubio V, Saltzman A, Schleicher M, Soto J, Tibbetts K, Tolonen C, Wade G, Talkowski ME, Genome Aggregation Database Consortium, Neale BM, Daly MJ, MacArthur DG: The mutational constraint spectrum quantified from variation in 141,456 humans. Nature 581(7809): 434-443, 2020. DOI: 10.1038/s41586-020-2308-7

32 Kowalski MH, Qian H, Hou Z, Rosen JD, Tapia AL, Shan Y, Jain D, Argos M, Arnett DK, Avery C, Barnes KC, Becker LC, Bien SA, Bis JC, Blangero J, Boerwinkle E, Bowden DW, Buyske S, Cai J, Cho MH, Choi SH, Choquet H, Cupples LA, Cushman M, Daya M, de Vries PS, Ellinor PT, Faraday N, Fornage M, Gabriel S, Ganesh SK, Graff M, Gupta N, He J, Heckbert SR, Hidalgo B, Hodonsky CJ, Irvin MR, Johnson AD, Jorgenson E, Kaplan R, Kardia SLR, Kelly TN, Kooperberg C, Lasky-Su JA, Loos RJF, Lubitz SA, Mathias RA, McHugh CP, Montgomery C, Moon JY, Morrison AC, Palmer ND, Pankratz N, Papanicolaou GJ, Peralta JM, Peyser PA, Rich SS, Rotter JI, Silverman EK, Smith JA, Smith NL, Taylor KD, Thornton TA, Tiwari HK, Tracy RP, Wang T, Weiss ST, Weng LC, Wiggins KL, Wilson JG, Yanek LR, Zöllner S, North KE, Auer PL, NHLBI Trans-Omics for Precision Medicine (TOPMed) Consortium, TOPMed Hematology & Hemostasis Working Group, Raffield LM, Reiner AP, Li Y: Use of >100,000 NHLBI Trans-Omics for Precision Medicine (TOPMed) Consortium whole genome sequences improves imputation quality and detection of rare variant associations in admixed African and Hispanic/Latino populations. PLoS Genet 15(12): e1008500, 2019. DOI: 10.1371/journal.pgen.1008500

33 Arnett FC, Edworthy SM, Bloch DA, Mcshane DJ, Fries JF, Cooper NS, Healey LA, Kaplan SR, Liang MH, Luthra HS, Medsger TA, Mitchell DM, Neustadt DH, Pinals RS, Schaller JG, Sharp JT, Wilder RL, Hunder GG: The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 31(3): 315-324, 1988. DOI: 10.1002/art.1780310302

34 Ingegnoli F, Castelli R, Gualtierotti R: Rheumatoid factors: clinical applications. Dis Markers 35(6): 727-734, 2013. DOI: 10.1155/2013/726598

35 Niewold TB, Harrison MJ, Paget SA: Anti-CCP antibody testing as a diagnostic and prognostic tool in rheumatoid arthritis. QJM 100(4): 193-201, 2007. DOI: 10.1093/qjmed/hcm015

36 Steiner G, Smolen J: Autoantibodies in rheumatoid arthritis and their clinical significance. Arthritis Res 4 Suppl 2(Suppl 2): S1-S5, 2002. DOI: 10.1186/ar551

37 Graham JE, Granger CV, Karmarkar AM, Deutsch A, Niewczyk P, Divita MA, Ottenbacher KJ: The Uniform Data System for Medical Rehabilitation: report of follow-up information on patients discharged from inpatient rehabilitation programs in 2002-2010. Am J Phys Med Rehabil 93(3): 231-244, 2014. DOI: 10.1097/PHM.0b013e3182a92c58

38 Jou JM, Lewis SM, Briggs C, Lee SH, De La Salle B, McFadden S, International Council for Standardization in Haematology: ICSH review of the measurement of the erythocyte sedimentation rate. Int J Lab Hematol 33(2): 125-132, 2011. DOI: 10.1111/j.1751-553X.2011.01302.x

39 Skol AD, Scott LJ, Abecasis GR, Boehnke M: Joint analysis is more efficient than replication-based analysis for two-stage genome-wide association studies. Nat Genet 38(2): 209-213, 2006. DOI: 10.1038/ng1706

40 Sharland DE: Erythrocyte sedimentation rate: The normal range in the elderly. J Am Geriatr Soc 28(8): 346-348, 1980. DOI: 10.1111/j.1532-5415.1980.tb01096.x

41 Miller A, Green M, Robinson D: Simple rule for calculating normal erythrocyte sedimentation rate. Br Med J (Clin Res Ed) 286(6361): 266, 1983. DOI: 10.1136/bmj.286.6361.266

42 Wener MH, Daum PR, McQuillan GM: The influence of age, sex, and race on the upper reference limit of serum C-reactive protein concentration. J Rheumatol 27(10): 2351-2359, 2000.

43 Coenen D, Verschueren P, Westhovens R, Bossuyt X: Technical and diagnostic performance of 6 assays for the measurement of citrullinated protein/peptide antibodies in the diagnosis of rheumatoid arthritis. Clin Chem 53(3): 498-504, 2007. DOI: 10.1373/clinchem.2006.078063

44 Fuchs HA, Brooks RH, Callahan LF, Pincus T: A simplified twenty-eight–joint quantitative articular index in rheumatoid arthritis. Arthritis Rheum 32(5): 531-537, 1989. DOI: 10.1002/anr.1780320504

45 Zeng Z, Bromberg Y: Predicting functional effects of synonymous variants: a systematic review and perspectives. Front Genet 10: 914, 2019. DOI: 10.3389/fgene.2019.00914

46 Horita N, Kaneko T: Genetic model selection for a case-control study and a meta-analysis. Meta Gene 5: 1-8, 2015. DOI: 10.1016/j.mgene.2015.04.003

47 Chan AY, Anderson MS: Central tolerance to self revealed by the autoimmune regulator. Ann N Y Acad Sci 1356(1): 80-89, 2015. DOI: 10.1111/nyas.12960

48 Tuncel J, Holmberg J, Haag S, Hopkins MH, Wester-Rosenlöf L, Carlsen S, Olofsson P, Holmdahl R: Self-reactive T cells induce and perpetuate chronic relapsing arthritis. Arthritis Res Ther 22(1): 95, 2020. DOI: 10.1186/s13075-020-2104-7

49 Zhu L, Ji F, Wang Y, Zhang Y, Liu Q, Zhang JZ, Matsushima K, Cao Q, Zhang Y: Synovial autoreactive T cells in rheumatoid arthritis resist IDO-mediated inhibition. J Immunol 177(11): 8226-8233, 2006. DOI: 10.4049/jimmunol.177.11.8226

50 Yap HY, Tee SZ, Wong MM, Chow SK, Peh SC, Teow SY: Pathogenic role of immune cells in rheumatoid arthritis: Implications in clinical treatment and biomarker development. Cells 7(10): 161, 2018. DOI: 10.3390/cells7100161

51 Bergström B, Lundqvist C, Vasileiadis GK, Carlsten H, Ekwall O, Ekwall AH: The rheumatoid arthritis risk gene AIRE is induced by cytokines in fibroblast-like synoviocytes and augments the pro-inflammatory response. Front Immunol 10: 1384, 2019. DOI: 10.3389/fimmu.2019.01384

52 Terao C, Yamada R, Ohmura K, Takahashi M, Kawaguchi T, Kochi Y, Human Disease Genomics Working Group, RA Clinical and Genetic Study Consortium, Okada Y, Nakamura Y, Yamamoto K, Melchers I, Lathrop M, Mimori T, Matsuda F: The human AIRE gene at chromosome 21q22 is a genetic determinant for the predisposition to rheumatoid arthritis in Japanese population. Hum Mol Genet 20(13): 2680-2685, 2011. DOI: 10.1093/hmg/ddr161

53 Shao S, Li XR, Cen H, Yin ZS: Association of AIRE polymorphisms with genetic susceptibility to rheumatoid arthritis in a Chinese population. Inflammation 37(2): 495-499, 2014. DOI: 10.1007/s10753-013-9763-3

54 Feng ZJ, Zhang SL, Wen HF, Liang Y: Association of rs2075876 polymorphism of AIRE gene with rheumatoid arthritis risk. Hum

Immunol 76(4): 281-285, 2015. DOI: 10.1016/j.humimm.2015.01.026

55 Li X, Li T, Chen M, Chai Y: [Association of AIRE gene polymorphisms with susceptibility to rheumatoid arthritis among ethnic Han Chinese from Shaanxi]. Zhonghua Yi Xue Yi Chuan Xue Za Zhi 33(3): 373-377, 2016. DOI: 10.3760/cma.j.issn.1003-9406.2016.03.022

56 Yang H, Li J, Jiang L, Jiang X, Zhou X, Xu N: The rs878081 polymorphism of AIRE gene increases the risk of rheumatoid arthritis in a Chinese Han population: a case-control study. Braz J Med Biol Res 51(12): e7944, 2018. DOI: 10.1590/1414-431X20187944

57 A Moneim NH, Mansour MF, Omar HH, Fouad MM, Metwally L, El-Abaseri TB, Abdelnaby MM: Association of autoimmune regulator gene polymorphism with susceptibility to rheumatoid arthritis in Egyptian population. Immunol Res 68(2): 90-96, 2020. DOI: 10.1007/s12026-020-09127-7

58 Rose AB: Intron-Mediated Regulation of Gene Expression. Curr Top Microbiol Immunol 326: 277-290, 2008. DOI: 10.1007/978-3-540-76776-3_15

59 Pandya JM, Lundell AC, Andersson K, Nordström I, Theander E, Rudin A: Blood chemokine profile in untreated early rheumatoid arthritis: CXCL10 as a disease activity marker. Arthritis Res Ther 19(1): 20, 2017. DOI: 10.1186/s13075-017-1224-1

60 Han JH, Suh CH, Jung JY, Nam JY, Kwon JE, Yim H, Kim HA: Association of CXCL10 and CXCL13 levels with disease activity and cutaneous manifestation in active adult-onset Still's disease. Arthritis Res Ther 17(1): 260, 2015. DOI: 10.1186/s13075-015-0773-4

61 Ruth JH, Rottman JB, Katschke KJ Jr, Qin S, Wu L, LaRosa G, Ponath P, Pope RM, Koch AE: Selective lymphocyte chemokine receptor expression in the rheumatoid joint. Arthritis Rheum 44(12): 2750-2760, 2001. DOI: 10.1002/1529-0131(200112)44:12<2750::aid-art462>3.0.co;2-c

*Received October 25, 2023*
*Revised November 21, 2023*
*Accepted November 22, 2023*