SCIENTIFIC EMINENCE GROUP

Research article

Open Access

Journal of Pharmacogenetics and Precision Medicine

JPPM@scientificeminencegroup.com

# Cytokine *IL-4* Polymorphisms as Predictors of Rheumatoid Arthritis Risk

**Li Wang[1,2,*], Xuguang Li[1,2,*], Xiaoli Liu[1,2], Huqiang Mai[4,*], Liang Wang[1,2], Hengxun Zhang[1,2,3], and Xuemei Li[1,2]**

[1]*Key Laboratory of Molecular Mechanism and Intervention Research for Plateau Diseases of Tibet Autonomous Region, School of Medicine, Xizang Minzu University, Xianyang 712082, Shaanxi, China*

[2]*Key Laboratory of High Altitude Hypoxia Environment and Life Health, School of Medicine, Xizang Minzu University, Xianyang 712082, Shaanxi, China.*

[3]*Department of Emergency, the Affiliated Hospital of Xizang Minzu University, Xianyang, Shaanxi 712082, China*

[4]*Trauma center of Dongfang people's Hospital, Dongfang, Dongfang, Hainan 572600, China*

## [*]Corresponding Author

[*] Li Wang, Key Laboratory of Molecular Mechanism and Intervention Research for Plateau Diseases of Tibet Autonomous Region, School of Medicine, Xizang Minzu University, Xianyang 712082, Shaanxi, China, Tel/Fax: +86-29-33755247, E-mail: wangli_xzmd@163.com

Xuguang Li, Key Laboratory of Molecular Mechanism and Intervention Research for Plateau Diseases of Tibet Autonomous Region, School of Medicine, Xizang Minzu University, Xianyang 712082, Shaanxi, China, Tel/Fax: 13149269912, E-mail: 792492066@qq.com

## Citation

Li Wang, Xuguang Li, Xiaoli Liu, Huqiang Mai, Liang Wang et al. (2023) Cytokine IL-4 Polymorphisms as Predictors of Rheumatoid Arthritis Risk. J Pharmacogenet and Precis Med 2: 1-7

## Publication Dates

Received date: May 15, 2023
Accepted date: June 15, 2023
Published date: June 19, 2023

## Abstract

**Background:** Although the relationships between several single nucleotide polymorphisms (SNPs) in IL-4 and rheumatoid arthritis (RA) risk has been examined, the associations between other SNPs in IL-4 and RA risk remains unknown and could not be deduced. Therefore, we explore the relationships between four IL-4 SNPs (rs2227284, rs2243267, rs2243270, and rs2243283) and RA risk in the Chinese Han population.

**Methods:** We genotyped the four SNPs from 493 RA patients and 493 healthy individuals by the Agena MassARRAY platform. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by logistic regression analysis to estimate the relationships between SNPs and RA risk. The interactions between SNP and SNP was analyzed using multifactor dimensionality reduction (MDR).

**Result:** Overall analysis found that three IL-4 SNPs (rs2227284, rs2243267 and rs2243270) were associated with decrease risk of RA. Stratification analysis indicated that rs2243267 and rs2243270 were correlated with reduced the risk of RA in female, age < 55, BMI < 24, drinking, and smoking; rs2227284 was associated with RA susceptibility in BMI < 24 and smoking; rs2243283 was associated with increased risk of RA in smoking.

**Conclusion:** Moreover, the four SNPs had strong linkage and interactions on RA risk. Our findings suggested that the four IL-4 SNPs (rs2227284, rs2243267, rs2243270, and rs2243283) serve as risk predictors of RA in the Chi-

nese Han population, and highlight the importance of considering heterogeneity in genetic association studies of RA risk.

**Keywords:** *IL-4*; Snps; Rheumatoid Arthritis; Risk; Mass Array

## Introduction

Rheumatoid arthritis (RA) is one of the most common chronic multifactorial inflammatory, systemic and autoimmune diseases, characterized by synovial inflammation and joint destruction leading to tissue damage, functional impairment, severe disability, and premature mortality [1,2]. Account for 0.5%-1% of the peoples affected by RA worldwide, and the age-standardised prevalence and disability adjusted life years rates of RA increased with age and were higher in females [3]. RA is more common in women, which are two to three times more prone to develop the disease than men [4]. Although the etiology of RA has not yet been clarified, significant amount of studies suggesting that RA is a complex disease influenced multiple genetic factors, environment factors, as well as gene-environment interaction, gender, infection, and immune system [5-8]. Recently, studies found that many cytokine genes variants were correlated with RA risk, as cytokines play a vital role in the pathogenesis of RA [9-11].

Interleukin 4 is encoded by the *IL-4* gene located in chromosome 5 (q31–33), which is produced primarily by activated Th2 type CD4+ T cells, monocytes, mast cells and basophilic granulocyte. IL-4 is a cytokine with various biological functions that induces immunoglobulin IgE production in B lymphocytes and serves as an important regulator of IgG isotype switching [12,13], and regulates the differentiations of precursor T helper cells into these of the Th2 subset that mediate humoral immunity and modulate antibody production [14]. IL-4 has an immunomodulatory effect on RA, which is a small cytokine with potential for RA therapy [15-17]. Numerous studies have been reported that the SNPs in *IL-4* were correlated with RA risk [9,18,19] .

Despite the relationships between several SNPs in *IL-4* and risk of RA has been examined, the relationships between other *IL-4* polymorphisms with RA risk in the Chinese Han population remains unknown and could not be deduced. Therefore, we enrolled 493 patients with RA and 493 healthy controls to explore the relationships between four SNPs (rs2227284, rs2243267, rs2243270, and rs2243283) in *IL-4* and susceptibility to RA in the Chinese Han population.

## Materials and Methods

### Sample size calculation and collection

The G*Power software was used to estimate the sample size of the case and control groups by independent samples T-test, and the parameters were set as: Tail = two, Effect size = 0.23, $\alpha = 0.05$, Power = 0.95, Allocation ratio = 1. We recruited 493 patients with RA and 493 healthy controls from the Affiliated Hospital of Xizang Minzu University. The RA patients were diagnosed according to the 2010 American College of Rheumatology (ACR) classification criteria for RA [20]. All RA patients were assessed according to the Disease Activity Score (DAS28) for 28 painful/swollen joints [21]. The healthy controls were randomly recruited from the general health examinations of the Affiliated Hospital of Xizang Minzu University during the same period. The controls with family history of autoimmune diseases or chronic inflammatory disease were excluded in this study. Simultaneously, we collected the basic information of all subjects, including age, gender, height (cm), weight (kg), body mass index (BMI, $kg/m^2$), status of smoking and drinking. Both patients with RA and these controls are genetically unrelated Chinese Han population.

### Ethical approval

The present study was approved by the Ethics Committee of the Affiliated Hospital of Xizang Minzu University, and was conducted according to the principles of the Declaration of Helsinki. All the participants signed written informed consents for blood samples collection and subsequent analysis at recruitment. All the participants included in this study provided written informed consent.

### DNA Extraction

We collected peripheral blood (5mL) from each patient with RA and control in vacutainers containing Ethylene diamine tetraacetic acid (EDTA) anti-coagulant. We used the Gold-Mag-Mini Whole Blood Genomic DNA Purification Kit (GoldMag. Co. Ltd., Xi'an, China) to extract genomic DNA from the whole blood samples, according to the manufacturer's specifications. The purity and concentration of the extracted DNA were tested by Nanodrop 2000 spectrophotome-

ter (Thermo Fisher Scientific, Waltham, MA, USA). Then, the DNA was stored at -20°C until further experiment use.

### Selection of Snps and Genotyping

We selected the interest gene (*IL-4*) based on published knowledge about the importance in the immune pathway and RA development [15, 16]. The four SNPs in the *IL-4* gene (rs2227284, rs2243267, rs2243270, and rs2243283) that were not reported to be associated with RA susceptibility were selected based on previously published articles [22-24], and the minor allele frequency (MAF) of these four SNPs is more than 0.05 in the global population from the 1000 Genomes Project database. The primers designed by the online software Agena Bioscience Assay Design Suite Version 2.0 (https://agenacx.com/online-tools/) were used to amplify the genomic DNA through PCR reaction. The four *IL-4* polymorphisms genotyping were performed using Agena MassARRAY platform with iPLEX gold chemistry (Agena Bioscience, San Diego, CA, USA), according to the manufacturer's instructions. The data management and analysis of genotyping results used the Agena Bioscience TYPER software (version 4.0).

### Statistical Analysis

The distributions of continuous and categorical variables between patients with RA and healthy controls were compared using the independent sample T-test and Pearson Chi-Square test, respectively. Genotype frequencies of the four SNPs in *IL-4* in controls were assessed for Hardy-Weinberg equilibrium (HWE) using chi-square test. The relationships between alleles, genotypes and haplotypes of the four SNPs in *IL-4* and susceptibility to RA was evaluated based on the odds ratio (OR) and 95% confidence interval (95% CI) calculated by logistic regression analysis in the PLINK software (version 1.07). We used the Haploview 4.2 software to conduct linkage disequilibrium (LD) haplotype block and evaluated the strength of linkage between each pair of SNPs based on D' and r-squared values. We used the multifactor dimensionality reduction (MDR) software (version 3.0.2) to analysis the SNP-SNP interactions on the risk of RA. Statistical analysis was performed using Microsoft Excel (Microsoft Corp., Redmond, WA, USA) and Statistical Package for the Social Sciences (SPSS) version 20 (SPSS, Chicago, IL). All statistical analyses were two sided and the *P* values less than 0.05 were considered as statistically significant.

## Results

The baseline characteristics of 493 patients with RA and the 493 healthy control individuals are described in Table 1. The average age, height, weight and BMI of the patients with RA were 54.35 ± 11.95 years old, 162.03 ± 5.91 (cm), 59.14 ± 8.78 (kg), and 22.46 ± 2.53 (kg/m$^2$), respectively; and the mean age, height, weight and BMI of the control group were 54.02 ± 8.84, 162.11 ± 7.50 (cm), 59.55 ± 11.34 (kg), and 22.48 ± 2.74 (kg/m$^2$), respectively. The t test results showed that there were no significant differences in age ($P = 0.615$), height ($P = 0.850$), weight ($P = 0.525$) and BMI ($P = 0.891$) between the case and control groups. The male subjects of patients with RA and healthy control group were 134 (27.2%) and 124 (25.2%), respectively. The female subjectswere 359 (72.8%) in the patients with RA and 369 (74.8%) for the healthy control group. In addition, we classified age and BMI according to the median age (55 years old) and Chinese BMI classification criteria (24kg/m$^2$). The chi-square test results showed that the distribution of gender, age and BMI, smoking, and drinking between RA patients and controls were adequately matched ($P = 0.469$, $P = 0.899$, $P = 0.627$, $P = 0.792$, and $P = 0.943$, respectively).

The basic information and allele frequency distribution of the four SNPs in *IL-4* in the patients with RA and healthy control subjects are demonstrated in Table 2. The chi-square test results indicated the genotype frequency distribution of the four SNPs in *IL-4* was in accordance with HWE in the control group ($P > 0.05$). The frequencies of rs2243267 allele G and rs2243270 allele A were significantly higher in the control group than in the case group ($P = 0.039$), as shown in Table 2. The logistic regression analysis results indicated that both rs2243267 and rs2243270 were significantly correlated with reduced risk of RA (OR = 0.79, 95% CI: 0.64–0.99).

We performed genetic models analysis to further explore the associations between four SNPs in *IL-4* with RA susceptibility, as shown in Table 3. Our results found that the carriers of the genotype GG of rs2227284 were related with the reduced of RA risk compared to the TT genotype before and after adjusting for sex, age, BMI, smoking and drinking (OR = 0.26, 95% CI: 0.07–0.95, $P = 0.041$). The SNP rs2227284 was also found to be correlated with RA susceptibility under the recessive model (GG vs. TT-GT: OR = 0.27, 95% CI: 0.07–0.97, $P = 0.026$). Moreover, we also found that rs2243267 (GG vs. CC:

OR = 0.26, 95% CI: 0.10–0.64, *P* = 0.004; GG vs. CC-GC: OR = 0.27, 95% CI: 0.11–0.66, *P* = 0.002; Log-additive: OR = 0.78, 95% CI: 0.62–0.98, *P* = 0.031) and rs2243270 (AA vs. GG: OR = 0.26, 95% CI: 0.10–0.64, *P* = 0.004; AA vs. GG-AG: OR = 0.27, 95% CI: 0.11–0.66, *P* = 0.002; Log-additive: OR = 0.78, 95% CI: 0.62–0.98, *P* = 0.031) had significantly decreased risk of RA under the codominant, recessive, and Log-additive model.

In order to reduce the potential influence of confounding factors (age, gender, BMI, smoking, and drinking) on the results, we conducted stratification analysis (Table 4). The two SNPs (rs2243267 and rs2243270) were found to be significantly associated with reduced risk of RA in female (Allele: OR = 0.72, 95% CI: 0.56–0.94, *P* = 0.013; Codominant: OR = 0.15, 95% CI: 0.04–0.53, *P* = 0.003; Recessive: OR = 0.16, 95% CI: 0.05–0.56, *P* = 0.001; Log-additive: OR = 0.70, 95% CI: 0.54–0.92, *P* = 0.010) , age < 55 (Codominant: OR = 0.20, 95% CI: 0.04–0.96, *P* = 0.044; Recessive: OR = 0.20, 95% CI: 0.04–0.98, *P* = 0.023), BMI < 24 (Allele: OR = 0.68, 95% CI: 0.52–0.89, *P* = 0.005; Codominant: OR = 0.12, 95% CI: 0.03–0.54, *P* = 0.005; Dominant: OR = 0.69, 95% CI: 0.50–0.94, *P* = 0.018; Recessive: OR = 0.13, 95% CI: 0.03–0.59, *P* = 0.001; Log-additive: OR = 0.65, 95% CI: 0.49–0.86, *P* = 0.003), drinking (Allele: OR = 0.62, 95% CI: 0.43–0.90, *P* = 0.010; Codominant: OR = 0.09, 95% CI: 0.01–0.77, *P* = 0.027; Dominant: OR = 0.65, 95% CI: 0.42–1.00, *P* = 0.048; Recessive: OR = 0.11, 95% CI: 0.01–0.85, *P* = 0.006; Log-additive: OR = 0.61, 95% CI: 0.41–0.90, *P* = 0.011) and smoking subgroups (Allele: OR = 0.54, 95% CI: 0.35–0.84, *P* = 0.005; Codominant: OR = 0.10, 95% CI: 0.01–0.84, *P* = 0.034; Dominant: OR = 0.55, 95% CI: 0.33–0.91, *P* = 0.021; Recessive: OR = 0.12, 95% CI: 0.01–0.99, *P* = 0.013; Log-additive: OR = 0.53, 95% CI: 0.33–0.83, *P* = 0.005). However, no association between these two SNPs (rs2243267 and rs2243270) and RA susceptibility was found in the female, age ≥ 55, BMI ≥ 24, nonsmoking, and non-drinking subgroup. We found that rs2227284 was associated with RA susceptibility in BMI < 24 (Allele: OR = 0.70, 95% CI: 0.52–0.94, *P* = 0.016; Dominant: OR = 0.70, 95% CI: 0.50–0.98, *P* = 0.035; Log-additive: OR = 0.66, 95% CI: 0.48–0.91, *P* = 0.009) and smoking subgroups (Allele: OR = 0.62, 95% CI: 0.41–0.94, *P* = 0.022; Dominant: OR = 0.63, 95% CI: 0.39–0.99, *P* = 0.045; Log-additive: OR = 0.61, 95% CI: 0.40–0.94, *P* = 0.024). Moreover, rs2243283 was found to be associated with increased risk of RA in smoking subgroups (GG vs. CC: OR = 3.04, 95% CI: 1.15–8.03, *P* = 0.025; GG vs. CC-GC: OR = 2.93, 95% CI: 1.12–7.64, *P* = 0.020).

The linkage analysis constructed a linkage haplotype block including the four *IL-4* SNPs (rs2243250, rs2227284, rs2243267, rs2243270, and rs2243283) in strong linkage disequilibrium with each other (Figure 1). However, no association was found between haplotypes of *IL-4* and RA susceptibility (Table 5).

The Dendrogram and Fruchterman-Reingold described the interactions between these SNPs, as shown in Figures 2. The results of the MDR analysis of the SNP-SNP interactions are demonstrated in Table 6. The results showed that the best model was four loci (rs2227284, rs2243267, rs2243270, and rs2243283) (Bal. Acc. CV Training = 0.536; Bal. Acc. CV Testing = 0.507; CVC =10/10; Testing Sensitivity = 0.605; Testing Specificity = 0.410; OR = 1.36; 95%CI: 1.04–1.78; *P* = 0.024).

## Discussion

To investigate other *IL-4* polymorphisms whether influence the susceptibility to RA in the Chinese Han population, we designed a case-control association study consisting of 493 patients with RA and 493 healthy controls. Overall analysis revealed that there SNPs (rs2227284, rs2243267 and rs2243270) in *IL-4* were correlated with decreased risk of RA. Stratification analysis results indicated that the two SNPs (rs2243267 and rs2243270) were found to be significantly associated with reduced risk of RA in female, age < 55, BMI < 24, drinking, and smoking subgroups. Moreover, rs2227284 was associated with RA susceptibility in BMI < 24 and smoking subgroups; rs2243283 was found to be associated with increased risk of RA in smoking subgroups. The four *IL-4* SNPs (rs2227284, rs2243267, rs2243270, and rs2243283) had strong linkage and interactions on RA risk.

*IL-4* is a major effector cytokines produced by activated CD4 + T cells and it promotes the polarization of Th2 cells in vitro, which was originally identified as B-cell stimulating factor and regulates isotype class switching of B cells toward IgG1 and IgE [25,26]. *IL-4* stimulates several cells proliferation, differentiation and activation and it acts by inhibiting the synthesis of proinflammatory cytokines or stimulates the synthesis of several cytokine inhibitors [27,28]. It has been reported

that *IL-4* exerts great anti-inflammatory role in RA [29].

Previous studies have reported that rs2227284 was significantly associated with susceptibility to many diseases, such as lung cancer [24], colorectal cancer [30], allergic rhinitis [31,32]. The SNP (rs2227284) was found obviously associated with renal cell carcinoma (RCC) risk in nonsmoking and drinking subgroup [22]. Our study is the first to find a significant association between rs2227284 and RA risk in overall, BMI < 24 and smoking subgroups. The two *IL-4* polymorphisms (rs2243267, rs2243270) were found obviously associated with RCC in age < 55 and drinking subgroup [22]. In this study, rs2243267 and rs2243270 were significantly associated with reduced risk of RA in overall, female, age < 55, BMI < 24, drinking, and smoking subgroups. Furthermore, rs2243283 was associated with risk of steroid-induced osteonecrosis of the femoral head (ONFH) [23], esophageal squamous cell carcinoma [33], and idiopathic-nephrotic syndrome [34]. In smoking subgroups, we found rs2243283 was related to risk of RA.

These findings of this study stratified analysis may suggest that RA genetic susceptibility varies by sex, age, BMI, smoking, and drinking status, and highlight the importance of considering heterogeneity in future genetic association studies of RA risk. Previous studies have confirmed gender differences in the epidemiological and clinical manifestations of RA, and *IL-4* may be a potential gene responsible for the pathogenesis of RA and leads to differences in RA between women and men [35]. Smoking has been implicated as one of the most important extrinsic risk factors for RA development and severity [36]. A prospective Chinese cohort study found that increasing alcohol consumption was associated with an increased risk of RA in women [37]. A National Health and Nutrition Examination Survey (NHANES) and meta-analysis in USA showed that nearly 33% of RA incidence was attributed to smoking, high BMI and low alcohol consumption [38]. This study is the first to report the association between four SNPs (rs2227284, rs2243267, rs2243270, and rs2243283) in *IL-4* and susceptibility to RA in the Chinese Han population. Therefore, further studies are needed to validate the findings of *IL-4* as a biomarker for RA with large samples.

## Conclusion

In conclusion, our results suggested that four *IL-4* polymorphisms (rs2227284, rs2243267, rs2243270, and rs2243283) ware associated with risk of RA in Chinese Han population. However, this study is a pilot study and further studies with large samples are warranted to confirm the results and evaluate the significance of our findings in the clinic.

## Acknowledgements

The authors thank all participants for their support and participation.

## Consent for publication

Written informed consent was obtained from the patient for publication of this report.

## Funding

This work was supported by the Natural Science Foundation of Tibet Autonomous Region, China (No. XZ2018ZRG-74(Z)) and National Natural Science Foundation of China (No. 81960291) and “Major Project Cultivation Plan” of Xizang Minzu University (No. 20MDT01), and Xizang Minzu University intramural scientific research project (No. 19MDQ03).

### Ethics Statement

The present study was approved by the Ethics Committee of the Affiliated Hospital of Xizang Minzu University, and was conducted according to the principles of the Declaration of Helsinki. All the participants signed written informed consents for blood samples collection and subsequent analysis at recruitment. All the participants included in this study provided written informed consent.

## Conflict of Interest

The authors declare that they have no competing interests in this work.

## Author Contribution

Conception: Lw And Xgl; Interpretation Or Analysis Of Data: Xil And Hqm; Preparation Of The Manuscript: Hxz; Revision For Important Intellectual Content: Xml; Supervision: Lw.

## References


1. Aletaha D, Smolen JS (2018) Diagnosis and Management of Rheumatoid Arthritis: A Review. JAMA 320: 1360-72.

2. Sparks JA (2019) Rheumatoid Arthritis. Ann Intern Med 170: ITC1-ITC16.

3. Safiri S, Kolahi AA, Hoy D, Smith E, Bettampadi D, Mansournia MA et al. (2019) Global, regional and national burden of rheumatoid arthritis 1990-2017: a systematic analysis of the Global Burden of Disease study 2017. Annals of the rheumatic diseases 78: 1463-71.

4. Favalli EG, Biggioggero M, Crotti C, Becciolini A, Raimondo MG, Meroni PL (2019) Sex and Management of Rheumatoid Arthritis. Clinical reviews in allergy & immunology 56: 333-45.

5. Weyand CM, Goronzy JJ (2021) The immunology of rheumatoid arthritis. Nature immunology 22: 10-8.

6. Atzeni F, Masala IF, di Franco M, Sarzi-Puttini P (2017) Infections in rheumatoid arthritis. Curr Opin Rheumatol 29: 323-30.

7. Dedmon LE (2020) The genetics of rheumatoid arthritis. Rheumatology (Oxford) 59: 2661-70.

8. Klareskog L, Ronnelid J, Saevarsdottir S, Padyukov L, Alfredsson L (2020) The importance of differences; On environment and its interactions with genes and immunity in the causation of rheumatoid arthritis. J Intern Med 287: 514-33.

9. Yucel B, Sumer C, Gok I, Karkucak M, Alemdaroglu E, Ucar F (2020) Associations between cytokine gene polymorphisms and rheumatoid arthritis in Turkish population. North Clin Istanb 7: 563-71.

10. Agonia I, Couras J, Cunha A, Andrade AJ, Macedo J, Sousa-Pinto B (2020) IL-17, IL-21 and IL-22 polymorphisms in rheumatoid arthritis: A systematic review and meta-analysis. Cytokine 125: 154813.

11. Xie Q, Xu WD, Pan M, Lan YY, Liu XY, Su LC et al. (2021) Association of IL-35 expression and gene polymorphisms in rheumatoid arthritis. Int Immunopharmacol 90: 107231.

12. Brown MA, Hural J (2017) Functions of IL-4 and Control of Its Expression. Crit Rev Immunol 37: 181-212.

13. Junttila IS (2018) Tuning the Cytokine Responses: An Update on Interleukin (IL)-4 and IL-13 Receptor Complexes. Front Immunol 9: 888.

14. Kubo M (2021) The role of IL-4 derived from follicular helper T (TFH) cells and type 2 helper T (TH2) cells. Int Immunol 33: 717-22.

15. Dong C, Fu T, Ji J, Li Z, Gu Z (2018) The role of interleukin-4 in rheumatic diseases. Clin Exp Pharmacol Physiol 45: 747-54.

16. Chen Z, Bozec A, Ramming A, Schett G (2019) Anti-inflammatory and immune-regulatory cytokines in rheumatoid arthritis. Nat Rev Rheumatol 15: 9-17.

17. Spieler V, Ludwig MG, Dawson J, Tigani B, Littlewood-Evans A, Safina C et al. (2020) Targeting interleukin-4 to the arthritic joint. J Control Release 326: 172-80.

18. Sun YH, Wei ST, Zong SH (2017) Correlation between IL-4 gene polymorphismas well as its mRNA expressionand rheumatoid arthritis. European review for medical and pharmacological sciences 21: 3879-85.

19. Elshazli RM, Elsaid AM, Shawky DM, Barakat LAA (2019) Genetic polymorphisms of ACE I/D, IL-1beta G > A and IL-4 VNTR among Egyptian subjects with rheumatoid arthritis. Arch Physiol Biochem 1-10.

20. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO 3rd, et al. (2010) rheumatoid arthritis classification criteria: An American College of Rheumatology/European League Against Rheumatism collaborative initiative. Annals of the rheumatic diseases 69: 1580-8.

21. Wells G, Becker JC, Teng J, Dougados M, Schiff M, Smolen J et al. (2009) Validation of the 28-joint Disease Activity Score (DAS28) and European League Against Rheumatism response criteria based on C-reactive protein against disease progression in patients with rheumatoid arthritis, and comparison with the DAS28 based on erythrocyte sedimentation rate. Annals of the rheumatic diseases 68: 954-60.

22. Rong H, He X, Wang L, He Y, Kang L, Jin T (2017) Associ-

ations between polymorphisms in the IL-4 gene and renal cell carcinoma in Chinese Han population. Oncotarget 8: 82078-84.

23. Jin T, Zhang Y, Sun Y, Wu J, Xiong Z, Yang Z (2019) IL-4 gene polymorphisms and their relation to steroid-induced osteonecrosis of the femoral head in Chinese population. Molecular genetics & genomic medicine 7: e563.

24. Tan N, Song J, Yan M, Wu J, Sun Y, Xiong Z et al. (2019) Association between IL-4 tagging single nucleotide polymorphisms and the risk of lung cancer in China. Molecular genetics & genomic medicine 7: e00585.

25. Zhu J (2015) T helper 2 (Th2) cell differentiation, type 2 innate lymphoid cell (ILC2) development and regulation of interleukin-4 (IL-4) and IL-13 production. Cytokine 75: 14-24.

26. Yoshimoto T (2018) The Hunt for the Source of Primary Interleukin-4: How We Discovered That Natural Killer T Cells and Basophils Determine T Helper Type 2 Cell Differentiation In Vivo. Front Immunol 9: 716.

27. Sahoo A, Wali S, Nurieva R (2016) T helper 2 and T follicular helper cells: Regulation and function of interleukin-4. Cytokine & growth factor reviews 30: 29-37.

28. Ho IC, Miaw SC (2016) Regulation of IL-4 Expression in Immunity and Diseases. Adv Exp Med Biol 941: 31-77.

29. Iwaszko M, Bialy S, Bogunia-Kubik K (2021) Significance of Interleukin (IL)-4 and IL-13 in Inflammatory Arthritis. Cells10.

30. Wen X, Xin X, Li J, Qiao L, Liu F, Guo Y et al. (2020) The correlation between IL-4 polymorphisms and colorectal cancer risk in a population in Northwest China. Eur J Cancer Prev 29: 95-9.

31. Li Y, Chen J, Rui X, Li N, Jiang F, Shen J (2018) The association between sixteen genome-wide association studies-related allergic diseases loci and childhood allergic rhinitis in a Chinese Han population. Cytokine 111: 162-70.

32. Moh'd Al-Rawashdeh B, Sada Alhanjori A, Ali E, Zihlif M (2020) Association of IL-4 Polymorphisms with Allergic Rhinitis in Jordanian Population. Medicina (Kaunas)56.

33. Wang J, Chen T, Tang W, Kang M, Chen S (2021) Associations of interleukin-4 and interleukin-4 receptor loci with esophageal squamous cell carcinoma susceptibility. Int Immunopharmacol 97: 107659.

34. Li W, Li L, He L, Du Y, Fu HD, Peng ZY et al. (2022) Cytokine Gene Polymorphisms in Chinese Children with Idiopathic Nephrotic Syndrome. Iran J Immunol 19: 9.

35. Yu C, Liu C, Jiang J, Li H, Chen J, Chen T et al. (2020) Gender Differences in Rheumatoid Arthritis: Interleukin-4 Plays an Important Role. J Immunol Res 2020: 4121524.

36. Chang K, Yang SM, Kim SH, Han KH, Park SJ, Shin JI (2014) Smoking and rheumatoid arthritis. Int J Mol Sci 15: 22279-95.

37. VanEvery H, Yang W, Olsen N, Bao L, Lu B, Wu S et al. (2021) Alcohol Consumption and Risk of Rheumatoid Arthritis among Chinese Adults: A Prospective Study. Nutrients 13.

38. Ye D, Mao Y, Xu Y, Xu X, Xie Z, Wen C (2021) Lifestyle factors associated with incidence of rheumatoid arthritis in US adults: analysis of National Health and Nutrition Examination Survey database and meta-analysis. BMJ Open 11: e038137.