Identification of the unique and common genes for asthma and chronic obstructive pulmonary disease: a case-control study in Kazakhstan

**Abstract:** Asthma and chronic obstructive pulmonary disease (COPD) are lung inflammatory diseases characterized by bronchial obstruction, which is often the cause of difficulties in conducting differential diagnosis between them. They belong to the polygenic diseases, which arise in a result of the gene-environment interactions. Identification of the unique and common genes for these diseases facilitates understanding of their complicated pathogenesis, and provides possible markers for their diagnosis and treatment. The aim of this study was to investigate the association of two single-nucleotide polymorphisms (-1082 A/G \textit{IL-10} and His161Arg \textit{IL17F}) with asthma and COPD in Kazakh population. The study groups consisted of 72 COPD patients, 71 asthmatics and 70 control individuals. Genotyping was performed on purified DNA using real-time polymerase chain reaction with specific primers and probes. Results revealed that the G allele and GG genotype frequencies of -1082 A/G \textit{IL-10} polymorphism were significantly different between the COPD patients and the controls. Furthermore, the G allele and GG genotype of His161Arg \textit{IL-17F} were significantly more common in the COPD patients than among the control individuals (p<0.05). No significant associations were observed for any of these examined SNPs with asthma. These data suggest that the -1082 A/G \textit{IL-10} and the His161Arg \textit{IL-17F} polymorphisms are associated with COPD susceptibility in the Kazakh population, and may be considered as potential biomarkers of this disease.

**Key words:** chronic obstructive pulmonary disease, asthma, IL-10, IL-17F, genetic polymorphism, Kazakhstan.

**Introduction**

Asthma and COPD are widespread diseases, leading to significant and constantly increasing economic and social damage [1-3]. Globally, about 300 million people suffer from asthma with 250,000 deaths per year [1]. According to rough estimates, from 0.2% to 37% of the population in different countries live with a diagnosis of COPD. Mortality from this disease varies from 3 to 111 deaths per 100,000 population, and unlike coronary heart disease and stroke this leading cause of death is still increasing [2; 3].

Asthma and COPD are characterized by airflow limitation, but the bronchial obstruction of COPD is irreversible and progressive. With a long-term presence of asthma, difficulties arise in the differential diagnosis of asthma and COPD due to the similarity of their clinical and functional parameters [4]. Various approaches are used for the diagnosis of asthma and COPD, with the definition of standard indicators, which do not always allow separation of these diseases. Unlike the vast number of proposed biomarkers, genetic polymorphisms have several advantages, including lack of variability in one individual, availability of material and relative ease of detection.

In addition to the genes unique to asthma and COPD, there is a large number of common genes involved in both diseases [5]. The interleukin 10 (IL-10) gene encodes an anti-inflammatory cytokine expressed by many cell populations, including monocytes, Th-2 cells, mast cells, B cells, activated and regulatory T cells [6]. According to the studies [6; 7], -1082 A/G \textit{IL-10} is the most important functional promoter polymorphism, which is localized in the binding site of the transcription factor, and can alter the production and secretion of IL-10. There are only a few studies [8-10] of association between the -1082A/G SNP in IL-10 gene and COPD and their results are conflicting. Seifart et al. [8] found that the G allele of this polymorphism is significantly more...
common in COPD patients than in healthy controls. As well a correlation between this polymorphism and higher lung function was obtained in COPD patients with severe α1-antitrypsin deficiency [9]. However, He et al. [10] did not reveal any association for this SNP with lung function in COPD. To assess the contribution of IL-10-1082A/G polymorphism to asthma the three meta-analyses were conducted in 2012, 2013, and 2014 years, showing its involvement in atopic asthma, asthma in Asians and adults [6; 11; 12].

Interleukin 17F (IL-17F) is an important cytokine engaged in airway remodeling and steroid resistance in asthma and COPD [13-15]. Kawaguchi et al. conducted the functional study of the His161Arg IL-17F polymorph variant in vitro for the first time [16]. They demonstrated that the wild type His161Arg is unable to activate the signaling pathway in which one of the MAP kinases (ERK1/2) is phosphorylated, resulting in suppression of cytokines and chemokines expression in bronchial epithelial cells. Hizawa et al. were first to identify the participation of His161Arg IL-17F polymorphism in predisposition to asthma and COPD in 1125 unrelated Japanese individuals [17]. It should be noted that the study of this polymorphism in COPD alone has not been conducted. Currently the three meta-analyses of the His161Arg polymorphism in IL-17F gene with asthma were performed [18-20], but only one of them has shown the association with asthma susceptibility in Asian population.

The purpose of this work is to examine the association of -1082 A/G polymorphism in IL-10 gene and His161Arg polymorphisms in IL-17F gene in patients with asthma and COPD in Kazakh population.

Materials and methods

Blood samples and questionnaires were obtained from patients with asthma and COPD, residing in Astana and Akmola region of Kazakhstan for not less than 5 years. Sampling was carried out in the Pulmonary Department of the City Hospital № 2, Astana, Kazakhstan. 71 patients with asthma, 72 patients with COPD and 70 healthy individuals were enrolled in the study. Each subject gave written informed consent. The study was conducted in accordance with the WMA declaration of Helsinki. Demographical data and smoking status were assessed through a questionnaire.

The clinical diagnosis of asthma was made by the expert doctors according to the Global Strategy for Asthma (GINA) recommendations, based on clinical, laboratory and instrumental workup (https://ginasthma.org). 36 men (50.7%) and 35 women (49.3%) with the age range from 31 to 68 years were examined among patients with asthma. COPD diagnosis was established in accordance with the Global Strategy for the treatment and prevention of COPD (GOLD) (https://goldcopd.org) using validated questionnaires for assessment of breathlessness and symptoms: the modified British Medical Research Council (mBMRC) scale and the COPD Assessment Test (CAT). The COPD group consisted of 72 patients (37 men (51.4%) and 35 women (48.6%) aged 43-75 years. Patients with severe respiratory diseases, such as lung cancer, tuberculosis and cystic fibrosis were excluded from the study.

70 healthy volunteers (controls) living in Astana were recruited for the study. Individuals of the control group had no signs of airway obstruction (FEV1 and FVC ≥ 80%, FEV1/FVC ≥ 70%). The criteria for the selection of healthy subjects were the absence of neurological, autoimmune, allergic, endocrine and metabolic diseases, and the lack of family history of atopic and respiratory symptoms. All participants, including asthma and COPD patients, healthy individuals were ethnic Kazakhs. All three groups matched each other with respect to age and gender status.

Isolation of DNA. Genomic DNA was extracted from EDTA treated peripheral blood samples using the standard phenol-chloroform method [21]. Quantitative and qualitative assessment of the DNA was performed by gel electrophoresis and spectrophotometry.

IL-10-1082A/G and IL-17F His161Arg genotyping. The -1082A/G polymorphism in the IL-10 gene (rs 1800896) and the His161Arg polymorphism in the IL-17F gene (rs763780) were detected using the TaqMan Allelic Discrimination assay with proprietary TaqMan probes and primers, produced in the National Center for Biotechnology of the Republic of Kazakhstan.

All polymerase chain reactions were carried out with a reaction mixture, consisting of 16 µl of Master Mix and 3 µl of Primer Mix. To perform RT-PCR FAM and ROX dyes were used. 45 cycles of amplification were conducted. At first, denaturation step was carried out at 95°C for 3 minutes, followed by two step denaturation performed at 95°C for 10 seconds, annealing and elongation performed at 60°C for 40 seconds. FAM dye corresponds to the T allele, and the dye ROX matched to the C allele. The discrimination of genotypes was carried out using BioRad CFX manager 3.1 software. To validate the results of genotyping approximately 5% of the samples were
randomly selected and re-genotyped. A 100% double coincidence of genotyping was achieved.

Statistical data analysis. All statistical tests were carried out using GraphPad InStat 7 Software (Graphpad Software Inc., San Diego, CA). Differences in basic characteristics of the participants were determined using the Students’ unpaired T-test and the Chi-square test. Hardy-Weinberg equilibrium was assessed using the χ2 test comparing genotype frequencies among cases and controls. Odds ratio (OR), 95% confidence intervals, and two-tailed p values were calculated for assessing the risk of the variant genotype towards the development of asthma and COPD. Statistical significance was set at p<0.05.

Results and discussion

In recent decades, a lot of information has been produced on asthma and COPD, but there are still a lot of unclear questions about the inheritance mechanisms of these complex diseases. The formation of COPD is most often associated with smoking and the presence of bronchial obstruction in childhood, but only 10-20% of the lifetime incidence of COPD occurs in people with a long history of smoking [22]. The accumulation of asthma cases in families is observed, and the coefficient of its heritability can reach up to 60% [23]. This data demonstrates that heredity plays an important role in the development of asthma and COPD.

A large number of common genes are implicated in formation of asthma and COPD [24], but their involvement to asthma and COPD varies in different populations. We investigated the association between -1082A/G polymorphism in IL-10 gene and His161Arg polymorphism in IL-17F gene with asthma and COPD in Kazakh population.

In total, 213 participants were enrolled in this study. Demographic data and clinical characteristics of the study participants are shown in Table 1.

Table 1 – Characteristics of the study population

| Parameters                              | Asthmatics | COPD patients | Controls |
|-----------------------------------------|------------|---------------|----------|
| Number of study participants (N)        | 71         | 72            | 70       |
| % male                                  | 36 (50.7%) | 37 (51.4%)    | 35 (50%) |
| Age, M±m                                | 52.3±10.6  | 54.5±14.3     | 53.7±11.3|
| Work experience at harmful work place, M±m | 5.4±1.6    | 17±4.2        | 0        |
| Index of smoking (PY), M±m              | 1.3±0.5    | **39.6±14.3   | 1.8±0.3  |
| Smokers/former smokers, n (%)           | 9 (12.7%)  | ****48 (66.7%)| 12 (17.1%)|
| Baseline FEV1% predicted (SD)           | 89.4±17.3  | ****52.3±4.05 | 95.1 (±7.3)|
| FEV1/FVC (SD)                           | 0.76 (±0.13)| **0.54 (±0.09)| 0.82 (±0.03)|
| Family history of asthma, n (%)         | 32 (45.1%) | -             | -        |
| Age at onset of diseases (y; median, range) | 31; 0-64   | 57.3; 39-75  |          |

Note: *, **, ***,***, ****p value <0.05, 0.01, 0.001, 0.0001 when compared to the control group;

Genotyping of the polymorphisms in IL-10 and IL17F among the asthma patients

No significant differences were observed between the cases and controls in the age and gender. The forced expiratory volume in the first second of expiration (FEV1, % predicted) and FEV1/FVC (FVC – forced vital capacity) showed significant differences in the COPD patients compared with the control group. The COPD patients and controls differed significantly with regards to the smoking status, index of smoking and work experience at harmful work place. The controls in our study matched to Hardy-Weinberg equilibrium (IL-10 rs1800896, $\chi^2=0.09$, p=0.76; IL-17F rs763780, $\chi^2=0.33$, p=0.57).

Association of polymorphisms in IL-10 and IL17F genes with COPD. Tables 2 and 3 show the allele and genotype distributions of -1082 A/G IL-10 and His161Arg IL17F in COPD patients and controls. We observed that the G allele and GG genotype of -1082 A/G IL-10 polymorphism have a significantly more frequencies in the COPD patients compared to the controls (p=0.005 and p=0.009, respectively). A significant difference in frequencies of G allele (OR=2.77; CI=1.23–6.21) and GG genotype (OR=9.26; CI=0.49–175.33) was found between COPD patients and control subjects for IL-17F gene His161Arg SNP.
Table 2 – Allele frequency of His161Arg IL-17F polymorphism among COPD patients and normal Kazakh population

| Cytokine polymorphism | Allele | COPD | Control | Fisher exact p-value | Odds ratio | Wald’s 95% CI |
|-----------------------|--------|------|---------|----------------------|------------|---------------|
|                       |        | N    | F       |                      |            |               |
| IL-10                 | A      | 95   | 0.660   | 0.005                | 2.16       | 1.25 – 3.72   |
|                       | G      | 49   | 0.340   |                      |            |               |
| IL-17F                | A      | 121  | 0.840   | 0.01                 | 2.77       | 1.23 – 6.21   |
|                       | G      | 23   | 0.160   |                      |            |               |

Table 3 – Genotype distributions of His161Arg IL-17F polymorphism among COPD patients and normal Kazakh population

| Cytokine polymorphism | Genotype | COPD | Control | Fisher exact p-value | Odds ratio | Wald’s 95% CI |
|-----------------------|----------|------|---------|----------------------|------------|---------------|
|                       |          | N    | F       |                      |            |               |
| IL-10                 | AA       | 34   | 0.472   | 0.009                | 0.47       | 0.24 – 0.92   |
|                       | AG       | 27   | 0.375   |                      | 1.40       | 0.70 – 2.82   |
|                       | GG       | 11   | 0.153   |                      | 4.03       | 1.07 – 15.12  |
| IL-17F                | AA       | 53   | 0.736   | 0.02                 | 0.41       | 0.17 – 0.99   |
|                       | AG       | 15   | 0.208   |                      | 1.78       | 0.72 – 4.40   |
|                       | GG       | 4    | 0.056   |                      | 9.26       | 0.49 – 175.33 |

The main biological functions of IL-10 as expected are the limit and termination of inflammatory responses and the regulation of differentiation and proliferation of immune cells, such as T cells, B cells, natural killer cells, antigen-presenting, mast cells and granulocytes. It was demonstrated that IL-10 participates in Tregs mediated suppression of allergic reactions, and also promotes immune homeostasis in the lung tissue [25]. Low levels of IL-10 are associated with the pathogenesis of asthma and COPD [26]. Our results showed that the GG genotype and G allele of -1082A/G polymorphism in IL-10 gene are more common in patients with COPD and patients with the presence of this genotype have a higher risk of developing a disease. As mentioned above, only a few studies have been devoted to the finding of association between -1082A/G polymorphism in IL-10 and COPD, and our data matches them. Study of Seifart C. et al. involved 469 unrelated Caucasian German individuals, including 113 COPD patients; the statistical significance was achieved, when COPD-smokers were compared with the population control [8]. Besides, DeMeo et al. found the association of IL-10 -1082A/G polymorphism with higher lung function in COPD patients with ZZ genotype of α1-antitrypsin gene [9].

Association of Th17-lymphocytes with a number of neutrophils, macrophages and proinflammatory cytokines is seen in the airways of COPD patients. It is known that the effects of Th17-lymphocytes are mediated by appropriate cytokines, including IL-17F [27]. Increasing the IL-17F level in the blood serum of COPD patients with acute exacerbation compared with stable COPD or control was obtained [28], as well as enhance of the IL-17F expression in local lung cells after cigarette smoke exposure in COPD patients [15]. We observed an association between His161Arg IL-17 polymorphism and COPD.

Genotyping of the polymorphisms in IL-10 and IL17F among the asthma patients. The genotypes and allele frequencies of the -1082A/G polymorphism in IL-10 gene and His161Arg polymorphism in IL17F gene are presented in Tables 4 and 5. None of the SNPs showed any association with asthma (p>0.05).

The alveolar macrophages of asthma patients produce significantly less of IL-10 [29]. Asthma severity is clearly dependent on IL-10 levels, which in return leads to the production of some pro-inflammatory cytokines, contributing to the formation of chronic inflammation in the lower respiratory tract [29]. Large number of associative studies of -1082A/G IL-10 polymorphism with asthma and its phenotypes were performed. The results of most of these studies were
summarized and evaluated in the three meta-analyses reported above, demonstrating the effect of this polymorphism on the development of asthma in adults, Asians and atopic asthma. However, we observed no significant associations between -1082A/G IL-10 polymorphism and asthma in Kazakh population.

### Table 4 – Allele frequency of -1082A/G L-10 polymorphism among asthma patients and normal Kazakh population

| Cytokine Polymorphism | Allele | Asthma | Control | Fisher exact p-value | Odds ratio | Wald’s 95% CI |
|-----------------------|--------|--------|---------|----------------------|------------|--------------|
|                       |        | N      | F       |                      |            |              |
| IL-10                 | A      | 109    | 0.768   | 113                  | 0.807      |              |
|                       | G      | 33     | 0.232   | 27                   | 0.193      | 0.42         |
|                       |        |        |         |                      | 0.79       | 0.45–1.40    |
| IL-17F                | A      | 133    | 0.937   | 131                  | 0.936      |              |
|                       | G      | 9      | 0.063   | 9                    | 0.064      |              |
|                       |        |        |         |                      | 0.98       | 1.02         |
|                       |        |         |         |                      |            | 0.39–2.64    |

### Table 5 – Genotype distributions of -1082A/G L-10 polymorphism among asthma patients and normal Kazakh population

| Cytokine Polymorphism | Geno-type | Asthma | Control | Fisher exact p-value | Odds ratio | Wald’s 95% CI |
|-----------------------|-----------|--------|---------|----------------------|------------|--------------|
|                       |           | N      | F       |                      |            |              |
| IL-10                 | AA        | 43     | 0.606   | 46                   | 0.657      |              |
|                       | AG        | 23     | 0.324   | 21                   | 0.300      |              |
|                       | GG        | 5      | 0.070   | 3                    | 0.043      |              |
|                       |           |        |         |                      | 0.43       | 0.80         |
|                       |           |         |         |                      |            | 0.40–1.59    |
|                       |           |         |         |                      | 1.12       | 0.55–2.28    |
|                       |           |         |         |                      | 1.69       | 0.39–7.37    |
| IL-17F                | AA        | 62     | 0.873   | 61                   | 0.871      |              |
|                       | AG        | 9      | 0.127   | 9                    | 0.129      |              |
|                       | GG        | 0      | 0.000   | 0                    | 0.000      |              |
|                       |           |        |         |                      | 0.97       | 1.02         |
|                       |           |         |         |                      |            | 0.38–2.73    |
|                       |           |         |         |                      | 0.98       | 0.37–2.65    |
|                       |           |         |         |                      | 0.99       | 0.02–50.39   |

Numerous studies demonstrating the important role of IL-17F in the pathogenesis of asthma is increased. The expression of IL-17F is observed in a wide range of asthmatics cells, such as activated CD4+ T lymphocytes, basophils, mast cells, Th17 lymphocytes, bronchial epithelial cells, etc. [13]. IL-17F can increase the allergic inflammation in airways and contributes the formation of severe asthma due to the induction of the chemokine ligand 20 (CCL20) by Th17 lymphocytes [13]. Moreover, the role of IL-17F in exacerbations frequent of neutrophilic asthma was shown [30]. In our study, we did not identify an association between His161Arg IL-17 polymorphism and asthma.

Kawaguchi et al. [16] first showed that His161Arg SNP is a protective variant of the IL-17F gene against asthma in Japan. Du et al. [18] investigated the His161Arg polymorphism of the IL-17F gene in Han ethnicity individuals living in China. This study showed no significant differences between patients with asthma and control in addictive models with significant differences in allele models. Also in this study, a meta-analysis of His161Arg IL-17F SNP in Asian populations was conducted and this polymorphism indicated association with asthma susceptibility. In another meta-analysis, Ke et al. reported no association between the His161Arg polymorphism in the IL-17F gene and the predisposition to asthma [19]. Further, a meta-analysis of 11 single nucleotide polymorphisms of IL-17F showed no significant connection between this SNP and asthma susceptibility in a study, implemented by Yan Jin et al. [20].

Recent achievements of asthma and COPD genetics suggest that one gene does not play the significant role in the total amount of etiologic factors in these diseases, and, as it can be seen from our study, smoking is also an important factor in the development of COPD. In addition, summation of pathological agents may lead to an increase in susceptibility to diseases, and the necessary condition for its finding is the establishment of a group of involved genes, as well as a detailed description of patients with indication of ethnicity.

**Conclusion**

This study examined the involvement of the IL-10 and IL17F genes in development of asthma
and COPD in Kazakh population. We concluded that His161Arg polymorphism in IL-17F gene and -1082A/G polymorphism in IL-10 are associated with COPD and may serve as the differential markers for this disease in population of ethnic Kazakhs.

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