The analysis of associations between cytokine network genes and inverse co-morbidity of bronchial asthma and tuberculosis

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Abstract
In the context of the studies of genetic causes of extremely rare co-existence of atopic bronchial asthma (BA) and tuberculosis (TB), an association study was carried out for the polymorphisms of IL1B, IL8, IL10, TNF, TNFRSF1B, and CXCL10 genes was carried out in 713 individuals from West Siberia. An association between the rs56061981 polymorphism of CXCL10 gene with TB was found. Also, an association of the rs1800872 variant of IL10 gene with both BA and pulmonary TB was identified. The results suggest the importance of IL10 gene in the development of BA and TB. Further studies of the IL10 signaling pathway will clarify the mechanisms by which the inverse co-morbidity between infectious and allergic diseases develops.

Introduction
Co-morbidity, a simultaneous development of several pathologic conditions in one individual, is a common phenomenon in clinical practice [1]. It is a pressing issue in healthcare as the presence of a concomitant disorder would reduce the efficacy of the treatment and may exacerbate the course of the main disease. According to the concept of syntropy/dystropy (often or rare co-morbidity, respectively), an accumulation of co-morbidity in families suggest a non-random nature in the combination of the diseases in individuals and their relatives and, therefore, the importance of genes, called “syntropic genes”, which are responsible for their simultaneous development [3]. In the basis of co-morbidity are such factors as age of the patients and the effects of shared environmental factors [4], as well as pleiotropic effects of genes [5].

The issue of co-morbidity is a specific subject of genetic studies. Using an example of cardiovascular diseases continuum, including a concomitant manifestation of coronary artery disease, type 2 diabetes, arterial hypertension, and hypercholesterinemia, it was shown that genetic profile of such co-morbidity is remarkably different from the genetic component of the diseases manifesting alone [6].

Unlike co-morbid diseases, dystropic, or inversely co-morbid disorders, are rare in human populations [3,7]. Various factors can participate in the suppression of one disease in the context of the development of another, including so-called dystropic genes [2]. The hypothesis about shared genes that can orchestrate mutual repulsion of diseases is getting more support in recent studies [8]. For instance, based on the epidemiological observations about the decrease of cancer risk in patients with neurodegenerative disorders [9], a transcriptomic meta-analysis was carried out that showed an essential overlap between differentially expressed genes de-regulated in opposite directions [10].

In patients with epidemiologically approved dystrophy of osteoporosis and osteoarthritis, shared genes participating in apoptosis and osteogenesis exhibit remarkably differential expression [11].

In the context of dystropic diseases, it is interesting to approach the problem from the “benefit/harm” point of view. It suggests that the increase of the risk of one group of diseases (“the harm”) leads to the decrease of the risk of other group of diseases (“the benefit”) [12]. In this regard, the identification of genes, proteins, signaling pathways, and other factors that reduce the risk of a disease in the presence of another disorder, is important for preventing disease manifestation and detection of new drug targets.

Atopic BA and pulmonary TB is a useful model for the study of the mechanisms of mutual exclusion of diseases, because they are known to extremely rare co-occur in the same patient [13,14]. Earlier, using systems biology approach, we identified shared genes for these diseases [15]; the genes that can potentially influence the development of dystrophic relationships between them. Once the genes have been identified, the next logical step would be to study their association with the diseases of interest. Hereewith, we report the results of such the study aimed to carry out the analysis of association of polymorphisms of IL1B, IL8, IL10, TNF, TNFRSF1B, and CXCL10 genes with BA and TB in Russians of West Siberia.

Material and methods
The study was approved by the Ethical Committee of the Research Institute of Medical Genetics. Signed informed consent was obtained from all the participants. A total of 154 patients with atopic BA were
studied (mean age 41.1 ± 13 years) including 118 females and 36 males. TB patients comprised 304 individuals (30 ± 16.1 years) including 107 females and 197 males. The control group included 255 healthy individuals (44.8 ± 21.7 years) including 194 females and 61 males. All participants were ethnic Russians settled in the city of Tomsk or Tomsk Region.

Seven polymorphisms were studied: IL10 rs1800872, IL8 rs4073, IL1B rs16944, TNF rs1800629, TNFRSF1B rs52589, CXCL10 rs4386624 and rs56061981. Genotyping was carried out using either allele-specific real-time PCR with TaqMan\textsuperscript{\textregistered} assays (Applied Biosystems) or restriction fragments length polymorphism approach (Table 1).

Fishier's exact test was used to assess the polymorphisms for compliance with Hardy-Weinberg equilibrium. Association between the polymorphisms and diseases was established using logistic regression accounting for sex and age as covariates. Additive, dominant and recessive genetic models were tested and the best fit was chosen using Akaike Information Criteria (AIC) [16]. Monte-Carlo permutations were used to address the multiple testing issue. Permutation p-value <0.05 were considered statistically significant. The analyses were carried out in R statistical environment.

Results and discussion

In healthy individuals, all the polymorphisms were present in accordance with the expectation under Hardy-Weinberg equilibrium except for the rs525891 of TNFRSF1B (p = 0.014). An association between the IL10 polymorphism rs1800872 and the development of BA and TB was found (Table 2). In both cases, additive model provided the best fit (the only statistically significant in case of BA) according to the AIC values. This suggests the increased risks of BA and TB associated with the rs1800872*A allele: OR = 1.55 [1.06-2.27], p = 0.022 for BA and OR = 1.72 [1.16-2.56], p = 0.008 for TB.

As a key anti-inflammatory cytokine, IL-10 is a subject in studies of multiple diseases. It is produced by various immune system cells, including macrophages, B- and T-cells [17]. The major source of IL-10 are sub-populations of T-helper cells such as Th1-, Th2-, Treg- and Th17-cells [18]. IL-10 governs immunoregulation and inflammation, can block NF-κB transcription factor activity, is involved in regulation Th17-cells [18]. There are sub-populations of T-helper cells such as Th1-, Th2-, Treg- and Th17-cells [17]. IL-10 governs immunoregulation and inflammation, can block NF-κB transcription factor activity, is involved in regulation of JAK-STAT signaling pathways. It suppresses expression of pro-inflammatory cytokines IL-1\textsuperscript{\textbeta}, IL-6, IL-8, TNF\textalpha, IL-12 [19]. An inhibition of the function of antigen-presenting cells can be the major mechanisms by which IL-10 regulates antigen-specific populations of Th-cell, and adaptive immune response as a whole, to limit pathological condition [20].

Complex regulation of the expression of IL-10 complicates understanding the mechanisms by which this cytokine exhibits its biological effects in different diseases. In particular, in the case of infectious disease, anti-inflammatory capacity of IL-10 may lead to a paradox situation whereby the initiation of inflammation is critical for effective defence, but its over-activity can lead to the development of inflammatory, autoimmune and oncologic diseases [21]. This mechanism is of interest for the explanation of the possible role of the IL10 gene in the development of dystrophic relationships between BA and TB – effective defence against mycobacteria prevents from the development of TB, but increases the risk of BA due to expansion of the inflammatory environment. This hypothesis required further experimental validation.

Heritability estimates for IL-10 levels obtained in twin studies lie in between 50% to 75% [22] suggesting essential genetic contribution in manifestation of this trait. Selective pressure on the IL10 gene alleles by various pathogens during evolution allowed formation of different haplotypic blocks influencing the levels of production of this cytokine [23]. The studied rs1800872 polymorphism is a G-to-A substitution in promoter region of the gene; it is a part in the haplotype comprised of rs1800896, rs180087, and rs1800872 polymorphisms that possibly influence IL10 gene expression.

The data on what particular allele or genotype of the rs1800872 is associated with BA and TB is controversial. In one study, the rs1800872'C allele and CC genotype are the risk factors for BA [24], while in another it is the alternative rs1800872'A allele [25]. Same controversies are seen for association between IL10 and TB, too [26]. The current study demonstrated that the risk of both BA and TB is elevated by the rs1800872'A allele associated with the decreased production of the cytokine [27].

Another association revealed in the current study concerns the rs56061981 polymorphism of CXCL10 chemokine gene and TB (Table 2). Additive model was the best according to AIC, though dominant model was very similar. The genetic variant is a G-to-A substitution in promoter region of the gene; it is a part in the haplotype comprised of rs1800896, rs180087, and rs1800872 polymorphisms that possibly influence IL10 gene expression.

ChemoKines play crucial role in anti-bacterial defence as they serve as early inflammatory mediators in response to infectious agent

### Table 1. Primers and restriction enzymes for the studied polymorphisms.

| Gene (polymorphism) | Primers | Restriction endonuclease and restriction fragments |
|---------------------|---------|---------------------------------------------------|
| IL10 (rs1800872)    | F: 5'-gatccagtggagcaacacct | Rsu I A: 311+182; C: 493 |
|                     | R: 5'-aagaagttgcttgcgggg | |
| IL1B (rs16944)      | F: 5'-gccctccgtcgtgatgga | Amad7 I A: 222; G: 166+56 |
|                     | R: 5'-ggctagggtaacagcacct | |
| IL8 (rs4073)        | F: 5'-caagttaacccgtaacagt | Mfe I T: 222; A: 141+81 |
|                     | R: 5'-gagaaaccgatagccaca | |
| TNFRSF1B (rs525891) | F: 5'-catgaagactctctctgg | Hp6180III T: 347; A: 280+67 |
|                     | R: 5'-tgggtgcttgctga | |
| TNF (rs1800629)     | F: 5'-agcatacgattgggacgact | Bsp19 I A: 107; G: 87+20 |
|                     | R: 5'-tctcctgactctgactcg | |
| CXCL10 (RS4386624)  | * | Xba I G: 499; A: 325+174 |
| CXCL10 (rs56061981) | * | |
intervention [28]. Chemokines induce effector cell migration into the focus of infection. A response to infection differs depending on the levels of expression of CXCL10 gene [29], thus, its promoter variants influencing the affinity of the sites for transcription factor binding and, therefore, affecting the gene expression, are of interest for the studies of genetic ground of susceptibility to infectious diseases. Earlier, other promoter variants of CXCL10 gene were shown to be associated with TB [30].

Thus, the study revealed an association between rs1800872 polymorphism of IL10 both with BA and TB, suggesting the involvement of shared molecular pathways in their pathogenesis. Also, possible impact of the rs56061981 polymorphism of CXCL10 gene on the development of TB was established for the first time. The results are of interest both for the understanding of the mechanisms of the phenomenon of dystropy and for the delineating genetic factors of susceptibility to BA and TB alone.

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