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Molecular Relationships between Bronchial Asthma and Hypertension as Comorbid Diseases

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Abstract:
Comorbidity, a co-incidence of several disorders in an individual, is a common phenomenon. Their development is governed by multiple factors, including genetic variation. The current study was set up to look at associations between isolated and comorbid diseases of bronchial asthma and hypertension, on one hand, and single nucleotide polymorphisms associated with regulation of gene expression (eQTL), on the other hand. A total of 96 eQTL SNPs were genotyped in 587 Russian individuals. Bronchial asthma alone was found to be associated with rs1927914 (TLR4), rs1928298 (intergenic variant), and rs1980616 (SERPINA1); hypertension alone was found to be associated with rs11065987 (intergenic variant), rs2284033 (IL2RB), rs11191582 (NT5C2), and rs11669386 (CARD8); comorbidity between asthma and hypertension was found to be associated with rs1010461 (ANG/RNASE4), rs7038716, rs7026297 (LOC105376244), rs7025144 (intergenic variant), and rs2022318 (intergenic variant). The results suggest that genetic background of comorbidity of asthma and hypertension is different from genetic backgrounds of both diseases manifesting isolated.

Keywords: Comorbidity, Bronchial Asthma, Hypertension, eQTL, SNP
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1 Introduction

Epidemiological study provide evidence that some diseases tend to develop together more often that it would be expected by chance, suggesting shared pathogenetic mechanisms [1]. Various terms are used to describe this phenomenon, such as co-morbidity, syntropy, multi-morbidity. Examples of common comorbid diseases include allergic disorders (bronchial asthma, allergic rhinitis, atopic dermatitis) [2], autoimmune phenotypes of so-called “the kaleidoscope of autoimmunity” [3], cardiovascular diseases continuum [4].

Importantly, such combinations are common not only in an individual, but in their relatives, thus implying hereditary factors presented by pleiotropic, co-regulated syntropic genes involved in shared pathways [5].

Bronchial asthma, a common disease of airways, is often co-morbid with cardiovascular disorders [6], [7], [8], especially in women [9], and late-onset patients [10]; however, the increased rates of hypertension in asthma patients are observed in the vast majority of the studied populations [11].
Mechanisms underlying this common co-morbidity remain unknown. Among the discussed reasons of the development of hypertension in asthma patients are the decrease of lung function, systemic inflammation, impairment of smooth muscle tonus regulation, vascular remodelling, adverse effects of therapy, as well as genetic factors [12].

Systems biology provides ground for understanding molecular basis of pathogenesis of co-morbid diseases and offers novel view on diseases classification. Rzhetsky et al. built a phenotypic network of diseases including 657 using 1.5 mln clinical records based on the co-morbidity between the diseases [13]. Both positive and negative correlations between the diseases was identified, e.g. schizophrenia and bipolar disorder (positive correlation) or aorta aneurism and schizophrenia, breast cancer and bipolar disorder (negative correlations). Another approach that used both data on disorders and genes from OMIM catalogue allowed building a network of 867 diseases with genes associated with at least two disorders [14]. Protein interaction networks can also be used to determine molecular causes of co-morbidity [15].

We hypothesise that co-morbidity between asthma and hypertension is largely based on underlying genetic variation. Recently, we used a bioinformatics approach to prioritise genes that are likely important for the co-morbidity based on the analysis of associative networks [16]. To provide further support for the hypothesis, in the current study we set out to carry out the analysis of association between asthma, hypertension and their comorbidity and functional SNPs linked to variation in gene expression (eQTL) for the genes established in the previous study.

2 Materials and Methods

2.1 Study Groups

A total of 587 individuals from West Siberia (Tomsk Region, Russia) were studied (Table 1). Participants were included in the study in case they were not related to each other and were of self-reported European ancestry. Recruitment was carried out in clinical facilities of the city of Tomsk and the city of Seversk. Diagnoses of bronchial asthma and essential hypertension were defined based on clinical and laboratory investigations of the participants in accordance with WHO accepted criteria. All patients with arterial hypertension exhibited not clinical or laboratory symptoms and signs of secondary hypertension. Individuals with the presence of systemic complex diseases such as type 1 diabetes; obesity III-IV degree and cancer were excluded from analysis. Control group comprised healthy individuals with normal arterial blood pressure and without bronchial asthma.

Table 1: Characteristics of the study groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Sample size (%)</th>
<th>mean ± SD</th>
<th>Age, years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>range</td>
</tr>
<tr>
<td>Hypertension</td>
<td>144</td>
<td>44.9 ± 8.9</td>
<td>39–60</td>
</tr>
<tr>
<td>Males</td>
<td>97 (67.4 %)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>47 (32.6 %)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asthma</td>
<td>145</td>
<td>44.9 ± 8.9</td>
<td>28–68</td>
</tr>
<tr>
<td>Males</td>
<td>39 (26.9 %)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>106 (73.1 %)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension+Asthma</td>
<td>146</td>
<td>56.3 ± 10.5</td>
<td>26–75</td>
</tr>
<tr>
<td>Males</td>
<td>40 (27.4 %)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>106 (72.6 %)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>152</td>
<td>47.7 ± 9.9</td>
<td>31–74</td>
</tr>
<tr>
<td>Males</td>
<td>40 (26.3 %)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>112 (73.7 %)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>587</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SD, standard deviation.

The study was approved by the Bioethics Committee of the Research Institute of Medical Genetics; a full verbal explanation of the investigation was given and written informed consent was obtained from all participants.
2.2 Genetic Analyses

Total genomic DNA was isolated from peripheral blood by phenol/chloroform extraction method. A total of 96 eQTL SNP were selected and genotyped by MALDI-TOF mass-spectrometry using Sequenom MassARRAY iPLEX (USA). Genotypes were established by automatic analysis using MassARRAY Typer 4 software.

2.3 Statistical Analysis

Statistical analysis was carried out in R programming environment. The analysis of associations between the SNPs and diseases was carried out using logistic regression adjusting for age and sex. Additive genetic model was tested. The Hardy-Weinberg equilibrium (HWE) was tested using Fisher’s exact test. The significance threshold was set at \( p < 0.05 \).

3 Results and Discussion

We studied association of eQTL SNPs of genes (TLR4, CAT, IL10, CST3, ICAM1, IRF6, AKT1, NFkB1, PNP, SELL, CCL5, IL2RB, IDS, FOS, NT5C2, BHLHE40 and etc.) selected by bioinformatics analysis [16] with bronchial asthma, hypertension and their combination. A total of 96 SNPs were studied. All of the SNPs met the expectations under HWE. For six SNPs (rs17350806, rs4987367, rs4743691, rs2284368, rs7548533, and rs4743692) genotyping rate was <80%; therefore, they were excluded from the analysis.

3.1 Polymorphisms associated with bronchial asthma

Three polymorphisms were found to be associated with isolated bronchial asthma (Table 2). Two of them (rs1927914 and rs1928298) are located in a proximity to TLR4 and are in strong LD \( (r^2 = 0.97) \) [17]. They influence TLR4 expression in various cell types including blood cells and oesophagus smooth muscles [18], [19], [20]. The gene encodes a pattern-recognition receptor essential in innate immunity by transducing microbial signals, such as LPS of gram-negative bacteria. The role of innate immunity in bronchial asthma pathogenesis is not fully understood; however, in patients with neutrophilic asthma, TLR4 along with related protein TLR2, are highly activated [21]. These receptors are also thought to be linked to fatal asthma exacerbations [22]. In the literature, there are data about the impact of TLR family genes polymorphisms on susceptibility to various phenotypes of bronchial asthma [23], [24], [25].

<table>
<thead>
<tr>
<th>SNP ID (localisation, functional consequence)</th>
<th>Genotype</th>
<th>BA (%)</th>
<th>Hypertension (%)</th>
<th>BA+ Hypertension (%)</th>
<th>Control (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1927914 (TLR4, upstream variant)</td>
<td>AA</td>
<td>51 (35.7)</td>
<td>62 (43.4)</td>
<td>75 (51.7)</td>
<td>75 (51.4)</td>
</tr>
<tr>
<td></td>
<td>AG</td>
<td>82 (57.3)</td>
<td>68 (47.6)</td>
<td>61 (42.1)</td>
<td>57 (39.0)</td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>10 (7.0)</td>
<td>13 (9.0)</td>
<td>9 (6.2)</td>
<td>14 (9.6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.0397</td>
<td>0.5915</td>
<td>0.5571</td>
<td></td>
</tr>
<tr>
<td>rs1928298 (intergenic)</td>
<td>TT</td>
<td>52 (37.4)</td>
<td>61 (43.9)</td>
<td>73 (52.5)</td>
<td>74 (51.1)</td>
</tr>
<tr>
<td></td>
<td>TC</td>
<td>76 (54.7)</td>
<td>66 (47.5)</td>
<td>57 (41.0)</td>
<td>58 (40.0)</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>11 (7.9)</td>
<td>12 (8.6)</td>
<td>9 (6.5)</td>
<td>13 (8.9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.0442</td>
<td>0.7059</td>
<td>0.6119</td>
<td></td>
</tr>
<tr>
<td>rs1980616 (SERPINA1, intron variant)</td>
<td>CC</td>
<td>96 (67.1)</td>
<td>80 (56.3)</td>
<td>83 (57.2)</td>
<td>82 (56.9)</td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>44 (30.8)</td>
<td>53 (37.3)</td>
<td>55 (37.9)</td>
<td>54 (37.5)</td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>3 (2.1)</td>
<td>9 (6.3)</td>
<td>7 (4.8)</td>
<td>8 (5.6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.0462</td>
<td>0.7530</td>
<td>0.6592</td>
<td>0.7530</td>
</tr>
<tr>
<td>rs11065987 (intergenic)</td>
<td>AA</td>
<td>51 (36.2)</td>
<td>54 (38.8)</td>
<td>42 (29.2)</td>
<td>47 (31.5)</td>
</tr>
<tr>
<td></td>
<td>AG</td>
<td>63 (44.7)</td>
<td>69 (49.6)</td>
<td>69 (47.9)</td>
<td>36 (24.2)</td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>27 (19.1)</td>
<td>16 (11.5)</td>
<td>33 (22.9)</td>
<td>66 (44.3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.2922</td>
<td>0.0076</td>
<td>0.7872</td>
<td>0.0076</td>
</tr>
</tbody>
</table>
Another gene found to be associated with bronchial asthma in the current study, SERPINA1, encodes a serine protease inhibitor. The chromosomal region where the gene resides (14q32.13) is associated with the levels of IgE, bronchial hyperreactivity and other signs of allergic inflammation in different populations [26], [27], [28]. Gene expression profiling of epithelial cells stimulated by key allergy cytokines IL-4 and IL-13 showed most remarkable increase of expression of serpin family genes SERPINB3 and SERPINB4 [29]. Their role in the pathogenesis of bronchial asthma is not known, but they can exhibit protective effects via inhibiting endogenous proteases associated with inflammatory response.

### 3.2 Polymorphisms associated with hypertension

A polymorphism rs11065987 associated with hypertension in the current study have been found to be associated in genome-wide association studies (GWAS) with a number of phenotypes related to the risks of cardiovascular diseases, including lipid levels [30], [31] and BMI [32]. This variant is considered as the marker of the locus of susceptibility to ischemic stroke and ischemic heart disease [33].

Another variant associated with hypertension in the current study resides in IL2-receptor gene IL2RB, which takes part in T-cell mediated immune response. Previously, IL2RB gene expression was found to be correlated with diastolic blood pressure levels in a large sample of 7017 individuals [34].

The rs11191582 SNP associated with hypertension in the current study is located in the gene-reach region near CYP17A1-CNNM2-NT5C2. The region contains a remarkable number of regulatory SNPs associated with the development of cardiovascular disorders in GWAS studies [35]. The SNP rs11191582 is a cis-eQTL for NT5C2, USMG5, and hsa-mir-1307 genes that are abundantly expressed in the whole blood [18].
Another polymorphism associated with hypertension in the current study, rs11669386, is located in an intron of the caspase recruitment domain family member 8 gene (CARD8). The gene is involved in apoptosis, inflammation and NF-kappaB signaling. CARD8 along with NLRP3 controls activity of caspase-1 and is involved in caspase-1-mediated activation of IL-1β [36]. In turn, both caspase-1 and IL-1β are crucial players in the development of coronary pathology in mouse model of Kawasaki disease [37].

3.3 Polymorphisms associated with the comorbidity between bronchial asthma and hypertension

The polymorphism rs1010461 associated with the comorbidity, is located in intron 1 of angiogenin gene (ANG) and is an eQTL for ANG and RNASE4 genes with shared promoter. The proteins encoded by ANG and RNASE4 genes have 38.7% similarity and belong to ribonuclease family (RNase). Their major function is induction of neovascularization [38]. Angiogenic activity of these genes may be important for interpretation of their associations with the comorbidity. Angiogenin is induced in inflammation and exhibit wound-healing effects and microbicidal activity [39]. In patients with arterial hypertension, the decrease of angiogenin and fibroblasts growth factor levels is observed along with the increase of the levels of endostatin, vascular endothelial grown factor, and IL-8, suggesting a disbalance of pro-/anti-angiogenic factors in this condition [40]. Increased vascularisation promotes bronchial asthma pathogenesis and correlates with several signs of clinical course of the disease including bronchial hyperreactivity and airflow limitation [41]. The levels of angiogenin and vascular endothelial grown factor are increased during asthma exacerbation and decreased followed by corticosteroid treatment [42]. This raises a possibility that this is corticosteroid treatment that promotes the development of hypertension in genetically predisposed asthma patients.

The comorbidity between bronchial asthma and hypertension was associated with three SNPs known to be eQTLs for TLR4 gene according to GTEx database: two LD-bound polymorphisms rs7038716 and rs7026297 as well as intergenic rs7025144 SNP.

Finally, rs2022318 SNP, an eQTL for CAT gene was found to be associated with the comorbidity between asthma and hypertension. Catalase encoded by the gene is an important antioxidant enzyme preventing harmful effects of active oxygen species. As a result, polymorphisms of this gene are widely studied in diseases though to be linked with oxidative stress. Promoter SNPs for this gene were found to be associated with cardiometabolic diseases [43], [44], bronchial asthma [45], type 1 diabetes and other diseases [46].

4 Conclusion

The important observation from the current study is the difference in SNPs associated with isolated bronchial asthma and hypertension or their comorbidity, thus suggesting different genetic background of the comorbidity. On the other hand, considering associations of TLR4 SNPs, this gene variation may be important both for the development of isolated bronchial asthma and comorbidity of bronchial asthma and hypertension. The most pronounced impact on the development of comorbidity between bronchial asthma and hypertension can be attributed to the TLR4, CAT and ANG/RNASE4 genes, suggesting the importance of inflammation, neovascularisation, and oxidative stress in co-pathogenesis of both diseases. Importantly, according to our bioinformatics analysis, TLR4 and CAT genes have the highest priority in the development of bronchial asthma and hypertension comorbidity [16]. Consistently, we observed associations of these genes with the comorbidity, thus providing evidence for productivity in the use of bioinformatics approaches for the planning of human association studies.

Currently not enough of GWAS are published aimed at the study of comorbidity. These works aimed to study psychiatric diseases [47], [48]. Our study, unlike GWAS, focuses on specific SNPs of genes found using bioinformatics approaches that can cause comorbidity of asthma and hypertension. This study has limitations, including no correction for multiple testing, small sample sizes, wide age range of the patients, and no correction for the use of therapy. Thus, the results of the current study must be treated with caution and considered as a starting point for further development of studies in this direction.

Future large-scale studies, such as GWAS, are needed to elucidate the molecular mechanisms of asthma and hypertension comorbidity; however, this will require large samples to evaluate the effects of genetic variants with moderate and small effects.
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References


