79. Gene–environment treatment and asthma

P468
Associations and interactions of genetic polymorphisms in innate immunity genes with early viral infections and susceptibility to asthma and asthma-related phenotypes
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Background: The innate immune system is essential for host survival because it recognizes invading pathogens and mounts defensive responses.

Objectives: Identify genetic associations of innate immunity genes and interactions with early viral infections (first 12 months of life) and asthma phenotypes in a high risk birth cohort

Methods: Three Canadian family-based studies and one Australian population-based case control study (n=5565) were used to investigate associations of 321 single nucleotide polymorphisms (SNPs) in 26 innate immunity genes with: atopy, asthma, atopic asthma and airway hyper-responsiveness (AHR). Interactions between innate immunity genes and early viral exposure to three common viruses (parainfluenza, respiratory syncytial virus and picornavirus) were examined in the Canadian Asthma Primary Prevention Study using both family-based transmission disequilibrium test and case-control methods.

Results: IL1R2 and TLR1 SNPs were associated with atopy after correction for multiple comparisons. There is significant evidence that SNP*virus interactions with these same SNPs modifies the risk for atopic asthma and AHR in a high risk birth cohort. In addition, an NFKBIA SNP was associated with atopic asthma. All three viruses demonstrated a skew in the distribution of SNP*viral interactions (based on QQ plots) for AHR at 7 years of age. RSV was associated with an increased number of SNP*viral interactions for atopic and atopic asthma at 7 years of age.

Conclusion: We have identified novel susceptibility genes for asthma and related traits and interactions between these genes and early life viral infections.

P469
GLCCI1 rs37972 gene’s polymorphism correlation with response to oral glucocorticosteroids treatment in severe asthmatics from the BIOAIR cohort
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A significant association of SNP rs37972 in GLCCI1 in a genomewide study was identified in children with asthma. Our goal was to evaluate biomarkers and pharmacogenetic determinants that may improve effectiveness of treatment in adults with SA. Eighty five SA patients and 66 with mild-to-moderate asthma (MA) were included in the BIOAIR study. After optimization of treatment, they underwent a double-blind 2 week oral steroid intervention (prednisolone 0.5 mg/kg/day). DNA was extracted from whole blood and the rs37972 (C/T) polymorphism in the GLCCI1 gene was analyzed using TaqMan allelic discrimination on the ABI Prism 7500 detection system. The oral steroid intervention resulted in a significant increase in FEV1 (% predicted) in SA (66.6%, 95% CI 2.4 – 10.8, p=0.002, steroid treatment vs placebo) but not in MA (–0.5%, 95% CI –3.4 – 2.4, p=0.38) or COPD (1.2%, 95% CI –1.7 – 4.0, p=0.35) (p=0.02 between group comparisons). The responsiveness to oral steroids was significantly better in patients characterized by the highest blood eosinophils (>0.44x10^9/L), the highest sputum eosinophils (>4%), the lowest sputum neutrophils (<49%) and the highest FeNO (>40ppb).

A functional GLCCI1 variant was weakly associated with reduced improvement of lung function in response to glucocorticosteroids (p=0.05) and correlated significantly with the number of eosinophils in induced sputum and FeNO (p<0.05).

In the majority of SA patients, systemic steroid treatment induces an improvement in lung function. The positive response to oral steroids may be associated with certain genotype and phenotypic markers that may improve therapeutic decisions.

P470
Two single nucleotide polymorphisms in TSLP gene promoter region are associated with asthma susceptibility in Chinese Han population
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Background: Asthma is a chronic inflammatory disease of the airway. Thymic stromal lymphopoietin (TSLP) can aggravate asthmatic lung inflammation. Objective: To analyze the polymorphism of two single nucleotide polymorphisms (SNPs) Rs2289276 and Rs2289278 in TSLP gene promoter region and to evaluate the association between these SNPs and asthma susceptibility in Chinese Han population.

Methods: 531 asthmatic patients and 540 normal controls were collected and the genotypes of SNPs Rs2289276 and Rs2289278 were detected with polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLPs). The statistical analysis was performed with SPSS 19.0. A functional GLCCI1 variant was weakly associated with reduced improvement of lung function in response to glucocorticosteroids (p=0.05) and correlated significantly with the number of eosinophils in induced sputum and FeNO (p<0.05).

In the majority of SA patients, systemic steroid treatment induces an improvement in lung function. The positive response to oral steroids may be associated with certain genotype and phenotypic markers that may improve therapeutic decisions.

Table 1. Genotypic and allelic association analysis of TSLP promoter single-nucleotide polymorphisms in Chinese asthmatic study

<table>
<thead>
<tr>
<th>SNPs ID</th>
<th>Asthma</th>
<th>Control</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rs2289276</td>
<td>C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>240</td>
<td>292</td>
<td></td>
</tr>
<tr>
<td>CT</td>
<td>224</td>
<td>198</td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>46</td>
<td>31</td>
<td>0.009</td>
</tr>
<tr>
<td>C</td>
<td>704 (69.0%)</td>
<td>782 (75.0%)</td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>316 (31.0%)</td>
<td>260 (25.0%)</td>
<td>0.002</td>
</tr>
<tr>
<td>Rs2289278</td>
<td>C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>342</td>
<td>306</td>
<td></td>
</tr>
<tr>
<td>CG</td>
<td>156</td>
<td>181</td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>28</td>
<td>41</td>
<td>0.043</td>
</tr>
<tr>
<td>C</td>
<td>840 (79.8%)</td>
<td>793 (75.1%)</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>212 (20.2%)</td>
<td>263 (24.9%)</td>
<td>0.009</td>
</tr>
</tbody>
</table>

Rs2289278 C allele was correlated with decreased FEV1%VC (P=0.05).
Conclusions: TSLP variants are significantly associated with bronchial asthma. TSLP might be a new therapeutic target molecule for asthma.

P471
Association of TGFBI and IL4R-Dr2 gene polymorphisms with asthma
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There are components of the asthma phenotype that appear to be strongly heritable. We have studied certain SNP variations in a set of genes related to the pathogenesis and evolution of asthma in patients with severe asthma and their immediate relatives, as well as in a control group of non-asthmatics patients.

Aims of this study: 1. To determine the existence of common genetic characteristics in individuals suffering from severe asthma. 2. To study whether there are common genetic patterns in relatives of patients with severe asthma.

Method: We selected patients diagnosed of severe persistent asthma according to the criteria proposed by the ATS Consensus for Definition of Severe/Refractory Asthma and their first-line relatives, symptomatic or non-symptomatic, according to their responses to the European Community Respiratory Health Survey Questionnaire. A control group of non-asthmatic patients was also included.

We obtained saliva samples to study 10 different SNPs located on different genes related to asthma (CHD3L1: rs1905908; ADRB2: rs1042713, rs1042714; DENND1B: rs1775456; ORMD3: rs121678; TMC06: rs2561910; ADAM33: rs2280091; IL4: rs2243250; TGB1: rs1800469; ILAR: rs1801275), by TaqMan SNP Genotyping Assay.

Results: We analyzed samples from 150 patients diagnosed of persistent severe asthma, 49 controls, 69 asymptomatic relatives and 83 symptomatic relatives. We found significant differences (p < 0.05) between the group of patients and relatives with respect to controls in two pairs of the alleles studied: TGB1F1 rs1800469; IL4R-Dr2: rs1801275.

Conclusions: We found a higher prevalence of TGB1F1 rs1800469 (AA and AG) in patients with severe asthma and their relatives; and IL4R-Dr2: rs1801275 (AA) in asthmatics.

P472
Investigating the role of IREB2 genetic variants in susceptibility to COPD
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The IREB2 gene encodes the iron-binding protein 2, which is a major regulator of iron homeostasis. Several studies have shown that the IREB2 locus is a contributor to COPD susceptibility. Previously, we observed significant associations of seven IREB2 genetic variants with increased risk of COPD in a large case-control study. Subsequent in-silico analysis showed that six of these SNPs were in tight linkage disequilibrium with two variants that lie within the promoter (rs256070) and the 3’UTR (rs12890351) of IREB2 gene. The promoter SNP is predicted to disrupt the binding of two transcription factors while the 3’UTR SNP is located in a region that is predicted to be a target site of mir-1285 and mir-5096. The aims of this study were to evaluate the functional effect of these variants on IREB2 expression.

To test the effect of the promoter SNP, two fragments (one for each allele) of the 5’region of IREB2 were inserted upstream of the luciferase gene in the pGL3 Basic vector and then transfected into the A549 cells. Our results show that there was no difference in luciferase expression from cells transfected with rs256070 wild type construct compared with the risk allele under basal conditions. Further analysis will be undertaken to examine the effect of the rs256070 under different stimulatory conditions. For the 3’UTR SNP, two fragments (for both alleles) spanning the potential miRNA target site was cloned downstream of the luciferase gene in pmiGLO vector and then transfected into HeptG2 cells that are known to express mir-1285. Luciferase assay showed that mir-1285 did not recognize the cloned sequence. Additional investigation will consider the regulatory role of mir-5096 on IREB2 expression.

P473
Case-control association analysis of candidate genes in asthma, rhinitis and COPD: A preliminary report
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This study aims to determine the genetic involvement in the susceptibility to asthma, rhinitis and COPD, by candidate gene association analysis, in a large and accurately defined series of Italian subjects, even considering exposure to some environmental contexts and life-styles.

The study population included 1075 subjects (aged 20-66 years) from the general population, enrolled in the frame of the Gene Environment Interactions in Respiratory Diseases (GEIRD) study between 2007 and 2010. Cases and controls were diagnosed during a clinical examination that included a detailed interview, pre/post bronchodilator spirometry, methacoline challenge, skin prick tests.

A panel of 384 Single Nucleotide Polymorphisms (Tag-SNP), representative of 63 candidate genes with a previous indication of possible association to the studied diseases, was genotyped by a customized GoldenGate Genotyping assay. Presently, genotyping of 725/1075 subjects are completed. A preliminary association study of candidate gene polymorphisms was conducted on these data, for the susceptibility to one or more of the studied phenotypes, by basic association test based on allele frequency comparison. Presence of association (unadjusted p < 0.005) was observed between GTP1P and non-atopic rhinitis, PDE4D and ever asthma with atopy, IL13 and past-asthma, TNS1 and chronic bronchitis. Moreover, a possible association (unadjusted p < 0.02) was also found for IL1RL2 with ever asthma, chronic bronchitis, atopic rhinitis and non atopic rhinitis. The analysis is going on to complete the genotyping of all the enrolled subjects and to perform haplotype analysis, to confirm the involvement of these genes in the studied diseases.

P474
Association analysis of β2-adrenergic receptor gene polymorphisms (Arg16Gly and Gln27Glu) with asthma in the Volga-Ural region of Russia
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The β2-adrenergic agonists are the most potent bronchodilators for the treatment of asthma. Genetic variation in the ADRB2 gene has been hypothesized to have a role in differential response to beta-agonist (BA) therapy in asthma. Two polymorphic variants rs1042713 (Gly16Arg) and rs1042714 (Gln27Glu) were genotyped in 618 patients with physician-diagnosed asthma, aged 2-60 years (192 Russians, 139 Tatars, 82 Bashkirs and 205 mixed origins), and 366 nonasthmatic individuals (123 Russians, 91 Tatars, 51 Bashkirs and 101 mixed origins) from the Volga-Ural region of Russia. Genotypes were determined by the PCR-RFLP method. Data were analyzed using the chi-square test with Haploview software.

We found significant association of Gln27Glu polymorphism with mild decrease in FEV1 (< 80% of the predicted value) in Russian patients. The frequencies of genotype ADRB2*2*Gln27Glu and allele ADRB2*2*Gln were increased in asthmatics of this group compared to controls (p = 0.03, OR = 2.25 [95% CI 1.05-4.82] and p = 0.02, OR = 1.97 [95% CI 1.13-3.50], respectively). The analysis of Arg16Gly polymorphism showed significant association with moderate asthma in Tatars. The higher frequency of the ADRB2*16Gly/16Arg heterozygotes was revealed in the patients compared with controls (p = 0.02, OR = 2.25 [95% CI 1.15-4.03]). In summary, these results suggest an important role for polymorphisms of gene ADRB2 in the development of asthma in the Volga-Ural region of Russia.
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Gene polymorphisms, gene expression and inflammatory markers in preschool children with and without wheeze

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Background: Although exact mechanisms underlying preschool wheeze are largely unclear, immune responses are likely to be involved.

Aims and objectives: We employed an integrative genomic approach relating gene polymorphisms, mRNA expression, and products in exhaled breath condensate (EBC) of markers involved in airway inflammation to each other and to recurrent wheeze in preschool children.

Methods: From the Asthma DEMention and Monitoring (ADEM) study, 202 children with recurrent wheeze (≥2 episodes, ISAAC questionnaire) and 50 healthy controls aged 2-4 years (mean 3.2 years) were included. Genetic variants, gene expression and protein levels of interleukin (IL) 4, IL8, IL10, IL13, Tumour Necrosis Factor alpha (TNFa) and Intercellular Adhesion Molecule 1 (ICAM1) were analysed in saliva or buccal cells (DNA), blood (RNA), and EBC. Statistical analysis was performed by logistic and linear regression.

Results: A IL-18 polymorphism rs4098 A-allele was positively relative to recurrent wheeze (p=0.02) and to increased mRNA expression of ICAM1 (p=0.01) which in turn was positively associated with soluble sICAM-1 in EBC (p=0.04). sICAM-1 in EBC was elevated in recurrent wheezers (p=0.03). IL10 polymorphisms rs1800872 and rs1800896 were associated with decreased IL10 mRNA expression (p<0.01). In EBC levels of IL4, IL10, and IL13 in EBC were elevated in recurrent wheezers compared to healthy controls (all p<0.01).

Conclusions: We studied several inflammation markers at different levels which may allow causative interpretation. This study indicates that ICAM1 associates as a significant marker in preschool wheeze.

P476

The role of IL-18 level and genetic polymorphism in patients with bronchial asthma and its relation to disease severity

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Background: Asthma is a complex multifactorial disease with an obvious genetic predisposition, immunological aberration, and involvement of environmental factors. Interleukin -18 (IL-18) is a unique cytokine that enhances innate immunity and both Th1- and Th2-driven immune responses.

Objective: The aim of this case control study was to evaluate the role of IL-18 and genetic polymorphism in asthmatic patients, its relation to asthma severity.

Subjects and Methods: Thirty patients with bronchial asthma and Thirty normal controls were enrolled in this study, and were matched to asthmatic group concerning age, gender and body mass index. The IL-18 polymorphism was analysed using allele specific polymerase chain reaction (PCR), at positions +607 in the promoter of the IL-18 gene.

Results: There was statistical significant increase in serum IL-18 in severe asthmatic patients in relation to the other two asthmatic groups and control group (F=1105.259, p=0.0001*). There was no significant difference between asthmatic patients and control group in –607C>A, IL-18 Allele distribution or frequencies. No significant association was observed with various disease characteristics.

Conclusion: Although the –607C>A is IL-18 Allele genetic analysis was insignificant between asthmatic and controls, we found significant increase in serum IL-18 and degree of asthma severity. So our results need to be replicated by further studies possibly with other IL-18 genetic variants.

P477

Opisthorchis felineus as environment factor modify genetic risk of asthma

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Asthma is a complex disease resulting from interactions between multiple genes and environmental factors. One of the environmental factors that have influence in risk of development asthma are helminthes. The Sibiria is endemic region of O. felineus as environment factor modify genetic risk of asthma.

P478

The influence of gene-gene interaction on the development of respiratory distress syndrome in the neonates

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Background: The investigation of gene-gene interaction is necessary for understanding of pathogenic mechanism in the development of respiratory distress syndrome (RDS).

Aims and objectives: We employed an integrative genomic approach relating gene-gene interaction in RDS.

The aim of this study was to evaluate the genes influence and their complex effect on the RDS development.

Methods: We conducted a case-control study of 151 neonates with RDS and 110 healthy neonates. The ILD, A1166C, G308A, C677T polymorphism of ACE, AT2R1, TNF-a, MTHFR genes were detected using PCR and RFLP analysis. Statistical analysis was performed to assess the analyzed effects (program SPSS 17.0 and MDR 2.0).

Results: The frequency of all investigated genotypes with mutant allele (except AC genotype of AT2R1 gene) was significantly higher among RDS cases.

Figure 1. Distribution of investigated gene polymorphic variants.

The statistical model including all investigated genotypes had high predictive value (Percentage Correct=72.8). We have found negative entropy which indicated independent effects of each gene.

Figure 2. Interaction dendrogram for the RDS dataset.

Conclusion: The investigated genes polymorphism is independent risk factor for the RDS development. The further research with including other prognostic factors may be useful for new approaches to preventive strategy.

P479

Polymorphisms in genes associated with the development of steroids-induced adverse events

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Glucocorticoids are widely used in the therapy of lung interstitial diseases or severe asthma because of their anti-inflammatory properties. Their actions are mediated through an intracellular receptor. Steroid - receptor complex translates to a nucleus and binds DNA fragments referred to as glucocorticoids-response elements (GREs). Transcription regulation is associated with both therapeutic results and unwanted effects e.g. hypertension, diabetes, osteoporosis.
Aim of the study: The aim of this study was to evaluate the frequency of polymorphisms in the selected genes (ESR2, ATP1B1, AT2R, Hind III) that may be responsible for the development of stereotypes-related complications in the population of Kashubia (northern Poland).

Patients and methods: Blood samples were collected from 250 participants randomly chosen from the population of Kashubia region. Presence of polymorphisms was determined by the means of PCR in the following locations: AT2R-3123 A/C, osteocalcin-rs1800247 C/T, ESR2-rs4986938 A/G, ATP1B1-rs1916264 C/T. In statistical analysis χ² test was used to determine whether the genotypes were in Hardy-Weinberg equilibrium.

Results: For all the genes polymorphisms were found in the specified locations. The frequencies of variants were as follow: AT2R- AA 36%, AC 19%, CC 45%; HindIII (osteocalcin)- CC 9%, TC 31%, TT 60%; ESR2- AA 10%, AG 99%, GG 51%; ATP1B1- TT 1%, TC 19%, CC 80%. In ESR2, ATP 1B and osteocalcin genes variants were in Hardy-Weinberg equilibrium.

Conclusion: Polymorphisms in the genes that may be related to adverse events of steroids are frequent enough to continue to study on the associations between polymeric variants and presence of side effects of steroids.

P480
Is GLCCI1 associated with response to inhaled corticosteroids in asthma patients?
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Inhaled corticosteroids (ICS) are the primary anti-inflammatory therapy for the control and management of asthma, but their effects are characterized by inter-individual variability, especially in more severe asthma. Tantisira K.G. et al. (New Engl J Med 2011; 365: 1173) reported a pooled result (p=0.0007) for the association of the GLCCI1 polymorphism, rs37973, with change from baseline in percent of the predicted FEV₁, following 4-8 weeks of treatment in white asthma patients (n=935, both adults and children) to three different ICS. Association of rs37973 with change from baseline in trough FEV₁ (ΔFEV₁), was explored in white asthma patients (N=739) from three Phase 2B GlaxoSmithKline-sponsored clinical studies (GSK Study FFA109684 (NCT0063746); FFA109685 (NCT0063278); FFA109687 (NCT0063382)) treated for up to 8 weeks with fluticasone furoate (administered once daily, N=591) or fluticasone propionate (administered twice daily, N=198) monotherapy. Under a generalized linear model framework, an additive genetic effect for rs37973 on ΔFEV₁ was not detected (p=0.24). The upper and lower quartiles for the ΔFEV₁ distribution defined a binary response variable and logistic regression failed to identify a significant effect for rs37973 on ΔFEV₁ (p=0.25, OR=1.42, 95% CI: 0.78, 2.59). This odds ratio estimate is not statistically different from the previously reported value of 2.36. Thus, despite a substantial sample size and investigation of a single ICS, we were unable to confirm or refute an association of rs37973 with response to ICS. The results of several follow-up trials should clarify the role of GLCCI1 in steroid response in asthma.

P481
Influence of ADRB2 gene polymorphism on cold airway hyperresponsiveness and asthma control depending on inhaled glucocorticoids use
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Previously published data suggest an association between c.46A>G SNP of ADRB2 gene, functional activity of β2-adrenergic receptors (β2-AR) and cold airway hyperresponsiveness (CAH) in patients with bronchial asthma (BA).

Objective: We aimed to investigate the impact of inhaled glucocorticosteroids (ICS) on the clinical state and CAH in BA patients depending on the c.46A>G (rs1042713) variants of ADRB2 gene.

Methods: 136 mild to moderate Caucasian asthmatics with mean age 37±10 were recruited. All the patients were assessed for asthma control, previous ISC use and underwent 3-min anoxic cold air hyperventilation (ICAH) challenge. Genotyping of the SNP was performed by PCR-RFLP analysis.

Results: ICS users number and daily dosage were comparable among distinct genotypes. AA homozygotes who did not receive therapy proved to have lower ACT score (14 (11; 14) vs. 18 (16; 19), p<0.001) and excessive FEV₁ fall after the ICAH (-22 (-51; -15) vs. -2.2 (-6.8; 1.08), p<0.001) in comparison with GG homozygotes. On the contrary, ICS users did not show such associations. Relations of their CAH level and disease control to genotype were not statistically significant. CAH prevalence was greater among AA homozygotes and decreased substantially if they received ICS treatment (p=0.01) while therapeutic effect on CAH in GG patients was not so noticeable.

Conclusions: The obtained results indicate diminished activity of β2-AR in the examined AA carriers as they more frequently exhibited CAH and had poorer asthma control. ICS therapy can reverse depressed β2-AR function in spite of the genetically induced defect and therefore provides particular benefit in AA patients.