231. Asthma and the genes: from GWAS to next generation transcriptome analyses

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Meta-analysis of genome-wide association studies of single nucleotide polymorphisms in selected genes of the WNT signaling pathway
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Background: The WNT signaling pathway is involved in a wide range of developmental events and maintenance of homeostasis in adult tissue, including lung development and health. WNT signaling genes have also been suggested to play a role in pathogenesis of lung diseases such as chronic obstructive pulmonary disease (COPD) and asthma.

Aims and objectives: The aim of this meta-analysis was to identify consistent disease markers for COPD, asthma, forced expiratory volume in one second (FEV1), and forced vital capacity (FVC) in nine genes of the WNT signaling cascade pathway (WNT10b, WIF1, WISP1, SFRP2, SFRP5, DKK1, Axin2, TCF7L2, and FZD3) using genome-wide association data from six European cohort studies.

320s

Oral Presentation
Room Lehar 1-2 - 10:45 - 12:45
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Aims and objectives: This study investigates how genetic factors contribute to gender differences in asthma susceptibility. This study investigated if and how genetic factors contribute to gender differences in asthma susceptibility.

Results and conclusions: We identified weak genetic associations (p-values between 0.002 and 0.046) in our meta-analysis for COPD (Axxma), asthma (SFRP2, TCF7L2, FEV1, FVC), atopy (P14), and DMRT1 (WNT10b). Notably in TCF7L2 six different SNPs were identified (p-values between 0.002 and 0.046) in association with asthma, FEV1, and FVC. In literature, WNT signaling genes were linked to COPD (Axxma), asthma (TCF7L2, SFRP2), and decreased FEV1 and FVC (TCF7L2).

Interaction of retinoid acid receptor-related orphan receptor alpha (RORA) and neuropeptide S receptor 1 (NPSR1) with gender: A recent study by RORA and NPSR1 in the context of gender differences in asthma susceptibility needs to be elucidated further.

Genome-wide prediction of childhood asthma and related phenotypes in a longitudinal birth cohort

Methods: A GWAS dataset based on the MAGIC/SISAAC II study population (651 cases; 652 controls; 57% males; mean ± SD age 10.3±2.1 years) was analysed after gender stratification. Additional genotyping for fine mapping of the associated region was performed with MALDI-TOF MS on 7 polymorphisms. Allele-specific effects on protein-DNA interactions were studied with electrophoretic mobility shift assays (EMSA) and changes in promoter activity were investigated with dual-luciferase reporter (DLR) assays.

Results: The gender-specific genome-wide data analyses identified polymorphisms in the DMRT1 locus to be associated with asthma only in males. Additional fine mapping confirmed that rs3812523 located 1592 base pairs upstream of the first ATG shows strongest association (P<0.05; OR 0.57; 95% CI 0.37-0.84; p=0.05). Functional analyses by EMSA revealed allele-specific differences in binding of transcription factors c-h and c-Fos to the potential promoter site. DLR assays revealed significant, allele-specific differences in promoter activity in HEK293 cells.

Conclusions: Using GWAS data we identified genetic variants in DMRT1 to be gender-specific risk factors for asthma development. Allele-specific transcription factor binding and consecutive changes in promoter activity seem to contribute to this effect. The role of DMRT1 in asthma pathogenesis needs to be elucidated further.

8165
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8164
A polymorphism in DMRT1 is associated with asthma in boys only and affects promoter activity by allele-specific transcription factor binding

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8166
Genetic variations in the TLR signaling pathway are associated with childhood asthma

Methods: A GWAS dataset based on the MAGIC/SISAAC II study population (651 cases; 652 controls; 57% males; mean ± SD age 10.3±2.1 years) was analysed after gender stratification. Additional genotyping for fine mapping of the associated region was performed with MALDI-TOF MS on 7 polymorphisms. Allele-specific effects on protein-DNA interactions were studied with electrophoretic mobility shift assays (EMSA) and changes in promoter activity were investigated with dual-luciferase reporter (DLR) assays.

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Conclusions: Using GWAS data we identified genetic variants in DMRT1 to be gender-specific risk factors for asthma development. Allele-specific transcription factor binding and consecutive changes in promoter activity seem to contribute to this effect. The role of DMRT1 in asthma pathogenesis needs to be elucidated further.
Objectives: To systematically analyze the association between SNPs in TLR signaling pathway genes and childhood asthma and atopy.

Methods: Common SNPs present in TLR signaling pathway were retrieved from HapMap database and LD analyses were performed to determine tagging SNPs. Association of 375 tagging SNPs with asthma were analysed in a genome wide association (GWA) dataset consisting of 651 asthmatics and 652 controls. SNPs were genotyped by Illumina HumanHap300Chip (n=109) or MALDI-TOF MS (n=19) or imputed (n=187). Algorithms were applied to rank associations and clustering of the associated genes on a virtual pathway map was performed by a systems biology approach and we assessed the putative functional relevance of associated SNPs by in silico analysis.

Results: We identified 41 genes involved in the TLR signaling and regulatory pathways, harbouring 145 SNPs (tagged by 375) with minor allele frequency >5% in the HapMap (CEU) population. SNPs located in 19 genes showed association with asthma at a significance level of at least p<0.05. Top ranked asthma-associated genes (e.g. IRAK-1, MKK-3, and ERK-2) mapped to distinct functional clusters within the TLR pathway and associated SNPs were located in promoter (n=16), intronic (n=116) and downstream regions (n=16).

Conclusion: SNPs in TLR signaling network genes show association with asthma and distinct clusters are associated with atopic and non-atopic asthma.

1867 Genetic variants in Protocadherin-1, bronchial hyperresponsiveness and asthma subphenotypes in German children

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Background: Recently, Protocadherin-1 (PCDH1), located on chromosome 5q31-33, in the vicinity of the cytokine gene cluster containing several known candidate genes for asthma and allergy (interleukin-4, interleukin-13, interleukin-15 and RAD50), was reported as a novel susceptibility gene for bronchial hyperresponsiveness (BHR) and asthma.

Objectives: We aimed to define linkage disequilibrium (LD) between the region comprising PCDH1 and the cytokine gene cluster. Next, for a comprehensive analysis of the PCDH1 locus we conducted detailed fine mapping of the PCDH1 region to identify potential effects of single nucleotide polymorphisms (SNPs) in BHR, asthma and related phenotypes.

Methods: Genotype information was acquired from Illumina HumanHap300Chip genotyping, MALDI-TOF MS genotyping and imputation. Associations were investigated in a population of at least 1,303 (651 asthmatics) from two German study populations (MAGICs and ISAAC II).

Results: No relevant LD between PCDH1 tagging SNPs and 98 SNPs within the cytokine cluster were detected. There were no significant associations with BHR, atopy, allergic rhinitis and atopic eczema. However, rs7719391 was associated with asthma (OR=0.85, p=0.039) and non-atopic asthma (OR=0.69, p=0.009). The exonic SNP rs7970504 previously reported to be associated with BHR and asthma, was significantly associated with non-atopic asthma (OR=0.70, p=0.019) in our study. Significant associations with non-atopic asthma were observed also for rs11167761 (OR=1.54, p=0.021), rs2974704 (OR=1.32, p=0.039), rs2427829 (OR=1.54, p=0.021) and rs521121 (OR=1.54, p=0.021).

Conclusions: PCDH1 polymorphisms may specifically affect the development of non-atopic asthma in children. These authors contributed equally. Mrs. Antoaneta Tonceva, Dr. Kathrin Suttner, and Mr. Sven Michel.

1868 Igf levels in asthmatics and non-asthmatics are affected by different SNPs in FCERIA

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1Department of Pediatric Pulmonology, Allergy and Neonatology, Hannover Medical School, Hanover, Germany; 2Research Unit of Molecular Epidemiology, Helmholtz Zentrum, Munich, Neuherberg, Germany; 3Hannover Unifed Biobank, Hannover Medical School, Hanover, Germany; 4University Children’s Hospital, Technical University, Dresden, Germany; 5Pathology, VU University Medical Center, Amsterdam, Netherlands; 6Department of Respiratory Medicine, Academic Medical Centre, Amsterdam, Netherlands; 7Clinical Epidemiology and Biostatistics, Academic Medical Centre, Amsterdam, Netherlands; 8Genome Analysis, Academic Medical Centre, Amsterdam, Netherlands; 9Respiratory Medicine, Admiral De Ruyter Hospital, Goes, Netherlands; 10Children’s Department, University Teaching Hospital, Landeskrankenhaus, Feldkirch, Austria; 11University Children’s Hospital, University Children’s Hospital, Vienna, Austria; 12Institute of Epidemiology and Medical Biometry, Ulm University, Ulm, Germany; 13University Children’s Hospital, Ludwig Maximilians University, Munich, Germany; 14Visiting Visi Chiesi at Stand B2.10.

Rationale: The pathophysiology of asthma is largely unknown. RNA-Seq allows detailed biological characterization of the airways. We hypothesized that the airway transcriptome is different between asthma and controls.

Aim: We investigated: a) the differences in transcriptomic profiles of whole bronchial biopsies between steroid-naive asthma and controls; b) the feasibility to map RNA from airway smooth muscle (ASM) captured by laser microdissection (LCM) suitable for RNA-Seq analysis.

Methods: 4 biopsies per subject (asthma/control: aim a=n=4/n=5; aim b=n=2/n=12) were incubated in RNAlater. Whole cryosection or LCM-captured ASM was put on a glass slide. The slides were hybridized with biotin-labelled RNA probes against cDNA targets. A total of 7556 probe sets was present in each array. Signals were quantified and normalized. The resulting data was analyzed using GEPAS software.

Table 1. Sample characteristics

<table>
<thead>
<tr>
<th>Whole biopsy</th>
<th>ASM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>88</td>
</tr>
<tr>
<td>RNA</td>
<td>2-27</td>
</tr>
<tr>
<td>cDNA</td>
<td>18-168</td>
</tr>
</tbody>
</table>

1Reference = UCSC hg19; 2subject.
into TRIzol. cDNA was obtained with Ovation RNA-Seq System and prepared for RNA-Seq (GS FLX+, 454).

**Results:** Sample characteristics are shown in Table 1. The 46 differentially expressed genes between asthma and controls were assigned to networks associated with cell cycle, morphology, and development.

**Conclusion:** Transcriptomic profiles of whole biopsies were different between asthma and controls. LCM-captured ASM is suitable for RNA-Seq. Regulation of airway biological processes in asthma and controls tends to be fundamentally different. These findings may help develop targeted asthma therapy.