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

1862

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.

Methods: The six European cohort studies included are: B58C (UK), ECRHS (multicentre), EGEA (France), GINI/LISA (Germany), NFBC1966 (Finland), and SAPALDIA (Switzerland). We identified a total of 105 single nucleotide polymorphisms (SNPs) in the nine genes (including a region 2 kb in size up- and downstream the gene). Effect estimates were analyzed using a fixed or random effect pooled testing (depending on homogeneity) for association in the overall study population.
Results and conclusions: We identified weak genetic associations (p-values between 0.002 and 0.046) in our meta-analysis for COPD (Axin2), asthma (SFRP2, TCF7L2, WIF1, DKK1), FEV1 (SFRP2, TCF7L2, DKK1), and FVC (TCF7L2, 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 (Axin2), asthma (TCF7L2, SFRP2), and decreased FEV1 and FVC (TCF7L2).

1863
Genome-wide prediction of childhood asthma and related phenotypes in a longitudinal birth cohort
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Aim: Childhood asthma varies greatly in clinical presentation and time course and underlying disease pathways are assumed to be heterogenous. We assessed the extent to which single nucleotide polymorphisms (SNPs) associated with childhood asthma in a genome-wide association study (GWAS) are predictive of asthma-related phenotypes.
Method: In 8365 children from a population based birth cohort, the Avon Longitudinal Study of Parents and Children, allelic scores were derived based on between 10 and 215443 SNPs ranked according to inverse of the p-value for association with physician diagnosed asthma in an independent GWAS (6176 cases and 7111 controls). We assessed the predictive value of allelic scores for asthma-related outcomes at age 7-9 years (physician's diagnosis, early wheezing phenotypes, pulmonary function, bronchial hyper-responsiveness (BHR) and atopy).
Results: Scores based on the 46 highest-ranked SNPs were associated with persistent ($P<10^{-11}$, area under ROC curve (AUC)=0.59) and intermediate onset ($P<10^{-3}$, AUC=0.58) wheeze. Among lower-ranked SNPs (ranks 21545-46416), there was evidence for associations with diagnosed asthma ($P<10^{-4}$, AUC=0.54) and atopy ($P<10^{-5}$, AUC=0.55). We found little evidence of associations with transient early wheezing, reduced pulmonary function or non-asthma phenotypes.
Conclusion: The genetic origins of asthma are diverse: some pathways are specific to wheezing syndromes while others are shared with atopy and BHR. Our study also provides evidence of aetiological differences among wheezing syndromes.
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1864
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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Introduction: Asthma affects boys at a 2:1 ratio during childhood while females are affected by asthma twice as often compared to males after puberty.
Aims and objectives: This study investigates if and how genetic factors contribute to gender differences in asthma susceptibility.
Methods: A GWAS dataset based on the MAGICS/ISAAC 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 OR=0.55 [95% CI: 0.41-0.74; $p=8\times10(-5)$]. Functional analyses by EMSA revealed allele-specific differences in binding of transcription factors c-Jun and c-Fos to the potential promoter site. DLR assays showed 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.

1865
Interaction of retinoid acid receptor-related orphan receptor alpha (RORA) and neuropeptide S receptor 1 (NPSR1) in asthma
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Rationale: Retinoid acid receptor-related orphan receptor alpha (*RORA*) was recently identified as a candidate gene for asthma by a genome-wide association study (Moffatt et al. NEJM 2010). To investigate whether *RORA* polymorphisms (SNPs) influence asthma susceptibility, we conducted a detailed genetic association study in the vicinity of the associated variant rs11071559. Furthermore, we hypothesized that RORA may have biological and genetic interactions with a previously implicated asthma gene, Neuropeptide S receptor 1 (*NPSR1*).
Methods: We genotyped 37 *RORA* SNPs in the Swedish birth cohort BAMSE (2033 children) and the cross-sectional PARSIFAL study (1120 European children), and performed allele and genotype association and *RORA-NPSR1* gene–gene interaction analyses. Regulation of RORA was investigated using NPSR1 over-expressing SH-SY5Y cells stimulated with NPS.
Results: Seven *RORA* SNPs were associated with doctor-diagnosed childhood asthma. The allele T in rs7164773 was under-represented in cases in BAMSE (52.6% vs. 44.7%, [OR 0.72; 95%CI 0.60-0.87], p=0.0005). In PARSIFAL, the allele T of rs11071559 was under-represented in cases (13.6% vs. 8.5%, [OR 0.58; 95%CI 0.37-0.92], p=0.02). These associations were confirmed in the combined BAMSE and PARSIFAL material ($p<0.005$ and $p<0.01$, respectively). Significant gene-gene interactions influencing the asthma risk were found between *RORA* SNPs and *NPSR1* SNPs. In cell line studies, NPS induced RORA mRNA expression.
Conclusions: Genetic variations in *RORA* are associated with childhood asthma and show epistasis with *NPSR1*. A direct regulatory effect further suggests interaction of RORA and NPSR1 pathways.

1866
Genetic variations in the TLR signaling pathway are associated with childhood asthma
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Background: Common single nucleotide polymorphisms (SNPs) in Toll-like receptors (TLRs) are associated with asthma and atopy, but very little is known about the relevance of SNPs in TLR regulatory and downstream signaling pathway molecules.

Objective: 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=169) 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 1405 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-5, interleukin-13* 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 and investigated 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 14 *PCDH1* tagging SNPs and 98 SNPs within the cytokine cluster was 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 rs3797054 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), rs3935792 (OR=1.32, p=0.039), rs2974704 (OR=0.43, p=0.009).
Conclusions: *PCDH1* polymorphisms may specifically affect the development of non-atopic asthma in children.
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1868

IgE levels in asthmatics and non-asthmatics are affected by different SNPs in *FCER1A*

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Background: Recently, three genome wide association studies (GWAS) demonstrated *FCER1A*, the gene encoding the α-subunit of the high-affinity IgE receptor, to be a major susceptibility locus for total serum IgE. The top association signal differed between the two studies from the general population and one study based on an asthma case-control design.
Objective: To investigate if different *FCER1A* single-nucleotide polymorphisms (SNPs) are associated with total serum IgE in the general population and asthmatics.
Methods: Nineteen SNPs were studied in *FCER1A* based on a detailed literature search and a tagging approach. SNPs were genotyped by the Illumina HumanHap300Chip (6) or MALDI-TOF-MS (13) in at least 1303 children (651 asthmatics) derived from the German ISAAC- and MAGIC studies.
Results: Similarly to two population-based GWAS the peak association with total serum IgE was observed for SNPs rs2427837, rs2251746 and rs2511211 (mean r^2 >0.8), with the lowest p-value of $4.37*10^{-6}$. The same 3 SNPs showed the strongest association in non-asthmatics (lowest p=0.0003). While these SNPs were also associated with total serum IgE in asthmatics (lowest p=0.003), additional SNPs (rs3845625, rs7522607 and rs2427829) demonstrated associations with total serum IgE in asthmatics only (lowest p=0.01). SNPs rs2427837, rs2511211, rs3845625 and rs2427829 were also associated with atopic asthma (lowest p=0.02).
Conclusions: These data suggest that SNPs in *FCER1A* specifically influence IgE levels in asthmatics on top of genetic determinants of "basal" IgE levels also present in *FCER1A* as previously identified by GWAS. Thus, *FCER1A* variants and IgE-related mechanisms could be involved in specific asthma phenotypes.
These authors contributed equally.

1869

Next-gen transcriptome analysis (RNA-Seq) of human bronchial biopsies and laser microdissected airway smooth muscle: Asthma vs. controls

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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 difference in transcriptomic profiles of whole endobronchial biopsies between steroid-naïve asthma and controls; b) the feasibility to obtain RNA from airway smooth muscle (ASM) captured by laser microdissection (LCM) suitable for RNA-Seq
Methods: 4 biopsies per subject (asthma/control: aim a n=4/n=5; aim b n=24/n=12) were incubated in RNAlater. Whole cryosection or LCM-captured ASM was put

Table 1. Sample characteristics

		Whole biopsy	ASM
Concentration (ng/μL)	RNA	30-310	2-27
	cDNA	204-321	18-168
RNA-Seq reads mapped (%)[1]	Asthma	87	
	Control	88	89[2]

[1]Reference = UCSC hg19; [2]1 subject.

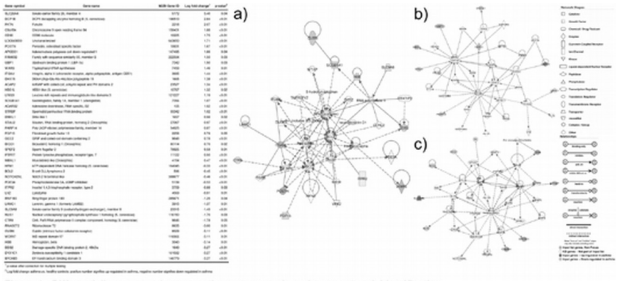

Figure 1 - Differentially expressed genes asthma vs. controls and gene network identification
Data was analysed based on the Poisson distribution. Gene network identification was performed with Ingenuity Pathway Analysis.

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.