## 212. Recent progress in mechanisms and diagnostics of COPD and asthma

**P1746**

**Selection of suitable housekeeping genes for real-time quantitative PCR in CD4+ lymphocytes from asthmatics with or without depression**

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**Objective:** No optimal housekeeping genes (HKGs) have been identified for CD4+ T cells from non-depressive asthmatic and depressive asthmatic adults for normalizing quantitative real-time PCR (qPCR) assays. The aim of present study was to select appropriate HKGs for gene expression analysis in purified CD4+ T cells from these asthmatics.
**Methods:** Three groups of subjects (Non-depressive asthmatic, NDA, n=10, Depressive asthmatic, DA, n=11, and Healthy control, HC, n=10 respectively) were studied. qPCR for 9 potential HKGs, namely RNA, 28S ribosomal 1 (RN28S1), ribosomal protein, large, P0 (RPLP0), actin, beta (ACTB), cyclophilin A (PPIA), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), phosphoglycerate kinase 1 (PGK1), beta-2-microglobulin (B2M), glucuronidase, beta (GUSB) and ribosomal protein L13a (RPL13A), was performed. Then the data were analyzed with three different applications namely BestKeeper, geNorm, and NormFinder.
**Results:** The analysis of gene expression data identified B2M and RPLP0 as the most stable reference genes and showed that the level of PPIA was significantly different among subjects of three groups when the two best HKGs identified

were applied. Post hoc analysis by Student-Newman-Keuls correction shows that depressive asthmatics and non-depressive asthmatics exhibited lower expression level of PPIA than healthy controls.
**Conclusions:** B2M and RPLP0 were identified as the most optimal HKGs in gene expression studies involving human blood CD4+ T cells derived from normal, depressive asthmatics and non-depressive asthmatics.

**P1747**
**Interleukin-17A in the pathogenesis of elastase-induced emphysema in mice**
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**Background:** Recent studies show that interleukin (IL) -17A is highly expressed in the lungs of patients with chronic obstructive pulmonary disease (COPD) and in the emphysematous lungs of mice after long-term cigarette smoke exposure. However, the role of IL-17A in the pathogenesis of emphysema is still unknown. In the present study, we examined the role of IL-17A in the development of elastase-induced emphysema using *Il-17A* gene-deficient (*Il-17a*$^{-/-}$) mice.
**Methods:** Porcine pancreatic elastase (PPE) or phosphate buffered saline (PBS) was administered intratracheally in *Il-17a*$^{-/-}$ and wild-type (WT, C57BL/6J) mice on day 0. IL-17A mRNA expression in the lungs was assessed with RT-PCR. Lung inflammation was determined by differential cell count in bronchoalveolar lavage fluid. On day 21, we measured lung compliance by forced oscillation method. Emphysema was assessed by alveolar mean liner intercept (Lm) determined by computer-assisted morphometric analysis.
**Results:** IL-17A mRNA expression was increased in WT mice lungs 6 hours after the administration of PPE. It was accompanied by neutrophilic inflammation in the lungs on day 2 – day 14, whereas neutrophil recruitment was significantly reduced in *Il-17a*$^{-/-}$ mice ($p < 0.05$). Lung compliance and emphysema (Lm) on day 21 in PPE-treated WT mice was significantly increased than in PBS-treated ones ($p < 0.05$). In contrast, *Il-17a*$^{-/-}$ mice administered with PPE showed significantly less increase in the compliance and Lm ($p < 0.05$, compared to WT).
**Conclusions:** These results suggest that IL-17A contributes to the development of elastase-induced neutrophilic inflammation and emphysema in mice.

**P1748**
**Promotor methylation of Bcl-2 in cigarette smoke extract-induced emphysema**
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**Objective:** Abnormal apoptotic events may play an important role in emphysema. We determined whether Bcl-2, a pivotal regulator of apoptosis, participates in emphysema. Furthermore, given the methylation in promoter CpG islands causing gene silencing, and existence of CpG islands in Bcl-2 promoter. We hypothesized that the deregulation of Bcl-2 in emphysema might be caused by methylation.
**Methods:** BALB/C mice were divided into four groups (n=10 per group): CSE, CSE+ 5-aza-2'-deoxycytidine(AZA, demethylation reagent), AZA, and control (phosphate buffered saline, PBS). CSE group was intraperitoneally injected with CSE once a week for 3 weeks to build emphysema models. AZA and PBS were also administered weekly as CSE. After 3 weeks of treatment, TUNEL assay was used to assess apoptotic index of pulmonary cells; western blotting or Realtime-RTPCR were used to detect expression of Bcl-2 and Cyt C in pulmonary tissue; bisulfite sequencing PCR (BSP) was used to observe the methylation status of Bcl-2 promoter.
**Results:** (1) The apoptotic index of pulmonary cells in CSE group[(14.2±0.6)%] was higher than CSE+AZA, AZA, Control groups[(4.9±0.5) %, (3.9±0.8)%, (4.5±0.6)%] ($p<0.05$). (2) The CSE induce-emphysema mice presented lower expression of Bcl-2 protein and RNA than other groups, and this goup showed higher expression of cytoplasmic Cyt C than other groups ($p<0.05$). (3) The CSE group showed higher methylation [(19.2±6.3)%] than CSE+AZA, AZA, Control groups[(5±1.0) %, (3±0.8)%, (4±0.6)%] ($p<0.05$).
**Conclusions:** The decreased anti-apoptic Bcl-2 might lead released cytoplasm Cyt C, which account for the increased apoptosis in emphysema. Furthermore, methylation might played a role in emphysema though deregulation of Bcl-2.

**P1749**
**Enhanced neutrophilic inflammation in IL-10-deficient mice exposed to cigarette smoke via TNF-α regulation**
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**Background:** We established a mouse model of short-term cigarette smoke (CS) exposure, which shares common molecular features with COPD patients. Interleukin (IL) -10 has a suppressive effect on inflammatory reactions and we previously presented that nasal administration of IL-10 attenuated CS-induced neutrophil recruitment to the lung (Higaki et al., ERS 2011). The effect of IL-10 on cigarette smoke-induced inflammation was investigated, using IL-10-deficient mice.
**Methods:** Both IL-10-deficient mice and wild-type mice were exposed to CS. Total cell counts, as well as cell defferentiations, in bronchoalveolar lavage (BAL) fluid were determined. TNF-α GM-CSF, and KC mRNA levels in lung tissue were estimated using quantitative RT-PCR. MMP-9 expression in lung tissue was investigated by immunohistochemical analysis.
**Results & discussion:** CS exposure significantly enhanced recruitment of neutrophils and macrophages to the lung ($p<.01$). CS exposure also increased the mRNA levels of TNF-α, KC, and GM-CSF in lung tissues in both genotypes ($p<.01$). IL-10 deficient mice revealed further enhancement in neutrophilic recruitment ($p<.05$) in comparison with wild-type mice, in parallel with the elevation in TNF-α levels ($p<.05$). Immunohistochemical analysis revealed that mpre MMP-9 positive cells were recruited to the lung in IL-10 deficient mice with CS exposure.
**Conclusion:** Our result indicated that anti-inflammatory effect of IL-10 on CS-induced inflammatory reactions, espicially neutrophilic recruitment to the lung, is probably via reducing TNF-α levels.

**P1750**
**Effect of genetic polymorphisms of some cytokines and xenobiotic-metabolizing enzymes on the lung function in patients with COPD**
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COPD is a chronic inflammatory lung disease characterized by decreased expiratory flow rate. The decrease of lung function in COPD depends on tissue remodelling due to xonobiotic- and inflammation-induced ROS-mediated tissue damage and impaired proteinase/antiproteinase balance. Since the activity and/or the protein level of cytokines and enzymes involved in inflammation and xenobitic and antioxidant detoxification are found to be associated with some gene variants, we aimed to evaluate the role of gene polymorphisms of three xonobiotic-metabolizing enzymes and three proinflammatory cytokines as factors involved in decline of lung function in COPD. We genotype altogether 164 patients with COPD and 174 non-affected by the disease control individuals for the following SNPs: *GSTP1+313A>G, IL6 –174G>C, TNFA –308G>A, IL1B –511C>T, IL1B +3953C>T* and for the null polymorphisms in *GSTM1* and *GSTT1*.
Our results displayed that the carriers of A allele of *GSTP1+313A>G* showed a tendency for higher FEV1%. compared to the carriers of GG genotype (p=0.097). Patients COPD stage III/IV having *GSTM1* null genotype demonstrated significantly lower FEV1% values (39.16%) than those with non-null genotype (43.91%, p=0.032). Moreover, patients with C containing genotypes of *IL6 –174G>C* SNP had significantly lower FEV1/FVC% (59.7%) compared to the patients with GG genotype (62.7%, p=0.034). The polymorphisms in *GSTT1*, *TNFA* and *IL1B* did not associate with respiratory indexes.
In conclusion, we suggest that the polymorphisms in the genes of some cytokines and xenobiotic-metabolizing enzymes, such *GSTP1*, *GSTM1* and *IL6* are factors that may affect the lung functions in COPD.

**P1751**
**Gender differences in 5- and 12/15-lipoxygenases products in bronchoalveolar lavage fluid from healthy never-smokers, smoker and COPD patients**
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**Background:** Chronic obstructive pulmonary disease (COPD) is a leading disease that is increasing particularly among females. Smoking represents the main risk factor for developing COPD and chronic inflammation persists after smoking cessation
**Aims:** We have investigated the effects of smoking, in relation to COPD, on lipid mediators in the inflammatory response in the lower airways
**Methods:** Bronchoalveolar lavage fluid (BALF) was obtained from healthy never-smokers, non-symptomatic smokers, and COPD patients of GOLD stage I-II (smokers and ex-smokers) of both genders. Different lipid mediators derived from the cytochrome P450, lipoxygenase (LOX) and cyclooxygenase (COX) pathways were analyzed by mass spectrometry
**Results:** Products of 12/15-LOX and 5-LOX clustered respectively when analyzed by multivariate analysis and were summed for further comparisons. 12/15-LOX products were selectively increased in females. 5-LOX products exhibited a distinct pattern with increases in smokers, but no gender-specificity. There was no difference in lipoxygenases products between healthy smokers and smoking COPD patients. However, in COPD ex-smokers the levels of 5-LOX products were decreased compared to COPD smokers

**Conclusions:** LOX activity in BALF shows gender-specific regulation in relation to both smoking and COPD. The observed shifts were 5-and 12/15-LOX-specific depending on gender and smoking status. This study will be continued with the analysis of other lipid mediators derived from 5 and 12/15-LOX, that are currently pharmacological targets in other diseases, such as asthma. These facts provide potential insight into the gender imbalance in COPD.

**P1752**
**The role of chaperone α-Bcrystallin (HspB5) in COPD pathogenesis**
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**Background:** α-Bcrystallin (HspB5) is a chaperone whose role as a marker of innate immunity activation as well as its therapeutic potential have recently been investigated in several inflammatory diseases – multiple sclerosis, myocardial ischaemia, Guillain Barre syndrome.
**Aim:** The aim of the study is to determine the role of α-Bcrystallin in COPD pathogenesis and inflammation.
**Materials:** Plasma levels of α-Bcrystallin were studied in 163 patients – 52 healthy non-COPD smokers; 20 COPD smokers I - II stage GOLD; 43 COPD smokers – III-IV stage (GOLD) and forty-eight patients with acute inflammatory respiratory disease. The plasma levels of α-Bcrystallin antibodies were determined by ELISA (human anti alpha-Bcrystrallin Abcam), and were confirmed with Western blotting.
**Results:** The mean levels of anti - α-Bcrystallin antibodies were: in non-COPD smokers – 0,291 OD; in COPD smokers – 0,352OD; in healthy non-COPD smokers – 0,433 OD. There was a statistically significant difference between COPD smokers and healthy non-COPD smokers (p-0,010). The same could be observed comparing the group of patients with acute inflammation and non – COPD healthy smokers (p-0,007). There was not a statistically significant difference in patients with mild/moderate and those with severe COPD.
**Conclusion:** α-Bcrystallin is increased in patients with inflammatory lung diseases. Though unspecific it could be used in a panel of markers discerning COPD smokers from healthy non- COPD smokers. Being a regulator of innate immunity and a therapeutic anti-inflammatory agent its exact role in COPD pathogenesis and therapy should further be explored.

**P1753**
**The incidence of alpha-1-antitrypsin (A1AT) deficiency alleles in Polish population – Preliminary results from newborn screening**
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Data concerning the prevalence of A1AT deficiency in Poland are very limited due either to small groups analyzed or unreliable methodology.
**Methods:** DBS samples were collected prospectively from 658 newborns between September 2011 - January 2012. AAT serum concentration was measured by nephelometry and PI-phenotype identified by by real-time PCR. The PI*S and PI*Z alleles were confirmed by isoelectrofocusing.
**Results:** Deficiency S or Z allele was observed respectively in 10 (1,5%) and 18 (2,8%) DBS samples (in total 28 (4,3%) samples). Calculated frequencies expressed per 1000 were for PI*Z 13,7 (95% CI: 5,8-21,5), PI*S 7,6 (95% CI: 1,7-13,5). The AAT gene prevalence calculated by Hardy-Weinberg equilibrium were: 1/1.04 for MM, 1/67 for MS, 1/17319 for SS, 1/37 for MZ, 1/4810 for SZ and 1/5345 for ZZ. The mean A1AT concentration was 216±50 mg/dl, in non-S non-Z individuals 220±48 mg/dl, in PI*S carriers 178±33 mg/dl, in PI*Z 138±17 mg/dl.
**Conclusion:** Our results suggest considerably higher prevalence of deficiency alleles in Polish population than currently available data. In particular, the frequency of ZZ deficiency genotype is considerably higher (current best estimate 1/9110).

**P1754**
**Role of *-511C>T/+3953C>T* haplotypes of *IL1B* gene as risk factors of COPD**
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COPD is multifactorial lung disease driven by abnormal inflammatory reaction. Therefore, inflammatory mediators are considered to be of distinct importance in pathogenesis of COPD. It is well recognized that the genetic factors play a role in susceptibility to COPD. Hence, polymorphisms in pro-inflammatory cytokines may confer a risk for the development of COPD. There are several case-control studies focused on the role of some of the described SNPs in *IL1B* in COPD, however no previous study has been conducted to evaluate the role of haplotypes of *-511C>T* and *+3953C>T* in *IL1B* as risk factors of COPD.
The aim of the current study was to investigated the role of the haplotypes of the -511C>T and +3953C>T in *IL1B* as candidate risk factors of COPD in Bulgarian population.
We genotyped 163 patients with COPD and 174 control individuals using Taqman genotyping assay for *IL1B -511C>T* SNP and PCR-RFLP-based method for *+3953C>T* SNP.
The frequencies of the *IL1B* haplotypes of the studied two loci of *IL1B (-511C>T* and *+3953C>T)* did not differ significantly between controls and COPD patients (p=0.099). However, the T_C haplotype, constructed by alleles found to determine enhanced expression of IL-1β, appeared to be associated with higher risk of COPD (OR 1.25, 0.88-1.79, p=0.231) compared to the most common C_C haplotype and with 1.70-fold higher risk of COPD (95% CI, 1.10-2.64, p=0.018) compared to the C_T haplotype, previously associated with lower *IL1B* expression.
Based on the results of the current study for the first time we propose that the T_C haplotype of *IL1B*-511C>T:+3953C>T, supposed to determine enhanced expression of IL-1b, is a predisposing factor for COPD in Bulgarian population.

**P1755**
**Rapid DNA extraction from dried blood spots for the alpha-1 antitrypsin deficiency detection by real-time PCR**
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**Background:** The use of dried blood spot (DBS) specimens for the genetic screening of alpha-1 antitrypsin (AAT) deficiency is often limited by low quantity and quality of extracted DNA. Our aim was to develop the method of simple, fast and efficient DNA extraction from DBS samples using commercial kit that effectively eliminates any natural PCR inhibitors allowing for successful AAT genotyping by real-time PCR and direct sequencing.
**Methods:** DNA extracted from 84 DBS samples from COPD patients was genotyped for PI*S and PI*Z AAT deficiency variants by real-time PCR. The results of AAT genotyping were validated by IEF phenotyping and concentration measurement of AAT protein in sera from the same patients. The diagnosis of rare/unknown AAT variants was established by direct sequencing.
**Results:** The proposed method of DNA extraction allowed successful DNA isolation from all analyzed DBS samples. Both quantity and quality of DNA were sufficient for further real-time PCR and genetic sequence analysis of all samples. The 100% concordance between AAT DBS genotypes and serum phenotypes in positive detection of two major deficiency alleles was achieved. Both assays, DBS AAT genotyping by real-time PCR and AAT phenotyping by IEF, positively identified PI*S and PI*Z allele in 8 out of 84 (9,5%) and 16 out of 84 (19%) patients, respectively.
**Conclusion:** The proposed procedure minimizes the hand-on-time of DBS samples preparation providing sufficient quantity and quality of genomic DNA to be used for either real-time PCR or genetic sequence analysis, as only good quality DNA template guarantees efficient and reproducible AAT genotyping results in any DBS screening protocol.

**P1756**
**Involvement of the respiratory and ocular systems in X-linked hypohidrotic ectodermal dysplasia**
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**Background:** X-linked hypohidrotic ectodermal dysplasia (XLHED, ectodysplasin deficiency), the most common of the ectodermal dysplasias, is classically described as affecting hair, sweat glands and dentition. What may be underappreciated is the effect ectodysplasin deficiency has on eccrine glands of the respiratory and ocular systems and the resulting chronic health issues.
**Methods:** 12 male children and 14 male adults with XLHED, age range 6 to 58 years, and 12 healthy controls were enrolled in this study conducted alongside a family conference in Germany (NCT01308333). All subjects were assessed for symptoms and signs of asthma by pulmonary function tests and measurement of exhaled nitric oxide (eNO), and for dry eye disease by investigating the tear film and the ocular surface. Standardized sweat duct counts and EDA genotype were included in correlation analyses.
**Results:** Respiratory symptoms and elevated eNO as a sign of pulmonary inflammation were present in the majority of XLHED subjects, in similar numbers of children and adults. In contrast, ocular symptoms were significantly more common in XLHED adults than in XLHED children. The presence of residual sweat ducts in XLHED subjects, suggestive of partial EDA expression, correlated with milder disease in two subjects with genetic abnormalities in exon 5 encoding the

collagen-like domain of ectodysplasin, but not in a third subject with a mutation affecting the TNF homology domain.
**Conclusions:** The high prevalence of asthma and dry eye syndrome in patients as young as 6 years suggests that screening evaluation, regular monitoring and consideration of therapeutic intervention should begin in early childhood.

**P1757**
**Effect of cigarette smoking on sputum proteomic profiles in patients with asthma and healthy volunteers**
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**Background:** Smokers with asthma have more severe symptoms and impaired short-term therapeutic response to corticosteroids, but the mechanisms accounting for these adverse effects are poorly understood. We hypothesize that the differences in sputum protein profiles provide insight into the pathophysiological effects of smoking in asthma.
**Methods:** High resolution label-free shotgun proteomics was employed to investigate sputum protein profiles in 43 asthmatic non-smokers, 50 asthmatic smokers, 10 healthy non-smokers and 10 healthy non-smokers. Protein expression was normalized to sputum albumin level.
**Results:** A total of 596 and 335 proteins were confidently detected (FDR<1%, Mascot) in asthmatic patients and healthy volunteers, respectively. Among them, 73 and 68 proteins were found differentially expressed between smokers and non-smokers for asthmatic patients and healthy volunteers, respectively ($p<0.05$, Benjamini corrected). Majority of differences observed were up-regulation in smokers. Functional enrichment analysis shows that peptidase inhibitor activity and acute inflammatory/defence response were over-representative in healthy smokers compared to healthy non-smokers ($p<0.05$, Benjamini corrected). In asthmatic smokers, there was an over-representation of oxidoreductase activity, thioredoxin fold (glutathione S-transferase), response to extracellular stimuli and lysosome related peptidase activity.
**Conclusions:** These results suggest that asthmatic patients may be hyper-responsive to cigarette smoke and that their airways may be susceptible to potential damage from lysosome related peptidase activity.

**P1758**
**Polymorphisms of MDR1, ADRB2 and IL13 genes are markers of therapy-resistant bronchial asthma (BA) in Russian patients**
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**Background:** BA is multifactorial disease caused by the interaction of genes and environment. Genetic polymorphisms influence BA development, progression and severity as well as response to BA therapy.
**Aim:** To assess severity of BA and effectiveness of BA pharmacotherapy in patients with different genetic background.
**Methods:** Genomic DNA was extracted from peripheral leukocytes. We investigated 4 SNPs by PCR-RFLP in 122 BA patients and in 103 healthy controls.
**Results:** Distribution of genotypes was similar to other European populations, except MDR1 gene. We revealed numerous associations of genetic variants with increased risk (IR): 3435CC with IR of BA (OR=3.92, 95%CI 1.74-8.79); 3435CC with IR of GCS doses more than 20 mg of prednisolon (OR=20.89, 95%CI 5.10-85.53); 3435CC with IR of therapy-resistant BA (OR=6.12, 95%CI 2.42-15.48); 16Gly with IR of respiratory failure (OR=17.31, 95%CI 2.01-149.28); 27GluGlu with IR of therapy-resistant BA (OR=3.35, 95%CI 1.16-9.66); 130Gln with IR of therapy-resistant BA (OR=2.09, 95%CI 1.01-4.30).

| Gene | SNP | dbSNP | Restrictase |
|---|---|---|---|
| Multidrug resistance 1 (MDR1) | C3435T | rs1045642 | MboI |
| Beta-2-adrenergic receptor (ADRB2) | Gly16Arg | rs1042713 | NcoI |
| Beta-2-adrenergic receptor (ADRB2) | Gln27Glu | rs1042714 | BseXI |
| Interleukin 13 (IL13) | Arg130Gln | rs20541 | AluI |

**Conclusion:** Analysis of MDR1, ADRB2 and IL13 polymorphisms is useful for both preventive care (revealing subjects with increased predisposition to BA) and pharmacotherapy optimization due to prediction of BA severity and risk of therapy-resistant BA.

**P1759**
**Genome wide association study of lung function in asthmatics**
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**Objective:** To investigate genes that may be important in determining lung function in subjects with asthma.
**Methods:** A genome-wide association study has been performed in subjects with asthma from the COMPASS study (Bleecker *et al,* Lancet 370: 2118-25, 2007). To reduce population stratification, homogenous populations were identified based on geographic region, self-reported race, and genetic ancestry using HapMap reference data. Standard quality control measures were applied to the resulting genotype data. Genome-wide association studies (GWAS) for percent predicted $FEV_1$ and $FEV_1$/FVC was performed on the genetically homogenous population from Eastern Europe using PLINK.
**Results:** To date, GWAS has been performed on 587,020 SNPs in Eastern Europeans (n = 885). For percent predicted $FEV_1$, the top three SNPs were in or near *SLITRK5* (SLIT and NTRK-like family, member 5) on chromosome 13 ($p=1.2\text{-}2.3\times10^{-06}$). Additional genes of interest for percent predicted $FEV_1$ include *ADAM7* (A Disintegrin And Metalloprotease 7) ($p=9.1\times10^{-06}$) and *ADRA1D* (alpha-1D-adrenergic receptor) ($p=1.1\times10^{-05}$). For $FEV_1$/FVC ratio, the top three genes are *FGF1* (fibroblast growth factor 1) ($p=2.2\times10^{-06}$), *EPHA5* (ephrin type-A receptor 5) ($p=2.8\times10^{-06}$) and *WNT3A* (wingless-type MMTV integration site family, member 3A) ($p=6.3\times10^{-06}$).
**Conclusions:** Evidence was found to suggest that genes involved in regulation of cellular growth (*ADRA1D* and *FGF1*) and asthma susceptibility (*EPHA5*) are associated with lung function in subjects with asthma.

**P1760**
**Assessment of allergic status of severe asthma with a new component resolved method using micro-array technique**
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Immunocap® ISAC biochip is a new biologic test using component micro-array technique for detection of specific IgE (sIgE) toward 103 molecular allergens. We have used this test to screen a group of severe asthmatics.
ISAC was performed in 21 consecutive patients with severe asthma (American Thoracic Society criteria). Lung function, exhaled nitric oxide (NO), blood eosinophilia, serum total IgE and skin prick tests for pneumallergens were systematically collected.
Population included 11 women and 10 men (mean age 52±16), median FEV1 72% predicted normal, mean NO 66±47 ppb. Seventeen (81%) patients had a negative ISAC and 4 (19%) had sIgE for at least one molecular allergen of the test. Three of the 4 positive patients had sIgE toward several allergenic sources (mites, pollens, animal dander). One patient was exclusively sensitized for Fel d1 (cat major allergen). Skin prick tests and ISAC were concordant in all patients. The other parameters, clinical, lung function and biology were not different in patients with positive ISAC compared to patients negative for the test.
ISAC is useful to confirm the non atopic status of a subgroup of severe asthmatics and to identify precisely the sensitization profile of severe allergic asthmatics.

**P1761**
**The roles of PCDH1 on epithelial barrier function in the airway**
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**Background:** PCDH1 is recently identified as a susceptibility gene of bronchial hypersensitivity (BHR). Although PCDH1 seems to be involved in the differentiation of the airway, the roles of PCDH1 on epithelial barrier functions have not been determined.
**Methods:** A human airway epithelial cell line, 16HBE, cells were cultured on Transwell chamber for 5days. Barrier function was evaluated by Trans Electric Resistance and dextran permeability. Expression of inflammatory cytokine was measured by RT-PCR. Morphology of cell junctions was analyzed by immunostaining using anti-PCDH1, anti-ZO-1, anti-Ocludin and an anti-E-cadherin antibody.
**Results:** The paracellular barrier function of 16HBE cell monolayer increased over time in culture. Similarly, the expression of PCDH1 increased for the period. The knockdown of PCDH1 significantly inhibited the paracellular barrier function. In addition, it inhibited the dsRNA-induced inflammatory cytokines expressions. Immunocytostaining revealed that PCDH might coexist with E-cadhein at the cell-cell contact sites.
**Conclusion:** Our results indicated that a susceptibility gene of PCDH1 plays an important roles on the immunological and physiological barriers of airway epithelium.

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**The preliminary proteomics analysis of bone marrow eosinophil progenitors in allergic asthmatic mice**

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**Introduction:** The early-stage airway inflammation of allergic asthma is associated with bone marrow (BM) progenitor cell commitment towards eosinophilic differentiation after allergen challenge. We sought to identify the differently expressed proteins by novel proteomic technology from the BM $CD_{34}^{+}$ progenitor cells of asthmatic mice and analyzed their bioinformatics.
**Methods:** The magnetic activated cell sorting separation coupled to fluorescence activated cell sorting separation strategy was used to harvest BM $CD34^{+}$ progenitor cells from naïve and ovalbumin-challenged asthmatic mice. Isobaric tags for relative and absolute quantitation combined with 2D nano LC-MS/MS technology was employed to profile proteome alterations in $CD_{34}^{+}$ progenitor cells. The analysis of bioinformatics was performed finally.
**Results:** Twenty-five proteins with 18 up-regulated and 7 down-regulated ones were identified. In the dysregulated proteins, 4 clusters of proteins were observed around collagen groups, ACTN1/Myosin groups, Mdh2 and Serpinh1, predominantly participating in pathways of focal adhesion, ECM-receptor interaction, tight junction and regulation of actin cytoskeleton.
**Conclusions:** Collagen group and ACTN1 related focal adhesion, ECM-receptor interaction and regulation of actin cytoskeleton could be the key pathway in bone marrow response of asthma.

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**Sputum protein profiles for monitoring lung pathophysiology in chronic lung diseases**

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**Background:** Sputum proteome, mainly derived from proteins secreted from airway epithelial cells, immune related cells and proteins released from lysed cells/pathogens, is a potential source for the investigation of lung pathology and pathophysiology.
**Objectives:** We hypothesize that sputum protein profiles from cystic fibrosis (CF) and chronic obstructive pulmonary disease (COPD) could provide novel insight into their pathophysiology.
**Methods:** High resolution shotgun proteomics was used to investigate sputum protein profiles in 29 patients with CF, 27 patients with COPD and 23 healthy volunteers.
**Results:** A total of 631 proteins were confidently detected and analysed in this study group. Sputum protein profiles of CF shows a dramatic difference compared to those of healthy volunteers while the profiles of COPD patients were highly variable. Functional enrichment analysis indicates that CF has increased protein expression in pathways of chromatin assembly, actin filament-based process, defense response, carbohydrate catabolic process, anti-apoptosis, and cell motion ($p<0.001$). A similar but less significant functional enrichment was found in COPD except that nicotinamide metabolic process was noticeable in COPD. We found that mortality in CF patients over the two consequent years was significantly associated with lower sputum $\alpha$1-antitrypsin in CF patients ($p<0.001$) but not MMP9, neutrophil elastase or MMP8 despite all of them showing a dramatic increase compared to healthy volunteers.
**Conclusion:** Sputum protein profile provides a comprehensive view of disease pathophysiology of chronic lung diseases. Some specific sputum protein expression could be useful for disease management and personalised treatment.