498. COPD inflammation and genes

P4793

Association of GSTT1 & M1 gene polymorphism with ageing in Northern Indian COPD and lung cancer patients

Rajni K. Shukla[1], Surya Kant[1], Sandeep Bhattacharya'[2], Balraj Mittal[3]. *[1]Pulmonary Medicine, [2]Physiology, C.S.M. Medical University, Lucknow/Uttar Pradesh, India; [3]Medical Genetics, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow/Uttar Pradesh, India*

Background: There is increasing evidence for a close relationship between aging and chronic inflammatory diseases. COPD is a chronic inflammatory disease of the lungs, which progresses very slowly and the majority of patients are therefore elderly. In this study, we assess whether age in metabolism of phase 2 enzyme gene polymorphisms GSTT1 & GSTM1 in northern Indian COPD and lung cancer Patients.
Material & methods: In this case only study, we have enrolled 422 study subjects (218 lung cancer & 204 COPD). COPD enrolled after spirometry evaluation and lung cancer patients confirmed by Histopathology. All genotyping were done by PCR-RFLP method.
Results: GSTM1 null was found to be significantly higher in COPD patients as compared with healthy controls (OR=2.08; 95%;CI=1.40-3.09; P=0.0001), but there were no significant differences in the distribution of genotypes of polymorphisms of GSTT1 null patients and healthy controls, and in lung cancer GSTT1 null polymorphism was found to significant associated (OR=0.47; 95%; CI=0.30-0.73;P=0.001). But GSTM1 null polymorphism was not significant associated with lung cancer patients. In subgroup analysis in age, we found GSTM1 N/P polymorphism significantly associated between age 50-60 years for COPD (43.5%/21.2%,p=0.015), but in case of Lung cancer none of GSTT1/M1 null polymorphism were associated (p=0.071,p=0.934).
Conclusion: We conclude that GSTM1 null polymorphism was associated with COPD. We also observe that GSTM1 null polymorphism was associated between aging group 50 -60 years COPD patients but not in case of Lung cancer. So we can conclude that aging have affect of COPD not in lung cancer patients.

P4794

Proteinase activated receptor-1 (F2R) polymorphisms and susceptibility to exacerbations in COPD

Manuela Plate'[1], Jennifer K. Quint[2], Jadwiga A. Wedzicha[3], Rachel C. Chambers[1], John R. Hurst[3]. *[1]Centre for Respiratory Research, University College London, United Kingdom; [2]Department of Non-communicable Disease Epidemiology, London School of Hygiene and Tropical Medicine, London, United Kingdom; [3]Academic Unit of Respiratory Medicine, Royal Free Campus, UCL Medical School, London, United Kingdom*

Introduction: COPD is a condition of global importance, characterized by accelerated lung function decline and an abnormal inflammatory response. Exacerbations (i.e. episodes of acute deterioration of respiratory health) account for much of the morbidity and mortality in COPD. The reasons why some patients are more susceptible to exacerbations is poorly understood, but familial clustering suggests that there may be a genetic basis. Proteinase activated receptor-1 (PAR_1) activation leads to the generation of several inflammatory mediators involved in COPD and our unpublished data have shown that functional polymorphisms of PAR_1 are protective in sarcoidosis.
Aims & objectives: The aim of this study was to investigate whether PAR_1 polymorphisms are associated with COPD exacerbation frequency (ExF).
Methods: Two PAR_1 SNPs (rs2227744 and rs32934) and a 13bp in/del (rs11267092) were genotyped in 136 infrequent and 67 frequent exacerbators.
Results: The genotypic distributions of all polymorphisms were in Hardy-Weinberg equilibrium. The rs2227744 SNP showed a statistically significant association with ExF. Frequency of the minor allele was 0.47 in infrequent and 0.37 in frequent exacerbators (OR 1.5, 95%CI 1.0-2.4, p=0.04). Considering exacerbations as a continuous variable, the presence of the minor allele was associated with a significantly lower exacerbation rate (3.03 vs 1.98 exacerbations/year, MWU p=0.04).
Conclusions: Taken together with our previous studies showing that the presence of the minor allele at SNP rs2227744 increases PAR_1 expression, these data suggest that this SNP may confer a degree of protection against exacerbations in COPD by increasing PAR_1 expression.
Funded by the BLF.

P4795

Heterozygosity for E292V in *ABCA3*, lung function and COPD in 64,000 individuals

Marie Bækvad-Hansen[1], Børge Nordestgaard[1], Morten Dahl[2]. *[1]Department of Clinical Biochemistry, Herlev Hospital, Copenhagen University Hospital, Herlev, Denmark; [2]Department of Clinical Biochemistry, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark*

Recessive mutations in the *ATP-binding-cassette-member A3* (*ABCA3*) gene are

associated with chronic lung disease in childhood, but frequency of chronic lung disease due to *ABCA3* mutations in the general population is unknown. We tested the hypothesis that individuals heterozygous for *ABCA3* mutations have reduced lung function and increased risk of COPD in the general population.
We resequenced 760 individuals and identified three novel (H86Y, A320T, A1086D) and four previously described mutations (E292V, P766S, S1262G, R1474W) in *ABCA3*. We genotyped the entire Copenhagen City Heart study (n=10,604) to assess the clinical importance of these mutations. To validate our findings we genotyped an additional 54,395 individuals from the Copenhagen General Population Study for the E292V mutation.
In the Copenhagen City Heart Study E292V heterozygotes had 5% reduced FEV_1% predicted (t-test: p=0.008) and 3% reduced FVC % predicted compared with noncarriers (p=0.04) and an increased odds ratio for COPD of 1.9 (95% CI: 1.1-3.1). In contrast, the A1086D mutation was associated with increased FEV_1% predicted (p=0.03) and FVC % predicted (p=0.008) in the Copenhagen City Heart Study. None of the other *ABCA3* mutations associated with lung function or COPD risk. In the larger Copenhagen General Population Study, and in the two studies combined, E292V heterozygotes did not have reduced lung function or increased risk of COPD ($p \geq 0.11$), while this was the case for the positive controls, *surfactant protein-B* 121ins2 heterozygotes and α_1*-antitrypsin* ZZ homozygotes.
Our results indicate that partially reduced ABCA3 activity due to E292V is not a major risk factor for reduced lung function and COPD in the general population.

P4796
Estimating the contribution of genetic variants in occupational chronic bronchitis
Leysan Akhmadishina[1], Gulnaz Korytina[1], Tatyana Viktorova[1,2]. *[1]Department ofGemomics, Institute of Biochemistry and Genetics, Ufa, Russian Federation; [2]Department of Biology, Bashkir State Medical University, Ufa, Russian Federation*

We hypothesized that occupational factors might affect the relationship between genetic predisposition and the risk of chronic bronchitis. Cases were 122 workers with a confirmed diagnosis of occupational chronic bronchitis and controls were 166 healthy workers matched to cases by age, sex and industrial exposure time. 55 SNPs were genotyped by PCR-RFLP. Significant associations with occupational chronic bronchitis risk were observed with VDBP 1307C>A, 1296T>G (Padj=0.00066, OR=2.54 95%CI 1.60-4.04), MMP1 519A>G (Padj=0.0001, Pcor=0.0002, OR=2.52 95%CI 1.36-4.69), ADAM33 13491C>G (Padj=0.0004, Pcor=0.0008, OR=2.52 95%CI1.40-4.52), IL8 -251T>A (Padj=0.0058, Pcor=0.0116, OR=2.87 95%CI 1.32-6.22), NQO1 465C>T (Padj=0.0004, Pcor=0.0008, OR=3.57 95%CI 1.35-6.72), UGT2B7 2146C>T (Padj=0.0021, Pcor=0.0042, OR=2.34 95%CI 1.35-4.04), CYP1A2 -2467delT (Padj=0.0041, Pcor=0.0082, OR=2.17 95%CI 1.20-3.91). Significant interaction were found for smoking status and UGT2B7 2146C>T (P=0.015), EPHX1 415A>G (P=0.04), GPX1 599C>T (P=0.037) and for PY and CYP2F1 c.14_15insC (P=0.05) for occupational chronic bronchitis. Also significant interaction were found for age of exposure time and IL1RN VNTR (P=0.02656), VDBP 1307C>A (P=0.02258), CYP1A1 3798T>C (P=0.01657). The formation of occupational chronic bronchitis in workers is determined not only by the composition of harmful dusts and duration of exposure but also the individual characteristics of the organism. The disease occurs predominantly in individuals with certain genetic constitution and is a consequence of the interaction of genetic and environmental factors.

P4797
SNPs in TSPYL-4, NT5DC1 genes are associated with susceptibility to COPD in a southern Chinese Han population
Yi Guo, Yi Gong, Guochao Shi, Kun Yang, Chunming Pan, Min Li, Qingyun Li, Qijian Cheng, Ranran Dai, Liang Fan, Huanying Wan. *Respiratory Diseases, Shanghai Rui Jin Hospital, Shanghai, China*

Background: The risk of developing chronic obstructive pulmonary disease(COPD) is partially genetically determined as well as environmental. Many published candidate gene studies had conflicting results due to different races and sample sizes.
Objectives: To investigate candidate genes and haplotype in susceptibility to COPD in a southern Chinese Han population.
Methods: We performed genotyping of DNA samples in 200 COPD patients and 250 control subjects according to 54 single nucleotide polymorphisms (SNPs) in 23 genes associated with development of COPD and/or pulmonary function identified by GWAS. We also made linkage disequilibrium (LD) and haplotype analysis according the results of genotyping.
Results: The frequencies of the SNP(rs3749893 of testis specific protein Y encoded like 4 (TSPYL-4) gene) G allele and SNP (rs1052443 of 5'-nucleotidase domain containing 1 (NT5DC1) gene) A allele were significantly higher in cases than in controls (p=0.032, p<0.05,OR=0.692, 95%CI=0.495-0.970; P=0.0205, p<0.05, OR=0.670, 95%CI=0.477-0.941, respectively). There were two blocks of SNPs (rs1052443 and rs3749893; rs11155242 and rs6937121) that had sufficient precision to warrant construction of a haplotype block. We constructed the TSPYL-4 and NT5DC1 haplotypes of cases and controls, but with no significantly difference between the two groups. rs3749893 A allele of TSPYL-4 and rs1052443 C allele of NT5DC1 were associated with a protective effect from the deterioration of pulmonary function.
Conclusion: TSPYL-4, NT5DC1 genes polymorphism are associated with susceptibility to COPD and pulmonary function in a southern Chinese Han population.

P4798
Genetics of detoxification and oxidative stress pathways in COPD
Qadar Pasha[1], B. Singh[2], A. Vibhuti[3], E. Arif[4]. *[1]Functional Genomics Unit, CSIR-Institute of Genomics and Integrative Biology, Delhi, India; [2]Respiratory Unit, RML Hospital, New Delhi, India; [3]FGU, CSIR-IGIB, Delhi, India; [4]FGU, CSIR-IGIB, Delhi, India*

Cigarette-smoking, although, is the most important risk factor, but only a small percentage of smokers develop symptomatic COPD. Does that mean the genetic predisposition plays a pivotal role? To address this query, we investigated polymorphisms on several genes but restrict here to cytochrome p450 1A1(CYP1A1), CYP1A, CYP2E1, N-acetyl transferase (NAT) and microsomal epoxide hydrolase (mEPHX) of detoxification and cytochrome b-245 alpha (CYBA), glutathione-S-transferase P1 (GSTP1) of oxidative stress pathways in a case-control design. We present data on haplotypes, gene-gene interactions and correlations with clinical and biolevels.
Haplotypes 462Val:3801C, 462Val:3801T of CYP1A1, −1293C:7632T, −1293C:9893C, −1293C:9893G and −1293C:7632T:9893C of the CYP2E1, 930G:242C of CYBA (p<0.05); 105V-114V of GSTP1 (p<0.001) and 113H-139H of mEPHX (p<0.05) were over-represented in patients. The same alleles-associated genotype-combinations between genes, GSTP1:CYBA and NAT2*6 and NAT2*7 alleles were prevalent in patients (p<0.01). Patients had significantly elevated malondialdehyde (MDA) level and decreased catalase (CAT), glutathione peroxidase (GPx) activities, glutathione (GSH) level (p 0.01). Genotypes, 462Ile/Val+Val/Val, 3801TC+CC of CYP1A1 and 930AG+GG of CYBA associated with increased MDA level, decreased FEV1, CAT activity and GSH level in patients (p 0.05). Likewise genotypes, I105V/V105V, A114V/V114V of GSTP1 and Y113H/H113H of mEPHX associated with increased MDA and decreased GSH levels in patients(p 0.05).
Gene polymorphisms affecting the function of proteins cause imbalance of detoxification and oxidative-stress pathways thereby contributing to pathogenesis.

P4799
New degradome markers in children and adults with chronic lung disease
Olga Tselousova[1,2], Gulnaz Korytyna[1], Tatyana Victorova[1,2]. *[1]Genomics, Institute of Biochemistry and Genetics, Ufa, Russian Federation; [2]Biology, Bashkir State Medical University, Ufa, Russian Federation*

The degradome proteases a large group of enzymes that act in the extracellular environment have been associated with multiple physiological and pathological processes in lung tissue. Through protein degradation and turnover proteases play an essential role in lung tissue remodeling and repair during the inflammatory response and may be important in the development of chronic lung disease.
In our case - control study we investigated common genetic mechanisms of susceptibility to chronic lung disease in children and adults.
We analyzed MMP genes in 220 children with chronic respiratory diseases and 230 control and in group of 362 adults with COPD and 483 control, respectivly. The folowing genotypes were detected: MMP1(-519A/G), MMP2 (-735C/T), MMP3(-11715A/6A), MMP9 (-1562C/T, 836A/G), MMP12 (-82A/G) in both groups of children and adults.
We observed significantly associated to chronic lung disease susceptibility in children and adults for the MMP3 (-11715A/6A) gene. The genotype 6A/6A were identified as a risk for chronic lung disease (OR=3,64; 95%CI 1,76-7,68 for children, OR=4,49; 95%CI 2,33-8,81 for adults). The MMP1*-519A/A genotype carriers in children had a significantly increased risk of chronic lung disease (OR=1,75; 95%CI 1,10-2,80).The MMP12*−82A/A genotype was identified as a risk for chronic lung disease in children (OR=1,75; 95%CI 1,10-2,80) but not for adults.
The genotype and allele frequencies of other MMP genes do not significantly differ in groups.
The results of our study suggest the genetic polymorphisms in degradome pathway genes may play a significant role in the development of chronic lung disease.

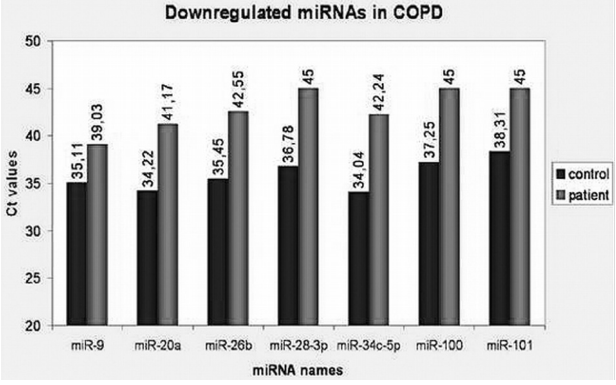
P4800
Longitudinal analysis of airway epithelium of COPD subjects reveals unique inflammatory gene networks up-regulated over time
Sriram Sridhar[1], Jay S. Fine[4], Christopher S. Stevenson[2], Hans Bitter[1], Ann E. Tilley[3], Ben-Gary Harvey[3], Robert Kaner[3], Michelle R. Staudt[3], Jennifer Fuller[3], Jacqueline Salit[3], Neil Hackett[3], Holly Hilton[1], Sreekumar Pillai[2], Gerhard Wolff[2], Sudha Visvanathan[2], Ronald G. Crystal[3]. *[1]Translational Research Sciences, Hoffmann La-Roche, Inc., Nutley, NJ, United States; [2]Inflammation Research, Hoffmann La-Roche, Inc., Nutley, NJ, United States; [3]Department of Genetic Medicine, Weill Cornell Medical College, New York, NY, United States; [4]Inflammation Research, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, United States*

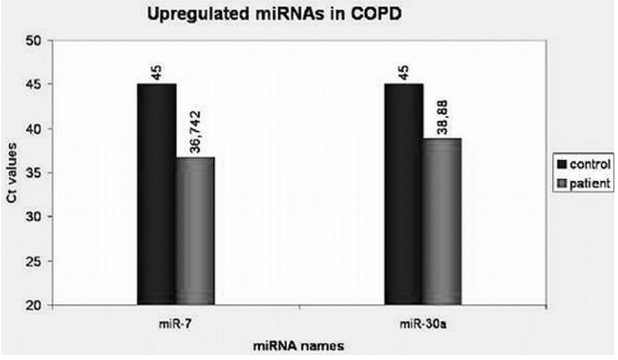
Introduction: The goal of this study was to determine temporal changes in regulatory gene networks in the small airway epithelium of subjects with COPD.
Methods: Small airway epithelial (SAE) cells were collected at bronchoscopy

from smokers with COPD (n=42), healthy nonsmokers (NS, n=30) and healthy smokers (HS, n=71). A subset of these subjects were subsequently sampled after 3 months (n=25 NS, n=11 HS, n=10 COPD). RNA from >95% pure populations of SAE was isolated and hybridized to Affymetrix microarrays. Weighted gene co-expression network analysis (WGCNA) was used to determine modules of correlated genes associated with clinical parameters at each timepoint, and temporal modules were compared to determine similarities. Functional categories were determined for modules using gene ontology analysis.
Results: WGCNA yielded an immune signaling module (e.g. TLR signaling and inflammasome genes), correlated with decline in FEV1/FVC ratio (baseline: R=-0.28, p = 0.002; month 3: R=-0.57, p=0.007). There was a significantly high degree of overlap in the immune-signaling module generated at each timepoint (173 genes, p=0) indicating the robustness of expression of this module. Another module associated with smoke exposure included genes involved in oxidative stress response and metabolic pathways.
Conclusions: Network analysis revealed a data-derived inflammation module associated with decline in lung function which is perturbed over time. This suggests that subtle induction of inflammatory signaling can be measured in the airway epithelium of COPD subjects in addition to pathways known to be induced by cigarette smoke exposure.

P4801
Investigation of the role of miRNAs as a biomarker in chronic obstructive pulmonary disease (COPD)
Fahri Akbas[1], Ender Coskunpinar[2], Engin Aynaci[3], Yasemin Musteri Oltulu[2], Pinar Yildiz[3]. *[1]Department of Genetics and Bioengineering., Fatih University, Istanbul, Turkey; [2]Department of Molecular Medicine, Istanbul University Institute of Experimental Medicine, Istanbul, Turkey; [3]Pulmonology, Third Clinic, Yedikule Chest Diseases and Thoracic Surgery Training Hospital, Istanbul, Turkey*

COPD as a complex disease with genetic and environmental compound, is one of the leading cause of death in worldwide. miRNAs have role in gene expression as responsible gene's down-regulated by blocking mRNA after transcription, translation inhibition and breaking of mRNA. Aim of the study is definition a biomarker that are based on the measurement miRNA level of plasma, is specific to COPD. 20 COPD and 10 healthy control cases were included in this study. The median age was 59±6,6 and 55±5,2 years for controls and COPD, respectively. miRNA-specific quantitative real-time-PCR based miRNA (miR-qRT-PCR) detection has been used. "Free PCR Array Data Analysis Software" has been used for data analysis. As a result of the study, two miRNA were significantly upregulated (miR-7 and miR-30a) and seven different miRNA were significantly downregulated in COPD patients (miR-9, miR-20a, miR-26b, miR-28-3p, miR-34c-5p, miR-100, miR-101).





As a conclusion, this is the first study using QRT PCR Array method to investigate the association between serum miRNAs and COPD. Upregulation of miR-7 and miR-30a might be potential biomarkers for early diagnosis of disease.

P4802
Cell-cell variation in expression of $bla_{CTX-M-14}$
Xiaorong Wang[1], Yu Kang[1], Shuchang An[1], Tong Zhao[2], Jichao Chen[3], Rongrong Fu[1], Zhancheng Gao[1]. *[1]Department of Respiratory & Critical Care Medicine, Peking University People's Hospital, Beijing; [2]Institute of Microbiology, Chinese Academy of Sciences, Beijing; [3]Department of Respiratory Medicine, The Central Hospital of China Aerospace Corporation, Beijing, China*

Background: Cell-cell variation in expression of their gene in isogenic population is frequently observed in microbes, which is recognized as one of the reasons for phenotype heterogeneity. The aim of this study is to investigate the hetorogeneous resistance level in genetically identical cells which was origenated from cell-cell variation in expression of $bla_{CTX-M-14}$.
Methods: The transcriptional region of CTX-M-14 with or without the entire coding sequence of CTX-M-14 which were amplified from clinic strain isolated from patients with lower respiratory tract infection were subcloned to upstream of green fluorescent protein (GFP) gene to regulate expression of GFP followed by standard method. Applying flow cytometry measurement (FCM) to analyze the expression pattern of $bla_{CTX-M-14}$ in single cell using GFP as the report genes. The expression pattern of cells after cultured with different ceftriaxone concentrations ranging from 0μg/ml to 2048μg/ml was also analyzed. Using the E-test method to measure the MIC to ceftriaxone.
Results: Variation in GFP expression from cell to cell was seen (The fluorescence intensity of different cells vary largely, ranging from 10^2 to 10^5). The resistance level to ceftroaxone was positively correlated with the expression level, with increment of the extracellular ceftroaxone concentrations, the proportion of cells with more GFP abundance increasing. We also found the epigenetic resistance phenotype mediated by the heterogeneous expression was non-strictly inheritable and have a transient property.
Conclusions: Heterogeneous expression of antibiotic genes other than nucleotide alterations in genetically identical cells may be one of the reasons for diverse antibiotic resistance level.

P4803
Polymorphisms of genes involved in extracellular matrix remodeling, xenobiotic metabolism, antioxidant pathways and chronic lung disease in children
Gulnaz Korytina[1], Leysan Akhmadishina[1], Elena Victorova[1,3], Tatyana Victorova[1,2]. *[1]Genomics, Institute of Biochemistry and Genetics, Ufa Scientific Center, Ufa, Russian Federation; [2]Biology, Bashkortostan State Medical University, Ufa, Russian Federation; [3]Genetics, George-August University of Goettingen, Goettingen, Germany*

We performed a candidate gene association study of 43 polymorphisms in genes coding genes functioning in extracellular matrix remodeling, xenobiotic metabolism and antioxidant pathways in 257 children with severe chronic lung disease (CLD) and 335 controls.
The frequencies of wild type/wild type genotype of CYP2F1 (c.14_15insC) gene were significantly higher in CLD patients than in the healthy control group (Padj=0.000001; OR=3.16 2.10-4.77). Association with CLD and CYP1A1 gene polymorphisms (3798T>C and 2454A>G) in additive model (Padj=0.0003; OR=1.72 1.28-2.33 and Padj=0.001; OR=2.31 1.28-2.33) was found. The patients with CLD showed significantly elevated frequencies of the GSTT1 gene deletion (Padj=0.0003; OR=1.98 95% CI 1.36-2.89). The GSTP1 (313A>G) polymorphism was associated with CLD (for AA genotype Padj = 0.0046, OR=1.65 1.17-2.34). Regression analysis showed that CAT (-262C>T) CC genotype is associated with a 1.84 -fold increase (95% CI 1.22-2.65; Padj=0.0009) and NQO1 (465C>T) is associated with a 1.89 -fold increase (95% CI 1.19-3.02; Padj=0.006) in additive model. Association with CLD and MMP3 (-1171 5A>6A), MMP12 (-82A>G), MMP9 (2660A>G) and TIMP3 (-1296T>C) gene polymorphisms in dominant model was found (Padj=0.0013, OR=2.75 1.43-5.31; Padj=0.007, OR=1.83 1.16-2.89; Padj=0.017, OR=1.54 1.08-2.21; Padj=0.033, OR=1.48 1.11-2.01)
Consequently, CYP2F1, CYP1A1, CAT, GSTP1, GSTT1, NQO1, MMP3, MMP9, MMP12 and TIMP3 genetic polymorphisms probably play a substantial part in susceptibility to severe pulmonary inflammation in children with CLD.

P4804
Association of xenobiotic metabolizing gene polymorphisms and chronic obstructive pulmonary disease in Indian population
Naushad Khan[1,2], Mradul Daga[1], Nitish Kamble[1], Govind Mawari[1], Istaq Ahmed[2], S.A. Husain[2]. *[1]Medicine, Maulana Azad Medical College, New Delhi, Delhi, India; [2]Biotechonology, Jamia Millia Islamia, New Delhi, Delhi, India*

Background and aim: Genetic susceptibility to the development of chronic obstructive pulmonary disease might depend on variation in the activities of enzymes that detoxify cigarette smoke products. We studied the relationship of GSTP1, GSTM1, and GSTT1 gene polymorphisms with COPD risk in a case- control study of Indian patients and controls.
Material and methods: A total of 186 patients with COPD and 160 healthy controls were included in the study. The Frequencies of GSTP1, GSTM1 alleles was determined by using conventional Multiplex PCR and GSTP1 by polymerase chain reaction and restriction fragment length polymorphisms technique.

Results: A significant case –control difference was observed for the presence of null GSTM1, (61.8% Vs 55.0%, P= 0.04). No difference was observed in the frequency of GSTT1 Null genotype and COPD susceptibility. (54.8% vs 50.6% OR: 1.26; CI: 0.87- 1.84; P value = 0.82) For GSTP1 polymorphism we found that Subjects homozygous variants Val/Val were at increased risk of developing COPD (OR: 2.58; CI: 1.2- 4.8) as compared to heterozygote variants Ile/Val (OR: 1.28 CI: 0.7-2.14) Also, the mutant allele frequency (Val) was significantly higher in patients as compared to controls and the difference was found to be statistically significant. (OR: 1.8 CI: 1.4- 4.2; P Value =0.001)
Conclusion: We propose that subjects with GSTM1 null allele and GST P1 homozygous isoleucine genotypes are at higher risk of COPD and are significant indicators of susceptibility to chronic obstructive pulmonary disease in Indian population.

P4805
Rare alpha-1 antitrypsin mutations in the Irish population
Tomás Carroll[1], Geraldine O'Brien[1], Catherine O'Connor[1], Ilaria Ferrarotti[2], Stefania Ottaviani[2], Maurizio Luisetti[2], Gerard McElvaney[1]. *[1]Department of Medicine, Royal College of Surgeons in Ireland, Dublin, Ireland; [2]Department of Molecular Medicine, University of Pavia, Italy*

AAT deficiency (AATD) results from mutations in the SERPINA1 gene, classically presenting with early-onset emphysema and liver disease. The most common mutation responsible for AATD is the Z mutation. AAT deficiency is under-diagnosed and prolonged delays in diagnosis are common. ERS and ATS guidelines advocate the screening of all COPD, poorly-controlled asthma, and cryptogenic liver disease patients, as well as first degree relatives of known AATD patients.
8,000 individuals have been screened following ATS/ERS guidelines as part of the Irish national targeted detection programme. AAT levels were determined by immunonephelometry. AAT phenotyping was performed by isoelectric focussing. Rare and novel mutations were identified by DNA sequencing of the SERPINA1 gene.
A number of rare SERPINA1 mutations including I, F, V, Xchristchurch, Zbristol, and Mmalton were identified. The I mutation (Arg39Cys) was present at a relatively high frequency (0.0038) in the targeted population, with over 60 cases described. Three Null SERPINA1 mutations were detected, including two novel mutations. In addition, the first individuals in Ireland homozygous for a Null mutation and for the Mmalton mutation were identified.
Current testing of suspected AATD cases is often limited and can miss rare and novel clinically significant SERPINA1 mutations. The rare mutations described in this study were not detected by a commonly used genotyping assay; however, the low AAT levels prompted their correct identification using more detailed genetic analysis. Our findings underline the need for a comprehensive diagnostic work up of all patients with low AAT levels including phenotyping, genotyping and if necessary, sequencing of the SERPINA1 gene.

P4806
Lung fibroblast function in patients with Birt-Hogg-Dubé syndrome
Yoshito Hoshika[1], Fumiyuki Takahashi[1], Shinsaku Togo[1], Muneaki Hashimoto[2], Toshiyuki Kobayashi[3], Hideyuki Kataoka[4], Masatoshi Kurihara[4], Etsuko Kobayashi[1], Katsutoshi Ando[1], Kuniaki Seyama[1], Kazuhisa Takahashi[1]. *[1]Respiratory Medicine, Juntendo University School of Medicine, Tokyo, Japan; [2]Molecular and Cellular Parasitology, Juntendo University School of Medicine, Tokyo, Japan; [3]Molecular Pathogenesis, Juntendo University School of Medicine, Tokyo, Japan; [4]Pneumothorax Research Center and Thoracic Surgery Division, Nissan Tamagawa Hospital, Tokyo, Japan*

Background: Birt-Hogg-Dubé syndrome (BHDS) is an autosomal dominant inherited disorder caused by germline mutations in the *FLCN* gene, and characterized by skin fibrofolliculomas, multiple lung cysts, spontaneous pneumothorax, and renal neoplasms. Pulmonary manifestations frequently develop earlier than other organ involvements and then frequently prompt to make a diagnosis of BHDS.
Objective: To clarify the mechanism of lung cyst formation, we isolated lung fibroblasts from patients with BHDS and analyzed their function because they are principal cells responsible for the production and maintenance of extracellular matrix.
Methods: Lung fibroblasts were isolated from lung tissue obtained from patients with BHDS (n=10) and lung cancer (n=7). Lung fibroblast function was evaluated by chemotaxis to fibronectin and three dimensional (3D)-gel contraction assay.
Result: We confirmed that lung fibroblasts express *FLCN* mRNA, but there was no difference in expression level between control and BHDS fibroblasts. BHDS fibroblasts showed reduced chemotaxis as compared with control fibroblasts (p=0.001). But there was no significant difference in contraction of 3D-collagen gels between two groups (p=0.1726). Expression of fibronectin, transforming growth factor (TGF)-b1 and type I collagen was significantly reduced in BHDS fibroblasts as compared to control fibroblasts when evaluated by real-time PCR. In support of these results obtained from primary cultured fibroblasts, normal fetal lung fibroblasts (HFL-1) exhibited reduced chemotaxis when *FLCN* expression in HFL-1 was knock-downed.
Conclusion: Our results suggest that FLCN haplo-insufficiency in the lung may cause fibroblast dysfunction leading to impaired tissue repair.

P4807
CCDC103 encodes a novel cilia dynein arm factor that is mutated in primary ciliary dyskinesia
Claudius Werner[1], Anita Becker-Heck[1], Jennifer R. Panizzi[2], Victoria H. Castleman[3], Dalal Al-Mutari[4], Eamonn Sheridan[4], Niki T. Loges[1], Heike Olbrich[1], Rahul Chodhari[5], Christopher O'Callaghan[6], Eddie M.K. Chung[5], Richard Reinhardt[7], Hannah M. Mitchison[3], Iain A. Drummond[2], Heymut Omran[1]. *[1]Klinik fuer Kinder- und Jugendmedizin -Allgemeine Paediatrie, Universitaetsklinikum Muenster, Germany; [2]Nephrology Division, Massachusetts General Hospital, Charlestown, United States; [3]Molecular Medicine Unit, University College London, United Kingdom; [4]Leeds Institute of Molecular Medicine, St. Jame's University Hospital, Leeds, United Kingdom; [5]General and Adolescent Paediatrics Unit, University College London, United Kingdom; [6]Department of Infection, Immunity and Inflammation, University of Leicester, United Kingdom; [7]Genome Centre Cologne, MPI for Plant Breeding Research, Cologne, Germany*

Primary ciliary dyskinesia (PCD) is a genetically heterogenous disorder characterized by chronic destructive respiratory tract disease. In about 50% of cases it is associated with situs inversus, because embryonic cilia play a critical role in establishing organ left-right asymmetry.
Zebrafish schmalhans mutants exhibit characteristic features of ciliopathy caused by a mutation in ccdc 103; in electron microscopy cilia lack inner (IDA) and outer (ODA) dynein arms.
Screening individuals for CCDC103 (the human ccdc103 ortholog) identified ten patients with mutations. We found homozygous loss-of-function mutations in six individuals (c.383_384insG) predicting a frame shift and premature termination of translation. In four affected individuals a homozygous transversion (c.A461C; p.H154P) was identified.
All affected individuals exhibited typical clinical findings for PCD. Three patients had situs inversus totalis, one had situs inversus abdominalis and two dextrocardia. High-speed videomicroscopy (HVM) of patient OP-1192II1 (c.383_384insG) showed ciliary immotiliy with only residual flickering. By contrast, in two patients with the p.H154P variant, HVM showed reduced beat amplitude and coordination and few immotile cilia.
In patient OP-1192II1, Immunofluorescence microscopy demonstrated distal ODA deficiency. Cells from PCD patient OP-1194II1 (p.H154P variant) displayed a normal localization of ODA components. Both patients showed a normal localization of the IDA component DNALI1.
Our findings indicate that CCDC103 mutations cause PCD in humans. Whereas the loss-of-function mutation results in ciliary immotility and distal ODA deficiency, the p.H154P variant presents as a hypomorphic mutation.

P4808
Recessive HYDIN mutations cause primary ciliary dyskinesia without situs abnomalities
Johanna Raidt[1], Heike Olbrich[1], Claudius Werner[1], Niki T. Loges[1], Nora F. Banki[2], Amelia Shoemark[3], Tom Burgoyne[3], Gabriele Köhler[4], Josef Schroeder[5], Gudrun Nürnberg[6], Peter Nürnberg[6], Richard Reinhardt[7], Heymut Omran[1]. *[1]Klinik fuer Kinder- und Jugendmedizin -Allgemeine Paediatrie, Universitaetsklinikum Muenster, Germany; [2]Department of Pediatrics, Semmelweis University, Budapest, Hungary; [3]Electron Microscopy Unit, Royal Brompton Hospital, London, United Kingdom; [4]Department of Pathology, Universitaetsklinikum Muenster, Germany; [5]Department of Pathology, University of Regensburg, Germany; [6]Institute for Genetics, Cologne Center for Genomics, Cologne, Germany; [7]Genome Centre Cologne, MPI for Plant Breeding Research, Cologne, Germany*

Primary ciliary dyskinesia (PCD) is a genetically heterogenous disease characterized by reduced muco-ciliary clearance. This is caused by defects in cilia motility. Impaired sperm flagella motility contributes to male infertility. In about 50% of cases PCD is associated with situs inversus or more rarely situs ambiguous as a result of embryonic cilia dysfunction leading to randomization of left-right body asymmetry.
70 years ago the hy3 mouse carrying hydin mutations has been described. These mutations lead to an abnormal composition of the central pair (CP) apparatus. Clinically, these mice suffer from lethal hydrocephalus.
Now we report HYDIN mutations in human PCD patients without hydrocephalus and normal body composition.
By using a homozygousity mapping strategy we identified a novel PCD locus on chromosome 16q21-q23 across the HYDIN locus. In three affected siblings genomic analyses showed homozygous c.3985G>T HYDIN mutations affecting the evolutionary conserved splice acceptor site of exon 27. We confirmed aberrant splicing with early stop of translation by cDNA analysis.
High-speed videomicroscopy (HVM) of respiratory cells showed a reduced bending capacity. Sperm motility was markedly decreased with only 8% of sperms showing minimal progressive motility.
Transmission electron microscopy (EM) appeared normal in most cross sections. 9 + 0 cilia and 8 + 1 cilia composition were found very rarely. EM tomography showed absence of the CP apparatus C2b projection resembling findings in Hydin-deficient mice.
Our results expand the knowledge on PCD genetics. Careful diagnostic evaluation is obligate in this PCD variant as HVM and EM findings are subtle.