498. COPD inflammation and genes

P4793
Association of GSTT1 & M1 gene polymorphism with ageing in Northern Indian COPD and lung cancer patients
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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=0.013), 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
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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 (PAR1) activation leads to the generation of several inflammatory mediators involved in COPD and our unpublished data have shown that functional polymorphisms of PAR1 are protective in sarcoidosis.

Aims & objectives: The aim of this study was to investigate whether PAR1 polymorphisms are associated with COPD exacerbation frequency (ExF).

Methods: Two PAR1 SNPs (rs2227744 and rs32934) and a 13bp in/del (rs11267092) were genotyped in 136 infrequent and 67 frequent exacerbators. 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 PAR1 expression, these data suggest that this SNP may confer a degree of protection against exacerbations in COPD by increasing PAR1 expression. Funded by the BLF.

P4795
Heterozygosity for E292V in ABCA3, lung function and COPD in 64,000 individuals
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Background: COPD inflammation and genes

Results:

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Background: COPD inflammation and genes

Results:
SNPs in TSPYL-4, NT5DC1 genes are associated with susceptibility to COPD

P4797

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 to the results of genotyping.

Results: The frequencies of the SNPs rs3749893 of tests specific protein Y encoded like 4 (TSPYL-4) gene G allele and SNP (rs1052443 of 5’-nucleotidase domain containing 1C) A allele were both significantly higher in cases than in controls (p=0.02, OR=0.59, 95% CI=0.34-0.99; p=0.015, OR=0.60, 95%CI=0.47-0.79, respectively). There were two blocks of SNPs (rs1052443 and rs3749893, rs1152542 and rs6037121) 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 are 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.
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 cells 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 correlation between inflammasome genes, correlated 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.

P4802
Cell-cell variation in expression of blaCTX-M-14
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Background: Cell-cell variation in expression of their gene in isogenic population is frequently observed in cancer, which is recognized as one of the reasons for phenotype heterogeneity. The aim of this study is to investigate the heterogeneous resistance level in genetically identical cells which was originated from cell-cell variation in expression of blaCTX-M-14.

Methods: The transcriptional region of CXT-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 blaCTX-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 204μg/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 107 to 109). The resistance level to ceftriaxone was positively correlated with the expression level, with increment of the extracellular ceftriaxone concentrations, the proportion of cells with more GFP abundance increasing. We also found the epitogenic 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
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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.14,15CMC) 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=0.0033; OR=1.19 1.13-2.39). The GSTT1 (313A>G) polymorphism was associated with CLD (for AA genotype Padj = 0.0046, OR=1.65 1.17-2.34).

Regression analysis showed that CYP1A1 (3798T>C) and GSTT1 (313A>G) gene polymorphisms were significantly associated with a 1.84-fold increase (95% CI 1.22-2.65; Padj=0.00009 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 MMP9 (-1171 5A>G) and TIMP3 (-1296T>G) in additive 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=1.88 1.11-2.71).

Consequently, CYP2F1, CYP1A1, CAT, GSTP1, GSTT1, NQO1, 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
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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 were determined by using conventional Multiplex PCR and GSTP1 by polymerase chain reaction and restriction fragment length polymorphisms technique.

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.
Poster Discussion

P4807

Primary ciliary dyskinesia (PCD) is a genetically heterogenous disorder charac-
terized 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. Zebralis schmalmans exhibit characteristic features of cilopathy caused
by a mutation in ccd103; in electron microscopy cilia lack inner (IDA) and outer
(ODA) dynein arms. Screening individuals for CDDC103 (the human ccdc103 ortholog) identified ten
patients with mutations. We found homozygous loss-of-function mutations in six
siblings across all PCD and cryptogenic liver disease patients, as well as at least six
reports on children of known affected parents. Rare and novel mutations were identified by DNA sequencing of the SERPINA1
gene.

A number of rare SERPINA1 mutations including I, F, V, X, 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 AAT 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.

P4808

Recessive HYDIN mutations cause primary ciliary dyskinesia without situs
inversum abnormalities

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Primary ciliary dyskinesia (PCD) is a genetically heterogenous disease character-
ized by reduced mucociliary 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 by3 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 homozygosity mapping strategy we identified a novel PCD locus on chromosome 16q21-q23 across the HYDIN locus. In three affected siblings
we found 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 compositions were found very rarely. EM tomogra-
phy 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.

882s

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