## 246. Genetic and molecular background in pulmonary fibrosis

**P1994**

**Mutations in SFTPC, SFTPA2 and TERT explain 60% of familial pulmonary fibrosis and correlate to specific disease phenotypes**

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Idiopathic Pulmonary Fibrosis (IPF) is a fatal lung disease, histologically characterized by diffuse interstitial remodeling and patchy inflammation. A significant percentage of IPF patients have a familial form of the disease. Separate reports have identified mutations in Surfactant Protein-C (SFTPC), Surfactant Protein-A2 (SFTPA2), Telomerase Reverse Transcriptase (TERT) or Telomerase RNA component (TERC) in these families.

We determined the frequency of mutations in SFTPC, SFTPA2, TERT and TERC in 20 patients with Familial Pulmonary Fibrosis (FPF).

Heterozygous non-tolerated sequence changes were detected in 12 out of 20 patients, consisting of 5 SFTPC, 2 SFTPA2 and 5 TERT mutations. Mutations segregated with disease in each family and haplotype analysis showed that identical mutations had arisen independently. Families with SFTPC and SFTPA2 mutations always had evidence of parent-offspring disease transmission, while in families with TERT mutation sibs were affected. Pediatric pulmonary disease occurred only in families with SFTPC mutations. Carriers of an SFTPA2 mutation also suffered from lung cancer. Families with a TERT mutation usually presented as typical IPF and did not show clear symptons associated with other known syndromes of telomere shortening.

This is the first report of a cohort of IPF families that is completely sequenced for candidate genes. We could identify a mutation in 60% of patients with FPF. These mutations correlated with a specific disease phenotype. The function of each of the mutated genes is very different, but all indicate towards a central role for the alveolar type II cell in disease pathogenesis.

**P1995**

**Association between polymorphisms in the P53 and P21 genes and IPF**

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**Introduction:** Idiopathic pulmonary fibrosis (IPF) is devastating and progressive lung disease. Its aetiology remains unclear but is thought to involve damage to the epithelium and abnormal repair. Alveolar epithelial cells near areas of remodelling show an increased expression of proapoptotic molecules (1,2). The purpose of this study was to investigate the role of genes involved in cell cycle control in IPF.
**Materials and methods:** We included 353 controls and 77 IPF patients and determined genotypes for five polymorphisms in the p53 gene and four polymorphisms in CDKN1A, the gene encoding p21. In PBMC from 16 healthy controls mRNA expression of p53 and p21 was determined.
**Results:** The rs12951053 and rs12602273 polymorphisms in the p53 gene were significantly associated with survival in IPF patients. Carriers of the minor allele had a 4-year survival of only 22% versus 57% in the non-carrier group (p=0.006). All four polymorphisms in CDKN1A significantly predisposed to IPF. The rs2395655 polymorphism was most associated with increased risk of developing IPF. In addition, the rs2395655G allele was associated with a rapid decline in lung function. The rs733590 polymorphism was significantly associated with p21 mRNA expression levels.
**Conclusion:** This study reports the novel finding that polymorphisms in the p53 gene are associated with survival and polymorphisms in the p21 gene predispose to IPF. This suggests cell cycle defects are involved in the pathology of IPF. Variations in the p53 and p21 genes can impair the response to cell damage and increase the loss of alveolar epithelial cells.
1 Kuwano, K. et al. Am J Respir. Crit Care Med. 1996; 154:477-483.
2 Plataki, M. et al. Chest 2005;127:266-274.

**P1996**

**Genetic variability in the IL1RN gene and the balance between IL1-Ra and IL-1β in IPF**

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**Introduction:** Idiopathic pulmonary fibrosis (IPF) is a rapidly progressive interstitial lung disease of unknown etiology. Interleukin (IL) -1β plays an important role in inflammation and has been associated with fibrotic remodelling. We investigated the balance between IL-1β and interleukin-1 receptor antagonist (IL-1Ra) in bronchoalveolar lavage fluid (BALf) and serum as well as the influence of genetic variability in the IL1B and IL1RN gene on disease susceptibility and cytokine levels.
**Materials and methods:** In 77 IPF patients and 349 healthy controls, single nucleotide polymorphisms (SNPs) in the IL1RN and IL1B gene were determined. Serum and BALf IL-1Ra and IL-1β levels were measured using a multiplex suspension bead array system and were correlated with genotypes.
**Results:** Both in serum and BALf a significantly decreased IL-1Ra/ IL-1β ratio was found in IPF patients compared to healthy controls. In the IL1RN gene, one SNP was associated with both the susceptibility to IPF and reduced IL-1Ra/IL-1β ratios in BALf.
**Conclusion:** Our results show that genetic variability in the IL1RN gene plays a role in the pathogenesis of IPF and that this role may be more important than until recently thought. IPF patients appear to have a relative shortage of IL-1Ra, which might contribute to a pro-inflammatory and pro-fibrotic environment in their lungs.

**P1997**

**The relationship of IL-4 cytokine gene polymorphisms, HRCT and histopathological score in patients with idiopathic pulmonary fibrosis**

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**Introduction:** Idiopathic pulmonary fibrosis (IPF) is a serious disease with unknown etiology, where an influence of cytokine gene polymorphisms is presumed. We compared HRCT alveolar (AS) and interstitial score (IS) and histoptahologic score with IL-4, and IL-4RA gene polymorphisms in IPF patients.
**Subjects and methods:** IPF was diagnosed in 46 patients according to ATS/ERS consensus statement. 43 patients had evaluable HRCT investigations, 14 patients had surgical lung biopsy. HRCT scans were evaluated using IS and AS scales by Gay et al. The histopathological evaluation of lung biopsies comprised: myofibroblast foci (MF), inflammation, eosinophils, granulomas and Ashcroft criteria for fibrosis grading. The IL-4 (-1098) (-590) (-33) and IL-4 RA +1902 gene polymorphisms were characterized utilizing a PCR- SSP method.
**Results:** AS was higher in IL-4 haplotypes 1 TTC and TTT carrierrs (p=0.0423). Ashcroft score was more advanced in IL-4 haplotype 2 GCC (p= 0,013) and MF counts were higher in TCC carriers (p=0,0736). IL-4 RA +1902 A1 G and IL-4 -590 A1 T correlated with higher AS (p=0,0335; p=0,0123). Ashcroft score was higher in IL-4 -1098 A2 G and IL-4 -33 A1 T carriers (p= 0,0443, p=0,0915).
**Conclusions:** We assume that IL-4 and IL-4RA polymorphisms might influence HRCT and histopathological phenotype of IPF. The correlation of functionally relevant IL-4 genes polymorphisms (especially IL-4 -33 T) with AS could mean, that new alveolar lesions with continuing fibrosis are more pronounced in these polymorphisms carriers. The positive correlation of IL-4 -33 A1 T with Ashcroft score might support a hypothesis of fibrogenetic role of IL-4 in IPF.

**P1998**

**Familial idiopathic pulmonary fibrosis and "genetic anticipation"**

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**Background:** Telomere dysfunction can be associated to "genetic anticipation", earlier age of onset and more rapid progression of disease in succeeding generations, well known in Dyskeratosis Congenita, due to heterozygous TERC/TERT mutations (essential components of telomerase), which have been reported in 8-15% of families with Idiopathic Pulmonary Fibrosis (IPF) and 1-3% of non familial IPF.
**Aims and objectives:** Retrospective study to assess whether a form of genetic anticipation can be found in families with IPF and to establish clinical features and outcome of familial IPF in comparison to non familial IPF
**Methods:** We reviewed all files of patients with familial IPF seen at our department (17 families consisting of a total of 37 individuals) and compared age at diagnosis, clinical progression, frequency of acute exacerbations and survival outcomes with a database of 162 patients with non familial IPF. We are sequencing TERT and TERC genes in patients with familial IPF.
**Results:** Among the familial IPF patients, 5 had their father or mother affected; in all these cases the son/daughter was diagnosed earlier than the parents (mean age 56,4 years vs 72,4) and had a more rapid progression (all patients had at least one disease progression during follow-up); mean age of diagnosis of siblings and non familial IPF patients was 60,9 years and 65,4 years respectively.
**Conclusions:** We hypothesize a form of genetic anticipation in families with IPF, with the latest generations being most severely affected. We are sequencing TERT and TERC genes in these patients to demonstrate that genetic anticipation in Familial IPF is associated to the inheritance of shorter-than-normal telomeres in association with the defective telomerase activity.

**P1999**

**Association of single nucleotide polymorphisms in 4 genes (VDR, COL1A1, CALCR and BGLAP) with susceptibility to steroid osteoporosis in patients with idiopathic pulmonary fibrosis (IPF)**

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Steroid osteoporosis is a serious medical and economic problem. At the same time, osteoporosis is a polygenic disorder.
**Aim:** To assess effectiveness of steroid osteoporosis prevention by antiresorptive agents (ARA - Bisphosphonates, Calcitonin) in patients with IPF with different genetic predisposition to osteoporosis.
**Subjects:** 114 patients with IPF, 19 males, 95 females, age 56.7±10.6 years, treated with Corticosteroids (CS).
**Methods:** Bone mineral density (BMD) measuring by DEXA, patients' questionnaires and genotyping were used. Genomic DNA was isolated from peripheral leukocytes. We investigated 5 SNPs by PCR-RFLP analysis in 4 genes: vitamin D receptor, collagen type1 alpha1, calcitonin receptor and osteocalcin.
**Results:** Severity of BMD loss and bone fractures occurrence strongly correlated with CS cumulative dose (p=0.010 and p=0.001, respectively). Multiple regression analysis showed significant influence of only VDR-FokI on BMD (p=0.009), and BGLAP was about significant (p=0.081). Environmental factors, firstly ARA intake, seems to have stronger influence on BMD than genes (adjusted $R^2$=0.065).

| Gene Acronym | Gene OMIM | dbSNP | Restriction Enzyme | Minor Allele Frequency |
|---|---|---|---|---|
| VDR | 601769 | rs1544410 | BsmI | 0.382 |
| VDR | 601769 | rs2228570 | FokI | 0.408 |
| COL1A1 | 120150 | rs1800012 | Van91I | 0.175 |
| CALCR | 114131 | rs1801197 | AluI | 0.276 |
| BGLAP | 112260 | rs1800247 | HindIII | 0.180 |

**Conclusion:** ARA administration is necessary for all patients with IPF, irrespectively of genotype. VDR-FokI analysis is useful to reveal subjects with increased risk of osteoporosis in order to more active BMD loss prevention. Further efforts are required to clarify weight of BGLAP.

**P2000**

**The genetic polymorphism of metalloproteinases MMP2, 7, 9 and MMP inhibitor TIMP2 in sarcoidosis**

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**Background:** Increased activity of metalloproteinases may play a role in the initiation and propagation of inflammation in sarcoidosis. It may also be one of the factors responsible for the development of lung fibrosis. The aim was to verify whether polymorphisms of MMP2 C735T, MMP7 A181G, MMP9 T1702A and tissue inhibitor of metalloproteinase (TIMP)2 G418C predispose to sarcoidosis.
**Material and methods:** 139 patients with sarcoidosis and 100 healthy subjects were included. MMPs and TIMP2 mRNA were measured in peripheral blood lysate using real time RT-PCR. DNA for genetic polymorphism was extracted from peripheral blood by GTC method. Protein concentrations in peripheral blood lysates were measured by ELISA, and MMP2 and 9 activities in BAL fluid were estimated by gel zymography.
**Results:** TT genotype in MMP9 T1702A was more frequent in sarcoidosis ($p<0.0001$, OR=13.71, 95%CI 7.02-26.80) and resulted in higher expression of MMP9 mRNA ($p<0.0001$). There was no relation to radiological stages, lung function test parameters, activity markers and the presence/absence of Löfgren syndrome. There were no differences in the distribution of MMP2, MMP7 and TIMP2 polymorphisms. MMP2, 7, 9 and TIMP2 mRNAs, as well as concentrations of these molecules were elevated ($p<0.0001$ for each). Gel zymography did not show differences in MMP2 and MMP9 activity in BAL fluid between different genotypes.
**Conclusions:** The TT homozygotes of MMP9 T1702A genotype may be predisposed to sarcoidosis. Elevated mRNAs of all these molecules suggest their inducibility.
This study was supported by the Medical University of Lodz (grant No 502 -16-807) and Polish Ministry of Science (grant NN 402 350838).

**P2001**

**Dose-dependent pro- or anti-proliferative effects of calcineurin inhibitors in bronchiolitis obliterans following allogeneic stem cell transplantation**

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**Background:** Bronchiolitis obliterans (BO) is a common complication after allogeneic stem cell transplantation (SCT), characterized by fibroproliferation, fibrotic occlusion of small airways, and poor prognosis. As BO is strongly associated with chronic graft-versus-host disease (GVHD), it is believed to be a pulmonary manifestation of chronic GVHD. The management of BO comprises the augmentation of immunosuppressive therapy, but treatment response is generally poor. Here, we investigated the effect of methylprednisolone (mPRED), cyclosporine A (CsA), and tacrolimus (FK506) on the proliferative capacity of fibroblasts isolated from surgical lung biopsies of SCT patients with histologically proven BO.
**Methods:** Primary cultures of human lung fibroblasts were grown from surgical lung biopsies obtained from 8 patients with BO after SCT. Fibroblasts were stimulated with increasing concentrations of each drug, and cell proliferation was assessed by [$^3$H]-thymidine incorporation.
**Results:** In fibroblasts derived from patients with BO after SCT low concentrations of CsA (0.01 mg/l, 0.1 mg/l) and FK506 (0.001 mg/l, 0.01 mg/l) significantly induced proliferation compared to untreated cells. Only high dose CsA (50 mg/l) and FK506 (5 mg/l) exerted an anti-proliferative effect in primary human lung fibroblasts derived from BO patients. mPRED caused an inhibition of proliferation in clinically relevant concentrations (10 mg/l, 50 mg/l).
**Conclusion:** Our data suggest that calcineurin inhibitors such as CsA and FK506 have no beneficial effect during the fibroproliferative phase of BO following allogeneic SCT.

**P2002**

**Enhanced expression of Fas ligand (FasL) in fibrotic interstitial lung diseases (ILDs). The possible role of membrane-bound FasL form**

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**Background:** The exact role of FasL and particularly its soluble and membrane-bound form in the development of chronic ILDs and lung fibrosis has not been extensively explored.
**Methods:** We aimed at analyzing membrane-bound FasL expression on alveolar macrophages (AM) and lymphocytes (AL) as well as soluble FasL (sFasL) levels in bronchoalveolar lavage (BAL) from ILDs patients: pulmonary sarcoidosis (PS), hypersensitivity pneumonitis (HP), silicosis, asbestosis, idiopathic pulmonary fibrosis (IPF), nonspecific interstitial pneumonia (NSIP), and healthy subjects (n=89,12,7,8,23,6,17, resp.).
**Results:** In IPF significantly increased percentage of AM FasL+ and CD8+FasL+ cells as well as sFasL levels in BAL were found. Increased sFasL levels were also observed in HP. NSIP and asbestosis were characterized by higher AM FasL+ relative number; CD8+FasL+ population was expanded in asbestosis only. There was significant decline in AL FasL+ percentage in PS and HP. Systemic steroid treatment, assessed in PS and IPF subgroups, did not affect FasL expression. Smokers with ILD tended to present lower sFasL levels, but not BAL cell FasL+ numbers. Vital capacity was negatively correlated with sFasL levels, AM FasL+ and CD8+FasL+ cell relative count. CD4+FasL+ and CD8+FasL+ percentage strongly correlated with BAL neutrophilia, an unfavourable prognostic factor of lung fibrosis.
**Conclusions:** The concurrent comparative BAL analysis for FasL expression indicates that FasL+ AM and AL (especially Tc cells) comprise an important element of the fibrotic process, mostly in IPF. FasL might play a crucial role in other fibrosis-complicated ILDs, like NSIP and asbestosis.

**P2003**

**Heme oxygenase–1 induced by quercetin attenuates TGF-β-stimulated collagen production in fibroblasts**

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Quercetin is a flavonoid with a wide variety of cytoprotective and modulatory functions. Heme oxygenase–1 (HO-1) is an inducible enzyme. Its reaction product, carbon monoxide (CO), confers cellular protection in a number of conditions and diseases associated with oxidative or inflammatory lung injury. Furthermore, quercetin was reported to be a potent inducer of HO-1 in several cell types. We hypothesized that quercetin suppresses the production of collagen in fibroblasts via the induction of HO-1. Here, we showed that quercetin suppresses transforming growth factor–β (TGF-β) induced collagen production in NIH3T3 cells and in normal human lung fibroblasts. This suppressive effect of quercetin was mediated by quercetin-induced HO-1. The suppression of collagen production was conferred by the reaction product of HO-1, CO, but not by bilirubin. Furthermore, the translocation of the nuclear factor E2–related factor–2 (Nrf2), an important transcription factor that regulates the expression of HO-1 from the cytoplasm to the nuclei, was demonstrated in NIH3T3 cells by exposure to quercetin. Assessment of the signal transduction pathway involved in TGF-β signaling showed that quercetin stimulated the Smad and mitogen-activated protein kinase pathway to varying degrees. Our results demonstrate that quercetin exerts suppressive effects on the expression of collagen by the induction of HO-1. Idiopathic pulmonary fibrosis is the most lethal diffuse fibrosing lung disease, and is characterized by the deposition of extracellular matrix. Quercetin or its derivatives, which effectively induced HO-1, will lead to new therapeutic strategies for promoting antifibrotic therapy in respiratory diseases.

**P2004**

**Different expression pattern of endothelin receptors in primary human lung fibroblasts derived from idiopathic pulmonary fibrosis compared to healthy controls**

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**Background:** Endothelin-1 (ET-1) has a considerable fibrogenic activity and it has been implicated in the pathogenesis of pulmonary fibrosis. Increased levels of ET-1 have been demonstrated in serum and bronchoalveolar lavage fluid of patients with idiopathic pulmonary fibrosis (IPF), and lung tissue of IPF patients show increased ET-1 immunoreactivity. The biological effects of ET-1 are mediated through two receptors – ET-A and ET-B. However, disease specific patterns of ET receptor expression in fibrotic and normal primary human lung fibroblasts had not been studied yet.
**Methods:** Primary human lung fibroblasts were isolated and propagated from lung parenchyma derived from patients with IPF (n=4) as well as from parenchyma derived from healthy controls (n=4). Isolated cells were grown to confluence. After transforming growth factor beta1 (TGF-beta1) stimulation total protein was harvested and immuno blot analysis was performed.
**Results:** In fibroblasts derived from patients with IPF the ET-A and ET-B receptors were equally expressed, whereas in control fibroblasts expression of both receptors was lower compared to IPF cells. Stimulation with TGF-beta1 caused a further increase of ET-A and ET-B receptor expression by IPF fibroblasts, but no such up-regulation was detected in control fibroblasts.
**Conclusion:** Our data demonstrate for the first time a difference in the pattern of

ET receptor expression between IPF and normal lung, and a disease-specific reaction upon stimulation with TGF-beta1. Our observations may have implications for our understanding of the roles of ET-1 and TGF-beta1 in the pathogenesis of IPF.

**P2005**

**Role of phosphatidylinositol-3-kinase (PI3K) in TGF-β-induced proliferation and differentiation of human lung fibroblasts into myofibroblasts**

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Molecular mechanisms and pathogenesis of idiopathic pulmonary fibrosis (IPF) remain unclear yet TGF-β-induced differentiation and proliferation of fibroblasts/myofibroblasts are recognized as primary events.
We investigated the role of PI3K/Akt pathway in TGF-β-induced proliferation of human lung fibroblasts and their differentiation into myofibroblasts. Moreover, we evaluated the expression of all PI3K class I p100 isoforms (α, β, δ and γ). By using selective inhibitors, we also dissected the functional role of these isoforms.
*Ex-vivo* human lung fibroblasts were stimulated with TGF-β in the presence or absence of PI3Ks pan-inhibitor LY294002 as well as of selective inhibitors. Cell proliferation was evaluated by cell counts and WST-1 proliferation assay. Western blot analysis and the Sircol assay were used for assessing a-Smooth Actin (SMA) expression and collagen production, respectively. RNA messenger and protein levels of p100 isoforms were evaluated by Q-RT-PCR and western blot analysis, respectively.
Here we show that LY294002 was able to abrogate the TGF-β-induced increase in cell proliferation,α-SMA expression and collagen production besides to inhibit Akt phosphorylation, thus demonstrating the central role of PI3K/Akt pathway in TGF-β-induced lung fibroblast proliferation and differentiation. Moreover, we show that PI3K p110δ and p100γ are functionally expressed in human lung fibroblasts, in addition to the ubiquitously expressed p100α and p110β. Finally, we demonstrate a major role of p110γ and p100α in the fibrotic process.
Overall, these results suggest that specific class I PI3K isoforms can be pharmacological targets in IPF.

**P2006**

**Inhibition of TGF-β1-induced extracellular matrix production in primary human pulmonary fibroblasts by rapamycin**

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Fibroblasts proliferation and extracellular matrix (ECM) accumulation play a key role in the development and the progression of pulmonary fibrosis. Rapamycin has been showed to decrease extracellular matrix in normal mesangial cells and human lung fibroblasts. This study is to examine the role of rapamycin on transforming growth factor β1 (TGF-β1)-induced lung fibrosis and to determine the related mTOR signaling pathways in primary human pulmonary fibroblasts.Primary human pulmonary fibroblasts were isolated from healthy lung transplantation donor. Growth arrested, synchronized cells were treated with TGF-β1 (10ng/ml) and various concentrations rapamycin (0.01, 0.1, 1, 10ng/ml) for 24h. mTOR, p-mTOR, S6K1 and p-S6K1 were assessed by Western blot analysis, type III collagen and fibronectin secreting detected by Elisa assay, type III collagen and fibronectin mRNA level determined by Realtime-PCR assay. TGF-β1 (10ng/mL) increased type III collagen, fibronectin secretion and mRNA level obviously compared to controls ($p<0.05$), rapamycin reduced the enhanced production of type III collagen, fibronectin mRNA and protein induced by TGF-β1 accompanying inhibition of S6K and mTOR phosphorylation.These data demonstrated that rapamycin inhibited TGF-β1- induced typeIII collagen,fibronectin mRNA and protein may be through mTOR/p70S6K pathway, rapamycin maybe has potential effect for being used in the treatment of pulmonary fibrosis.

**P2007**

**Familial cases of idiopathic pulmonary fibrosis: Clinical observation**

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**Background:** Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive, often fatal interstitial lung disease of unknown etiology. Familial idiopathic pulmonary fibrosis (FIPF) is defined when two or more affected individuals are identified in one family. FIPF accounts for 0.5-2.2% of all IPF cases.
**Results:** We found 10 FIPF patients in 5 families among totally observed 475 IPF cases. There were 3 pairs of siblings (2 males and 4 females) and 2 pairs of mother and daughter. FIPF was diagnosed according to ATS/ERS criteria, histologically proven in 9 of 10 subjects. The mean age of IPF manifestation appears to be low: 37±5.3 years. All patients were treated by systemic corticosteroids, 4 of 10 - in combination with cytotoxic agents. 2 of 10 (mother and daughter in one family) have rather favorable course of IPF and are alive. The rest 8 cases had rapid severe progression of disease. Among them two patients are still alive, but demonstrate severe deterioration of their conditions, 6 - died within five years since first symptoms of disease occurred. The cause of death was progressive respiratory failure in 4 cases, respiratory failure with pneumonia in one case and lung cancer in another case.
**Conclusion:** 2.1% of our cohort of IPF patients has familial history of pulmonary fibrosis. Although rare, such cases represent an important subgroup in which genetic susceptibility to lung fibrosis plays a significant role. FIPF appears histologically indistinguishable from non-familial forms, but characterized by younger age of onset, resistance to treatment, progressive course and unfavorable prognosis. Molecular genetic analysis could improve our understanding of the pathogenic mechanisms of IPF.

**P2008**

**Paraquat-induced epithelial-mesenchymal transition: Role of Rac1b/Akt/Twist**

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**Objective:** To examine whether paraquat (PQ), a well-known reactive oxygen species (ROS) producer, could induce epithelial-mesenchymal transition (EMT) which involving in ROS-mediated pulmonary fibrosis and possible mechanisms.
**Method:** Human alveolar epithelial (A549) cells were cultured and exposed to sub-lethal doses of PQ, specific signaling pathway inhibitors and siRNAs for ROS signaling pathway. Intracellular ROS was measured with DCF-DA. Protein and RNA was evaluated by Western blot and real-time PCR, respectively.
**Results:** Intracellular ROS increased after various concentration paraquat stimulus for 5 minutes ($p<0.05$), while only PQ at the concentration of 20μM (PQ20) induced EMT manifested as increased fibronectin, decreased E-cadherin and a fibroblast-like cell appearance. mRNA of Twist, a key transcriptional factor for EMT, increased after PQ20 stimulus for 30minutes ($P<0.05$), accompanied with a rapid Twist nuclear translocation in A549 cells, while transfected with Twist cDNA antisense vector abolished PQ-induced EMT. Mitochondrial complex I inhibitor rotenone significantly inhibited PQ20-induced ROS. Small GTPase Rac1b involving in mitochondrial ROS mediated EMT was induced 5 minutes after PQ20 treatment, and siRNA targeted Rac1b blocked ROS generation and Akt phosphorylation induced by PQ20 ($p<0.05$). In addition, wortmannin, a PI3K inhibitor, could also block Akt phosphorylation and significantly reduce Twist nuclear translocation in A549 cells 2 hours after PQ20 treatment.
**Conclusion:** This study demonstrated that PQ could induce A549 cells EMT through Twist upregulation, which relied on Rac1b-dependent ROS generation/Akt signaling pathway and provided a better understanding of pulmonary fibrosis.

**P2009**

**The prevalence of neoplastic transformation in idiopathic pulmonary fibrosis (IPF) lungs. A report from a transplanted IPF population**

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Idiopathic pulmonary fibrosis (IPF) is known to be associated with increased risk of lung cancer but the potential pathological abnormalities that could precede the development of lung cancer are not well known. The aim of our study was to investigate the prevalence of high grade dysplasia/foci of neoplastic transformation (precancerous changes) and their relationship with metaplastic changes in honeycomb areas.
The lungs of 66 patients who underwent lung transplantation for IPF were studied. Scores were used to quantitate the degree of honeycomb changes and squamous, cuboidal and bronchial cell metaplasia in honeycombed areas. The presence or absence of precancerous changes in the same areas was recorded. Twelve of the 66 patients (18%) showed foci of neoplastic transformation/high grade dysplasia ("cancer" group). The "cancer" group (mean age at transplant 53±9 years; F:M=3:9) had similar smoking history, age and duration of disease than the "no cancer" group (mean age at transplant 57±6 years; F:M=17:37). Although all lungs showed metaplasia, the score of squamous ($p=0.0001$), cuboidal ($p=0.018$) and bronchial cell (0.018) metaplasia was significantly higher in the "cancer" than in the "no cancer", while the honeycomb score was similar in the two groups.
In conclusion, contrary to previous reports, we have found that the presence of metaplasia, specially squamous, in the honeycombed areas, is associated to the development of precancerous changes in IPF, independently to smoking history.